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Population Genetic Structure of Bull Trout
in the Upper Flathead River Drainage

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Abstract. -- Samples of bull trout, *Salvelinus confluentus*, were obtained from 24 locations in the upper Flathead River drainage. Whenever possible, individuals from two or more year classes were collected from a location so we could examine spatial and temporal patterns of genetic diversity. Electrophoretic analysis of the products of 45 protein coding loci indicated little genetic variation within populations. There was also relatively little genetic divergence among year classes of a population or among populations from the same drainage. In contrast, there was substantial genetic divergence among populations from the North, Middle, and South Fork Flathead, Swan, and Stillwater drainages. We do not advocate supplementation as a mitigation tool, but if it is to be used in the upper Flathead River drainage the available data indicate that transfer of fish among drainages should be avoided. Because only two loci were widely polymorphic it is difficult to assess the potential genetic impacts of within drainage transfers. In this situation we prefer a conservative approach and suggest such transfers be kept to a minimum.

INTRODUCTION

Bull trout, *Salvelinus confluentus*, were originally considered conspecific with Dolly Varden, *S. malma*. Recent osteological, morphological, and biochemical genetic studies, however, strongly support that these two fishes are distinct species (Cavender 1978; Haas and McPhail 1991; Crane et al., in press).

Historically, bull trout had an extensive distribution. They existed in the upper Sacramento River drainage, California, northwards to the upper Yukon and MacKenzie river drainages, Canada. With the exception of the St. Mary's River, Montana (unpublished data), they are restricted to waters west of the Continental Divide below the 49th parallel but above this point exist on both sides of the Divide.

Bull trout are now considered to be in serious decline throughout much of their native range. They are thought to be extinct in California (Hesseldenz 1985) and are considered to be a species of special concern throughout most of their remaining distribution in the United States and Alberta, Canada (Johnson 1987; Howell and Buchanan 1992). They have recently been petitioned to be protected as an endangered species in the United States under the Endangered Species Act.

Many interrelated factors are thought to be responsible for the decline in bull trout abundance. Its piscivorous nature led commercial and sports fishermen and fisheries managers to view it as a threat to more 'desirable' fish species such as Pacific salmon, *Oncorhynchus* spp., rainbow trout, *O. mykiss*, and cutthroat trout, *O. clarki*. In some areas, a bounty was placed on bull trout to aid early eradication efforts. Dam construction has blocked spawning migrations

and agricultural, logging, and mining operations are believed to have made spawning, nursery, and adult habitats no longer suitable for bull trout (e.g. Howell and Buchanan 1992; Platts et al. 1993; Pratt and Huston 1993).

The introduction of brook trout, *S. fontinalis*, brown, *Salmo trutta*, and rainbow trout is also believed to have aided the decline of bull trout. These fishes are thought to be capable of displacing bull trout especially under degraded conditions. There is also evidence that hybridization with brook trout can be common in certain situations and that this may aid displacement of bull by brook trout (Leary et al. 1993).

Conservation of bull trout is the goal of state, federal, tribal, and provincial management agencies. Knowledge of the population genetic structure of the species is essential in order for this to be accomplished effectively (e.g. Allendorf and Leary 1988; Meffe and Vrijenhoek 1988; Quattro and Vrijenhoek 1989). Previously, we used electrophoretic analysis of proteins to investigate the broad scale population genetic structure of bull trout in the Columbia and Klamath River drainages (Leary et al. 1993). The results indicated that there tended to be little genetic variation within populations but substantial differences among them. There was also no geographic pattern to the amount of genetic divergence observed among the populations. Populations widely separated from each other at times appeared very similar while in other cases populations relatively close were very different. Preserving the genetic diversity of bull trout in this area, therefore, requires the continued existence of many populations throughout the region.

