Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Razorback Sucker Broodstock Evaluation and Genetic Monitoring

2012 Annual Report

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

September 2012

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Multi-Species Conservation Program

Razorback Sucker Broodstock Evaluation and Genetic Monitoring

2012 Annual Report

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September 2012

ACRONYMS AND ABBREVIATIONS

GR	Green River
GV	Grand Valley
µL	microliter(s)
mM	millimolar
NFH	National Fish Hatchery
NFHTC	National Fish Hatchery and Technology Center
OR	Ouray
PCR	polymerase chain reaction

Symbols

°C	degrees Celsius
%	percent

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BACKGROUND

Ouray National Fish Hatchery (NFH) is a complex that consists of two facilities—Ouray (OR) and Grand Valley (GV). The Ouray facility (Vernal, Utah) maintains razorback sucker (*Xyrauchen texanus*) broodstocks that were developed with wild individuals from the Green River (GR). Beginning in 1989, a mix of 15 females and 13 males were spawned annually for 3 years to create three year-classes (YC) (GR-OR-F_0: 89YC, GR-OR-F_0: 90YC, and GR-OR-F_0: 91YC).

The Grand Valley facility (Grand Junction, Colorado) maintains broodstocks that were initially developed with individuals from the mainstem upper Colorado River (CR-GV- F_0: 89YC), including Etter Pond (EP-GV- F_0: 93YC). In addition, individuals from the San Juan (SJ) River arm of Lake Powell were spawned at Ouray in 1992, with both adults and offspring being transferred to Grand Valley (SJ-GV-F_1: 92YC) in 1995. Lake Mohave and Green River individuals were added to the broodstock to increase the number of mating pairs (i.e., diversity) (U.S. Fish and Wildlife Service 2003; Upper Colorado River Endangered Fish Recovery Program [UCREFRP] Biology Committee Meeting summary 2003). Therefore, the Grand Valley stocks are a mix of individuals from different populations.

To prevent inbreeding, the staff at Grand Valley have separated and tracked individual family lots; however, this mating strategy requires a large amount of space and time and is extremely time consuming. The purpose of this study was to determine the genetic diversity and pairwise relatedness of individuals from the Ouray National Fish Hatchery Grand Valley Unit and compare those estimates to the broodstocks held at Dexter National Fish Hatchery and Technology Center and wild Lake Mohave individuals. This information will be used to determine if randomly selecting individuals for spawning without tracking family lots can be accomplished without altering genetic diversity, including inbreeding.

MATERIALS AND METHODS

Tissues

A total of 96 razorback suckers were collected from Grand Valley during the April 2011 inventory and consisted of five year-classes (2004, 2005, 2006, 2007, and 2008). All individuals had a small portion of their fin clipped, after which they were returned to the population alive. These fin clips were then stored in 95 percent (%) ethanol until DNA extraction.

Extraction, Polymerase Chain Reaction, and Genotyping

Genomic DNA was extracted using Qiagen DNeasy® 96 Blood and Tissue Kits following the manufacturer's instructions, after which samples were stored at -80 degrees Celsius (ºC). Polymerase chain reaction (PCR) amplifications (10 microliters [µL]) consisted of 0.175 µL AmpliTaq Gold® DNA polymerase; 1X GeneAmp® 10X PCR buffer; 2.5 millimolar (mM) $MgCl_2$; 1.5 mM dNTPs; 0.5 µL each, forward and reverse primers; 3.5 µL ddH_20; and 2 µL DNA. Forward primers (table 1) were labeled with one of four fluorescent dyes (6-FAM, PET, NED, and VIC). All PCR reagents and primers were purchased from Applied Biosystems, Foster City, California. Amplification for all samples consisted of a touchdown protocol performed in an ABI 9700 GeneScan™ thermal cycler. The thermal profile included a denaturing step of 95 ºC for 9 minutes (to activate the polymerase), followed by 33 cycles of 94 ºC for 45 seconds, an initial annealing temperature of 56 ºC for 45 seconds, and an extension temperature of 72 ºC for 60 seconds. The annealing temperature decreased by 0.2 ºC for every cycle. The final extension cycle was 15 minutes at 70 ºC. PCR products were processed on an ABI 3130xl genetic analyzer using the GeneScan™ 500 LIZ® size standard. Composite genotypes for individual fish were compiled with GeneMapper™ 4.0 software (Applied Biosystems).

