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**GENETIC COMPOSITION OF THE RAINBOW TROUT
SPAWNING RUN IN GEORGETOWN LAKE, MONTANA**

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ABSTRACT

For a number of years, rainbow trout, Oncorhynchus mykiss, from the Arlee, Eagle Lake, and Kamloops strains have been introduced into Georgetown Lake, Montana to support a sport fishery. Horizontal starch gel electrophoresis of two proteins was used to estimate the genetic contribution of each strain to the 1990, 1991, and 1992 spawning runs in Georgetown Lake. The 1990 data indicated no evidence of spatial or temporal differences in the genetic composition of the spawning run. Likewise, no genetic differences were detected among the 1990, 1991, and 1992 samples. Thus, all samples were combined into a single Georgetown Lake sample. The results indicate that about 46% of the genes in the fish in the spawning run originated from Arlee rainbow trout, about 54% from Eagle Lake rainbow trout, and about 0% from Kamloops rainbow trout. Compared to the number of fish from each strain introduced into Georgetown Lake from 1985 to 1990 the success rate of each strain in terms of a genetic contribution to the spawning run is: Arlee 1.031, Eagle Lake 2.559, Kamloops 0.000. If these values are standardized to the Eagle Lake strain by dividing by 2.559, then Arlee are 40% as successful as Eagle Lake rainbow trout and Kamloops 0% as successful as Eagle Lake.

INTRODUCTION

Georgetown Lake, Montana was created in 1894 by the impoundment of Flint Creek. It supports a very popular recreational fisheries for brook trout, Salvelinus fontinalis, kokanee salmon, Oncorhynchus nerka, and rainbow trout, O. mykiss. The brook trout and kokanee salmon fisheries have long been maintained solely by natural reproduction. In contrast, the rainbow trout fishery has mainly relied upon the introduction of hatchery fish. Recently, however, significant spring spawning runs of rainbow trout have been observed in Stuart Mill Bay and the North Fork of Flint Creek.

Montana Department of Fish, Wildlife, and Parks personnel have introduced rainbow trout from three different hatchery populations into Georgetown Lake in recent years. The Arlee strain is maintained by the Montana Department of Fish, Wildlife, and Parks at the Jocko River State Trout Hatchery, Arlee, Montana. In the hatchery, this population spawns from mid-October to mid-December. The Eagle Lake strain is maintained by the United States Fish and Wildlife Service at the Ennis National Fish Hatchery, Ennis, Montana and is spawned from early January through mid-April. The Kamloops strain is also maintained at the Ennis National Fish Hatchery and is spawned from mid-January to mid-March. The contribution of each of these strains to the spring spawning run is largely unknown. We address this issue in this report using protein electrophoretic data collected from pectoral fins obtained from adult fish in the 1990, 1991, and 1992 spawning runs. We also examined progeny, presumably from the 1991 spawning season.

MATERIALS AND METHODS

Samples

In 1990 pectoral fins were obtained from adult rainbow trout during the early, middle, and late portions of the spawning season from both North Fork Flint Creek (April 27, N=50; May 10, N=47; May 23, N=50) and Stuart Mill Bay (April 13, N=90; April 26, N=49; May 10, N=50). With this sampling scheme we were able to address the possibility of both a spatial and temporal difference in the composition of the spawning run. Pectoral fins were also obtained from the Georgetown Lake spawning run in 1991 (May 29, N=90) and 1992 (April 17, N=47). On April 17, 1992, 24 juvenile rainbow trout were collected from North Fork Flint Creek at the Montana Department of Fish, Wildlife, and Parks cabin (T5N R13W S7). These fish are presumably the progeny of fish that spawned in 1991.

Electrophoresis

Horizontal starch gel electrophoresis was used to determine each fish's genetic characteristics at a gene coding for lactate dehydrogenase (LDH-B2*) and another coding for a dipeptidase (PEPA-1*). These genes were chosen because previous electrophoretic analysis indicated the Arlee, Eagle Lake, and Kamloops strains are all genetically very different at them and the proteins could be adequately resolved from pectoral fins.

Data Analysis

Contingency table chi-square analysis was used to determine if genetic differences existed among samples collected from the same area, but at different times, and between areas in the 1990 collections. If evidence of differences were found, this would indicate a temporal change, a spatial change, or both forms of change in the genetic composition of the spawning run during a single

season. This analysis was also used to determine if genetic differences existed among fin collections from the various years. Such a difference would indicate that the genetic characteristics of the spawning run were changing from year to year. Finally, we compared the juvenile collection to adults to determine if there was any evidence of genetic differences between them. If so, this would indicate that certain components of the spawning run were more successful at producing progeny to one year of age than others.

