

AN ABSTRACT OF THE THESIS OF

JEAN-MARIE BLANC for the MASTER OF SCIENCE
(Name) (Degree)

in FISHERIES presented on Dec. 14, 1972
(Major) (Date)

Title: GENETIC ASPECTS OF RESISTANCE TO MERCURY

POISONING IN STEELHEAD TROUT (SALMO GAIRDNERI)

Abstract approved: Redacted for privacy
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Newly hatched steelhead alevins were obtained from a factorial breeding experiment in which 24 males were each mated to each of 10 females. These alevins were tested for tolerance to intoxication by methylmercuric chloride (CH_3HgCl), and mercury analyses were performed on samples of dead and tolerant alevins after the bioassay. The principal conclusions of this study were listed as follows:

1. A high mortality (about 80% of the tested alevins) resulted within two weeks from constant exposure to CH_3HgCl (8 ppb of mercury) in the water.
2. Tolerance to intoxication was measured by the time to death as well as by the percentage survival and had a high heritability (about 0.5).
3. The mercury concentration of the alevins that died from the

bioassay depended mainly on time to death with a negligible genetic influence.

4. The rate of mercury accumulation had a significant heritability (about 0.2) and was a factor influencing tolerance to intoxication.

5. Maternal effects and non-additive genetic effects on tolerance to intoxication and mercury accumulation were little or negligible.

Genetic Aspects of Resistance to Mercury
Poisoning in Steelhead Trout
(Salmo gairdneri)

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1973

APPROVED:

Redacted for privacy

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Date thesis is presented Dec. 14, 1972

Typed by Mary Jo Stratton for J. M. Blanc

ACKNOWLEDGMENTS

The author is indebted to Drs. R. C. Simon, J. D. McIntyre and K. E. Rowe for their helpful suggestions; and to B. McPherson for his important advice about the techniques and interpretation of mercury analyses.

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GENETIC ASPECTS OF RESISTANCE TO MERCURY POISONING IN STEELHEAD TROUT (SALMO GAIIRDNERI)

INTRODUCTION

Interest in the incidence of environmental contamination by mercury has been rapidly increasing in the past ten years. To gain an understanding of the effects of mercury on animals, especially fishes, toxicity bioassays have been conducted with various mercurial compounds. The interpretation of these experiments is based partly on the assumptions that individuals of a given species have approximately the same tolerance to specific lethal conditions and that the resistance of the population is not going to change in the near future. Such assumptions, however, do not seem to be consistent with experimental results. Among fishes of the same age within a given species, several investigators have observed a large variation in the rate of mercury accumulation (Bache et al., 1971; Hannerz, 1968) and in the tolerance to the toxicant (Akiyama, 1970; Amend et al., 1969). On the other hand it has been demonstrated that in a contaminated environment plant and animal populations are exposed to a selective pressure that may cause a progressive increase in tolerance to the contaminating toxicants (Bradshaw et al., 1965; Crow, 1957; Ferguson, 1967). Therefore genetic studies are necessary in an attempt to predict the short-term evolution of fish populations living in polluted waters, on

the basis of the fundamental concepts of quantitative inheritance (Falconer, 1960).

This study is concerned with the potential for genetic modification of steelhead (Salmo gairdneri) populations through the selective action of mercury. Accordingly, the specific objective was to estimate the heritability of resistance to mercury toxicity for steelhead and to relate that resistance to the mercury accumulation in the fish. A brief account of some of the methods and results has been presented earlier (McIntyre and Blanc, in press).

MATERIALS AND METHODS

Newly hatched steelhead alevins obtained from a diallel mating experiment (described in the next paragraph) were maintained during a two-week interval in water containing methyl-mercuric chloride (CH_3HgCl) at a concentration approximating 8 ppb of mercury. The number of alevins that died each day, the number of survivors at the end of the bioassay and the average mercury content in samples of dead and surviving alevins were recorded.