Table 1.—Details of the 13 microsatellite loci used to screen captive (Dexter National Fish Hatchery and Technology Center and Ouray NFH) and wild (Lake Mohave) *Xyrauchen texanus*

Locus	T_A (°C)[1]	Repeat Mofiff	Primer Sequence	Reference
*Dlu*416	57	$(GATA)_{26}$	F: TATTAATCAACATAACTGACAAAG R: TTCTGAAATGATGAAAAAGTC	Tranah et al., 2001
*Xte*23	56_{TD}	$(ATCT)_{22}X_8(ATCT)_1(GTCT)_4X_4(GTCT)_6$	F: GTTATGTTTGAATGAAAGGT R: TCAGAGTAGAATATCAAGG	Dexter Unpublished
*Xte*15[2]	56_{TD}	$(ATCT)_{14}(GTCT)_{14}X_8(GTCT)_3X_8(GTCT)_5$	F: CATTAGCACACTGGAATCTC R: TAGTCTTACCCAGATGAACAG	Dexter Unpublished
*Dlu*476	57	$(GATA)_{35}$	F: ATGGTTGGCTACTTTAACAATCAA R: TACACCTCCAATCTCGTTTCATAA	Tranah et al., 2001
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*Dlu*4184	57	$(GATA)_{15}$	F: C CATGCATGCACCAATGTAGAAAT R: CAGCAGTGCCCATATGATTACACA	Tranah et al., 2001
*Dlu*439	57	$(ACAG)_7(GATA)_{25}$	F: GAGACAGTCCACACTTCACATTGT R: TTCCATAATACACTCTTGGCATAG	Tranah et al., 2001
*Dlu*4201	57	$(GATA)_{21}$	F: CCAACCTTCTGAACAACTGTAAAT R: GTGGTAAAGAGGTCTGCCTGTAT	Tranah et al., 2001
*Dlu*4300	57	$(GATA)_{22}$	F: CACACCTGTTAGTGAGCTCCTCTC R: AAACCAATAAAGCAATAGATAGAA	Tranah et al., 2001
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US6	57	$(TCTA)_{15}$	F: AAGTGTGTGCCAAAGCATCA R: GCCTTGTTAAGGGCATATGAA	Cardall et al., 2006
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1. Annealing temperature described in reference paper; TD refers to Touch Down PCR program (see Materials and Methods in this report).
2. This is different from the Dowling and Marsh (2010) *Xte*15.

Data Analysis

GENEALEX v6 (Peakall and Smouse 2006) and MLRELATE (Kalinowski et al. 2006) were used to calculate relatedness (probability that two individuals share alleles, R_{XY}). The relationship categories used were unrelated ($R_{XY} < 0.09$), cousins ($R_{XY} = 0.1$ to 0.18), half sibs ($R_{XY} = 0.19$ to 0.38), and full sibs ($R_{XY} > 0.39$).

RESULTS/DISCUSSION

All descriptive statistics (heterozygosity, Hardy-Weinburg equilibrium, number of alleles, and allelic richness) with comparisons to samples taken at the Dexter National Fish Hatchery and Technology Center (NFHTC) and Lake Mohave (wild) are reported and discussed in attachment 1. In summary, the samples from the Grand Valley unit had fewer alleles (lower allelic richness) than the stocks of the Dexter and wild Lake Mohave samples. These results were consistent with those obtained from wild Upper Basin samples taken from the Green and Colorado Rivers (Dowling and Marsh 2010).

In an analysis of pairwise relatedness in the Grand Valley stocks, 79% of the pairs were unrelated, 12% were cousins, 6% were half sibs, and 2% were full sibs (figure 1); however, the full sib and half sib estimates may be inflated. The 2008 year-class had multiple samples taken from the same lot number, which were identified as full sibs. For example, lot number 0810 was represented by 9 of the 39 (23%) 2008 year-class samples. Likewise, lot number 0806 was represented by 7 (18%) of the 39 samples. These two lots together represent 41% of the samples analyzed from the 2008 year-class with the other 59% being from 9 other lots; however, if these are not inflated estimates, individuals within the Grand Valley broodstocks are more related than individuals within any of the Dexter stocks (figure 1). Even if the unrelated and cousin categories are combined, approximately 1 in 10 pairs drawn at random are either full sibs or half sibs.

RECOMMENDATIONS

1) These results indicate that individuals should not be drawn at random without any other information.

    a. Family lots and unique matings should continue to be tracked.

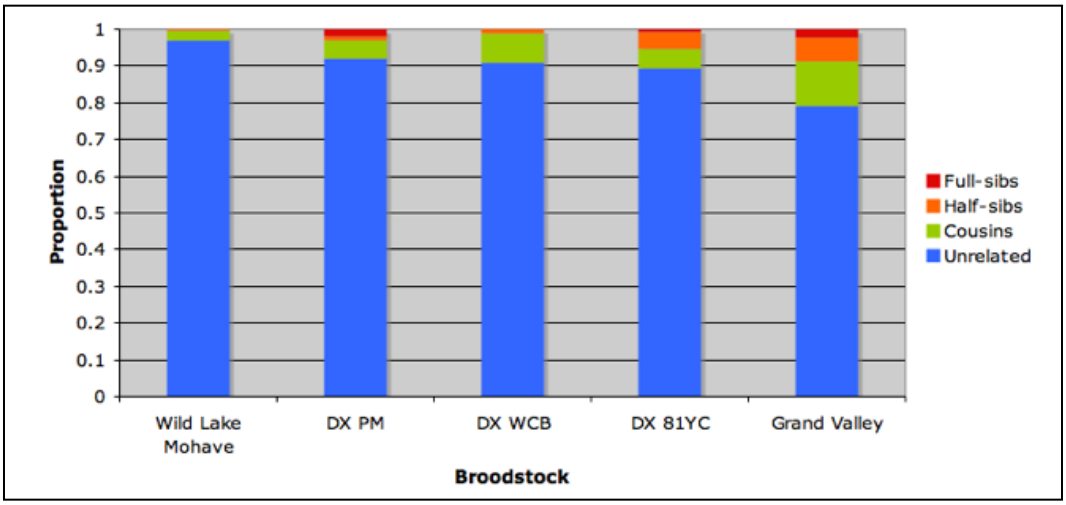

Figure 1.—Pairwise relatedness proportion estimates of the different broodstocks at Dexter NFHTC, Grand Valley Unit (Ouray NFH), and wild Lake Mohave.

