

ECOLOGY OF UPPER KLAMATH LAKE SHORTNOSE AND LOST RIVER SUCKERS

5. Molecular evolution and ecology of Klamath Basin suckers

B. Evidence for a lethal homozygous genotype at the Ankyrin_G locus in Klamath Basin suckers (Catostomidae)

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ABSTRACT

Three nuclear loci, coding for Ankyrin_G, Collagen 1, and Aldehyde dehydrogenase, were examined in four sucker species from Klamath and Rogue river basins.

Frequencies of genotypes and alleles differed markedly between pre-adult and adult suckers for the Ankyrin_G locus but were usually similar for the other two loci. All adult Klamath Basin suckers greater than 163 mm FL (n=242), had only two Ankyrin_G genotypes, AA and AB, with no BB genotypes. However, all three Ankyrin_G genotypes were found in larvae (n = 325) and juveniles up to 71 mm SL (n = 71). When selection was simulated by removing the BB genotype from younger life stages, larva and juvenile Ankyrin_G allelic frequencies resembled adult frequencies.

Four populations representing two of the species, Klamath largescale and shortnose suckers, were examined in greater detail. Genotypic frequencies were in Hardy-Weinberg equilibrium for Collagen 1 and Aldehyde dehydrogenase in all four populations but not for Ankyrin_G in three of four populations. Similarly, differences in allelic frequencies between life stages were not significantly different in seven of eight comparisons with Collagen 1 and Aldehyde dehydrogenase while they were significantly different in three of four Ankyrin_G comparisons. When young Ankyrin_G BB homozygotes were removed from the analysis, Collagen 1 and Aldehyde dehydrogenase allelic frequencies changed by less than 4% but Ankyrin_G allelic frequencies changed 8-26% and 11 of 12 life stage comparisons were not significantly different. The simulation suggested a stable allelic frequency across generations and the distribution of genotypes suggested a heterozygous advantage perhaps caused by lethality of the homozygous BB genotype in pre-adults. The Ankyrin_G BB genotype may therefore represent a previously unsuspected source of significant early life history mortality.

INTRODUCTION

The Klamath Basin contains four endemic species of suckers representing three genera. Two are generally considered lacustrine (Scoppettone and Vinyard, 1991) and are federally listed endangered species (U. S. Fish and Wildlife Service, 1988), *Deltistes luxatus*, Lost River sucker (LRS), and *Chasmistes brevirostris*, shortnose sucker (SNS). Two are generally considered riverine and are not federally protected species, *Catostomus snyderi*, Klamath largescale sucker (KLS), and *C. rimiculus*, Klamath smallscale sucker (KSS). The distribution of the lacustrine species and *C. snyderi* is centered on Upper Klamath Lake and Lost River subbasins with some expatriated individuals showing up in downstream reservoirs. The distribution of *C. rimiculus* is disjunct in the lower Klamath R. below Keno, OR and in the adjacent Rogue R.

Biologists have consistently had difficulty identifying species of Klamath suckers and Miller and Smith (1981) contend that hybridization has occurred making identification more arduous. Consequently, the federal recovery plan (U. S. Fish and Wildlife Service, 1993) for the lacustrine species includes the need to characterize and conserve genetic diversity of populations of all four Klamath Basin suckers. The initial goals of this molecular survey were to isolate and characterize species markers, identify hybrids, and investigate population structure. We surveyed 28 (10,373 base pairs (bp)) low copy number anonymous nuclear loci in the four Klamath Basin species and in selected species outside Klamath Basin. Only four polymorphic loci were found and none showed fixed species markers for Klamath Basin suckers (Wagman and Markle 2000) although the Ankyrin_G locus appeared useful for catostomid phylogeny. In the following, we show that Ankyrin_G has genotypic and allelic patterns suggesting a heterozygous advantage possibly acting through a lethal homozygous genotype in pre-adults.

Catostomid genetics and the three proteins

Catostomids are allotetraploids (Uyeno and Smith, 1972; Tsoi, et al., 1989), presumably from an ancestral, tetraploid hybridization event in China (Tsoi et al., 1989) approximately 50 mya. (Uyeno and Smith, 1972). Allotetraploids possess two complete diploid genomes that segregate independently resulting in disomic inheritance (Dawson, 1962 and Ronfort et al., 1998). The ancestral condition for any catostomid locus is tetraploid and disomic. Potentially derived states are diploidization and tetraploid

tetrasomic inheritance. Tetrasomic inheritance produces multivalent or quadrivalent meiotic structures and are common in autotetraploids such as salmonids (Allendorf and Thorgaard, 1984), but are unknown in catostomids. Uyeno and Smith (1972) report no multivalents in meiotic chromosome spreads in *Erimyzon* and Tsoi et al. (1989) did not see them in *Myxocyprinus*. Ferris and Whitt (1978) report a large number of heterozygous loci and ratios of isozyme activities consistent with allotetraploids. Each of the loci described below had two alleles and consequently, we assume the three loci have become diploidized and segregate independently.

Ankyrin_G is a member of the ankyrin multiple gene family of proteins. These genes exhibit tissue specificity, sequence divergence (Bennett, 1992) and are characterized by their 33- amino acid motif, the ANK repeat. They are peripheral membrane proteins that interconnect integral proteins with the spectrin rich membrane skeleton. The gene family currently consists of three genes Ankyrin_B, Ankyrin_R and Ankyrin_G (also known as Ankyrin 3, the short form of the molecule). Ankyrin_R is associated with erythrocyte membrane skeleton and is localized in post-mitotic neurons of the brain (Lambert and Bennett, 1993). Ankyrin_B has two splice variants of 220kDa and 440 kDa (Kunimoto, et al., 1991) and both are associated with unmyelinated and premyelinated axons (Chan et al, 1993). Ankyrin_G, is large, 480 kDa, (hence, "G" for giant) and has a splice variant of 270 kDa. Both molecules associate with the voltage-dependent sodium channel at the nodes of Ranvier, the axonal initial segments and the neuromuscular junction (Kordeli et al., 1995). Ankyrin_G has 5 major domains (Figure 1). From the amino terminal, the molecule is composed of a membrane binding domain, a short spacer region connected to a spectrin binding domain, a serine rich region that connects to a variable length extended tail region that ends at the carboxyl terminal. Construction of size variants is suspected to be by splicing different regions from the extended tail region (Kordeli et al., 1995).

The Ankyrin_G clone contained a 60 bp open reading frame (ORF), containing a 20 amino acid translated sequence that was 88% identical (18/20) to human Ankyrin_G (Kordeli, et al., 1995) with an overall similarity of 99% (Table 3). The clone sequence exhibited a higher identity score (94% and 93%) to that of the 270-kDa rat Ankyrin_G isoform and mouse Ankyrin_G. Their region of identity is one amino acid shorter than the human Ankyrin_G long form (480 kDa). This region (480-533) corresponds to the 4081-

4098 nucleic acid region of the human Ankyrin_G sequence in the extended tail region, upstream of the carboxyl end of the protein (Figure 1). This region is spliced out of the smaller 270-kDa size variant (Kordeli et al., 1995). The two Klamath Basin alleles, A and B, differed by a single base pair transversion of cytosine to adenine at nucleotide position 357 of the clone (Table 1).

Collagen 1 had 59% identity (73% overall homology, 20/27 aa) to Collagen 1 gene from *Ephydatia muelleri* (Wagman and Markle 2000). We detected two alleles caused by a 10-bp deletion between positions 260-270 (Wagman and Markle 2000). Aldehyde dehydrogenase had 45% identity (69% overall homology, 26/37 aa) to a tumor associated aldehyde dehydrogenase from *Ratus norvegicus*. We detected two alleles caused by a 4-bp deletion between positions 303-307 (Wagman and Markle 2000).

MATERIALS AND METHODS

Adult Klamath and Rogue suckers used for this study are listed in Wagman and Markle (2000, Appendix 1). The adult sampling targeted spawning groups. Attempts were made to collect 30 individuals from each spawning site and collections included many different age groups. In one case, non-spawning fish were sampled that could represent multiple spawning groups (Eagle Ridge adults from Upper Klamath Lake). These fish have been grouped with Upper Klamath Lake samples. Larval suckers were collected with a dip net or drift nets from April to June, 1996 and 1997 from 14 sites approximating known spawning sites (Appendix 1). Larvae were preserved in 70% ethanol or frozen in 1-ml ultrapure water placed on dry ice and could not be identified to species. Juvenile suckers were collected with castnets in a stratified random sampling regime from Upper Klamath Lake (Simon et al., 2000) during September and October of 1997 (Appendix 1). Juvenile suckers were identified using gillrake and vertebral counts. Catalogued adult specimens are referenced by museum catalog number (OS = Oregon State University Fish Collection) or tissue archive number (#). Juvenile and larval specimens are referenced by field site number.