The matings were accomplished in May 1971 with 10 female and 24 male steelhead that were obtained from the Fish Commission of Oregon from the North Santiam River. The gametes of each fish were placed into separate polyethylene bags, kept cool over ice in styrofoam coolers and transported to Corvallis, Oregon. Within 8 hours, 240 matings were performed by fertilizing 24 subsamples of eggs from each of 10 females. Each male fertilized a single subsample of eggs from every female. The fertilized eggs were put into 240 separate cells in a Heath incubator. On July 7 two samples of approximately 20 eggs each were removed from each cell of the incubator and placed into separate cells in the bioassay apparatus that was contained in a constant temperature room at 11°C . This apparatus consisted of a series of four enameled troughs with interconnections to permit a closed water circuit. Dechlorinated water was propelled

by a small pump and a constant supply of air was introduced into a reservoir within the system. The total volume of water was 213 liters and the flow was maintained between 500 and 1,000 ml per minute. Each trough contained 120 cylindrical cells made from 2 in. PVC pipe that had been cut to a length of 2 in. These cells were cemented together with plastic cement and closed at the bottom by a continuous plastic screen. The block thus obtained was supported by glass rods at 1/2 in. above the bottom of the trough so that the water could freely circulate under it and irrigate each cell. Placement of the 480 samples of eggs into the cells was done in a randomized fashion to minimize any factorial effect related to location in the apparatus.

Hatching occurred between July 8 and July 17 and the bioassay was initiated on July 19. The eggs and alevins were maintained with negligible mortality until July 20. After the onset of the experiment, analyses showed diminishing mercury levels which probably arose from a combination of fish absorption, evaporation and adsorption on the container walls and cells. Additional toxicant was retained in a carbon filter that was operated for 24 hours to reduce metabolic wastes. Hence, it was necessary to add CH_3HgCl to the water at irregular intervals to maintain the concentration at approximately 8 ppb. Mercury concentration was checked at least once a day and was found to fluctuate between 6 and 10 ppb with an average of 8 ppb.

Mortality began on July 21, increased until July 24 and then decreased progressively until August 2. During this period, the dead alevins in each cell were counted daily, placed into plastic bags and stored in a freezer for mercury content analysis (one bag was used for each cell, and 478 samples were thus stored). On August 2 the experiment was concluded and the number of survivors in each cell was recorded. Samples of 10 alevins were taken out of the 62 cells that showed the highest survival and frozen. An attempt was then made to maintain the other survivors in dechlorinated water, but these alevins suffered from fungi infections and died within two months.

During the summer of 1972 the 540 frozen samples of alevins were analyzed for total mercury content. Each sample was thawed, weighed, digested at about 50°C using nitric and sulphuric acids, oxidized with potassium permanganate and hydrogen peroxide and analyzed by flameless atomic absorption spectrophotometry (Uthe et al., 1970, modified by McPherson, personal communication, see Appendix I). Tests showed that this method recovered about 90% of the total mercury with a relative error less than 10%. The wet weight of the samples, however, could not be obtained with high precision, due to the presence of variable amounts of residual water on the skin of the alevins. It was estimated that the mercury content of each sample was estimated with a standard error equal to about 10% of the true concentration.

RESULTS

Time to Death

Approximately 81% of the total population (7,635 alevins) died between the second and fourteenth day of the bioassay with an apparently log-normal distribution (Table 1). The results were closely similar in the three first troughs of the apparatus, longer survival times being observed in the fourth one (see Appendix II). A logarithmic transformation was therefore applied to time of death (counted in days from the beginning of the bioassay) to approximate a normal distribution. The transformed data (variable x) were treated by standard methods of analysis of variance and, from the expected mean squares for this analysis, the variance components attributable to the sources of variation studied were estimated with their respective standard errors (Becker, 1967; Snedecor and Cochran, 1967) as presented in Table 2. These results indicate that the interaction effect is not significant and that the variances σ_M^2 and σ_F^2 are not significantly different. The additive genetic variance can therefore be estimated as $\sigma_A^2 = 2(\sigma_M^2 + \sigma_F^2) = 9.54 \times 10^{-3}$ which leads to the following estimated heritability:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} = 0.62$$

Table 1. Evolution of a population of steelhead alevins maintained in water containing methyl-mercuric chloride (8 ppm of mercury).

Date		Time to death (days)	No. of dead fish each day	No. of survivors
July	19	0	0	9,441
	20	1	0	9,441
	21	2	59	9,382
	22	3	157	9,225
	23	4	996	8,229
	24	5	3,191	5,038
	25	6	1,156	3,882
	26	7	966	2,916
	27	8	712	2,204
	28	9	214	1,990
	29	10	85	1,905
	30	11	29	1,876
	31	12	20	1,856
August	1	13	25	1,831
	2	14	25	1,806

Table 2. Variance and covariance components estimated for the logarithm of time to death (x) and the probit of percentage of survival (y).

