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Effects of Water-Hardening Eggs in a Betadine or Erythromycin Solution on Hatching Success, Development, and Genetic Characteristics of Rainbow Trout

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Abstract.—We investigated genetic and developmental effects of using a Betadine (povidone–iodine) or erythromycin solution to water-harden eggs from rainbow trout (*Oncorhynchus mykiss*). Electrophoretic data indicated that no genetic differences existed between control fish and fish from erythromycin-treated eggs. In contrast, a small genetic difference existed between control fish and those from Betadine-treated eggs. The mean counts of five bilateral meristic characters were similar between fish from control and treatment groups, indirectly suggesting that the treatments did not influence development rate. Reduced hatching success was observed from eggs hardened in Betadine solution. Erythromycin appears to have disrupted the development of the fish, as indicated by the increased asymmetry (differences in left- and right-side counts) exhibited by meristic characters. These deleterious effects of the treatments, however, were not large, and in most situations the effects will be far outweighed by the value of these treatments as a means of controlling disease transmission.

The prevention and control of diseases are major concerns of hatchery managers. To minimize the transmission of diseases from parents to offspring, eggs of salmonid fishes are often water-hardened in a solution of Betadine or erythromycin. These treatments are valuable for disease control, but the possibility that they may adversely affect other characteristics of the fish is largely uninvestigated. This possibility is a growing concern of hatchery personnel. Therefore, we compared hatching percentages, developmental characteristics, and genetic characteristics of rainbow trout (*Oncorhynchus mykiss*) produced from eggs water-hardened in water (control), a Betadine solution, and an erythromycin solution.

An individual's genetic composition (genotype) can influence its susceptibility to pathogens and toxicants (Frelinger 1972; McKenzie and Clarke 1988). Diseases and toxicants, therefore, can alter the genetic characteristics of a population by imposing selective mortality. That is, individuals with susceptible genotypes are more likely to die from these factors than are individuals with resistant genotypes. Thus, a change in the genetic characteristics of a population exposed to a toxicant under controlled conditions indicates that selective mortality was associated with exposure.

In salmonid fishes, it is not unusual to find that the counts of a bilateral meristic character differ between the left and right sides of an individual. This condition is generally termed asymmetry. Because each side of a fish is genetically identical, development is programed to produce equal counts on both sides. The existence of asymmetry, therefore, indicates that development has deviated from the targeted outcome. This results when individuals are not able to precisely control and integrate the processes responsible for the differentiation of a character on each side. Levels of asymmetry thus provide information about the ability of individuals to develop normally. Increased asymmetry indicates increased perturbation of development.

Methods

Spawning and rearing procedures.—On September 9, 1987, gametes were obtained from ten 4-year-old female and ten 2-year-old male rainbow trout of the Arlee strain. This strain is maintained by the Montana Department of Fish, Wildlife, and Parks at the Jocko River State Trout Hatchery, Arlee, Montana, and its history was presented by Leary et al. (1983). Prior to fertilization, eggs from all females (≈250 each) were pooled and thoroughly mixed. Milt from all males (1 mL each) was pooled and used to fertilize the eggs. The eggs were divided into three groups 30 s after fertilization. (Although replication would have strengthened the reliability of the data, space con-

straints precluded replicates.) A control group of eggs ($N = 860$) was hardened for 1 h in hatchery water (pH 7.95). Another group of eggs ($N = 956$) was water-hardened for 1 h in a Betadine solution containing 125 mg of active iodine per liter of hatchery water. Baking soda was added (1.06 g/L) to buffer the solution to pH 8.0. The third group of eggs ($N = 836$) was hardened for 1 h in a solution of hatchery water and erythromycin (13.2 mg/L, pH 7.95). Water-soluble erythromycin was obtained from Gallimycin (0.23 g erythromycin/g; Abbott Laboratories). The eggs were then placed in a partitioned trough for incubation and rearing of juveniles. During the first 21 d of incubation, the eggs were treated with formalin to prevent fungal growth. The percentage of eggs hatched in each group was determined by numerical counts. On March 24, 1988, fish from each treatment group were sacrificed for electrophoretic and meristic analyses. Previous data (Leary et al. 1984a) indicated that the meristic characters used in this study are established by this age in Arlee rainbow trout.