DNA extraction, library construction, clone isolation, sequencing, PCR amplification, PAGE (polyacrylamide gel electrophoresis) and SSCP (single strand conformational polymorphism) procedures are described in Wagman and Markle (2000).

Ankyrin_G (Figure 3), Collagen 1, and Aldehyde dehydrogenase loci had no more than two alleles in a specimen, each had only two alleles in Klamath Basin, and each produced three genotypes (Table 2). Denaturing PAGE profiles for the size variant alleles (Collagen 1 and Aldehyde dehydrogenase) produced three distinct genotypes without staining variation among the alleles. SSCP profiles for Ankyrin_G (Figure 3) exhibited two single-stranded bands for each allele. Bands stained differently according to their overall charge with the slow band of the A allele (higher in the gel) and the fast band of the B allele (lowest in the gel) staining less intensely than their complements. Scoring of Ankyrin_G genotypes was based on the fast band of the A allele and the slow band of the B allele.

Standard population genetic statistics were generated from the data using the web-based Genepop (version 3.0). We tested for Hardy-Weinberg equilibrium (HWE) using a Hardy-Weinberg exact probability test (web-based Genepop, version 3.0) which employs a Markov chain reiteration of a 1000 re-sampling of the data to determine the probability that chance alone could produce a deviation between the observed and expected values at least as great as the deviation actually realized. If the probability is large then chance alone could account for the deviation (Hartl and Clarke, 1997). Statgraphics (version 3.0) also performed comparisons between loci and species.

RESULTS

Ankyrin_G

Allele A was present in all adult suckers tested (Wagman and Markle, 2000) and was the original Ankyrin_G clone. Allele B was only found in Klamath and Rogue drainages and was found in all four Klamath species (appendix 2a, and Wagman and Markle, 2000). The BB homozygote genotype was absent in all 242 adults sampled (Tables 3, Figure 2), but present in 32% of larvae (104/325) and 50% of juveniles (Table 3, Figure 2). The largest identified BB homozygote was a 71 mm (TL) SNS from Rose Andersen Dam on the Lost River and the smallest adult was 163-mm fork length (FL) KLS from Sycan River (OS 15898-D, tissue #123). Samples in the 60-160 mm size class were generally unavailable for analysis, only two samples are in this range. We tested for sex related

differences in adults and found no significant difference between allele frequencies and sex (ANOVA; $p = 0.178$ for A and $p = 0.201$ for B).

Basin wide frequencies of larval AA homozygotes were identical to pooled adult frequencies (10%) but frequencies of heterozygotes were less (58% *versus* 90%) while frequencies of BB homozygotes were much greater BB (32% *versus* 0%, Table 3, Figure 2). Removal of the BB genotype from the larval data set brings the pooled larval heterozygote frequency (0.85) in line with the pooled adult frequency (0.90) (Table 3). Allele frequencies were significantly different between larvae and adults (ANOVA; $p = 0.00001$ for the A allele). After the removal of the larval BB genotypes, frequencies for the A allele between these life stages were no longer significant (ANOVA; $p = 0.2048$ between adults and larvae). Removal of larval BB genotypes from other sub-areas produced similar results (appendix 2A). SNS juvenile suckers from Upper Klamath Lake also had BB homozygotes. The A allele frequencies between juveniles and adults were significantly different (ANOVA; $p = 0.00001$). Again, removal of the BB genotypes brought genotypic and allelic frequencies of juveniles in line with pooled SNS adults (Table 3). The frequency of allele A was no longer significantly different (ANOVA; $p = 0.1057$).

Collagen 1

Basin wide genotypic frequencies were similar between larvae and adults of Klamath Basin suckers with a preponderance of AA homozygotes in adults (0.74) and larvae (0.69) (Table 3). Allelic frequencies were also nearly identical (Table 3, ANOVA; $p = 0.5603$ based on a comparison of the A allele frequency). After removal of the BB genotypes for Ankyrin_G, allelic frequencies remained not significantly different (ANOVA; $p = 0.2486$). Allele A frequencies were significantly different between SNS juveniles from Upper Klamath Lake and adults (ANOVA; $p = 0.0219$) but are similar after removal of Ankyrin_G BB genotypes (Table 3; ANOVA; $p = 0.3499$ for the A allele frequency comparison between juveniles and adults).

Aldehyde dehydrogenase

Genotypic frequencies were identical between larvae and adults of Klamath Basin suckers with a preponderance of AA homozygotes in both (0.57) (Table 3). Allelic frequencies were also identical (Table 3; ANOVA; $p = 0.6370$ for the A allele). After removal of the BB genotypes for Ankyrin_G, allelic frequencies remained not significantly

different (ANOVA; $p = 0.9205$). SNS juveniles from Upper Klamath Lake are similar to adult SNS before removal of BB genotypes (ANOVA; $p = 0.3316$ for the A allele) and after removal of the AnkyrinG BB genotypes (Table 3; ANOVA; $p = 0.3872$ for the A allele).

Population-level analyses

Because population phenomena can not be analyzed across species we restricted the data set to four single-species analyses: KLS, upper Williamson River at Rocky Ford; KLS, Sycan River; KLS, Gerber Reservoir/Barnes Valley Creek; and SNS, Upper Klamath Lake. The first three data sets were adult-larval comparisons and the fourth was an adult-juvenile comparison.

A Hardy-Weinberg exact probability test of genotypic frequencies from different life stages showed that different life stage populations for Collagen 1 and Aldehyde dehydrogenase had a high probability of being in HWE (Table 2). In contrast, Ankyrin_G genotypes were not in HWE except in the Sycan River and the coefficients were highly significant (Table 2). Comparisons of allelic frequencies between life stages indicated that Collagen 1 and Aldehyde dehydrogenase were not significantly different in seven of eight comparisons (Table 5). The exception was Collagen 1 in SNS from Klamath Lake where the adult population was fixed for the AA genotype. Allelic frequencies were significantly different between life stages for Ankyrin_G except for Gerber Reservoir KLS (Table 5). When data were reanalyzed after removal of individuals that were Ankyrin_G BB homozygotes, results were more uniform across loci (Table 6). Aldehyde dehydrogenase allelic frequencies either did not change or changed by 1% and there were still no significant differences between life stages. Collagen 1 allelic frequencies changed 2-4% and there were still no significant differences between life stages except for juvenile SNS from Upper Klamath Lake (Table 6). Ankyrin_G allelic frequencies changed 8-26% in larvae and juveniles (Table 6) and changed all comparisons with adults to not significantly different.

DISCUSSION

Two of the three loci examined, Collagen 1 and Aldehyde dehydrogenase, were generally similar in genotype and allelic frequencies between young and older life history stages (Table 3). However, all four species of adult suckers were missing the BB genotype of Ankyrin_G while larvae had 32% and juveniles 50% BB genotypes (Table 3).

The high frequency of Ankyrin_G heterozygotes should have resulted in about 20% BB homozygotes.

The results might have been due to sampling error. If sampling error occurred we would expect other, unlinked loci to deviate from HWE. Collagen 1 and Aldehyde dehydrogenase appeared to be unlinked to Ankyrin_G because removal of the BB homozygotes did not change their genotypic or allelic frequencies by more than 4% (Table 3). Collagen 1 and Aldehyde dehydrogenase were in HWE in seven of eight comparisons between different life stages (Table 2). The exception, Collagen 1 in Upper Klamath Lake SNS, was fixed for the AA genotype in adults (Table 2). The lack of linkage with Ankyrin_G and high probability that these two loci are in HWE argues against a sampling error.