Source of variation	Notation	Variances		Covariances (x, y)	
		Estimation for x	Estimation for y	Notation	Estimation (from cell means)
Male	σ_M^2	2.21×10^{-3} ($\pm 0.71 \times 10^{-3}$)	0.256 (± 0.085)	cov_M	23.5×10^{-3} ($\pm 7.5 \times 10^{-3}$)
Female	σ_F^2	2.56×10^{-3} ($\pm 1.14 \times 10^{-3}$)	0.211 (± 0.098)	cov_F	20.8×10^{-3} ($\pm 9.9 \times 10^{-3}$)
Interaction	σ_{MF}^2	0.45×10^{-3} ($\pm 0.37 \times 10^{-3}$)	0.109 (± 0.054)	cov_{MF}	14.1×10^{-3} ($\pm 2.9 \times 10^{-3}$)
Replicate	σ_R^2	4.57×10^{-3} ($\pm 0.46 \times 10^{-3}$)	0.337	cov_{RW}	10.7×10^{-3}
Residual	σ_W^2	5.60×10^{-3}	1.000		
Total (between-cells)	-----			cov_p	69.1×10^{-3}
Total	σ_P^2	15.39×10^{-3}	1.913		

Approximate standard errors are included in parentheses.

with the standard error, S. E. (h^2) = 0.18 (Becker, 1967). Since time to death could not be defined for the survivors, a source of error arises from the fact that the sample size in each cell is variable and is correlated with the average time to death (see below). Furthermore, the results were obtained from a biased sample of the alevin population, which causes the variances to be underestimated. Another important source of error arises from the difficulty of recording the deaths of such large numbers of alevins at less than 24-hour intervals. This procedure created a class effect which resulted in an underestimation of the residual component of variance σ_W^2 .

Percentage Survival

To avoid some of these biases as well as to better estimate the selective value of the alevins, a similar analysis was carried out on the basis of the percentage survival which was measured for each cell. A large variation was found, 176 cells having no survivors and the other ones ranging from 1 to 20 survivors. To obtain a variable (y) having a normal distribution and a relatively stable variance, the probit transformation was used on the percentages survival that were greater than zero (Bliss, 1935). The transformed data were found to be linearly correlated to the mean logarithms of time to death of the corresponding cells. The regression equation of the first variable (y) on the second (x) was estimated as $\hat{y} = 9.863x - 3.878$ and was

used to discriminately estimate the probit of the percentage survival for those 176 cells that showed a complete mortality. Very low values were thus estimated that could not have been directly measured, due to the relatively small number of alevins per cell. A variance analysis was then carried out on the whole set of data and the variance components were estimated. According to the definition of the probit transformation the residual variance σ_W^2 was given the value 1.000 (Table 2). The interaction variance σ_{MF}^2 was low but significant. Since there was no significant difference between σ_M^2 and σ_F^2 , the additive genetic variance was estimated from both variances as $\sigma_A^2 = 0.934$ and the heritability was therefore $h^2 = 0.49$ with standard error, S. E. $(h^2) = 0.14$. There is no apparent major difference between these results and those relating to time of death. Even when considering the fact that a part of the cell probits was estimated by regression and was therefore equivalent to transformed times of death, it was hypothesized that time to death and percentage survival are two different expressions of the same fundamental character which is the tolerance to the lethal conditions of the bioassay.

To test that hypothesis the two variables were considered simultaneously in a covariance analysis (Becker, 1967). Estimation of the mean products was performed on the basis of the percentages survival that were directly measured, thus avoiding a bias in the covariances attributed to the major sources of variation studied

(Table 2). This method, however, resulted in an underestimation of the standard errors of these covariances. Furthermore, since the within-cell covariance was not defined, the phenotypic correlation was computed from the total variances and covariance between cells as

$$r_p(x, y) = \frac{\text{cov}_p(x, y)}{\sqrt{\sigma_p^2(x) \sigma_p^2(y)}} = 0.61.$$

The additive genetic covariance may be computed as $\text{cov}_A(x, y) = 2(\text{cov}_M + \text{cov}_F) = 88.6 \times 10^{-3}$, leading to the approximate estimation of the additive genetic correlation

$$r_A(x, y) = \frac{\text{cov}_A(x, y)}{\sqrt{\sigma_A^2(x) \sigma_A^2(y)}} = 0.94.$$

No reliable estimation can be obtained for the environmental correlation due to excessive errors in estimating the corresponding variances and covariance. The important result, however, is the evidence of a high additive genetic correlation between the percentage survival and the time to death of the alevins that do not survive, showing that these characters are mainly controlled by the same gene complex.