b. Alternatively, individuals selected for spawning should be genetically screened prior to spawning to determine relatedness and inbreeding levels. This could be done as a rapid response study in which results are reported within 7 days of Dexter NFHTC receiving the samples. The results would be a list of pairs that are determined to be either half or full sibs, thus avoiding future inbreeding.

LITERATURE CITED

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ATTACHMENT 1

2011 Annual Report

2011 Annual Report

Dexter National Fish Hatchery and Technology Center

Razorback Sucker Broodstock Evaluation and Genetic Monitoring

Inter-agency Acquisition R11PG30006

Photo Credit U.S.F.W.S

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30 September 2011

EXECUTIVE SUMMARY

The Razorback sucker, *Xyrauchen texanus*, is an endangered endemic fish from the Colorado River system. Wild populations are in rapid decline, with an estimated 200 wild fish remaining in Lake Mohave. Because of these declines, *X. texanus* has been reared in captivity at nine facilities in both the upper and lower Colorado Basins. Of these facilities Dexter National Fish Hatchery and Technology Center (NFHTC) and Ouray National Fish Hatchery (NFH), are responsible for a majority of the spawning activities.

Currently, the genetic relationship of the wild and captive stocks is unknown. In keeping with Dexter's Genetics Management and Captive Propagation Plan the genetic diversity of both the Ouray NFH (including Grand Valley) should be determined. In FY2010, a microsatellite analysis of the Dexter, Ouray, and Grand Valley broodstocks was partially completed and a comparison of mitochondrial diversity in the Dexter captive stocks versus the wild Lake Mohave population was not undertaken. Thus, the objectives for FY2011 were two fold: 1) continue to document the genetic status of the Dexter captive broodstocks by determining the mitochondrial diversity of the stocks and comparing it to the diversity of wild Lake Mohave fishes and 2) characterize the genetic status of the Ouray and Grand Valley captive stocks using microsatellites.

CONCLUSIONS

Overall the Dexter captive stocks were genetically diverse and almost identical to the wild Lake Mohave population as measured by microsatellites. The Ouray and Grand Valley stocks are also diverse, but had lower allelic richness when compared to the lower basin (Dexter and wild Lake Mohave) samples, a pattern similar to what has been observed in wild upper basin populations. In a comparison of mitochondrial diversity, both the Dexter 1981 year-class and

Dexter wild caught broodstock were as diverse as the wild Lake Mohave population, with the exception of a few rare haplotypes.

RECOMMENDATIONS

1) The Dexter NFHTC stocks are diverse and can be viewed as the secondary lower basin population, with the wild Lake Mohave population being the primary population. The wild caught broodstock should be the primary stock used for production and recovery.

2) Complete analyses of all upper basin stocks are needed before recommendations can be made. These additional analyses include calculating pairwise relatedness of the Ouray and Grand Valley stocks (FY2012 objective). This additional information will help determine what steps may be necessary to increase the diversity of the Ouray and Grand Valley stocks.

INTRODUCTION

The Razorback sucker, *Xyrauchen texanus*, is an endangered endemic fish from the Colorado River system. Wild populations are in rapid decline, with an estimated 200 wild fish remaining in Lake Mohave. Because of these declines, *X. texanus* has been reared in captivity at nine facilities in both the upper and lower Colorado Basins (Mueller, 2006). Of these facilities, Dexter National Fish Hatchery and Technology Center (NFHTC) and Ouray National Fish Hatchery (NFH) are responsible for a majority of the spawning activities with other facilities receiving eggs/larvae for grow-out or holding facilities of backup broodstocks (see Table 1 in Mueller, 2006).

Dexter NFHTC (DX) in Dexter, New Mexico maintains three broodstocks: a Lake Mohave (LM) 1981 year class (LM-DX-F_0: 81YC) developed from 136 wild caught adults, of these 49 are still alive on station; a paired mating (PM) future broodstock (LM-DX-F_1: PM) which are the product of paired matings of Lake Mohave wild caught adults spawned at Willow Beach NFH between 1994 and 2003; and a wild caught (WCB) future broodstock (LM-DX-F_0: WCB) which is a mix of 5 year classes of wild-caught larval fish from Lake Mohave at 8 sites between 1999 and 2004. Dexter's stocks provide an essential link to the original wild fish from the Lake Mohave area, and may be needed for future recovery efforts to provide fish for augmentation in Lake Mohave.