The results might have been caused by selection against the BB homozygous Ankyrin_G genotype. Ideally, selection should be followed in a cohort. Our pooled samples included multiple species, populations, and age classes. Because larvae could not be identified to species, our best approximations of a cohort-level analysis were three larval-adult data sets from sites thought to contain a single species and one juvenile-adult data set of SNS from Upper Klamath Lake (Table 2). Genotypic frequencies were in Hardy-Weinberg equilibrium for Collagen 1 and Aldehyde dehydrogenase in all four populations but not for Ankyrin_G in three of four populations. Similarly, differences in allelic frequencies between life stages were not significantly different in seven of eight comparisons with Collagen 1 and Aldehyde dehydrogenase while they were significantly different in three of four Ankyrin_G comparisons (Table 5). When young Ankyrin_G BB homozygotes were removed from the analysis, 11 of 12 life stage comparisons of the three loci were not significantly different. (Table 6). These results indicate that the genotypes and allelic frequencies of young Klamath Basin suckers do not resemble adults until Ankyrin_G BB homozygotes are removed as would happen with selection against the BB genotype.

The Ankyrin_G heterozygote AB genotype was the most common genotype in Klamath Basin (Tables 2 and 5), and suggests a heterozygote advantage or overdominance (Hartl and Clarke, 1997). Because the Ankyrin_G homozygote BB genotype was completely absent in adults, but present in larvae and juveniles up to at least 71 mm, at least one copy of the A allele seems crucial. Further support for the

importance of the A allele was found in outgroups where we have found at least one copy of the A allele in all suckers examined to date (Wagman and Markle, 2000).

The Ankyrin_G system in Klamath Basin suckers may be similar to the heterozygous advantage seen in the thalassemic disease, human sickle cell anemia (Livingstone, 1960, 1989). Populations with a heterozygous advantage will not be in HWE even when one allele is lethal in the homozygote state because stabilizing selection maintains overdominant alleles, as seen in the sickle cell example (Hartl and Clark, 1997). Without stabilizing selection the lethal allele is quickly reduced from the gene pool. Heterozygote advantages appear in many groups (*Pinus ponderosa*; Farris and Mitton, 1984; *Bufo boreas*; Samollow and Soule, 1992; HLA class II type infection of Hepatitis B, Thursz, 1997; *Pleuronectes platessa*, Beardmore and Ward, 1977; Pigeons; Frelinger, 1972; *Dendragapus obscurus*; Redfield 1974; *Colias* sp., Watt, 1977; *Crassostrea virginica*; Singh and Zouros, 1978 and Zouros et al, 1980).

Ankyrin_G is part of a multi-genic family exhibiting multiple splice variants (Kordeli et al., 1995). The polymorphism in Klamath Basin suckers (allele B) is in the extended tail region of the molecule (Figure 1), the region modified in splice variants. The transversion found in allele B is in a sequence that could be involved in a splicing donor site (Lewin, 1983) or be linked to a downstream (3') mutation within the open reading frame (Table 1). The Ankyrin_G molecule is localized in nervous tissue and is associated with a voltage-dependent sodium channel at the nodes of Ranvier (Kordeli et al., 1995). Sodium is exchanged for NH₄⁺ and H⁺ at the gills (Moyle and Cech, 1988) and environmental changes in ammonia and pH could be related to the function of the Ankyrin_G alleles.

Upper Klamath Lake experiences high, potentially lethal, levels of un-ionized ammonia and pH during summer (Kann and Smith, 1999). The juvenile SNS used in this study were collected in 1997 after very high un-ionized ammonia levels had been reached and at least one Ankyrin_G BB juvenile had over-wintered (the largest, 71 mm, caught at Rose Andersen Dam on the Lost River, April, 1997). Water quality conditions are a logical link to the dynamics of Ankyrin_G in Klamath Basin suckers. Conditions responsible for the selection against the BB genotype and selection for the AB genotype may differ but the conditions may be unique to the Rogue and Klamath basins since the B allele has not been found outside the basins (Wagman and Markle, 2000).

Understanding the stabilizing selection for the Ankyrin₆ B allele may be important in conservation and recovery of endangered suckers because it represents a previously unsuspected source of significant early life history mortality. The success or failure of a fish year class is usually attributed to factors such as starvation, predation, or dispersion to unfavorable habitats in the first year of life (Houde, 1987, 1989, Bailey and Houde, 1989, Sinclair, 1988). The endangered lacustrine suckers are long lived (> 30 y) and highly fecund (up to 57,000 eggs/yr. in female SNS and 235,000 eggs/yr. in LRS) (Scopettone and Vinyard, 1991), so year class failure is to be expected. However, we know of no case where early life history mortality in a fish has been attributed to a naturally occurring lethal homozygous genotype. Current efforts to monitor year class strength are based on juvenile sampling in early fall when fish are 60-120 mm FL. Thus, the timing of mortality of the BB genotype has an important bearing on interpretation of year class monitoring efforts.

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Table 1. Ankyrin_G clone sequence (535bp), bold, smallcase areas are primers, underlined area is Ankyrin_G open reading frame; **c *** is the site of the transversion.

5'-AGCCCCAAAGTGCGTCAGCCCACCGGGAAAATGCCGGTATGCCAGATTAC
CTGT**ccagtcctgggaccataccat**ATGTTGCAGGACTTCTTCTATTCTATT
CTATTTGCACAAGGAATGTTTGGATGAAAAATAAATAATCCATGAGTCTTTAT
CAATTACAAAAGAAAAATATTCCTCACTCCTTTTATCCAACCTTTCTTTTTTTT
TTCATTAGATTTTTGTGACAGGGGAAATCTAAAATCGATACTTCCCCTGACA
CACTTAAGATGAAGATATAAAATAACCAGTTTAAGAGAAATGTGAAACATTGA
CACATTTATTGTGCCGAAAACGTTTGCACAGTTCAATGCAC**CAAATCTCTCTG**
*
AACCTTAATTATCTTTGCTTGACCAGAAAAGGGCAATCTGCTGCATTAGTTT
TCTAACTTCTCTGAAAAAATATGTAATAAAATATTCTCATAGTTTTT**Catctta**
tgcttcaggccctcaaAGTCCCTGTGAAAGAACAGACCTTCGAATGGCTA
TTGTGGCA-3'

Table 2: Comparison of genotypic frequencies between life stages at four sites. p values are from a Hardy-Weinberg exact probability test and estimate the probability that a population is in Hardy-Weinberg equilibrium.

Site	Life stage	n	Genotype	Ankyrin _G	Collagen 1	Aldehyde dehydrogenase
Sycan River	Larvae	40	AA	0.075	0.41	0.53
			AB	0.575	0.55	0.40
			BB	0.35	0.04	0.07
			p	0.1807	0.1263	1.0
	Adults	7	AA	0.14	0.43	0.43
			AB	0.86	0.43	0.57
			BB	0	0.14	0
			p	0.1608	1.0	1.0
Rocky Ford	Larvae	22	AA	0	0.59	0.82
			AB	0.55	0.41	0.18
			BB	0.45	0	0
			p	0.1432	0.5381	1.0
	Adults	19	AA	0.05	0.74	0.89
			AB	0.95	0.21	0.11
			BB	0	0.05	0
			p	0.0002*	0.371	1.0
Upper Klamath Lake	Juveniles	69	AA	0.02	0.78	0.78
			AB	0.48	0.22	0.22
			BB	0.50	0	0
			p	0.0513	1.0	1.0
	Adults	27	AA	0.07	1.0	0.74
			AB	0.93	0	0.22
			BB	0	0	0
			p	0.0000*	NA	0.4538
Gerber Reservoir	Larvae	22	AA	0.04	0.59	0.64
			AB	0.82	0.36	0.32
			BB	0.14	0.05	0.04
			p	0.0075*	1.0	1.0
	Adults	21	AA	0.10	0.86	0.71
			AB	0.90	0.14	0.29
			BB	0	0	0
			p	0.0003*	1.0	1.0

Table 3. Pooled genotypic, and allelic data for all adults, SNS juveniles and larvae for Ankyrin_G, Collagen 1 and Aldehyde dehydrogenase loci before and after removal of Ankyrin_G BB genotypes.