Mercury Accumulation and Its Relation to Survival

Total mercury analyses provided the average mercury content in ppm for the fish that died in each cell. The concentrations ranged from 0.6 to 17.1 ppm, with a distribution which was grossly

log-normal. A major maximum was found between 1 and 2 ppm along with a lesser maximum between 9 and 11 ppm (see Appendix III). A similar bimodal distribution was found by Hannerz (1968) though not explained. To bring some improvement to that distribution as well as to stabilize the variance due to measurement errors, the logarithmic transformation was again used. Though the transformed data (variable z) showed an important deviation from normality, standard methods of analysis of variance were applied to test the genetic significance of mercury concentration in fish, considered as a maximum tolerance limit that could not have been reached without the onset of death. Components of variance were obtained (Table 3) which show a very important influence of environmental factors, a negligible interaction effect and a large but not significant superiority of the female component relative to that of the male, the latter being not significantly different from zero. From both components, the additive genetic variance may be estimated as $\sigma_A^2 = 12.80 \times 10^{-3}$ and the heritability as $h^2 = 0.12$ with standard error, S. E. (h^2) = 0.07. The statistical significance of the additive genetic effect, if any, is therefore doubtful, particularly if we consider the possible existence of a maternal effect. When comparing these results to those relative to survival, it appears that the average mercury concentration in the alevins can hardly be considered as a threshold that could explain the genetic variation in survival.

Table 3. Variance components estimated for the logarithm of mercury concentration in alevins that died during the bioassay (z) and covariance components estimated between z and x and between z and y.

Source of variation	Variances (z)		Covariances		
	Notation	Estimation	Notation	Estimation for (z, x)	Estimation for (z, y)
Male	σ_M^2	0.94×10^{-3} ($\pm 1.76 \times 10^{-3}$)	cov_M	1.85×10^{-3} ($\pm 0.94 \times 10^{-3}$)	25.7×10^{-3} ($\pm 9.9 \times 10^{-3}$)
Female	σ_F^2	5.46×10^{-3} ($\pm 3.23 \times 10^{-3}$)	cov_F	3.03×10^{-3} ($\pm 1.69 \times 10^{-3}$)	29.0×10^{-3} ($\pm 15.3 \times 10^{-3}$)
Interaction	σ_{MF}^2	0.62×10^{-3} ($\pm 6.73 \times 10^{-3}$)	cov_{MF}	-0.33×10^{-3} ($\pm 1.33 \times 10^{-3}$)	-64.7×10^{-3} ($\pm 14.0 \times 10^{-3}$)
Residual (replicate)	σ_{RW}^2	99.79×10^{-3}	cov_{RW}	15.68×10^{-3}	119.4×10^{-3}
Total (between cells)	σ_p^2	106.81×10^{-3}	cov_p	20.23×10^{-3}	109.4×10^{-3}

Approximate standard errors are included in parentheses.

For a better comparison, however, covariance analyses (Becker, 1967) were performed between the log-transformed concentration (z) and each of the two variables characterizing the tolerance to the toxicant (x and y). The covariance components (Table 3) can be used to compute the phenotypic (between cells) correlation (r_p) and additive genetic correlation (r_A): (1) concerning mercury concentration and time to death, $r_p(z, x) = 0.61$ and $r_A(z, x) = 0.88$; and (2) concerning mercury concentration and percentage survival, $r_p(z, y) = 0.30$ and $r_A(z, y) = 1.00$. The above results have only an indicative value, since they have been obtained with substantial irregularities which are not taken into account in the standard errors listed in Table 3. The main source of error is probably the fact that the logarithm of the mean mercury concentration per cell does not follow a normal distribution and does not have the same statistical properties as the mean logarithm of individual measurements. Also, the lack of within-cell data caused the phenotypic variance and covariances to be underestimated, which is important to remember when considering the heritability of mercury concentration and the phenotypic covariances. However, beyond the doubt caused by these errors, relationships exist between the mercury concentration in the fish that died during the bioassay and both variables characterizing the resistance to the toxicant. The interpretation of these relationships is probably complex.