In this paper, we use protein electrophoresis to examine the population genetic structure of bull trout on a finer scale by focusing on populations in the upper Flathead River drainage, Montana and British Columbia (Figure 1). Bull

trout in this region are thought to largely be migratory with adults residing in lakes and moving to tributary rivers or streams to spawn. In the Stillwater River portion of the drainage, adult bull trout inhabit Stillwater and Whitefish lakes and spawn in the Stillwater River and Swift Creek, respectively. Adults from Flathead Lake historically spawned in tributaries to the South, Middle, and North Forks of the Flathead River. The construction of Hungry Horse Dam isolated the South Fork spawning tributaries from Flathead Lake in 1951. Adult fish using these tributaries now reside in Hungry Horse Reservoir above the dam. It is not known whether bull trout migrated from Flathead Lake into Swan River tributaries to spawn or the fish using these tributaries originated from Swan Lake. Regardless, Big Fork Dam isolated the Swan River from Flathead Lake in 1902 and adults now use Swan Lake.

Methods

Sample Collection

A backpack electroshocker was used to obtain samples, mainly of juvenile bull trout, from 24 locations in the upper Flathead River drainage (Table 1, Figures 1-5). Criteria for selecting sample locations were that sampling was not perceived to have an adverse impact on the population and that the sites should encompass most of the geographic range of the known spawning streams in the North, Middle and South Fork Flathead and the Swan River drainages. When possible, individuals from two or more age classes were collected to allow us to examine temporal as well as spatial genetic divergence. The total length (mm) was used to determine the age of the fish collected using the criteria of Fraley and Shepard (1989).

Electrophoresis

Horizontal starch gel electrophoresis was used to determine each fish's genotype at 45 loci coding for enzymes present in muscle, liver, or eye tissue (Table 2). Electrophoresis followed the procedures of Leary and Boone (1990). Stains used to reveal the position of particular enzymes in the gels after electrophoresis followed the recipes of Harris and Hopkinson (1976) and Allendorf et al. (1977). Nomenclature of loci and alleles follows the recommendations of Shaklee et al. (1990). Allelic mobilities are relative to the product produced from the common allele at the homologous locus in Arlee rainbow trout maintained by the Montana Department of Fish, Wildlife, and Parks at the Jocko River State Trout Hatchery, Arlee, Montana. This convention makes it easy for us to electrophoretically compare various salmonid fish taxa.

Data analysis

Chi-square analysis was used to determine if observed genotypic distributions at the polymorphic loci in each sample statistically conformed to expected random mating proportions (Hardy-Weinberg proportions). Contingency table chi-square analysis was used to determine if allele frequencies were statistically heterogeneous at the polymorphic loci between samples from different year classes from the same location and among locations in the five major river drainages: North, South, and Middle Fork Flathead, Stillwater, and Swan. If no significant differences were found between year classes from the same location, they were combined into a single sample. Year classes between which significant differences were detected were treated as separate samples in the following analyses. The total amount of genetic diversity detected among all the samples was partitioned into the proportion due to genetic variation within samples and

to genetic differences between year classes within a location, among locations within the five major drainages, and among samples from the different drainages using the procedure of Chakraborty (1980). Since only two loci were commonly and highly polymorphic a plot of the frequency of the common allele at each locus was used to examine the relative amount of genetic divergence among samples.

Results

Hybridization with brook trout

At nine of the loci analyzed, brook trout and bull trout rarely share alleles in common (Leary et al. 1983). Some fish in samples from the Swan River drainage were heterozygous for alleles characteristic of both the bull and brook trout at all these loci indicating they were first generation hybrids (Table 3). In the field, only fish considered to be bull trout were kept so the proportion of hybrids in the samples is certainly an underestimate of the proportion in the different year classes and only qualitative statements about the occurrence of hybridization can be made. The available evidence indicates that hybridization occurs widely throughout the drainage and is much more frequent in Lion Creek than other areas sampled in the drainage. Within Lion Creek there is also some suggestion that the amount of hybridization may vary substantially from year to year.

Bull trout genetic diversity

Evidence of genetic variation was detected at only sAAT-1*, CK-A2*, IDDH*, mIDHP-1*, and LDH-A1* among the samples. Only IDDH* and mIDHP-1* were frequently polymorphic. Variation at LDH-A1* was observed only in the sample of adults from

Hungry Horse Reservoir. Variation at sAAT-1* and CK-A2* was largely restricted to samples from the South Fork Flathead drainage and the variant allele at these loci was usually detected at frequencies less than 0.05. Thus, the data set mainly involves examining patterns of genetic diversity at IDDH* and mIDHP-1*.