Life stage	n	Species	AA	AB	BB	Freq A	Freq B
Ankyrin_G- With Ankyrin_G BB genotypes							
Adults	119	SNS	0.13	0.87	0	0.56	0.44
Adults	45	KLS	0.04	0.96	0	0.52	0.48
Adults	47	KSS	0.02	0.98	0	0.51	0.49
Adults	31	LRS	0.23	0.77	0	0.61	0.39
Adults	242	all adults	0.1	0.9	0	0.55	0.45
Larvae	325	Larvae	0.1	0.58	0.32	0.39	0.61
Juveniles	69	SNS	0.01	0.49	0.5	0.26	0.49
Ankyrin_G- Without Ankyrin_G BB genotypes							
Larvae	221	Larvae	0.15	0.85	0	0.58	0.42
Juveniles	36	SNS	0.03	0.97	0	0.51	0.49
Collagen 1- With Ankyrin_G BB genotypes							
Adult	119	SNS	0.91	0.08	0.01	0.95	0.05
Adult	45	KLS	0.71	0.2	0.09	0.81	0.19
Adult	47	KSS	0.15	0.06	0.79	0.18	0.82
Adult	31	LRS	0.97	0.03	0	0.98	0.02
Adult	242	Total	0.74	0.09	0.17	0.78	0.17
Larvae	325	Larvae	0.69	0.22	0.09	0.8	0.2
Juvenile	69	SNS	0.78	0.22	0	0.89	0.11
Collagen 1- Without Ankyrin_G BB genotypes							
Larvae	221	Larvae	0.72	0.2	0.08	0.82	0.18
Juvenile	38	SNS	0.84	0.16	0	0.92	0.08
Aldehyde Dehydrogenase- With Ankyrin_G BB genotypes							
Adult	119	SNS	0.73	0.25	0.02	0.86	0.14
Adult	45	KLS	0.73	0.27	0	0.87	0.13
Adult	47	KSS	0.02	0.47	0.51	0.26	0.74
Adult	31	LRS	0.58	0.29	0.13	0.73	0.27
Adult	242	Total	0.57	0.3	0.13	0.73	0.27
Larvae	325	Larvae	0.57	0.29	0.14	0.71	0.29
Juvenile	69	SNS	0.78	0.22	0	0.89	0.11
Aldehyde Dehydrogenase- Without Ankyrin_G BB genotypes							
Larvae	221	Larvae	0.58	0.28	0.13	0.73	0.27
Juvenile	38	SNS	0.76	0.24	0	0.88	0.12

Table 5. Allelic frequency comparisons between life stages at four sites before selection. p values represent the results of an ANOVA testing for frequency differences for the A allele.

Site	Life stage	n	Allele	Ankyrin _G	Collagen 1	Aldehyde dehydrogenase
Sycan River	Larvae	40	A	0.36	0.68	0.73
			B	0.64	0.32	0.28
	Adults	7	A	0.57	0.64	0.71
			B	0.43	0.36	0.29
			p	0.008*	0.0866	0.9337
Rocky Ford	Larvae	22	A	0.27	0.80	0.91
			B	0.73	0.20	0.09
	Adults	19	A	0.53	0.84	0.95
			B	0.47	0.16	0.05
			p	0.003*	0.5851	0.5015
Upper Klamath Lake	Juveniles	69	A	0.25	0.89	0.89
			B	0.75	0.11	0.11
	Adults	27	A	0.54	1.0	0.85
			B	0.46	0	0.15
			p	0.0000*	0.008*	0.4457
Gerber Reservoir	Larvae	21	A	0.45	0.77	0.80
			B	0.55	0.23	0.20
	Adults	21	A	0.55	0.93	0.86
			B	0.45	0.07	0.14
			p	0.1873	0.0676	0.5637

Table 6: Allelic frequency comparisons between life stages at four sites after removal of the Ankyrin_G BB individuals from the larval data. p values represent the results of an ANOVA testing for frequency differences for the A allele.

Site	Life stage	n	Allele	Ankyrin _G	Collagen 1	Aldehyde dehydrogenase
Sycan River	Larvae	26	A	0.56	0.71	0.73
			B	0.44	0.29	0.27
	Adults	7	A	0.57	0.64	0.71
			B	0.43	0.36	0.29
			p	0.8492	0.6044	0.9096
Rocky Ford	Larvae	12	A	0.50	0.80	0.91
			B	0.50	0.20	0.09
	Adults	19	A	0.53	0.84	0.95
			B	0.47	0.16	0.05
			p	0.4361	0.38	0.3016
Upper Klamath Lake	Juveniles	34	A	0.51	0.91	0.90
			B	0.49	0.09	0.10
	Adults	27	A	0.54	1.0	0.85
			B	0.46	0	0.15
			p	0.4314	0.0213*	0.4611
Gerber Reservoir	Larvae	19	A	0.53	0.81	0.81
			B	0.47	0.19	0.19
	Adults	21	A	0.55	0.93	0.86
			B	0.45	0.07	0.14
			p	0.6202	0.0822	0.4301

Ankyrin_G Molecule 480 kDa structure

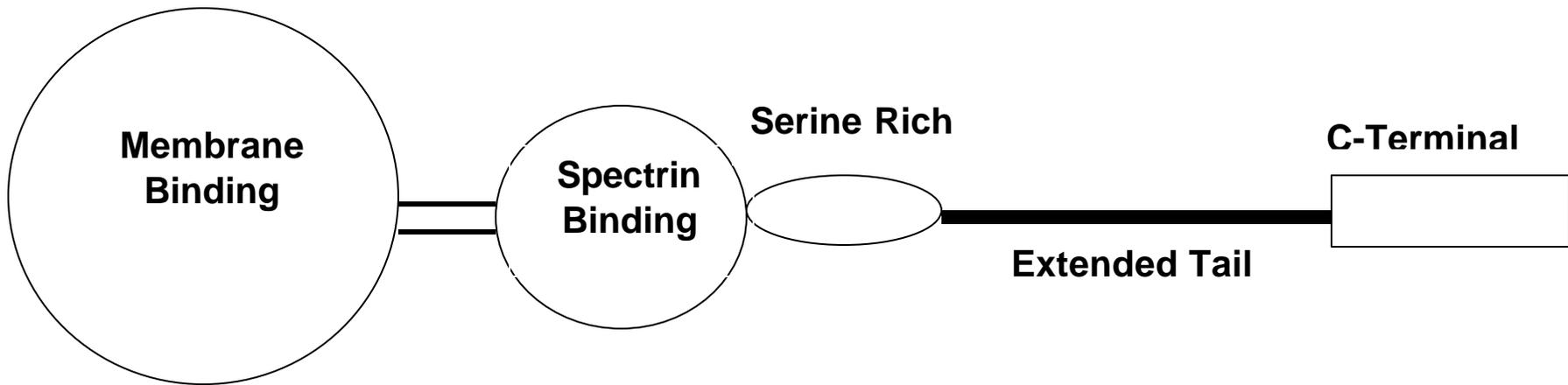


Figure 1. Ankyrin_G molecule (Kordeli et al., 1995) re-printed with permission from the author and journal.

