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Mercury Concentrations in Pacific Hake, *Merluccius productus* (Ayres), as a Function of Length and Latitude

Abstract. *Mercury concentrations in Pacific hake increase with fish size and with the latitude of collection. While the mercury-size trend is consistent with data for other species, the latitudinal trend is opposite to that reported for other fishes over the same geographical area. Consequently, latitudinal trends of mercury concentrations in fishes do not necessarily indicate trends of mercury concentrations in water. Food habits and metabolism may cause the observed variations.*

The discovery that mercury concentrations in some fishes exceeded levels allowed by regulatory agencies, and that mercury-polluted fishes caused many deaths in Japan, focused great concern on the use and control of mercury. We report a study of mercury in Pacific hake collected off the western U.S. coastline in 1969 to 1970 (*1*).

Mercury occurs in the environment both as a result of natural processes and as a pollutant from human activities. Jenne (*2*) concluded that high concentrations in western U.S. waters were more closely associated with the occurrence of mercury in rocks than with industrial discharges. However, even though northern California and Oregon have been the principal mining regions for mercury in the United States (*3*), Jenne also observed that the mercury in western waters was generally lower than in U.S. waters as a whole. On a global scale, Weiss *et al.* (*4*) estimated that natural degassing of the earth mobilizes more mercury than mining, smelting, or fossil fuel burning. They also suggested that man has increased the mercury content of the surface ocean by increasing the exposure of earth crust to the atmosphere. Nonetheless, it appears that increased mercury concentrations in marine fish by pollution has been proved only for relatively restricted bodies of water such as Minimata Bay (*5*) or local coastal areas (*6*).

Conversely, Young (*7*) reported that flatfish trawled from the vicinity of submarine outfalls in southern California showed no unusual accumulation of mercury even though the mercury content of discharge is many times higher than seawater, and sediments near the outfalls contain much higher mercury concentrations than similar sediments nearby. Barber *et al.* (*8*) concluded that the concentration of mercury in benthic fish depended more on species-specific metabolic factors and individual size than on the water content of mercury. A positive correlation of mercury concentration with size (age) has been reported for many fishes (*9–11*). This has generally been explained as an inability of fish to eliminate mercury at the rate at which it is assimilated.

Both the ecological availability of mercury to fish and the kinetics of turnover depend on chemical form. Methylmercury(II) (CH_3Hg^+) appears to be much more mobile in aquatic systems (*12*) and more readily assimilated by fish (*13*) than inorganic forms. Biological retention times are longer for methylmercury than inorganic mercury (*13, 14*) and a large proportion of the mercury found in fish is methylated (*15*).

Pacific hake, *Merluccius productus* (Ayres), spawn in winter along the coast of southern California (*16*). The young feed primarily on crustaceans, although a wide variety of prey have been identified in stomach contents (*17*). Adults migrate northward along the coastline during late spring and summer, feeding largely upon euphausiids supplemented by fish and pandalid shrimp (*18*). Many individuals migrate as far northward as the coast of Washington. During fall, hake appear to disperse offshore and to migrate southward. Adults are estimated to attain an age of 10 to 12 years and are presumed to migrate annually. A distinct nonmigratory population of the same species resides in Puget Sound.

Pacific hake were collected during their summer migration (*19*). Whole fish were taken directly from the trawl nets, placed in plastic bags, and frozen immediately after capture. They were stored on board ship at about −10°C and in the laboratory at about −25°C. Care was taken to avoid thawing the samples or rupturing the plastic bags during storage and transport.

For analysis the individual fish were selected at random, their lengths were

Table 1. Mercury concentrations in Pacific hake and results of linear least squares regressions on length for different latitudes. Standard errors are shown for means and for regression slopes.