Observed genotypic distributions significantly departed from expected random mating distributions only in the Coal Creek and Stillwater River samples (Table 4). Considering the number of comparisons, the deviation in Coal Creek at mIDHP-1* is most likely a chance departure from conformity and has little biological relevance. In contrast, all fish in the Stillwater sample were heterozygous at IDDH*. The simplest explanation for this dramatic departure from random mating proportions is that most, if not all, the fish in the sample were full-sibs produced from a mating between alternate homozygotes. The only possible allele frequencies in a full-sib family are 0, 0.25, 0.50, 0.75, and 1.00. The frequency of mIDHP-1*350 in the Stillwater River sample of 0.80, therefore, is also compatible with the fish representing a full-sib family.

Allele frequencies within spawning tributaries were not always temporally stable. Significant differences among year classes were detected in the Bear Creek, Goat Creek, Schafer Creek, and White River samples (Table 5). With the exception of Schafer Creek, all these comparisons involved three year classes and in these cases the observed heterogeneity is mainly due to the youngest year class. Pairwise comparisons indicate that allele frequencies at the heterogenous locus significantly differ between the youngest and the other year classes in these samples. Allele frequencies, however, were statistically homogenous between the other year classes. Thus, these two year classes were combined into a single sample in subsequent analyses.

Previously, we found that bull trout populations in the Columbia River

drainage were characterized by low amounts of genetic variation within populations and substantial genetic divergence among them (Leary et al. 1993). This also pertains to the populations sampled from the geographically more restricted upper Flathead River drainage. Average expected heterozygosity among the samples ranged from zero to 0.022 indicating little genetic diversity within populations (Table 6). Statistically significant allele frequency differences exist among the samples within all five major drainages indicating the existence of genetically divergent populations within each (Table 6). When the total amount of genetic diversity detected among all the samples is partitioned in a hierarchical fashion a geographic pattern to the amount of genetic divergence among populations emerges. Only 62.8% of the total genetic diversity detected is due to genetic variation within populations indicating a substantial amount of genetic divergence among them. Genetic differences among year classes within a stream account for only 1.4% of the total genetic diversity, differences among populations within a drainage 7.3%, and differences among populations from different drainages 28.5%. Thus, most of the genetic divergence exists between drainages with decreasing amounts due to differences within drainages and between year classes.

A plot of IDDH*100 and mIDHP-1*350 allele frequencies indicates that most of the between drainage divergence is due to genetic differences between populations in the North Fork Flathead and Stillwater River drainages and those in the Middle and South Fork and Swan River drainages (Fig. 6). Populations in the former two drainages occupy unique regions in the two dimensional space. In contrast, there is considerable overlap among the regions occupied by the latter three drainages.

Discussion

The available data indicate that at times year classes of bull trout may be produced from a small number of spawners. This is the simplest explanation for the observed temporal instability of allele frequencies in some streams and the large departure of observed genotypic distributions from expected random mating proportions in the Stillwater River sample. This may also account for the apparent variability in the extent of hybridization with brook trout among year classes in Lion Creek. Thus, the genetic characteristics of some bull trout populations in the upper Flathead River drainage now appear to be largely controlled by stochastic nonadaptive processes which potentially can threaten their viability.

We are not advocates of hatchery supplementation as a means of mitigating reduced fish abundance. We feel initial efforts should focus primarily on mitigating the true causes of decline such as habitat degradation rather than simply trying to increase abundance with expensive hatchery operations. We recognize, however, that there is likely to be some support for supplementation as a mitigation tool in the upper Flathead River drainage as some populations become precariously close to extinction. Thus, we will address the relevance of the data to a supplementation program.

When interpreting the data it is necessary to keep in mind that it mainly involves a comparison of allele frequencies at two widely polymorphic loci. In this situation, the power of detecting genetic differences is quite weak. Thus, when differences are apparent it is safe to assume that they are real and relevant but the converse is not a safe assumption. That is, lack of evidence for genetic divergence should not be interpreted to mean that no differences

exist. At this time the data indicate no, but weak, evidence of substantial genetic divergence.

As the amount of gene flow decreases among populations the amount of genetic divergence and the probability of local adaptations among them increases. The available evidence indicates that a substantial amount of genetic divergence exists among populations from the different drainages. It is possible, therefore, that populations in the different drainages may possess some degree of local adaptation. Because of this we cannot recommend that a perceived supplementation plan propose transferring fish from one drainage to another. Interbreeding between the native and introduced fish may serve as a means of disrupting local adaptation and decreasing the productivity and viability of the native populations.