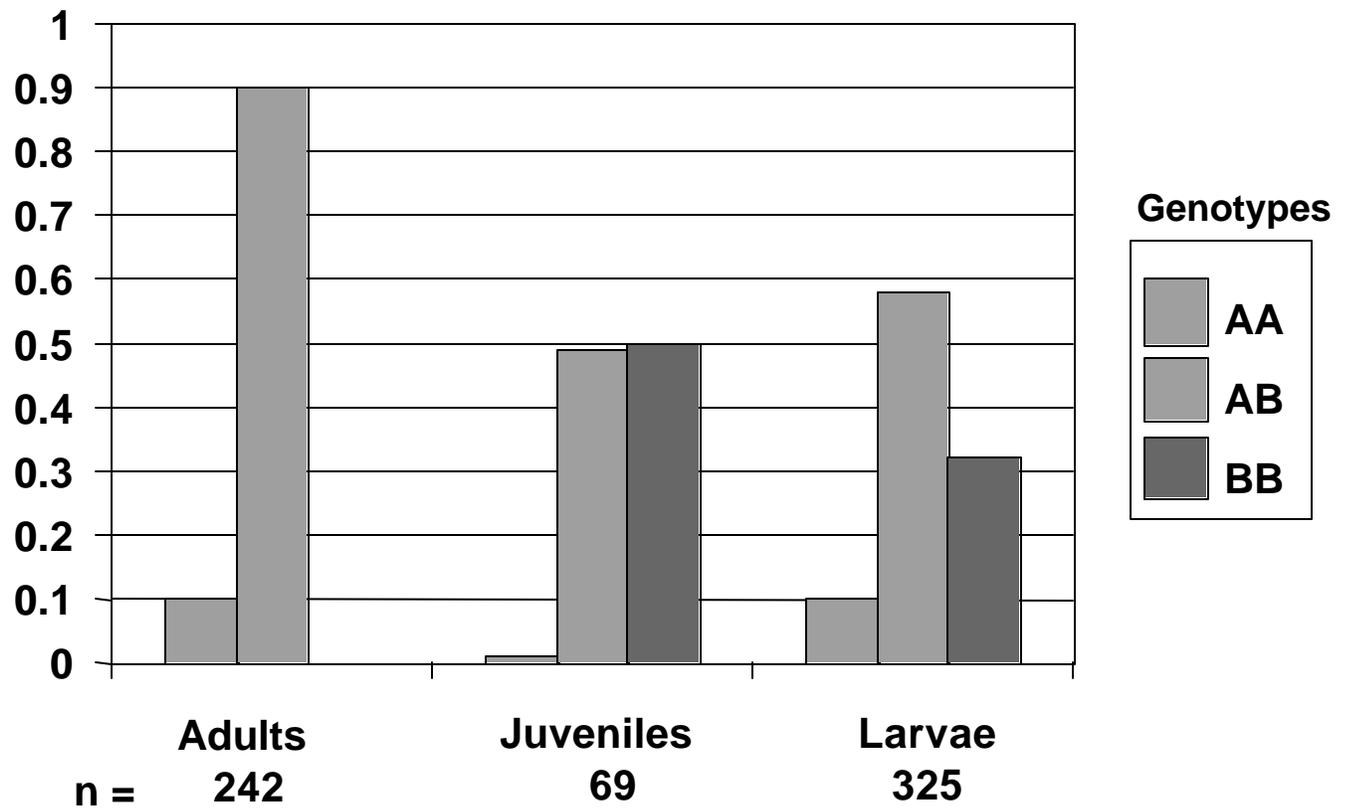


Figure 2. Genotypic frequencies of Klamath basin adults, SNS juveniles and larvae.

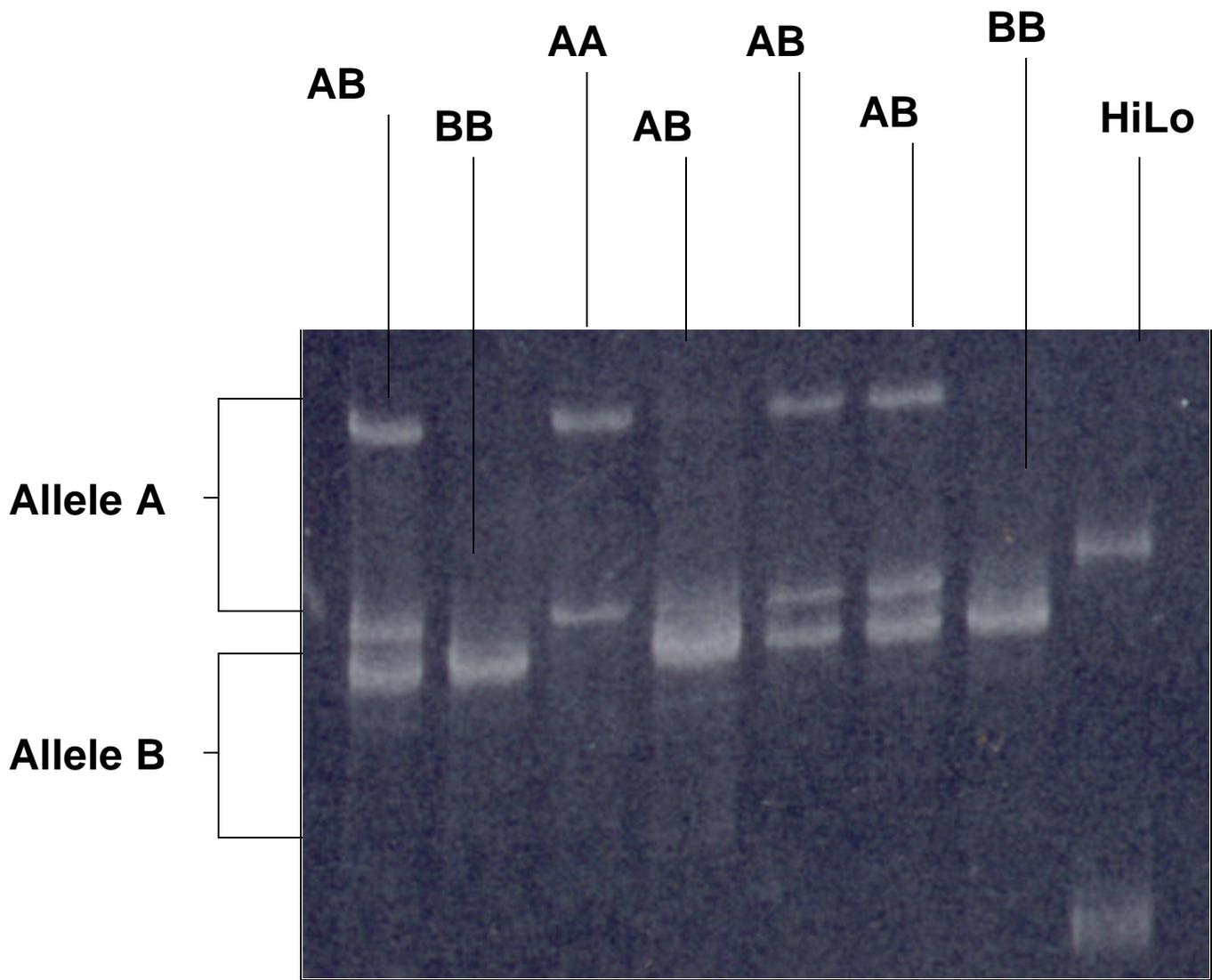


Figure 3. SSCP profile of genotypes of Klamath basin suckers. HiLo are size markers.

Appendix 1: Larval and juvenile suckers used in this study. Samples are listed by drainage, site, latitude and longitude, site code, individual number, Capture date, Developmental stage, and Total Length (mm).

Sprague River, Pole Creek: N42⁰ 21.156',W121⁰ 02.100'

SP3-

10, 6/19/96,ML,18;	21, 6/19/96,ML,20
11, 6/19/96,ML,20	22, 6/19/96,ML,20
12, 6/19/96,ML,19	23, 6/19/96,ML,20
13, 6/19/96,ML,21	24, 6/19/96,ML,22
14, 6/19/96,ML,22	25, 6/19/96,ML,21
15, 6/19/96,ML,21.5	26, 6/19/96,ML,20
16, 6/19/96,ML,22	27, 6/19/96,ML,22
18, 6/19/96,ML,20	28, 6/19/96,ML,23
19, 6/19/96,ML,22	29, 6/19/96,ML,20.5
20, 6/19/96,ML,22.5	30, 6/19/96,ML,21

Sprague River, between Beatty and Bly: N42⁰ 26.822',W121⁰ 14.260'

SP2-

11, 5/13/97, PML, 14

Sprague River, Mile Marker 12: N42⁰ 33.61',W121⁰ 38.92'

SP7-

1, 5/14/97, PML, 15	13, 5/14/97, PML, 18
2, 5/14/97, PML, 15	14, 5/14/97, PML, 15
3, 5/14/97, ML, 18	15, 5/14/97, ML, 21
4, 5/14/97, ML, 18	16, 5/14/97, ML, 18
5, 5/14/97, ML, 19	17,5/14/97,ML,19
6, 5/14/97, ML, 20	18,5/14/97,PML,16
7, 5/14/97, PML, 13	20,5/14/97,ML,21
8, 5/14/97, ML, 17	21,5/14/97,ML,25
9, 5/14/97, PML, 14	22,5/14/97,ML,21
10, 5/14/97, ML, 23.5	23,5/14/97,ML,21
11, 5/14/97, ML, 18	24,5/14/97,PML,12
12, 5/14/97, ML, 17	

Sycan River, Drews Road: N42⁰ 29.15',W121⁰ 16.77+H65'

SY1-

1, 5/14/96, FML, 15	13, 6/19/96, FML, 13.5
2, 5/14/96, FML, 16	15, 6/19/96, FML, 13.5
3, 5/14/96, FML, 13	16, 6/19/96, FML, 13
5, 5/14/96, FML, 13	19, 5/14/96, FML, 14
7, 5/14/96, FML, 12.5	20, 5/14/96, FML, 13
8, 5/14/96, FML, 13	21, 5/14/96, FML, 13
9, 5/14/96, FML, 13.5	22, 5/14/96, FML, 9
11, 5/14/96, PFML, 16	30, 6/19/96, PFM, 14.0