To study the accumulation of toxicant in the fish through time, the regression of the logarithm of the average mercury concentration in the dead fish of each cell (z) on their mean logarithm of time to death (x) was computed (Snedecor and Cochran, 1967). This regression was found to be approximately linear and was estimated as $\hat{z} = 2.041x - 0.906$ which is equivalent to:

Expected ppm mercury in dead fish =

$$\frac{1}{10^{0.906}} (\text{time to death in days})^{2.041}.$$

Thus, the quantity of mercury accumulated per day is not constant but increases through time. However, a slight tendency was noticed for the mercury concentration to stabilize in the cells having a high survival, which suggests that the mercury accumulation curve would be sigmoid rather than parabolic. A similar depiction of mercury accumulation in fish through time has been noted by Hannerz (1968). Since in the present study the correlation $r_p(z, x)$ previously estimated as 0.61 was not high enough to allow definition of clear relationship, the linear regression between the log-transformed variables was used in first approximation.

The large variation of mercury concentration between samples of alevins having the same average time to death was obviously the result of differences in the rate of mercury accumulation. To appreciate the effect of genetic and environmental factors on this

character, the deviation of the logarithm of the average mercury concentration of the dead fish in each cell from the regression line ($z-\hat{z}$) was computed. Since normality was approximated, the significance of deviations was tested using an analysis of variance from which the components of variance were estimated (Table 4). It was found that the greatest part of the variance was due to environmental factors which could be related to the circulation of water in the experimental troughs. First, the variability between successive cells that were ranked along the water flow was lower than the variability between cells ranked in the perpendicular direction. Besides, whenever the design of the apparatus allowed to predict a difference in the circulation of water between two areas of a trough, a corresponding difference was found in the rate of mercury accumulation. There was no significant interaction effect and the components of variance due to male and female effects were similar enough to allow the estimation of the additive genetic variance from both components: $\sigma_A^2 = 12.28 \times 10^{-3}$. The heritability is therefore $h^2 = 0.18$ with the standard error S. E. (h^2) = 0.08. A significant additive genetic effect on the speed of mercury accumulation is indicated. This may explain a part of the genetic variability in tolerance to the toxicant.

Because of this significance of additive genetic effects, covariance components were computed (Table 4) between the deviation of the logarithm of mercury concentration from its regression on the logarithm of time to death ($z-\hat{z}$) and (1) the logarithm of time to death

Table 4. Variance components estimated for the deviation of the logarithm of mercury from its regression on time to death ($z - \hat{z}$) and covariance components estimated between $z - \hat{z}$ and x and between $z - \hat{z}$ and y .

Source of variation	Variances ($z - \hat{z}$)		Covariances		
	Notation	Estimation	Notation	Estimation for ($z - \hat{z}, x$)	Estimation for ($z - \hat{z}, y$)
Male	σ_M^2	2.59×10^{-3} ($\pm 1.69 \times 10^{-3}$)	cov_M	-2.68×10^{-3} ($\pm 0.92 \times 10^{-3}$)	-20.8×10^{-3} ($\pm 9.9 \times 10^{-3}$)
Female	σ_F^2	3.55×10^{-3} ($\pm 2.10 \times 10^{-3}$)	cov_F	-2.16×10^{-3} ($\pm 1.26 \times 10^{-3}$)	13.3×10^{-3} ($\pm 11.1 \times 10^{-3}$)
Interaction	σ_{MF}^2	4.92×10^{-3} ($\pm 4.09 \times 10^{-3}$)	cov_{MF}	-1.35×10^{-3} ($\pm 0.90 \times 10^{-3}$)	-77.4×10^{-3} ($\pm 11.2 \times 10^{-3}$)
Residual (replicate)	σ_{RW}^2	55.63×10^{-3}	cov_{RW}	5.91×10^{-3}	62.1×10^{-3}
Total (between-cells)	σ_P^2	66.69×10^{-3}	cov_P	-0.28×10^{-3}	-49.4×10^{-3}

Approximate standard errors are included in parentheses.

Note: Since \hat{z} is the regression estimation of z from x , $\text{cov}_P(z - \hat{z}, x)$ is theoretically null. In this table, however, this total covariance is computed as the sum of the covariance components, which slightly differs from the total mean product.