Electrophoresis.—Horizontal starch gel electrophoresis was used to obtain estimates of allele frequencies at 12 previously known polymorphic loci (i.e., variable genes) coding for proteins in muscle, liver, or eye tissue. Fifty individuals were randomly chosen from each treatment group. Electrophoresis followed the procedures of Allendorf and Utter (1979). Buffers used to make gels, and stains used to reveal the positions of particular proteins in the gels after electrophoresis, were described by Allendorf et al. (1977) and Harris and Hopkinson (1976). Nomenclature of loci and alleles follows the procedures of Leary et al. (1987), with a few modifications to conform to the standard nomenclature developed for protein-coding loci in fish (Shaklee et al. 1990). The following enzymes, with the loci that encode them and enzyme numbers (IUBNC 1984) in parentheses, were analyzed: creatine kinase (*CK-A1, CK-C1*; 2.7.3.2), isocitrate dehydrogenase (*mIDHP-2, IDHP-1,2*; 1.1.1.42), L-lactate dehydrogenase (*LDH-B2, LDH-C*; 1.1.1.27), cytosolic malate dehydrogenase (*sMDH-B1,2*; 1.1.1.37), *N*-acetyl-β-glucosaminidase (*bGLUA*; 3.2.1.30), phosphoglucomutase (*PGM-2*; 5.4.2.2), and superoxide dismutase (*SOD-1*; 1.15.1.1).

In rainbow trout, a common allele at some pairs of loci produces a protein with identical function and electrophoretic mobility. For example, *IDHP-1* and *IDHP-2* both produce an isocitrate dehydrogenase present in liver, and the proteins produced from the common allele at these loci occupy the same position in the gel after electrophoresis. Such pairs of loci are commonly termed isoloci, and their existence can be detected only when one or both loci are polymorphic. In such situations, however, it is not possible to determine at which locus of the pair a variant allele exists. To estimate allele frequencies at the isoloci examined (*IDHP-1,2* and *sMDH-B1,2*), therefore, we considered each pair to be a single gene with four instead of two copies per individual.

Chi-square analysis with contingency tables was used to determine if allele frequencies varied significantly between control and treatment samples. Such variation would indicate that genetic differences existed between the control and one or both treatment groups despite their common parentage. Such differences would most likely have resulted from selective mortality, because environmental variation among treatment groups was minimized.

We used average observed heterozygosity of individuals as an estimate of the amount of genetic variation in the samples. For any particular locus, an individual that possesses two different alleles is said to be heterozygous and genetically variable at that locus. Individuals that possess two copies of the same allele at a locus are termed homozygous and are genetically invariant at the locus under consideration. Average observed heterozygosity is computed by (1) determining the number of genes examined at which an individual was heterozygous, (2) averaging these values over all individuals in the sample, and (3) dividing this value by the total number of genes examined. Thus, an average observed heterozygosity of 0.200 indicates that, on average, individuals in the sample were heterozygous at 20% of the loci examined. Because average observed heterozygosity in this study was based only on examination of polymorphic loci, the values obtained are substantially larger than those usually reported for rainbow trout populations. The Wilcoxon two-sample test was used to determine if average observed heterozygosity differed significantly between control and treatment samples. Individuals were considered heterozygous at the isoloci according to the criterion described by Leary et al. (1983).

Meristic counts.—The counts for five bilateral meristic characters were determined on the left and right side of each fish in samples used for electrophoretic analysis. These characters were the rays in the pectoral and pelvic fins, gill rakers on the lower and upper first branchial arches, and mandibular pores. Asymmetry in the samples was

TABLE 1.—Allele frequencies at polymorphic loci in Arlee strain rainbow trout from eggs subjected to three water-hardening treatments: water (control), Betadine (povidone–iodine) solution, and erythromycin solution. Asterisks denote a significant difference at $P < 0.01$**.