31, 5/14/97, PFM, 14.0
32, 5/14/97, PFM, 13.5
33, 5/14/97, PFM, 14.0
34, 5/14/97, PFM, 14.0
35, 5/14/97, PFM, 13.0
47, 5/14/97, PFM, 14.0
48, 5/14/97, PFM, 13.5
50, 5/14/97, PFM, 13.0
51, 5/14/97, PFM, 14.0
52, 5/14/97, PFM, 14.0
53, 5/14/97, PFM, 15.0
54, 5/14/97, PFM, 12.0

55, 5/14/97, PFM, 14.0
56, 5/14/97, PFM, 14.0
57, 5/14/97, PFM, 13.5
58, 5/14/97, PFM, 13.0
61, 6/4/97, PFM, 14.0
63, 6/4/97, PFM, 14.0
67, 6/4/97, PFM, 12.5
72, 6/4/97, ML, 19.0
73, 6/4/97, PFM, 15.5
76, 6/4/97, PFM, 14.0
77, 6/4/97, ML, 24.0
79, 6/4/97, PFM, 12.0

Williamson River, Rocky Ford: N42⁰ 53.679',W121⁰ 27.821'

WL1-

13, 6/2/97, FM, 13.0
14, 6/2/97, FM, 13.0
15, 6/2/97, FM, 12.0
16, 6/2/97, PFM, 12.0
18, 6/2/97, FM, 12.5
19, 6/2/97, FM, 11.5
20, 6/2/97, FM, 12.0
21, 6/2/97, FM, 12.0
22, 6/2/97, FM, 13.0
24, 6/2/97, FM, 12.0
26, 6/2/97, PFM, 15.5

27, 6/2/97, FM, 13.0
28, 6/2/97, FM, 14.0
31, 6/2/97, FM, 12.0
33, 6/2/97, FM, 12.0
34, 6/2/97, FM, 13.0
35, 6/2/97, FM, 10.0
36, 6/2/97, PFM, 15.5
37, 6/2/97, PFM, 14.0
38, 6/2/97, PFM, 14.0
40, 6/19/96, PFM, 14.5
44, 6/19/96, ML, 17.0

Williamson River, Kirk Bridge: N42⁰ 44.914',W121⁰ 49.967'

WL3-

14, 6/2/97, PF, 12.5
15, 6/2/97, PF, 18
16, 6/2/97, PF, 14
17, 6/2/97, PF, 12
18, 6/2/97, PF, 17
19, 6/2/97, PF, 15
21, 6/2/97, PF, 18
22, 6/2/97, PF, 17.5
23, 6/2/97, PF, 17
24, 6/2/97, PF, 14
25, 6/2/97, PF, 17

26, 6/2/97, PF, 17
27, 6/2/97, PF, 16
29, 6/2/97, ML, 17
30, 6/2/97, PFML, 15
31, 6/2/97, PFML, 15
32, 6/2/97, ML, 18
33, 6/2/97, PFML, 15
34, 6/2/97, ML, 19
35, 6/2/97, PFML, 14
36, 6/2/97, ML, 18
38, 6/2/97, ML, 19

Williamson River, Chiloquin boat ramp: N42⁰ 34.25',W121⁰ 52.74'

WL2-

1, 5/16/96, PL, 12.5
2, 5/16/96, PL, 12

3, 5/16/96, PL, ?
4, 5/16/96, PL, 12
5, 5/16/96, PL, 11.5

8,5/16/96,PL,13.5
9,5/16/96,PL,11.5
12,5/16/96,PL,12
14,5/16/96,PL,13
17,5/16/96,PL,13
18,5/16/96,PL,14
22,5/16/96,PL,12
25,5/14/97,PL,8.0
26,5/14/97,PL,10.0
27,5/14/97,PL,11.0
28,5/14/97,PL,10.0
29,5/14/97,PL,11.0
30,5/14/97,PL,10.0
31,5/14/97,PL,10.0
32,5/14/97,PL,9.0
33,5/14/97,PL,10.0
34,5/14/97,PL,12.0
35,5/14/97,PL,10.0
36,5/14/97,PL,11.0
37,5/14/97,PL,10.0
38,5/14/97,PL,9.0

39,5/14/97,PL,9.0
40,5/14/97,PL,10.0
41,5/14/97,PL,9.0
42,5/14/97,PL,11.0
43,5/14/97,PL,10.0
44,5/14/97,PL,12.0
45,5/14/97,PL,9.0
46,5/14/97,PL,11.0
47,5/14/97,PL,10.0
48,5/14/97,PL,9.0
49,5/14/97,PL,10.0
50,5/14/97,PL,9.0
62,5/16/96,PL,12.0
63,5/16/96,PL,12.0
64,5/16/96,PL,12.0
5,5/16/96,PL,12.0
66,5/16/96,PL,11.5
67,5/16/96,PL,11.0
68,5/16/96,PL,11.0
70,5/16/96,PL,10.5

Upper Klamath Lake, Silver Building Spring: N42⁰ 23.55',W121⁰ 49.17'

U2-

1,5/14/96,PL,15
2,5/14/96,PL,14
5,5/14/96,PL,12
6,5/16/97,PL,12
7,5/16/97,PL,13
9,5/16/97,PL,13
11,5/16/97,PL,14
12,5/16/97,PL,13.5
13,5/16/97,PL,11
14,5/16/97,PL,10

15,5/16/97,PL,10
16,5/16/97,PL,9.5
17,5/16/97,PL,10
18,5/16/97,PL,10.5
20,5/16/97,PL,9
21,5/16/97,PL,11
22,5/16/97,PL,11
23,5/16/97,PL,11
24,5/16/97,PL,10

Upper Klamath Lake, Ouxy Spring: N42⁰ 23.93',W121⁰ 49.41'

U3-

2,5/15/97,PL,12
3,5/15/97,PL,13
4,5/15/97,PL,13
5,5/15/97,PL,12.5
6,5/15/97,PL,12.5
7,5/15/97,PL,13.5
8,5/15/97,PL,14
9,5/15/97,PL,14
10,5/15/97,PL,13

11,5/15/97,PL,13.5
12,5/15/97,PL,13
13,5/15/97,PL,13
14,5/15/97,PL,13.5
15,5/15/97,PL,13.5
16,5/15/97,PL,12.5
17,5/15/97,PL,12.8
18,5/15/97,PL,13
19,5/15/97,PL,12.5

20,5/15/97,PL,12.5

21,5/15/97,PL,13.9

Upper Klamath Lake, Stone House: N42° 15.28',W121° 50.49'

UKSH-

1,5/21/97,ML,18
 2,5/21/97,ML,20
 3,5/21/97,ML,19
 4,5/21/97,PML,14
 5,5/21/97,PML,18
 6,5/21/97,PML,15
 7,5/21/97,PML,14

8,5/21/97,ML,20
 9,5/21/97,ML,18
 11,5/21/97,PML,14
 12,5/21/97,PML,15
 13,5/21/97,PML,16
 14,5/21/97,PML,17

Samples are listed by Field number:

Upper Klamath Lake: Juveniles: *Chasmistes brevirostris*:

A97354-1	A97381-3	A97475-1
A97361-1	A97382-1	A97477-1
A97361-2	A97382-2	A97477-2
A97361-3	A97382-3	A97477-3
A97361-4	A97382-4	A97477-4
A97361-5	A97382-5	A97477-5
A97361-6	A97383-1	A97477-6
A97361-7	A97386-1	A97477-7
A97361-9	A97386-2	A97477-8
A97361-10	A97398-1	A97477-9
A97361-11	A97398-2	A97477-10
A97361-12	A97436-1	A97477-11
A97361-13	A97436-2	A97480-1
A97362-1	A97438-1	A97480-2
A97362-2	A97440-1	A97480-3
A97362-3	A97440-2	A97480-4
A97365-1	A97445-1	A97480-5
A97365-2	A97449-1	A97480-6
A97365-4	A97449-2	A97480-7
A97365-5	A97456-1	A97480-9
A97365-6	A97456-2	A97480-10
A97371-1	A97456-3	A97490-1
A97371-2	A97456-4	A97545-1
A97381-1	A97474-1	A97674-1
A97381-2	A97474-2	