(x), and (2) the probit of percentage survival (y). The first covariance partitioning leads to estimate the phenotypic (between cells) correlation (r_p) and the additive genetic correlation (r_A) as $r_p(z-\hat{z}, x) = -0.01$ and $r_A(z-\hat{z}, x) = -0.89$. The corresponding values obtained from the second variance partitioning are $r_p(z-\hat{z}, y) = -0.17$ and $r_A(z-\hat{z}, y) = -0.64$ respectively. Both computations in addition result in positive environmental components of covariance. The fact that $r_p(z-\hat{z}, x)$ is practically equal to zero is due to the definition of the first variable as a deviation from a regression on the second one, which causes the total sum of squares to be exactly null. The value $r_p(z-\hat{z}, y) = -0.17$ is therefore a much better estimation of the phenotypic correlation between the rate of mercury accumulation and resistance to the toxicant. A statistical consequence of this result is a compound relationship between the time to death and the mercury concentration finally attained. On one hand, a longer survival time leads to a greater accumulation of toxicant. On the other hand, and to a much lesser extent, a greater accumulation of mercury results in shorter survival time. Therefore, the regression computed between mercury concentration and time to death underestimates the real accumulation of toxicant through time. However, since $r_p(z-\hat{z}, y)$ is small, this bias is probably of little importance, particularly when compared to the more serious errors due to the distribution of z and to the lack of within-cell components of variance and covariances.

To complete the results relative to mercury accumulation and its relation with the tolerance to the toxicant, the samples of survivors (10 each) taken out of the 62 cells with best survival were analyzed for total mercury concentration. Mercury concentration was found to range between 2.7 and 21.2 ppm with a mean of 12.29 ppm. A logarithmic transformation was used on these data to equalize the variance due to measurement errors. The mean concentration computed on a logarithmic scale was 10.84 ppm. Extrapolating without caution the regression line of the logarithm of mercury concentration (z) on the logarithm of time to death (x) would predict that at the end of the bioassay (fourteenth day) the logarithmic mean of mercury concentration in the fish should be 27.1 ppm. This is much higher than the average actually observed among survivors. The discrepancy may be mainly explained by the tendency indicated earlier of the mercury concentration to reach a plateau at the end of the experiment. It is also possible that the survivors accumulated mercury somewhat slower than did the other fish. Since the mercury concentration among survivors was recorded only for a small number of cells, no meaningful partitioning of variance could be obtained. By subsampling the groups of survivors, the within-cell variance could be roughly evaluated as 10 to 20% of the total phenotypic variance. The relative insignificance of the variance caused by the individual within-cell variation and the measurement errors was furthermore confirmed by the strong

correlation (0.95) existing between the logarithm of mercury concentration in the mortalities and in the survivors. Finally, no significant correlation could be found between mercury concentration in the survivors and the tolerance to the toxicant, due perhaps to the small number of cells studied and the large variability of mercury concentrations. It was observed, however, that the alevins from the cells having more than 70% survival contained slightly more mercury than the average of all cells. This may be related to the earlier finding of a positive environmental correlation between the rate of mercury accumulation through time ($z - \hat{z}$) and the tolerance to the toxicant (x or y).

DISCUSSION

Bioassays designed to analyze the variability of resistance to a toxicant are neither simple to perform or to interpret. Very large samples are required, which causes measurements to be unmanageably numerous. The large size of the experimental apparatus itself is partly responsible for problems encountered in performing the bioassay. Furthermore, synergistic effects of the toxicant with other environmental factors may cause secondary phenomena including interactions among fishes, particularly within the experimental cells. These phenomena can hardly be separated from the main toxic effects, even if a control experiment is conducted. Although no actual control experiment could be maintained in this study due to space limitations, the capacity of the apparatus to keep the eggs and the alevins alive in the absence of mercury was demonstrated.

The significant amounts of mortality of steelhead alevins that resulted from exposure to the methyl-mercuric chloride concentration used in the bioassay demonstrated the extreme sensitivity of these fish to this compound. To evaluate the sensitivity of an individual or a group of individuals, several measurements can be used which are not a priori equivalent one to the other. In this experiment however, tolerance to the toxicant appeared to be represented by the time to death as well as by the percentage survival, provided that adequate

transformations were used on the raw data to allow a meaningful statistical treatment. On that basis, reliable conclusions can be obtained from a study of time to death whenever the measurement of the actual survival rates is impossible or lacks precision. In this experiment, the number of alevins per cell was not large enough to provide a good estimation of very high or very low values of percentage survival.