The much smaller amount of genetic divergence detected among populations within drainages suggests that appreciable amounts of gene flow among them naturally occurs and that supplementation programs can safely ignore within drainage genetic differences. Although this is an attractive conclusion from a practical perspective, it is only weakly supported. At this time, therefore, we would advocate a conservative approach and suggest that within drainage transfers be kept to a minimum.

From a genetics perspective, the potential costs of widespread within drainage transfers cannot now be reliably assessed. Additional polymorphic loci need to be examined to increase the power of the data set. We do not perceive that screening the products of additional protein coding loci will prove to be a useful approach to detect other polymorphisms as this portion of the genome appears to be quite invariable throughout the range of bull trout. Thus, we will primarily focus on examination of mitochondrial and nuclear DNA extracted from

the same individuals used in this study as methods of detecting other polymorphisms.

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TABLE 1. - Location of juvenile bull trout samples (location), collection date (month, day, year), and number per year class obtained from 24 locations in the upper Flathead River drainage, Montana and British Columbia.

Location	Date	Number per year class				
		1989	1990	1991	1992	1993
North Fork Flathead						
Big Creek	11/17/92			25	26	
Coal Creek	06/29/93				24	
Whale Creek	06/28/93				29	
Trail Creek	11/19/92			28	25	
upper North Fork	07/19/93		11	10		
Howell Creek	07/20/93			16		
Middle Fork Flathead						
Ole Creek	08/23/93				16	
Bear Creek	08/24/93			23	20	16
Granite Creek	08/24/93			17		
Dolly Varden Creek	08/31/93					25
Schafer Creek	08/31/93		18			25
South Fork Flathead						
Hungry Horse Reservoir	11/10/92	32 Adults				
Wounded Buck Creek	09/07/93		19	16		
Sullivan Creek	09/07/93	18	16	25		
Spotted Bear River	09/08/93			14		12
Big Salmon Creek	08/09/93			27	28	
White River	08/10/93			19	21	27
Youngs Creek	07/30/93		25			
Swan River						
South Lost Creek	08/03/93			18	19	
Goat Creek	11/24/92		14	20	15	
	09/28/93				15	
Lion Creek	08/03/93		16	18	25	
Elk Creek	11/17/92		20	25	20	
Stillwater River						
Stillwater River	07/12/93				25	
Swift Creek	12/15/92				24	

Table 2. - Enzymes and loci examined. EC represents enzyme commission number (IUBNC 1984). Tissues : E=eye, L=liver, M=Muscle. Buffer indicates the buffer system or systems that gave the best electrophoretic resolution for each enzyme.