Lost River, Rose-Andersen Dam: N42° 00.454',W121° 31.765'

L1-

2J, 4/22/97,Juvenile, 67

4J, 4/22/97,Juvenile, 71

Lost River, Below Harpold Dam: N42⁰ 10.201',W121⁰ 27.19'

L3B-

10,5/13/97,PML,14	2a,5/15/96,NoData,16
11,5/13/97,PML,12	3a,5/15/96,ND,13
12,5/13/97,PML,13	3b,5/13/97,ND,15
13,5/13/97,PML,14	4a,5/15/96,ND,14
14,5/13/97,ML,15	4b,5/13/97,ND,12
16,5/13/97,PML,12	5a,5/15/96,ND,13
17,5/13/97,PML,14	6a,5/15/96,ND,15
20,5/13/97,PML,13	7a,5/15/96,ND,14
21,5/13/97,PML,13	7b,5/13/97,ND,14
23,5/13/97,PML,13	8a,5/15/96,ND,16

Lost River, Above Harpold Dam: N42⁰ 10.201',W121⁰ 27.19'

L3A-

1,6/19/96,ML,16	14,6/19/96,PML,13
2,6/19/96,ML,15.5	15,6/19/96,ML,19
3,6/19/96,ML,16	16,6/19/96,ML,15
10,6/19/96,ML,15	17,6/19/96,ML,16
11,6/19/96,ML,18	18,6/19/96,PML,15
12,6/19/96,PML,13	19,6/19/96,PML,13
13,6/19/96,PML,15	

Lost River, Bonanza, outside of Big Springs: N42⁰ 11.950',W121⁰ 23.916'

L2-

2,5/13/97,PML,10.5	8,5/13/97,PML,13
3,5/13/97,PML,14	10,5/13/97,PML,12.5
4,5/13/97,PML,12	11,5/13/97,PML,13
5,5/13/97,PML,9.5	
6,5/13/97,PML,13	
7,5/13/97,PML,10	

Lost River, Barnes Valley Creek: N42⁰ 10.7',W121⁰ 03.9'

G1-

13,5/14/96,FML,11.5	26,5/14/96,FML,13
15,5/14/96,FML,13	27,5/14/96,FML,12
16,5/14/96,FML,13	29,5/14/96,FML,13
17,5/14/96,FML,12.5	30,5/14/96,FML,13
18,5/14/96,FML,13	33,5/14/96,FML,12.5
19,5/14/96,FML,13.5	35,5/14/96,FML,13
21,5/14/96,FML,12.5	37,5/14/96,FML,13
22,5/14/96,FML,12.5	38,5/14/96,FML,13
23,5/14/96,FML,14	39,5/14/96,FML,12.5
24,5/14/96,FML,14	40,5/14/96,FML,13
25,5/15/96,FML,13	42,5/14/96,FML,13

Lower Klamath River, Spencer Creek: N420 09.081',W1220 0.698'

LK2-

1,6/5/97,PML,17	14,6/5/97,PML,13
2,6/5/97,PML,13	15,6/5/97,PML,15
3,6/5/97,PML,13	16,6/5/97,PML,15
4,6/5/97,PML,14	17,6/5/97,PML,14.5
6,6/5/97,PML,14	18,6/5/97,PML,14
9,6/5/97,PML,15	19,6/5/97,PML,15
10,6/5/97,PML,14	20,6/5/97,PML,16
11,6/5/97,PML,15	21,6/5/97,PML,16
12,6/5/97,PML,18	23,6/5/97,PML,16
13,6/5/97,PML,18	24,6/5/97,PML,15

Lower Klamath River, Jenny Creek: N420 07.106',W1220 21.976'

LK1-

8,6/20/96,PL,13
9,6/20/96,PL,16.5
10,6/20/96,PL,14
11,6/20/96,PL,16
12,6/20/96,PL,17
14,6/20/96,PL,15
15,6/20/96,PL,17
16,6/20/96,PL,16
18,6/20/96,PL,19
19,6/20/96,PL,14
21,6/20/96,PL,15
23,6/20/96,PL,14
24,6/20/96,PL,1

Appendix 2: Genotypic and allelic frequencies for all life stages, identified species and sites.

A) Ankyrin_G, B) Collagen 1, C) Aldehyde dehydrogenase

A) Ankyrin_G genotypic and allelic frequencies by species, life stage and site. Selection is simulated by removal of the BB genotype individuals from the larval data set.

Species	site	With Ankyrin _G BB genotypes						Without Ankyrin _G BB genotypes				
		n	AA	AB	BB	Freq A	Freq B	n	AA	AB	Freq A	Freq B
SNS	all	119	0.13	0.87	0	0.56	0.44					
KLS	all	45	0.04	0.96	0	0.52	0.48					
KSS	all	47	0.02	0.98	0	0.51	0.49					
LRS	all	31	0.23	0.77	0	0.61	0.39					
Total	all	242	0.10	0.90	0	0.55	0.45					
Larvae	all	325	0.10	0.58	0.32	0.39	0.61	221	0.15	0.85	0.58	0.42
SNS	Sprague	5	0.20	0.8	0	0.60	0.40					
KLS	Sprague	16	0	1.0	0	0.50	0.50					
LRS	Sprague	5	0.20	0.8	0	0.60	0.40					
Larvae	Pole Creek	21	0.10	0.52	0.38	0.36	0.64	13	0.15	0.85	0.58	0.42
Larvae	Mile Marker 12	23	0.09	0.91	0	0.54	0.46	23	0.09	0.91	0.54	0.46
Larvae	total	44	0.09	0.73	0.18	0.45	0.55	36	0.11	0.89	0.56	0.44
KLS	Sycan	7	0.14	0.86	0	0.57	0.43					
Larvae	total	40	0.08	0.58	0.34	0.36	0.64	26	0.12	0.88	0.56	0.44
KLS	Rocky Ford	19	0.06	0.94	0	0.53	0.47					
Larvae	Rocky Ford	22	0	0.55	0.45	0.27	0.73	12	0	1.0	0.50	0.50
Larvae	Kirk Bridge	22	0.14	0.32	0.54	0.30	0.70	10	0.30	0.70	0.65	0.35
SNS	Lower Williamson	12	0.08	0.92	0	0.54	0.46					
KLS	Lower Williamson	1	0	1.0	0	0.50	0.5					
LRS	Lower Williamson	1	0	1.0	0	0.50	0.5					
Larvae	Chiloquin boat	46	0	0.54	0.46	0.27	0.73	25	0	1.0	0.50	0.50

ramp												
SNS	Upper Klamath Lake (UKL)	10	0	1	0	0.5	0.50					
KSS	UKL	1	0	1	0	0.5	0.50					
LRS	UKL	14	0.29	0.71	0	0.64	0.36					
Larvae	Silver Building Spring	19	0.21	0.63	0.16	0.53	0.47	16	0.25	0.75	0.63	0.37
Larvae	Ouxy Spring	20	0.25	0.75	0	0.63	0.37	20	0.25	0.75	0.63	0.37
Larvae	Stone House	13	0	0.31	0.69	0.15	0.85	4	0	1.0	0.50	0.50
Larvae	total	52	0.18	0.61	0.21	0.48	0.52	40	0.23	0.77	0.61	0.39
SNS	Juveniles	69	0.02	0.48	0.50	0.25	0.75	34	0.03	0.97	0.51	0.49
SNS	Gerber	20	0.10	0.90	0	0.55	0.45					
KLS	Gerber	1	0	1.0	0	0.50	0.50					
Larvae	Barnes Valley Creek	22	0.04	0.82	0.14	0.45	0.55	19	0.05	0.95	0.53	0.47
SNS	Lost River	1	0	1.0	0	0.50	0.50					
LRS	Lost River	3	0	1.0	0	0.50	0.50					
Juveniles	Rose Andersen Dam	2	0	0	1.0	0	1.0	0	0	0	0	0
Larvae	Below Harpold Dam	20	0.05	0.55	0.4	0.33	0.67	12	0.08	0.92	0.54	0.46
Larvae	Above Harpold Dam	13	0.92	0.08	0	0.96	0.04	13	0.92	0.08	0.96	0.04
Larvae	Big Springs, Bonanza	9	0.11	0.89	0	0.56	0.44	9	0.11	0.89	0.56	0.44
Larvae	total	44	0.33	0.47	0.19	0.57	0.43	36	0.39	0.61	0.69	0.31
SNS	Clear Lake	62	0.18	0.82	0	0.59	0.41					
LRS	Clear Lake	8	0.25	0.75	0	0.63	0.37					
SNS	Topsy	3	0	1.0	0	0.50	0.50					

KLS	Topsy	1	0	1.0	0	0.50	0.50					
KSS	Topsy	18	0.06	0.94	0	0.53	0.47					
Larvae	Spencer Creek	20	0	0.2	0.8	0.1	0.9	4	0	1.0	0.50	0.50
SNS	Copco	5	0	1.0	0	0.50	0.50					
Larvae	Jenny Creek	13	0	1.0	0	0.50	0.50	13	0	1.0	0.50	0.50
KSS	Rogue River	28	0	1.0	0	0.50	0.50					
SNS	Hatchery	1	0	1.0	0	0.50	0.50					

B) Collagen 1-Genotypic and allelic frequencies by species and site.