The extremely large variability found in the tolerance to CH_3HgCl was partly due to the fact observed later that the mercury was not equally distributed throughout the experimental apparatus. About half of this variability, however, was attributed to additive genetic effects. This relatively high proportion may be related to the apparent lack of natural selection for resistance to mercury poisoning among the steelhead populations from the Santiam River. It also implies that the mean resistance of these steelhead can be expected to increase if mercury-related mortality occurs. But the amount of this increase depends partly on the fate of the survivors. The heritability estimated in this study is meaningful only to the extent that apparently resistant alevins can fully survive and contribute to the gene pool of the next generation. Though adults are likely to be less sensitive than alevins (Akiyama, 1970), long-term studies are necessary to provide some knowledge about the effects of mercury intoxication throughout the entire life-cycle of the fish, including the gametic stage.

Interpretation of the results is further restricted and compromised by the expectation that genetic resistance to mercury will not be due to the same gene combinations at concentrations and exposure times different from those of the present experiment.

The evidence of an important genetic contribution to mercury resistance raises several questions concerning the physiological mechanisms through which this contribution acts. It may be the result of factors preventing the accumulation of toxicant in the body, as well as factors increasing the amount of toxicant that can be tolerated in the tissues prior to death. This study shows that genetic mechanisms of the first type do exist, since the rate of mercury accumulation through time is partly heritable and is negatively correlated to survival. Environmental factors, however, seem to be the cause of a positive covariance component between the two characters, and therefore to affect these characters through some physiological mechanisms that are not the result of genetic influence (Falconer, 1960). Unfortunately, this study does not provide enough information for further analysis since several environmental factors could vary simultaneously with the flow of water among the cells of the experimental apparatus. In particular, the distribution patterns of oxygen and mercury were probably closely related. Due to the action of mercury on the gills (see below), oxygen might have become a limiting factor, in which case survival would have increased in the

well irrigated cells in spite of an increase in the accumulation of mercury. It is also possible that the healthier alevins had a higher metabolic rate causing them to absorb more mercury. Further experiments are obviously necessary.

In general, the rate of accumulation is the result of two simultaneous processes, namely uptake and elimination. In the case of mercury, however, it has been demonstrated that elimination is very slow as compared with uptake (Hannerz, 1968; Miettinen et al., 1969). Correspondingly in this study the alevins appeared to accumulate mercury continuously, though with variable rate. The rate of accumulation probably decreased in the last days of the experiment among the fish that survived to that time. Such a tendency unfortunately could not be clearly demonstrated in the absence of individual analyses of these fish. The fact that mortality became negligible at the end of the bioassay possibly suggests that the alevins which finally survived had reached an equilibrium stage where the uptake of mercury was exactly balanced by its elimination.

It appears from this study that the variability existing in the rate of mercury accumulation explains only a part of the variability in the survival. The possible existence of factors determining the maximum amount of toxicant compatible with survival must therefore be considered. From that standpoint, the results presented here appear somewhat disappointing. The average mercury concentration at time

of death seems to be mainly determined by environmental factors without significant genetic control, and its correlation with survival cannot be clearly interpreted since the time to death directly influences the mercury concentration whatever the actual cause of death. This does not prove, however, that no genetic control of the maximum amount of toxicant allowing for normal function of some particular organ exists. According to several investigators (Bäckström, 1969; Hannerz, 1968; Rucker and Amend, 1969), mercury accumulates mainly in the liver, the spleen, the kidneys and the gills, while the concentrations found in the brain and muscles are greatly less. The damage caused by mercury to various organs, on the other hand, is not proportional to its accumulation. It appears that the fish die mainly from suffocation caused by damage to the gills (Akiyama, 1970; Amend et al., 1969), although toxic effects are also observed in the kidneys and nervous system (Bäckström, 1969). Therefore, the average mercury concentration in the whole fish is not a good indicator of the degree of intoxication, which partly depends on the distribution of mercury among the organs. The distribution pattern and its evolution through time then should be studied from a genetic standpoint when possible in relation to survival.