Enzyme	Loci	EC	Tissue	Buffer
Adenylate kinase	<u>AK-1*</u> , <u>AK-2*</u>	2.7.4.3	M	AC
Alcohol dehydrogenase	<u>ADH*</u>	1.1.1.1	L	RW
Aspartate aminotransferase	<u>sAAT-1*</u> , <u>sAAT-2*</u> , <u>sAAT-3,4*</u>	2.6.1.1	L M	AC, RW AC, RW
Creatine kinase	<u>CK-A1*</u> , <u>CK-A2*</u> , <u>CK-B*</u>	2.7.3.2	M E	RW SR
Dipeptidase	<u>PEPA*</u>	3.4.-.-	E	SR
Fumarate hydratase	<u>FH-1*</u> , <u>FH-2*</u>	4.2.1.2	L	AC
Glucose - 6 - phosphate isomerase	<u>GPI-A*</u> , <u>GPI-B1*</u> , <u>GPI-B2*</u>	5.3.1.9	E M	SR RW
Glyceraldehyde - 3 - phosphate dehydrogenase	<u>GAPDH-3,4*</u>	1.2.1.12	E	AC+
Glycerol - 3 - phosphate dehydrogenase	<u>G3PDH-1*</u>	1.1.1.8	L	RW
N-acetyl-beta-glucosaminidase	<u>bGLUA*</u>	3.2.1.30	L	RW
Iditol dehydrogenase	<u>IDDH*</u>	1.1.1.14	L	RW
Isocitrate dehydrogenase	<u>mIDHP-1*</u> , <u>mIDHP-2*</u> , <u>sIDHP-1*</u> <u>sIDHP-2*</u>	1.1.1.42	M L E	AC+ AC AC+
Lactate dehydrogenase	<u>LDH-A1*</u> , <u>LDH-A2*</u> <u>LDH-B1*</u> , <u>LDH-B2*</u> , <u>LDH-C*</u>	1.1.1.27	M E	RW SR
Malate dehydrogenase	<u>sMDH-A1,2*</u> <u>sMDH-B1,2*</u>	1.1.1.37	L M	AC AC+
Malic enzyme	<u>mMEP-1*</u> , <u>mMEP-2*</u> <u>sMEP-1*</u> , <u>sMEP-2*</u>	1.1.1.40	M L	AC AC
Phosphogluconate dehydrogenase	<u>PGDH*</u>	1.1.1.44	M	AC
Phosphoglucomutase	<u>PGM-1*</u> , <u>PGM-2*</u>	5.4.2.2	M	AC, RW
Pyruvate kinase	<u>PK-3*</u> , <u>PK-4*</u>	2.7.1.40	E	AC+
Superoxide dismutase	<u>sSOD-1*</u>	1.15.1.1	L	RW
Tripeptide aminopeptidase	<u>PEPB*</u>	3.4.-.-	E	SR

AC = N-(3-aminopropyl)-morpholine and citric acid buffer (Clayton and Tretiak 1972).

AC+ = Same as AC except 2 drops of 2-mercaptoethanol and 15mg beta-nicotinamide adenine dinucleotide are added just before degassing to every 200ml gel buffer

RW = Tris-citric acid buffer (Ridgway et al. 1970).

SR = Tris-citric acid buffer (Gall and Bentley 1981).

Table 3. - Numbers of bull trout and first generation hybrids of bull and brook trout in samples from three locations in the Swan River drainage, Montana.

Location	Year Class	Bull trout	Hybrids
Elk Creek	1990	18	1
	1991	25	0
	1992	22	0
Goat Creek	1990	14	0
	1991	20	0
	1992	29	1
Lion Creek	1990	16	0
	1991	10	8
	1992	22	3

Table 4.- Observed and expected random mating genotypic distributions in samples from Coal Creek and the Stillwater River. * = $P < 0.05$, *** = $P < 0.001$

Sample		Locus and genotypic distribution			Chi-Square
		IDDH*			
		<u>100/100</u>	<u>120/100</u>	<u>120/120</u>	
Coal	observed	12	12	0	
	expected	13.50	9.00	1.50	2.667
Stillwater	observed	0	25	0	
	expected	6.25	12.50	6.25	25.000***
		MIDHP-1*			
		<u>350/350</u>	<u>600/350</u>	<u>600/600</u>	
Coal	observed	1	2	21	
	expected	0.17	3.65	20.18	4.830*
Stillwater	observed	15	10	0	
	expected	16.00	8.00	1.00	1.563

Table 5.- Allele frequencies at the polymorphic loci in samples providing evidence of temporal instability of allele frequencies among year classes from the same spawning tributary. Variant alleles not listed are sAAT-1*92, CK-A2*140, IDDH*120, and mIDHP-1*600. Chi-square is contingency chi-square statistic for homogeneity of allele frequencies among samples. D.f. = degrees of freedom. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Sample	Year Class	Allele frequencies			
		<u>sAAT-1*23</u>	<u>CK-A2*100</u>	<u>IDDH*100</u>	<u>mIDHP-1*350</u>
Bear Creek	1991	1.000	1.000	0.913	1.000
	1992	1.000	1.000	0.850	1.000
	1993	1.000	1.000	1.000	0.969
Chi-square		----	----	6.168*	2.727
D.f.				2	2
Goat Creek	1990	1.000	1.000	1.000	0.964
	1991	1.000	1.000	1.000	0.976
	1992	1.000	1.000	1.000	0.750
Chi-square		---	---	---	13.940***
D.f.					2
Schafer Creek	1990	1.000	1.000	0.694	0.917
	1993	1.000	1.000	0.920	0.720
Chi-square		---	---	7.393**	5.113*
D.f.				1	1
White River	1991	0.947	0.947	0.868	0.868
	1992	1.000	0.952	0.881	0.905
	1993	1.000	0.981	0.778	1.000
Chi-square					
D.f.		5.055	0.897	2.241	6.945*
		2	2	2	2

Table 6. - Allele frequencies at the polymorphic loci in samples of bull trout from the upper Flathead River drainage. Variant alleles not listed are sAAT-1*92, CK-A2*140, IDDH*120, mIDHP-1*600, and LDH-A1*null. Chi-square and D.f. as in Table 5. H_e = average expected heterozygosity.