Locus	Allele	Betadine treatment (frequency)	Betadine–water χ^2 [a]	Water treatment (frequency)	Erythromycin–water χ^2 [a]	Erythromycin treatment (frequency)	df[b]
CK-A1	*100	0.990	9.955**	0.880	2.198	0.940	1
	*76	0.010		0.120		0.060	
CK-C1	*100	1.000	1.006	0.990	2.749	0.950	1
	*38	0.000		0.010		0.050	
bGLUA	*100	0.470	0.059	0.450	0.511	0.400	1
	*72	0.530		0.550		0.600	
mIDHP-2	*100	0.663	0.744	0.720	0.140	0.690	1
	*140	0.337		0.280		0.310	
IDHP-1,2	*100	0.785	1.831	0.805	2.117	0.810	3
	*114	0.085		0.085		0.065	
	*71	0.005		0.015		0.005	
	*40	0.125		0.095		0.120	
LDH-B2	*100	0.929	0.047	0.920	0.244	0.900	1
	*76	0.071		0.080		0.100	
LDH-C	*100	0.612	2.554	0.720	0.842	0.660	1
	*95	0.388		0.280		0.340	
sMDH-B1,2	*100	0.810	0.016	0.815	0.563	0.785	1
	*83	0.190		0.185		0.215	
PGM-2	*100	0.840	1.490	0.898	0.684	0.860	1
	*90	0.160		0.102		0.140	
SOD-1	*100	0.770	0.028	0.780	0.250	0.750	1
	*152	0.230		0.220		0.250	
Average observed heterozygosity		0.348		0.359		0.358	

[a] Chi-square statistic from contingency tables for homogeneity of allele frequencies between treatments.
[b] Degrees of freedom for both comparisons.

quantified by (1) determining the number of characters examined for which an individual was asymmetric (i.e., had different counts for left and right sides) and (2) averaging these values over all individuals in the sample. The Wilcoxon two-sample test was used to determine if levels of asymmetry and mean total counts (left plus right) of the characters differed significantly between control and treatment samples. Because levels of asymmetry and the counts of meristic characters in salmonid fishes are affected by genetics and the environment (Leary et al. 1984a, 1984b, 1985a), such differences could result from selectively generated genetic differences among the groups or from a direct effect of one or more of the water-hardening treatments on development.

Results

Hatching Success

The percentage of eggs that hatched differed significantly ($\chi^2 = 6.991$, df = 1, $P < 0.01$) between those hardened in hatchery water (63.7%) and those hardened in Betadine solution (57.6%). In contrast, hatching percentage was statistically homogeneous ($\chi^2 = 0.085$, $P > 0.90$) between control eggs and those hardened in erythromycin solution (63.0%). Thus, Betadine was associated with a slight reduction in hatching success (9.6%) relative to that of the control group, whereas erythromycin had no detectable effect on hatching success.

Genetic Comparisons

Electrophoretic data indicated that slight genetic differences existed between the control and Betadine-treated groups. Chi-square analysis revealed that the allele frequencies at *CK-A1* were heterogeneous between the samples (Table 1). This difference could have reflected a true genetic difference between the groups or a chance departure from homogeneity due to sampling error. To distinguish between these interpretations, we compared the probability associated with the chi-square statistic to the modified level of significance proposed by Cooper (1968). The modified significance level accounts for the possibility of encountering chance departures from homogeneity when multiple tests are performed between

TABLE 2.—Mean (SD) total counts and levels of asymmetry exhibited by five bilateral meristic characters in Arlee strain rainbow trout from eggs subjected to three water-hardening treatments: water (control), Betadine (povidone–iodine) solution, and erythromycin solution.

Trait	Betadine treatment	Betadine–water P[a]	Water treatment	Erythromycin–water P[a]	Erythromycin treatment
Asymmetry[b]	1.40 (0.85)	0.787	1.36 (0.87)	0.019	1.74 (0.93)
Meristic characters					
Lower gill rakers	23.00 (1.54)	0.465	22.78 (1.12)	0.089	22.32 (1.42)
Mandibular pores	16.28 (1.46)	0.624	16.12 (1.40)	0.317	16.40 (1.33)
Pectoral fin rays	29.20 (1.50)	0.734	29.34 (1.38)	0.395	29.10 (1.39)
Pelvic fin rays	20.10 (0.54)	0.234	20.02 (0.65)	0.056	19.86 (0.57)
Upper gill rakers	17.38 (1.38)	0.035	16.82 (1.16)	0.435	17.02 (1.16)

[a] Probability of the Wilcoxon two-sample test for homogeneity of means between treatments.
[b] Asymmetry = number of meristic characters examined for which an individual had different counts on the left and right sides.

samples. It is computed by dividing the standard 0.05 level of significance by the number of tests performed; in this case, ten tests were performed, yielding a modified significance level of 0.005 ($\chi^2_1 = 7.879$). Because the chi-square value for *CK-A1* had a probability less than the modified significance level, we concluded that this reflected a genetic difference between the control and Betadine-treated groups. However, this difference did not appreciably alter the overall amount of genetic variation in the Betadine group because it statistically ($P > 0.90$) had the same average observed heterozygosity as the control group (Table 1).