Species	site	n	AA	AB	BB	Freq A	Freq B
SNS	all	119	0.91	0.08	0.01	0.95	0.05
KLS	all	45	0.71	0.20	0.09	0.81	0.19
KSS	all	47	0.15	0.06	0.79	0.18	0.82
LRS	all	31	0.97	0.03	0	0.98	0.02
Total	all	242	0.74	0.09	0.17	0.78	0.17
Larvae	all	325	0.69	0.22	0.09	0.80	0.20
SNS	Sprague	5	1.0	0	0	1.0	
KLS	Sprague	16	0	1.0	1.0	0.91	0.09
LRS	Sprague	5	0.80	0.20	0	0.90	0.10
Larvae	Pole Creek	20	0.75	0.25	0	0.88	0.12
Larvae	Mile Marker 12	24	0.83	0.17	0	0.92	0.08
Larvae	total	44	0.80	0.20	0	0.90	0.10
KLS	Sycan	7	0.285	0.428	0.285	0.50	0.50
Larvae	Drews Road	40	0.45	0.52	0.03	0.71	0.29

KLS	Rocky Ford	19	0.74	0.21	0.05	0.84	0.16
Larvae	Rocky Ford	21	0.57	0.43	0	0.79	0.21
Larvae	Kirk Bridge	22	1.0	0	0	1.0	0
SNS	Lower Williamson	12	1.0	0	0	1.0	0
KLS	Lower Williamson	1	1.0	0	0	1.0	0
LRS	Lower Williamson	1	1.0	0	0	1.0	0
Larvae	Chiloquin Boat ramp	46	0.87	0.13	0	0.93	0.07
SNS	Upper Klamath Lake	10	1.0	0	0	1.0	0
SNS- Juv	Upper Klamath Lake	69	0.78	0.22	0	0.89	0.11
KSS	Upper Klamath Lake	1	1.0	0	0	1.0	0
LRS	Upper Klamath Lake	14	1.0	0	0	1.0	0
Larvae	Silver Building Spring	19	1.0	0	0	1.0	0
Larvae	Ouxy Spring	20	1.0	0	0	1.0	0
Larvae	Stone House	13	0.92	0.08	0	0.96	0.04
SNS	Gerber	20	0.85	0.15	0	0.93	0.07
KLS	Gerber	1	1.0	0	0	1.0	0
Larvae	Barnes Valley Creek	22	0.59	0.36	0.05	0.77	0.23
SNS	Lost River	1	1.0	0	0	1.0	0
LRS	Lost River	3	1.0	0	0	1.0	0
Larvae	Rose Andersen Dam	2	1.0	0	0	1.0	0
Larvae	Below Harpold	20	0.55	0.40	0.05	0.75	0.25
Larvae	Above Harpold	13	1.0	0	0	1.0	0
Larvae	Big Springs, Bonanza	9	0.67	0.33	0	0.83	0.17
SNS	Clear Lake	62	0.90	0.08	0.02	0.94	0.06
LRS	Clear Lake	8	1.0	0	0	1.0	0
SNS	Topsy Reservoir	3	1.0	0	0	1.0	0
KLS	Topsy Reservoir	1	0	1.0	0	0.50	0.50

KSS Larvae	Topsy Reservoir Spencer Creek	18 20	0.33 0	0.17 0.30	0.50 0.70	0.42 0.15	0.58 0.85
SNS Larvae	Copco Jenny Creek	5 13	0.80 0	0.20 0	0 1.0	0.90 0	0.10 1.0
KSS	Rogue River	28	0	0	1.0	0	1.0
SNS	Hatchery	1	1.0	0	0	1.0	0

C) Aldehyde dehydrogenase genotypic and allelic frequencies by species and site.

Species	site	n	AA	AB	BB	Freq A	Freq B
SNS	all	119	0.73	0.25	0.02	0.86	0.14
KLS	all	45	0.73	0.27	0	0.87	0.13
KSS	all	47	0.02	0.47	0.51	0.26	0.74
LRS	all	31	0.58	0.29	0.13	0.73	0.27
Total	all	242	0.57	0.3	0.13	0.73	0.27
Larvae	all	325	0.57	0.29	0.14	0.71	0.29
SNS	Sprague	5	0.60	0.20	0.20	0.70	0.30
KLS	Sprague	16	0.63	0.37	0	0.81	0.19
LRS	Sprague	5	0.40	0.40	0.20	0.60	0.40
Larvae	Pole Creek	20	0.35	0.45	0.20	0.58	0.42
Larvae	Mile Marker 12	24	0.79	0.21	0	0.90	0.10
Larvae	total	44	0.58	0.33	0.09	0.74	0.26
KLS Larvae	Sycan Drews Road	7 40	0.43 0.52	0.57 0.40	0 0.08	0.71 0.73	0.29 0.28
KLS Larvae	Rocky Ford Rocky Ford	19 22	0.89 0.82	0.11 0.18	0 0	0.95 0.91	0.05 0.09

SNS	Lower Williamson	12	0.83	0.17	0	0.92	0.08
KLS	Lower Williamson	1	1.0			1.0	
LRS	Lower Williamson	1	1.0			1.0	
Larvae	Chiloquin boat ramp	46	0.54	0.43	0.02	0.76	0.24
SNS	Upper Klamath Lake	10	0.70	0.30	0	0.85	0.15
SNS-	Upper Klamath Lake	69	0.78	0.22	0	0.89	0.11
Juveniles							
KSS	Upper Klamath Lake	1	0	0	1.0	0	1.0
LRS	Upper Klamath Lake	14	0.79	0.14	0.07	0.86	0.14
Larvae	Silver Building Spring	19	0.58	0.37	0.05	0.76	0.24
Larvae	Ouxy Spring	20	0.45	0.35	0.20	0.63	0.38
Larvae	Stone House	13	0.62	0.31	0.07	0.77	0.23
SNS	Gerber	20	0.70	0.30	0	0.85	0.15
KLS	Gerber	1	1.0			1.0	
Larvae	Barnes Valley Creek	22	0.64	0.32	0.04	0.80	0.20
SNS	Lost River	1	0	1.0	0	0.50	0.50
LRS	Lost River	3	0.33	0.67	0	0.67	0.33
Larvae	Rose Andersen Dam	2	0.50	0	0.50	0.50	0.50
Larvae	Below Harpold Dam	20	0.60	0.40	0	0.80	0.20
Larvae	Above Harpold Dam	13	1.0	0	0	1.0	0
Larvae	Big Springs, Bonanza	9	0.67	0.33	0	0.83	0.17
Larvae	total	44	0.73	0.25	0.02	0.85	0.15
SNS	Clear Lake	62	0.79	0.19	0.02	0.89	0.11
LRS	Clear Lake	8	0.375	0.375	0.25	0.56	0.44
SNS	Topsy Reservoir	3	0.34	0.66	0	0.67	0.33
KLS	Topsy Reservoir	1	1.0	0	0	1.0	0
KSS	Topsy Reservoir	18	0.06	0.77	0.17	0.44	0.56
Larvae	Spencer Creek	20	0	0	1.0	0	1.0

SNS Larvae	Copco Jenny Creek	5 13	0.40 0	0.60 0.15	0 0.85	0.70 0.08	0.30 0.92
KSS	Rogue River	28	0	0.29	0.71	0.14	0.86
SNS	Hatchery	1	1.0	0	0	1	