Ouray NFH is a complex that consists of two facilities, Ouray (OR) and Grand Valley (GV). The Ouray facility (Vernal, Utah) maintains broodstocks that were developed with wild individuals from the Green River (GR). Beginning in 1989, a mix of 15 females and 13 males were spawned annually for three years to create three year classes (GR-OR-F_0: 89YC, GR-OR-F_0: 90YC, and GR-OR-F_0: 91YC).

The Grand Valley facility (Grand Junction, Colorado) maintains broodstocks that were initially developed with individuals from the mainstem upper Colorado River (CR-GV- F_0: 89YC) including Etter Pond (EP-GV- F_0: 93YC). In addition, individuals from the San Juan River arm of Lake Powell were spawned at Ouray in 1992 with both adults and offspring being transferred to Grand Valley (SJ-GV-F_1: 92YC) in 1995. Lake Mohave and Green River individuals were added to the broodstock to increase the number of mating pairs (i.e., diversity) (USFWS, 2003; UCREFRP Biology Committee Meeting summary, 2003). Therefore, the Grand Valley stocks are a mix (MX) of individuals from different populations.

Currently, the genetic relationship of the wild and captive populations is based on a mitochondrial DNA study using small sample sizes (Dowling et al., 1996a). In keeping with Dexter's Genetics Management and Captive Propagation Plan (USFWS, 2003), the overall objectives are: 1) document the microsatellite and mitochondrial genetic status of Dexter's captive broodstocks; 2) document the genetic status of the upper Colorado River basin broodstocks (Ouray including Grand Valley) and determine if those stocks are different from the Lower basin population (Dexter); 3) characterize the pairwise relatedness of individuals so that a studbook system can be established for the breeding of non-related individuals; 4) update Dexter's 2003 Razorback Sucker Genetics Management and Captive Propagation Plan; 5) annually monitor the stocks. In all, these goals ensure that future management of broodfish and production fish can provide a genetically appropriate product for restoration activities in the entire (upper and lower) Colorado River Basin.

In FY2010, an analysis of microsatellite data from objectives 1 and 2 (document the genetic diversity of Dexter and the wild Lake Mohave) was partially completed. The mitochondrial portion of objectives 1 and 2 was not completed. Thus, the objectives of FY2011

were two fold: 1) continue to document the genetic status of the Dexter captive broodstocks by determining the mitochondrial diversity of the stocks and comparing it to the diversity of wild Lake Mohave fishes and 2) characterize the genetic status of the Ouray and Grand Valley captive stocks using microsatellites.

MATERIALS AND METHODS

Tissues

A total of 657 razorback suckers collected from Dexter, Ouray, Grand Valley, and wild Lake Mohave were used in this study. Samples from Dexter NFHTC included the LM-DX: 81YC (n = 43), LM-DX: PM (n = 71), LM-DX: WCB (n = 248) captive stocks. The Grand Valley samples were collected during the 2003 and 2011 inventories (April) and consist of five different year classes [1992, 1994, 1995 (n = 93) and 2004, 2005, 2006, 2007, 2008 (n = 96)]. The samples (n = 69) from Ouray NFH (Utah) consist of a mix of nine different year classes (1989, 1990, 1991, 1993, 1994, 1995, 1996, 1997, 1998). Wild caught Lake Mohave individuals (n = 37) were clipped at Willow Beach NFH during the annual razorback sucker roundup in 2000.

All individuals had a small portion of their fin clipped after which they were returned to the population alive. These fin clips were then stored in 95% ethanol until DNA extraction.

Extraction, PCR, and Genotyping

Genomic DNA was extracted using Qiagen DNeasy® 96 Blood and Tissue Kits following the manufacturer's instructions, after which samples were stored at -80 ºC. PCR amplifications (10 µl) consisted of 0.175 µl AmpliTaq Gold® DNA polymerase; 1X GeneAmp® 10X PCR buffer; 2.5 mM $MgCl_2$; 1.5 mM dNTPs; 0.5 µl each, forward and reverse primers; 3.5 µl ddH_20; 2 µl DNA. Forward primers were labeled with one of four fluorescent dyes (6-FAM, PET, NED,

VIC). All PCR reagents and primers were purchased from Applied Biosystems, Foster City, CA. Amplification for all samples consisted of a touchdown protocol performed in an ABI 9700 GeneScan™ thermal-cycler. The thermal profile included a denaturing step of 95ºC for 9 min. (to activate the Amplitaq Gold®), followed by 33 cycles of 94ºC for 45s, an initial annealing temperature of 56ºC for 45s, and an extension temperature of 72ºC for 60s. The annealing temperature decreased by 0.2ºC for every cycle. The final extension cycle was 15 min. at 70ºC. PCR products were processed on an ABI 3130xl genetic analyzer using GeneScan™ 500 LIZ® size standard. Composite genotypes for individual fish were compiled with GeneMapper™ 4.0 software (Applied Biosystems).