Unlike Betadine, erythromycin did not detectably alter the genetic characteristics of the fish. The allele frequencies at all the loci were statistically homogeneous between the control and erythromycin-treated groups (Table 1). Furthermore, both groups had comparable levels of average observed heterozygosity ($P > 0.50$; Table 1).

Meristic Comparisons

Neither water-hardening treatment appeared to have affected developmental rate. Only the mean number of upper gill rakers differed significantly between the control and Betadine-treated samples (Table 2). This difference, however, was not significant at the modified significance level of 0.01. Thus, we concluded that it was more likely a chance departure from homogeneity than indicative of a meristic difference between the groups due to Betadine affecting developmental rate. None of the mean meristic counts significantly differed between the control and erythromycin-treated groups (Table 2).

In contrast to the mean meristic counts, levels of asymmetry exhibited by the counts differed significantly between the control and erythromycin-treated samples (Table 2). Those fish from eggs hardened in the erythromycin solution had greater asymmetry. Thus, erythromycin in this experiment was associated with greater perturbation of development relative to that of control fish. On the other hand, Betadine did not detectably perturb development because fish from Betadine-treated eggs had a level of asymmetry comparable to that of control fish (Table 2).

Discussion

Our preliminary evidence indicates some slight deleterious effects of water-hardening rainbow trout eggs in a solution of Betadine or erythromycin. Betadine appears to have slightly altered the genetic characteristics of the fish. Fish from eggs treated with Betadine had a lower frequency of the *CK-A1*76* allele than did the control group. (Allele designations are preceded by an asterisk [*].) We do not know precisely why this genetic difference existed but the reduced hatching success of Betadine-treated eggs leads us to suspect selective mortality. Apparently, individuals possessing the *CK-A1*76* allele were more susceptible to adverse effects of Betadine during early development than were fish without this allele. Another possibility is that these differences reflected an experimental nuance and were not causally related to Betadine.

Levels of asymmetry in fishes can be affected by genetic (Leary et al. 1984b, 1985a) and environmental (Valentine et al. 1973) variation. Because few or no genetic differences were observed among the treatment groups, and because environmental differences should have been minimal, we concluded that the greater asymmetry of fish from erythromycin-treated eggs was mainly the consequence of exposure to the antibiotic during early development.

The counts of meristic characters in salmonid

fishes are determined during certain critical periods of development (Tåning 1950). The critical periods of the characters used in this study are unknown for Arlee rainbow trout, but they probably begin within days after water-hardening. Within 24 h after exposure to erythromycin, levels of the antibiotic in salmonid eggs fall below that sufficient to inhibit growth of the bacterium responsible for kidney disease (Bullock 1982). Thus, erythromycin does not appear to stay in the egg for an appreciable amount of time. Therefore, the increased asymmetry of meristic characters in fish from erythromycin-treated eggs probably resulted from an early perturbation of development that was caused by erythromycin and that persisted into later developmental stages.

Our results suggest that Betadine treatment may result in selective mortality, and consequently reduced hatching success, and that erythromycin may disrupt development. We stress that these results should not be interpreted to mean that these treatments should not be used to control disease transmission in hatchery populations. The effects of these treatments on survival and development appear to be small. Thus, in most situations, these effects will be of little consequence to the value of the fish produced and will be outweighed by the advantages of disease control. For populations in which hatching success or the development of individuals is already impaired because of genetic or environmental stresses (Leary et al. 1985b), water-hardening of eggs with Betadine or erythromycin solutions could reduce survival and increase the proportion of seriously deformed individuals to the point that the value of the brood stock is seriously diminished (Leary et al. 1985c).

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