Previous data indicate the Eagle Lake strain is genetically very different from the Arlee and Kamloops strain as it is the only one of the three that contains the PEPA-1*115 allele (Table 1). The Kamloops strain is also very distinctive because it has a very high frequency of the LDH-B2*76 allele (Table 1). Because of these large differences, we can use the data from these two genes to obtain estimates of the genetic contribution of each strain to the samples. If the frequency of PEPA-1*115 in Eagle Lake fish introduced into Georgetown Lake is the same as in the hatchery, then the proportion of Eagle Lake genes in a sample is the observed PEPA-1*115 frequency divided by the hatchery frequency. Next, the proportion of Arlee and Kamloops genes in a sample is estimated by solving the following two simultaneous equations:

$$\text{observed frequency } \underline{\text{LDH-B2*100}} = 0.964(x) + 0.250(y) - \text{proportion}$$

Eagle Lake genes in sample

$$\text{observed frequency } \underline{\text{LDH-B2*76}} = 0.036(x) + 0.750(y),$$

where x represents the proportion of Arlee genes in a sample and y the proportion of Kamloops genes. First, the second equation is solved for y in terms of x, this is substituted into the first equation, and a value of x is obtained yielding an estimate of the Arlee genetic contribution. The sum of the Arlee and Eagle Lake contributions subtracted from one yields an estimate of the Kamloops genetic contribution.

RESULTS AND DISCUSSION

Contingency table chi-square analysis revealed no significant differences in allele frequencies at LDH-B2* and PEPA-1* among samples collected in the same area but at different times during 1990 (Table 2). Likewise, no evidence of genetic differences were detected between the 1990 North Fork Flint Creek and Stuart Mill Bay combined samples (Table 2). Thus, all six samples were combined into a single 1990 sample.

No significant differences in allele frequencies were detected between adult rainbow trout in the 1991 spawning run and juveniles presumably produced from the run (Table 3). These two samples, therefore, were combined into a single 1991 sample.

Allele frequencies were statistically homogeneous among the 1990, 1991, and 1992 samples (Table 4). Thus, the composition of the spawning run appears to have been stable over this time period.

We estimated the genetic contribution of the three strains to the spawning run in each year separately and in the combined sample. Arlee rainbow trout were estimated to have between a 32 and 53 percent genetic contribution among the three years and a 46 percent contribution to the combined sample (Table 5). Eagle Lake rainbow trout tended to have a slightly higher contribution ranging from 47 to 67 percent among the years and 54 percent to the combined sample (Table 5). Kamloops rainbow trout had little or no detectable contribution to the spawning run (Table 5).

The above estimates should be interpreted only qualitatively. An assumption made in their calculation is that the allele frequencies in Arlee, Eagle Lake, and Kamloops rainbow trout surviving to adulthood in Georgetown Lake are the same as in the hatchery. This assumption is probably not strictly correct for a variety of reasons. For example, Montana Department of Fish,

Wildlife, and Parks records indicate that in some years the fish of each strain introduced into Georgetown Lake probably came from only one or two egg takes. In Arlee and Eagle Lake rainbow trout we have observed genetic differences among egg takes within a season (Leary et al. 1989). Thus, in some years the allele frequencies in the introduced fish may differ from those in the strains as a whole. This will introduce some error into the estimates. For example, if the frequency of PEPA-1*115 was higher in the introduced fish than 0.295, then we have overestimated the Eagle Lake contribution. Likewise, if the frequency of this allele was less than 0.295 in the introduced fish, then we have underestimated the Eagle Lake contribution.

The estimates in Table 5 should also not be interpreted as indicating the proportion of fish from each strain in the spawning run. Fish from each strain have been introduced into Georgetown Lake for a number of years. During the spawning season it is likely that fish of the different strains reproduce with each other. Subsequently, when these fish attain maturity they will enter the spawning run and initiate the formation of a hybrid swarm. Thus, the estimates in table 5 indicate the proportion of genes originating from each strain in the fish in the spawning run and not the proportion of fish from each strain.

Qualitatively, the following conclusions can be made from the data. First, Kamloops rainbow trout have had very little genetic contribution to the spawning run. The majority of the genes in the fish in the spawning run originated from Arlee and Eagle Lake rainbow trout with the latter strain have a slightly greater contribution than the former.

The last issue to address is how do the estimates of the genetic contribution of each strain to the spawning run compare to the proportion of fish from each strain introduced into Georgetown Lake in recent years. From 1985 to 1990, Montana Department of Fish, Wildlife, and Parks records indicate that Arlee

rainbow trout have been the predominant fish introduced and Eagle Lake rainbow trout the least predominant (Table 6). We can estimate a relative success rate, in terms of a genetic contribution to the spawning run, by dividing the proportion of fish from each strain introduced into Georgetown Lake from 1985 to 1990 into the genetic contribution of each strain to the combined 1990 to 1992 sample. The results are: Arlee 1.031, Eagle Lake 2.559, Kamloops 0.000. These values can then be standardized to the success of Eagle Lake rainbow trout by dividing each by 2.559. The results indicate Arlee are 40% and Kamloops 0% as successful as Eagle Lake in contributing genes to the spawning run. Thus, Kamloops rainbow trout in these terms are by far the least successful strain and Eagle Lake rainbow trout by far the most successful strain in Georgetown Lake.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Leary, R.F., F.W. Allendorf, and K.L. Knudsen. 1989. Genetic differences among rainbow trout spawned on different days within a single season. Progressive Fish Culturist 51:10-19.