Lastly, this study appears to give some basis for increased confidence that valuable fish populations may be maintained by natural selection in spite of increasing environmental contamination. This

selection however, by influencing other characteristics, may render the populations less viable or desirable. One important consideration in an ecological context is the relative amount of mercury that is concentrated in the tissues of surviving individuals. Though resistance to mercury seems to result partly from factors preventing its accumulation in the fish, a doubt remains as to what extent an increase of this resistance might be accompanied by an increase of the amount of toxicant tolerated in the fish tissues prior to death. Some tissues could become a dangerous food product for consumer species including man. In the light of these considerations the need for better knowledge of the potential influence of pollutants on fish can be appreciated.

CONCLUSIONS

The principal conclusions of this study that are supported by statistical evidence are listed below. To be placed in proper perspective they must be compromised to the extent suggested in the discussion.

1. A high mortality (about 80% of the tested alevins) resulted within two weeks from constant exposure to CH_3HgCl (8 ppm of mercury) in the water.
2. Tolerance to intoxication was measured by the time to death as well as by the percentage survival and had a high heritability (about 0.5).
3. The mercury concentration in the alevins that died from the bioassay depended mainly on time to death with a negligible genetic influence.
4. The rate of mercury accumulation had a significant heritability (about 0.2) and was a factor influencing tolerance to intoxication.
5. Maternal effects and non-additive genetic effects on tolerance to intoxication and mercury accumulation were little or negligible.

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APPENDICES

APPENDIX I

PREPARATION OF ALEVIN SAMPLES FOR
TOTAL MERCURY ANALYSIS

Each sample (1 to 20 alevins) was weighed and put into a 125 ml Erlenmeyer flask. The flask was stoppered with a rubber stopper protected by a piece of "Parafilm." The following steps were then performed:

1. Three to five ml of concentrated nitric acid (HNO_3 70%) were added and the flask was put into a 50°C water bath for one hour.
2. After 10 min. refrigeration at about 1°C , 5 to 8 ml of concentrated nitric-sulphuric acid mixture (HNO_3 70% + H_2SO_4 , at equal volumes) were added and the flask was put into the 50°C water bath for two to three hours. This resulted in the complete digestion of the sample.
3. After 10 min. refrigeration, 10 to 15 ml of potassium permanganate solution (KMnO_4 6%) were added carefully in two portions with slow stirring. The flask was then put into the water bath for 1-1/2 hours.
4. The flask was taken out of the water bath, and about 2 ml of potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$ 5%) were added. The flask was left at room temperature for the next half hour.
5. Hydrogen peroxide (H_2O_2 10%) was then added dropwise until the brown coloration due to permanganate disappeared.

6. The sample was then filtered on glass wool to remove the fat, and distilled water was added to adjust the volume to 100 ml in a volumetric flask.

7. After homogenization, 10 ml of the obtained sample were analyzed by following the standard procedure supplied by Coleman Instruments Co. for apparatus model MAS-50.

APPENDIX II

COMPARED MORTALITIES IN THE FOUR TROUGHS
OF THE BIOASSAY APPARATUS

Time of death (days)	Trough number			
	1	2	3	4
2	31	23	5	0
3	50	75	24	8
4	274	392	219	111
5	740	943	1,031	477
6	340	235	279	302
7	142	94	160	570
8	115	88	83	426
9	81	60	51	22
10	37	21	25	2
11	10	12	5	2
12	3	7	9	1
13	7	7	7	4
14	7	9	8	1
Mean time of death (days) derived from logarithms:	5.415	5.130	5.415	6.283
No. of survivors	472	403	471	460
No. of alevins tested	2,309	2,369	2,377	2,386
Percentage survival	20.4%	17.0%	19.8%	19.3%

APPENDIX III

DISTRIBUTION OF THE MERCURY CONCENTRATION FOUND
IN 478 SAMPLES OF ALEVINS THAT DIED
DURING THE BIOASSAY

Mercury concentration (ppm)	No. of samples	Mercury concentration (ppm)	No. of samples
0.0 - 0.9	12	9.0 - 9.9	34
1.0 - 1.9	70	10.0 - 10.9	36
2.0 - 2.9	57	11.0 - 11.9	13
3.0 - 3.9	48	12.0 - 12.9	10
4.0 - 4.9	53	13.0 - 13.9	10
5.0 - 5.9	37	14.0 - 14.9	3
6.0 - 6.9	31	15.0 - 15.9	3
7.0 - 7.9	33	16.0 - 16.9	0
8.0 - 8.9	27	17.0 - 17.9	1