Amplification of mitochondrial (mtDNA) cytochrome-b (cyt-b) followed the PCR protocol outlined above using the primers, LE^{RBSSEQ} (Dowling et al., 2005) and HA (Dowling et al., 2005; Schmidt et al., 1998). PCR products were purified using the Exo-SAP (Fermentas) procedure using 1/4 reactions following manufactures instructions, and the sequencing reactions used the Big Dye® v3.1 cycle sequencing kit (ABI) using 1/8 reactions and were run on an ABI 3130xl Genetic Analyzer. Sequence data was edited using Sequencher v4.9 (Gene Codes), aligned by hand in Se-Al v2.0a11 (http://tree.bio.ed.ac.uk/software/seal) and compared with reference haplotypes (Dowling, TE pers. comm.) using PAUP* v4.0b10 (Swofford, 2001).

Table 1. Details of the 13 microsatellite loci used to to screen captive (Dexter NFHTC and Ouray NFH) and wild (Lake Mohave) *Xyrauchen texanus*.

Locus	T_A (°C)[1]	Repeat Mofiff	Primer Sequence	Reference
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1. Annealing temperature described in reference paper; TD refers to Touch Down PCR program (see Materials and Methods in this report).
2. This is different from the Dowling and Marsh (2010) *Xte*15.

Data Analysis

GENEPOP v4.0 (Raymond and Rousset, 1995; Rousset, 2008) was used to test for departures from Hardy-Weinberg (HW) equilibrium and conduct global tests of linkage equilibrium among all pairs of loci and populations. The test for HW equilibrium used the method of heterozygote deficiency (Rousset and Raymond, 1995) which is a global test that tests either the population(s) or locus but not both simultaneously. The test of linkage equilibrium tests for association between genotypes at each pair of loci (i.e., composite linkage disequilibrium; Weir, 1996).

GENEALEX v6 (Peakall and Smouse, 2006) was used to calculate expected heterozygosity (H_E; Genetic Diversity) and observed heterozygosity (H_O) on a per locus basis.

FSTAT v2.9.3.1 (Goudet, 1995) was used to calculate allele frequencies and descriptive statistics, including allelic richness (A_R) and average inbreeding coefficients (F_{IS}) for

microsatellites, in addition to A_R and gene diversity for mitochondrial DNA. Allelic richness was calculated using the methods described by Petit et al. (1998), which uses rarefaction and repeated random sub-sampling to provide unbiased estimates of A_R (Leberg, 2002). This is important due to the fact that tests using highly variable loci are sensitive to differences in sample size (i.e., more individuals sampled = increased likelihood new alleles are found).

ARLEQUIN v3.1 (Excoffier et al., 2005) was used to examine the differences in genetic variation: 1) among basins (upper Ouray-Grand Valley and lower Dexter-wild); 2) among stocks within basins; and 3) among samples within stocks. To accomplish this, a hierarichical analysis of molecular variance (AMOVA; Weir and Cockerham, 1984) was used to calculate: 1) F_{ST}; 2) F_{CT}; and 3) F_{SC} respectively.

The Bayesian clustering method of STRUCTURE v2.3.2 (Pritchard et al., 2000) was used to investigate the number of *X. texanus* genetic clusters (K). The admixture model that assumes gene flow among populations and allows for correlated allele frequencies across populations was applied. This model assigns a proportion of each individual's genome to each of the genetic clusters pursuing solutions that maximize HWE and linkage equilibrium within clusters. Ten iterations were performed for each K with the true K assumed to be between 1 and 8. All runs had a burn-in of 100,000 preliminary iterations followed by 100,000 iterations of data collection. The method of Evanno et al. (2005) that uses the second order rate of change between K and K+1 clusters (ΔK) was used to estimate the number of genetic clusters, as implemented in STRUCTURE HARVESTER (Earl, 2011). The K with the largest ΔK value is assumed to be the correct K.

RESULTS

Microsatellites

Averaged across all loci, F_{IS} (within population measure of departure from Hardy-Weinberg expectations) estimates were low ranging from -0.039 (MX-GV: 92-95YC) to 0.006 (LM: Wild) with all of the captive estimates having a negative value indicating heterozygote excess (Appendix 1). The positive value of the LM: Wild population indicates heterozygote deficiency. In tests of Hardy-Weinberg equilibrium, only the MX-GV: 92-95YC was significant ($P = 0.000$) for heterozygote excess (Appendix 1). Mean observed heterozygosity (H_O) was high for all wild and captive populations and ranged from 0.864 (MX-GV: 92-95YC) to 0.910 (LM-DX: PM); *Xte*27 had the lowest estimates ranging from $H_O = 0.418$ (MX-GV: 92-95YC) to $H_O = 0.865$ (LM: Wild). Tests of linkage disequilibrium (gametic) did not show significant associations in the LM: Wild population, while the Dexter and Ouray (including Grand Valley) populations showed statistically significant associations at multiple loci, even after Bonferroni correction (Rice, 1989). The specific associations were as follows: LM-DX: 81YC (*Dlu*4300/*Xte*27; *Dlu*476/*Dlu*4300; *Dlu*4184/*Dlu*4201); LM-DX: WCB (*Xte*15/*Xte*27; *Dlu*476/*Xte*27; *Dlu*4184/*Xte*27; *Dlu*4201/*Dlu*4283); and LM-DX: PM (*Xte*15/*Dlu*439; *Dlu*4184/*Dlu*4201; *Xte*15/US6; US6/*Dlu*4283). The number of statistically significant associations (78 comparisons) were much higher for the Ouray populations with MX-GV: 92-95YC having 40, MX-GV: 04-08YC having 23, and GR-OR: 89-98YC having 59. These associations were not consistent across all populations. However, when species go through bottlenecks the effect of genetic drift is enhanced, resulting in the nonrandom associations between loci (i.e., loci that are not physically linked on a chromosome, appear to be linked and associated; Allendorf and Luikart, 2007). Likewise, linkage disequilibrium can occur when there is non-random mating, which is the case in captive propagation programs, and when there is

admixture of populations with different allele frequencies. All of these events have occurred in both the Ouray and Grand Valley stocks. Therefore, none of the 13 loci were removed for further analyses given the reasons stated.