North latitude	Number of fish analyzed	Mean mercury concentration [μg (g wet)$^{-1}$]	Regression on length	
			Slope (μg g^{-1} m^{-1})	r^2
32°52′	6	0.085 ± .007	0.42 ± .03	.98
37°03′	13	0.179 ± .008	0.81 ± .07	.94
38°41′	17	0.168 ± .007	0.37 ± .04	.86
39°16′	37	0.130 ± .007	0.58 ± .02	.94
40°08′	29	0.180 ± .008	0.77 ± .05	.91
41°32′	20	0.197 ± .010	0.80 ± .11	.74
42°23′	22	0.239 ± .005	0.59 ± .04	.93
43°23′	17	0.242 ± .007	0.67 ± .05	.93
45°49′ to 45°59′	30	0.286 ± .006	0.99 ± .07	.87
46°14′ to 46°55′	57	0.371 ± .005	0.83 ± .04	.88
47°10′	8	0.371 ± .007	0.45 ± .07	.88
48°00′ to 48°28′	15	0.383 ± .006	1.09 ± .03	.56
Puget Sound*	10	0.323 ± .011	0.70 ± .03	.98

*Not included in pooled data.

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measured, and the skin was removed from the frozen specimens. Approximately 30 g (wet) of muscle tissue was analyzed for mercury by flameless atomic absorption spectrophotometry with nitric acid and hydrogen peroxide digestion (*20*). Analytical procedures were tested by analyzing certified standards from the National Bureau of Standards and by interlaboratory comparison of results for selected samples (*21*).

Mercury concentration increased with size of Pacific hake at each latitude as shown by the positive slope of concentration versus length (Table 1). The values of r^2 imply that 56 to 98 percent of the variation in mercury concentration is related to variation in length. However, when all the data for the migratory population are pooled, the r^2 value is only .47 (Table 2), and the residuals from the regression display a positive correlation with latitude. Indeed, regression of mercury concentration on latitude yields an r^2 of .77, suggesting that the variation in mercury is more dependent on latitude than on length. Length and latitude are not independent, however, since fish from the northern stations are larger, on the average, than fish from the southern stations. Nevertheless, mercury concentration increases both with length and latitude (Fig. 1).

Multiple regression of mercury on length and latitude yields an r^2 of .89 and the coefficients for length and latitude are both highly significant (Table 2). Residuals from the multiple regression are

Table 2. Results of multiple regressions on length and latitude for pooled data. The pooled average mercury concentration is 0.249 ± .006 μg per gram (wet weight) (N = 271).

Independent variables	Regression slope (μg g^{-1} m^{-1} or μg g^{-1} degree^{-1})	r^2
Length	1.07 ± .07	.47
Latitude	0.0237 ± .0008	.77
Length plus latitude	0.59 ± .04 0.0193 ± .0006	.89

random when plotted against length but when plotted against latitude increase north of 45°N. This trend is also shown in Fig. 1 by the greater crowding of concentration lines toward the north.

Residuals for mercury on length for separate latitudes are sometimes concave downward. Because curves for age versus length are also concave downward (*16*), the rate of accumulation of mercury declines with age. For this reason, and because the residuals versus length are not random, nonlinear regression could yield a better fit to the data. Although such procedures would be useful for prediction of mercury concentration in individual fish, simple linear regressions demonstrate the dependence of concentration on length and latitude.

Dependence of mercury concentrations in Pacific hake on latitude is surprising in view of the migratory habits of the species. For example, an individual measuring 0.5 m in length collected at 37°N contained 0.2 μg of mercury per gram of body weight, and an individual of the same size taken at 47°N contained 0.4 μg/g (Fig. 1). If these individuals were from the same population, and if that population migrated annually, their mercury concentration would fluctuate by a factor of 2 in an annual cycle. Although we have not measured mercury concentrations in hake as a function of season, we deem such fluctuations highly improbable. Biological half-lives for methylmercury in fish are typically hundreds of days (*13, 14*), and the overall dependence of mercury on size (age) is normally attributed to lifelong net accumulation. The fact that the individuals were analyzed in random order rules out analytical error as a cause for the observed pattern. The residuals show no correlation with collection vessel or collection date.