Sample	Allele Frequencies					H _e
	sAAT-1*23	CK-A2*100	IDDH*100	mIDHP-1*350	LDH-A1*100	
North Fork Flathead						
Big Creek	1.000	1.000	0.979	0.225	1.000	0.010
Coal Creek	1.000	1.000	0.750	0.083	1.000	0.013
Whale Creek	1.000	1.000	0.983	0.190	1.000	0.008
Trail Creek	1.000	1.000	0.981	0.094	1.000	0.005
Upper Flathead	1.000	1.000	0.929	0.571	1.000	0.015
Howell Creek	1.000	1.000	0.844	0.313	1.000	0.017
Chi-square	---	---	39.788***	48.320***	---	
D.f.			5	5		
Middle Fork Flathead						
Ole Creek	1.000	1.000	0.750	0.781	1.000	0.017
Bear Creek (91,92)	1.000	1.000	0.884	1.000	1.000	0.005
(93)	1.000	1.000	1.000	0.969	1.000	0.001
Granite Creek	1.000	0.971	0.941	0.971	1.000	0.005
Dolly Varden Creek	1.000	1.000	0.960	1.000	1.000	0.002
Schafer Creek (90)	1.000	1.000	0.694	0.917	1.000	0.014
(93)	1.000	1.000	0.920	0.720	1.000	0.013
Chi-square	---	8.128	27.037***	48.914***	---	
D.f.		6	6	6		
South Fork Flathead						
Hungry Horse	0.984	0.969	0.781	0.891	0.984	0.016
Wounded Buck Creek	1.000	1.000	0.857	1.000	1.000	0.006
Sullivan Creek	1.000	1.000	0.992	1.000	1.000	0.000
Spotted Bear River	1.000	1.000	0.904	1.000	1.000	0.004
Big Salmon Creek	1.000	1.000	0.946	1.000	1.000	0.002
White River (91,92)	0.975	0.950	0.875	0.887	1.000	0.015
(93)	1.000	0.981	0.778	1.000	1.000	0.009
Youngs Creek	1.000	0.760	0.720	1.000	1.000	0.019
Chi-square	10.175	83.673***	43.723***	52.080***	8.115	
D.f.	7	7	7	7	7	
Swan						
South Lost Creek	1.000	1.000	1.000	1.000	1.000	0.000
Goat Creek (90,91)	1.000	1.000	1.000	0.971	1.000	0.001
(92)	1.000	1.000	1.000	0.750	1.000	0.009
Lion Creek	1.000	1.000	1.000	0.937	1.000	0.003
Elk Creek	1.000	1.000	1.000	0.977	1.000	0.001
Chi-square	---	---	---	46.934***	---	
D.f.				4		
Stillwater						
Stillwater River	1.000	1.000	0.500	0.800	1.000	0.022
Swift Creek	1.000	1.000	0.717	0.542	1.000	0.022
Chi-square	---	---	4.739*	7.440**	---	
D.f.			1	1		

Figure 1. - Upper Flathead River drainage. ● = location of Swift creek and Stillwater River Samples.

Figure 2. - North Fork Flathead River drainage. ● = sample locations.

Figure 3. - Middle Fork Flathead River drainage. ● = sample locations

Figure 4. - South Fork Flathead River drainage. ● = sample locations

Figure 5. - Swan River Drainage. ● = sample locations

Figure 6. - Plot of IDDH*100 and mIDHP-1*350 allele frequencies. ■ = North Fork Flathead River samples. □ = Middle Fork Flathead River samples. ● = South Fork Flathead River samples. ○ = Swan River Samples. ▲ = Stillwater River Samples.