The percentage of polymorphic loci in the data set was 100% for all populations. Total number of alleles per locus (N_A) ranged from $N_A = 4$ (*Xte*27) to $N_A = 33$ (*Dlu*4300). Allelic richness (A_R), slightly lower than N_A due to being adjusted for sample size, was high for most loci and was lowest in *Xte*27 (Appendix 1). Averaged across all loci, A_R was highest in LM-DX: WCB ($A_R = 16.2$) and lowest in MX-GV: 92-95YC ($A_R = 11.6$) with the LM: Wild and Dexter populations being higher (range 13.4-16.2) than the Ouray populations (range 11.6-12.5).

The AMOVA indicated that there was significant genetic differentiation 1) between the upper (Ouray and Grand Valley) and lower (Dexter and Wild) basins ($F_{ST} = 0.0243$, $p < 0.0000$); 2) between stocks within each group (i.e., basins) ($F_{SC} = 0.009$, $p < 0.0000$); and 3) among samples within stocks ($F_{CT} = 0.0154$, $p < 0.0000$). The major source of this variation however, is due to differences among samples within stocks, i.e., individuals (97%), and not between stocks within basins (0.89%) or among basins (1.54%).

In the STRUCTURE analysis, the number of genetic clusters (K) was estimated to be 2 based on the ΔK method (Figure 1). These clusters corresponded to: 1) Lake Mohave captive and wild populations and 2) Green River Ouray captive population and the mixed Grand Valley population (Figure 2).

Figure 1. Graphical representation of the STRUCTURE analyses showing ΔK results (Evanno et al., 2005) as implemented in STRUCTURE HARVESTER (Earl, 2011).

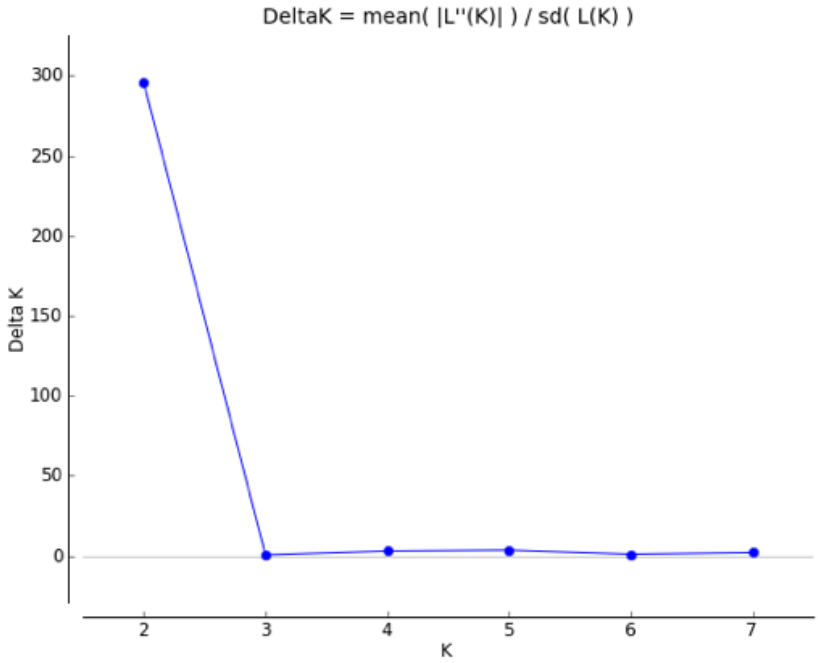
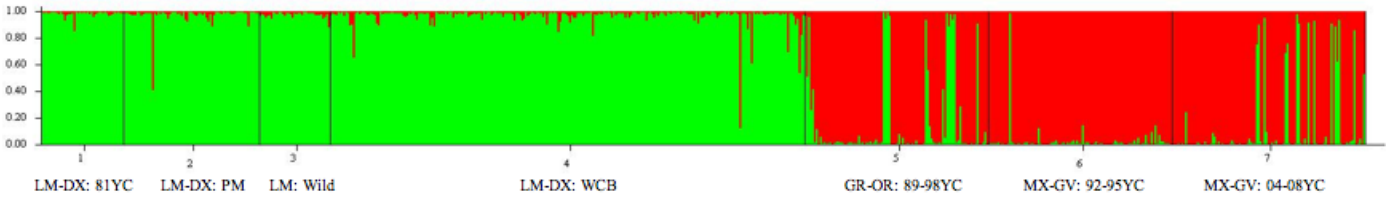


Figure 2. Graphical representation of the STRUCTURE analyses - assignment probability (y axis) of each individual (x axis) into one of two genetic clusters (green or red). Each individual = single vertical bar.