TABLE 1

Allele frequencies at LDH-B2* and PEPA-1* in the 1987 year class of Arlee, Eagle Lake, and Kamloops rainbow trout.

<u>Locus</u>	<u>Alleles</u>	<u>Strain and allele frequencies</u>		
		<u>Arlee</u>	<u>Eagle Lake</u>	<u>Kamloops</u>
<u>LDH-B2*</u>	<u>100</u>	0.964	1.000	0.250
	<u>76</u>	0.036	-	0.750
<u>PEPA-1*</u>	<u>100</u>	1.000	0.705	1.000
	<u>115</u>	-	0.295	-

TABLE 2

Allele frequencies in samples of rainbow trout collected from North Fork Flint Creek and Stuart Mill Bay, Georgetown Lake during the spring of 1990. Chi-square is contingency table chi-square analysis for homogeneity of allele frequencies among samples. D.F. is degrees of freedom.

Sample	<u>Loci and allele frequencies</u>			
	<u>LDH-B2*</u>		<u>PEPA-1*</u>	
	<u>100</u>	<u>76</u>	<u>100</u>	<u>115</u>
Flint Creek				
4/27/90	0.970	0.030	0.890	0.110
5/10/90	1.000	-	0.830	0.170
5/23/90	0.950	0.050	0.867	0.133
Chi-square		4.626		1.507
D.F.		2		2
Stuart Mill				
4/13/90	0.989	0.011	0.872	0.128
4/26/90	1.000	-	0.852	0.148
5/10/90	0.990	0.010	0.840	0.160
Chi-square		1.072		0.588
D.F.		2		2
Flint Creek (combined)	0.973	0.027	0.859	0.141
Stuart Mill (combined)	0.992	0.008	0.863	0.137
Chi-square		3.822		0.026
		1		1

TABLE 3

Allele frequencies in a sample of adult rainbow trout collected from Georgetown Lake during the 1991 spawning season and one year old juveniles collected in the spring of 1992. Chi-square and D.F. as in Table 2.

<u>Sample</u>	<u>Loci and allele frequencies</u>			
	<u>LDH-B2*</u>		<u>PEPA-1*</u>	
	<u>100</u>	<u>76</u>	<u>100</u>	<u>115</u>
Adults	0.983	0.017	0.816	0.184
Juveniles	0.958	0.042	0.750	0.250
Chi-square		1.112		1.037
D.F.		1		1

TABLE 4

Allele frequencies in samples of rainbow trout collected from Georgetown Lake during 1990, 1991, and 1992. Chi-square and D.F. as in Table 2.

<u>Sample</u>	<u>Loci and allele frequencies</u>			
	<u>LDH-B2*</u>		<u>PEPA-1*</u>	
	<u>100</u>	<u>76</u>	<u>100</u>	<u>115</u>
1990	0.984	0.016	0.861	0.139
1991	0.978	0.022	0.802	0.198
1992	0.979	0.021	0.809	0.191
Chi-square		0.354		5.212
D.F.		2		2

TABLE 5

Estimated proportion of Arlee, Eagle Lake, and Kamloops rainbow trout genes in the 1990, 1991, and 1992 Georgetown Lake spawning season.

<u>Sample</u>	<u>Strain and percent composition</u>		
	<u>Arlee</u>	<u>Eagle Lake</u>	<u>Kamloops</u>
1990	53	47	0
1991	32	67	1
1992	34	65	1
Combined	46	54	0

TABLE 6

Total number and percentage of Arlee, Eagle Lake, and Kamloops rainbow trout introduced into Georgetown Lake from 1985 to 1990.

<u>Year</u>	<u>Arlee</u>		<u>Eagle Lake</u>		<u>Kamloops</u>	
	<u>Number</u>	<u>Percent</u>	<u>Number</u>	<u>Percent</u>	<u>Number</u>	<u>Percent</u>
1985	100,860	39.3	83,062	32.4	72,522	28.3
1986	65,026	34.3	53,287	28.1	71,273	37.6
1987	65,004	32.1	64,975	32.1	72,347	35.8
1988	67,143	100.0	-	-	-	-
1989	64,994	69.2	-	-	28,963	30.8
1990	63,407	43.4	-	-	82,526	56.6
Total	426,434	44.6	201,324	21.1	327,631	34.3