Perhaps not all individuals migrate over the full range represented by our samples, and hake of the same size have different migratory ranges. This hypothesis implies that adults collected in winter on the spawning ground would have highly variable mercury concentrations, depending on the northward extent of their prior migrations. It would not, however, explain the reason for the increase in mercury concentration toward the north for hake of a given size. Zinc and cadmium, also in group IIB of the periodic table, show no such latitudinal trend, and radioactive zinc was maximal near the mouth of the Columbia River (*22*).

Hall *et al.* (*10*) found that mercury in halibut (*Hippoglossus stenolepis*) declined from Washington State northward to the Bering Sea, and Hall *et al.* (*11*) found mercury in sablefish (*Anoplopoma fimbria*) declined toward the north over the same range as our hake. This contrast precludes the possibility that the latitudinal trends in the concentrations of mercury in fishes reflect variations of concentration in the water. More probably they depend on species-specific metabolism and food habits, as suggested by Barber *et al.* (*8*). Without detailed knowledge of such factors it is not possible to conclude that fishes are indicators of water pollution patterns.

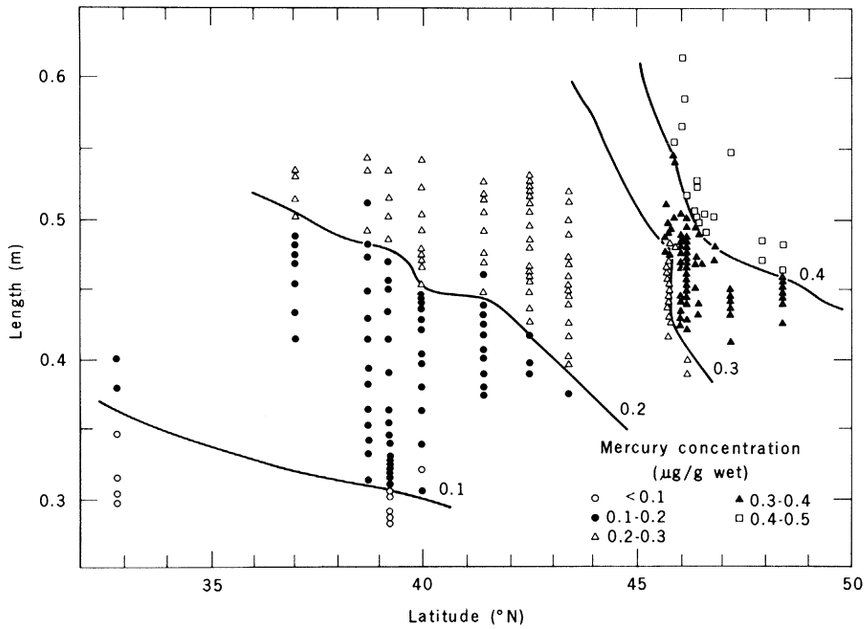

Fig. 1. Mercury concentrations in hake plotted and contoured as a function of length and latitude. The range symbols and the contours show dependence of mercury concentrations on both length and latitude of collection.

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Biochemical Evidence for Keratinization by Mouse Epidermal Cells in Culture

Abstract. *More than 70 percent of the urea-extractable proteins from mouse stratum corneum or from differentiated cells of mouse epidermis grown in culture are two proteins of molecular weight 68,000 (keratin 1) and 60,000 (keratin 2), which are present in equimolar amounts on polyacrylamide gels. These proteins are the subunits of the keratin filaments, because when isolated from stratum corneum or cells grown in culture they form native-type epidermal keratin filaments in vitro. These observations provide biochemical evidence that epidermal cells grown in culture synthesize the major differentiation products of the epidermis.*

Cultures of specialized cells have been extremely valuable for studies of cell biology, differentiation, and pharmacology and appear promising for investigations of chemical carcinogenesis. Primary epidermal cell cultures have been used for studies of differentiation and carcinogenesis (*1*). Recent reports have shown that human epidermal cell lines may also be valuable for such studies (*2*), which suggests that propagation of cells from diseased epidermis might shed light on the pathogenesis of the disease process. In previous reports of epidermal cell culture from any species, the determinants of differentiation have been the histochemical, morphological, or nonspecific chemical nature of the cells or cell products. This report presents specific biochemical evidence that the differentiation products of mouse epidermal cells in vitro are keratin proteins identical to those obtained from the fully differentiated stratum corneum of mouse skin.