Mitochondrial DNA

A total of 18 cyt-b haplotypes were observed in the two Dexter stocks (Table 2, Appendix 2). The LM-DX: 81YC stock had 9 of the 18 haplotypes with three of them (F, H, V) being rare and found in single individuals. The LM-DX: WCB stock had 17 of the 18 haplotypes with four of them (G, I, K, U) being rare and only found in single individuals. Haplotype E had the highest frequency in both stocks.

Table 2. Mitochondrial (mtDNA) cytochrome subunit-b (cyt-b) haplotype frequencies in the Dexter *Xyrauchen texanus* captive stocks [LM-DX: 81YC (n = 95) and LM-DX: WCB (n = 253)]. Haplotype designations follow that of Dowling (2005).

	mtDNA cyt-b haplotype																	
Captive Stock	A	B	C	E	F	G	H	I	J	K	M	P	R	S	U	V	Z	BB
LM-DX: 81YC	-	0.042	-	0.316	0.011	-	0.011	-	-	-	-	0.063	-	0.316	0.137	0.011	-	0.095
LM-DX: WCB	0.028	0.095	0.012	0.617	0.051	0.004	0.016	0.004	0.008	0.004	0.008	0.008	0.067	0.043	0.004	-	0.012	0.020

DISCUSSION

Microsatellites

Overall the captive stocks were high in genetic diversity (A_R range 11.6 – 16.2) and did not show signs of inbreeding as indicated by high heterozygosity (H_O range 0.864 – 0.910) and low F_{IS}. However, diversity was lower in the Ouray and Grand Valley stocks than in either the Dexter stocks or wild Lake Mohave samples (Appendix 1). Turner et al. (2009) developed 10 *X. texanus* microsatellite loci and is the only published paper describing microsatellite diversity in *X. texanus*. The small sample size of the study (n = 16) makes comparisons difficult, however the 10 loci in that study and 5 additional loci were used in a subsequent unpublished study (Dowling and Marsh, 2010) that can be used to compare measures of genetic diversity. Dowling and Marsh (2010) found that adult samples taken from the lower Colorado River locations had higher allelic richness (Lake Mohave A_R = 9.47; Lake Mead A_R = 8.31) than the upper Colorado River and Green River (Powell A_R = 6.82; Green-Yampa A_R = 6.22; upper Colorado A_R = 3.44), which is consistent with the current findings. In the lower portion of the Colorado River, average allelic richness in larval *X. texanus* taken from Lake Mohave between 1997 and 2004 was 17.1 (Saltzgiver et al., 2011), similar to the average of 16.1 for LM-DX: PM, LM-DX: WCB, and LM: Wild (LM-DX: 81YC excluded, A_R = 13.4, collected as wild adults).

In the analyses of genetic variation between groups, stocks and among samples using STRUCTURE, it was determined that there are two genetic clusters, upper basin (Ouray and Grand Valley) and lower basin (Lake Mohave and Dexter, Figure 1, 2). However, both clusters

had some genetic signatures of the other cluster. For example, the LM-DX: WCB had a few individuals that were more like the upper basin (red) than the lower basin (green, Figure 2). Likewise, some individuals in the MX-GV: 04-08YC were more like the lower basin than the upper basin, which is expected as these stocks have had lower basin individuals added to increase genetic diversity. However, wild populations show the same patterns. Dowling and Marsh (2010) in a similar STRUCTURE analysis (K = 2 and K = 3) found that some individuals from Powell, Green-Yampa, had genetic signatures of Mead and Mohave with the upper Colorado River samples being unique. This indicates that the upper Colorado River population has been genetically isolated due to isolated individuals in backwater ponds (Dowling and Marsh, 2010).

Mitochondrial DNA

In all, 18 cyt-b haplotypes were observed in the two Dexter stocks with LM-DX: 81YC having fewer haplotypes than LM-DX: WCB (Tables 2 and 3). A comparison of haplotype diversity in wild Lake Mohave individuals collected by Dowling et al. (2005) and the LM-DX: WCB, indicates that the LM-DX: WCB stock contains most of the cyt-b genetic variation contained in the wild Lake Mohave population. This stock (LM-DX: WCB) contained 17 of the 28 wild cyt-b haplotypes observed in 2,432 larval samples collected between 1997 and 2003 by Dowling et al. (2005). The 11 wild haplotypes not observed in the LM-DX: WCB stock, were rare in the Dowling et al. (2005) study, occurring only in a few individuals and years. For example, haplotypes D, T, Y, and AA were observed in single individuals over the seven-year study; haplotypes L, N, O, W, and X were observed in 2 - 4 individuals over the seven years, many of which were only observed in one or two years (e.g., L – 1997; N – 1997 and 1999; O – 1997 and 2001). If given enough input from the Lake Mohave and time, the stocks at Dexter

NFHTC could capture the rare haplotypes, however due to the presence of quagga mussels (*Dreissena rostriformis bugensis*) in Lake Mohave, larval fish can no longer be translocated out of Lake Mohave.