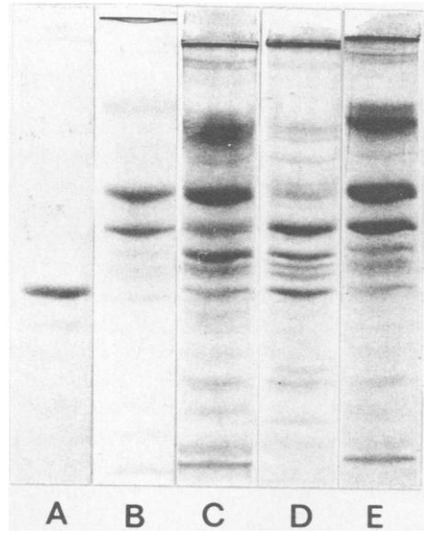

Fig. 1. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of urea-soluble proteins. Mouse muscle actin, mouse stratum corneum, or cell pellets were extracted in 8*M* urea, 0.05*M* tris-HCl buffer (*p*H 9.0), and 0.1*M* 2-mercaptoethanol for 4 hours at 23°C and centrifuged to remove debris. The supernatant was equilibrated in cathode buffer containing 0.5 percent (weight/volume) SDS and 0.1*M* 2-mercaptoethanol and heated at 95°C for 2 minutes. A portion containing 50 to 100 μg of protein was electrophoresed for 4 hours at 2 mA per gel on 0.6 by 10 cm 9 percent T and 3 percent C gels (*3*). Gels were stained with fast green and scanned at 610 nm with an Isco model 659 gel scanner. (A) Mouse muscle actin, (B) mouse stratum corneum, (C) mouse epidermal cells before culture, (D) attached cells from mouse epidermal cultures 4 hours after plating, and (E) unattached cells from mouse epidermal cultures 4 hours after plating. The bands of high molecular weight in (B) to (E) are noncovalently bound aggregates of the two keratin proteins.

Epidermal cells from newborn BALB/c mice were isolated (*1*) and cultured in medium 199 (National Institutes of Health Media Unit) containing 11 percent fetal calf serum (Reheis Chemical Co., Kankakee, Illinois) and 1 percent antibiotic-antimycotic solution (Gibco, Grand Island, New York). Cells were plated at 1×10^5 per square centimeter into 60- or 100-mm plastic tissue culture dishes and maintained at 37°C in a humidified atmosphere of 5 percent CO_2 and 95 percent air. Four hours after plating, unattached cells were collected from each dish by centrifugation of the medium and a saline wash at 1400 rev/min for 5 minutes. At subsequent times unattached cells, sloughed into the medium from the monolayer, were collected as above, and attached cells were harvested by scraping and centrifugation. Cell pellets were extracted with 8*M* urea buffer (legend, Fig. 1) and electrophoresed on sodium dodecyl sulfate polyacrylamide gels (*3*). Authentic stratum corneum, which was removed from newborn mouse skin during the isolation of cells for culture, was extracted similarly. Gels were prepared and evaluated as described in the legend of Fig. 1. Proteins were isolated from preparative gels and assayed for their ability to form 80 Å filaments in vitro (*4*).

Figure 1B represents a gel containing bands for two major proteins extracted from stratum corneum migrating and having molecular weights of 68,000 [upper band, keratin 1 (K_1)] and 60,000 [lower band, keratin 2 (K_2)]. These two

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