Table 3. Comparison of mitochondrial (mtDNA) cytochrome subunit-b (cyt-b) descriptive statistics for the two Dexter *Xyrauchen texanus* captive stocks and wild Lake Mohave samples collected by Dowling et al. (2005).

Captive Stock/Population	N	# Haplotypes	Gene Diversity	Allelic Richness[1]
LM-DX: 81YC	95	9	0.78	9.0
LM-DX: WCB	253	17	0.60	14.8
Dowling 1980's	27	9	0.69	8.0
Dowling early 1990's	22	5	0.59	5.0
Dowling late 1990's	223	18	0.66	6.8

1. Current study is based on 95 samples and Dowling et al. (2005) is based on 22 samples.

Two previous papers (Dowling et al., 1996a; Dowling et al., 1996b) also examined *X. texanus* mtDNA diversity in Lake Mohave; however, both papers used restriction endonuclease analysis to define haplotypes and are not comparable to the sequencing data in this study or Dowling et al. (2005). Dowling et al. (2005) however, did sequence some of the same Lake Mohave individuals from the 1980's and define haplotypes to compare to more recent data (Table 3). In comparing Lake Mohave adult individuals from the 1980's (Dowling et al., 2005 and LM-DX: 81YC), 4 haplotypes (B, E, F, S) were shared between the two studies; haplotypes that differed were rare alleles. Haplotypes H, P, U, V, and BB were observed in the LM-DX: 81YC and not in the Dowling et al. (2005) samples; likewise, haplotypes A, C, J, R, T were observed in the Dowling et al. (2005) study, but not LM-DX: 81YC.

CONCLUSIONS

Overall the Dexter captive stocks were genetically diverse and almost identical to the wild Lake Mohave population as measured by microsatellites. The Ouray and Grand Valley stocks are also diverse, but had lower allelic richness when compared to the lower basin (Dexter and wild Lake Mohave) samples, a pattern similar to what has been observed in wild upper basin populations. In a comparison of mitochondrial diversity, both the Dexter 1981 year-class and

Dexter wild caught broodstock were as diverse as the wild Lake Mohave population, with the exception of a few rare haplotypes.

RECOMMENDATIONS

1) The Dexter NFHTC stocks are diverse and can be viewed as the secondary lower basin population, with the wild Lake Mohave population being the primary population. The wild caught broodstock should be the primary stock used for production and recovery.

2) Complete analyses of all upper basin stocks are needed before recommendations can be made. These additional analyses include calculating pairwise relatedness of the Ouray and Grand Valley stocks (FY2012 objective). This additional information will help determine what steps may be necessary to increase the diversity of the Ouray and Grand Valley stocks.

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Appendix 1. Summary statistics of the 13 microsatellite loci used to screen *Xyrauchen texanus* captive and wild stocks: Lake Mohave (LM); Green River (GR); Mix of Green River and Lake Mohave (MX); Dexter NFHTC (DX); Ouray NFH (OR); Ouray NFH Grand Valley Unit (GV)

		Lower Colorado River Basin				**Upper Colorado River Basin**		
		LM-Wild	**LM-DX: 81YC**	**LM-DX: PM**	**LM-DX: WCB**	**GR-OR: 89-98YC**	**MX-GV: 92-95YC**	**MX-GV: 04-08YC**
Locus	Statistic[1]	n = 37	n = 43	n = 71	n = 248	n = 69	n = 93	n = 96
*Dlu*416								
	N_A	17	18	19	25	12	14	15
	A_R	16.1	16.3	16.1	17.8	11.6	11.8	11.4
	H_O	0.892	0.907	0.958	0.915	0.986	0.914	0.917
	H_E	0.918	0.914	0.923	0.930	0.884	0.869	0.850
	F_{IS}	0.042	0.019	-0.030	0.018	-0.104	-0.049	-0.067
	Size Range	155-259						
*Xte*23								
	N_A	21	14	20	25	22	16	19
	A_R	19.4	12.8	17.0	17.2	18.7	14.5	16.9
	H_O	0.946	0.884	0.958	0.931	0.855	0.957	0.958
	H_E	0.932	0.871	0.927	0.932	0.922	0.918	0.924
	F_{IS}	-0.001	-0.003	-0.026	0.003	0.063	-0.037	-0.032
	Size Range	245-459						
*Xte*15								
	N_A	19	17	24	27	14	15	18
	A_R	17.9	15.1	19.7	19.3	12.1	12.9	14.6
	H_O	0.919	0.860	0.958	0.935	0.877	0.903	0.969
	H_E	0.925	0.879	0.939	0.940	0.874	0.887	0.911
	F_{IS}	0.020	0.033	-0.013	0.006	-0.017	-0.005	-0.058
	Size Range	256-394						
*Dlu*476								
	N_A	18	15	20	21	10	12	13
	A_R	16.6	14.1	17.4	16.5	9.2	10.9	10.7
	H_O	0.973	0.907	0.972	0.927	0.828	0.839	0.853
	H_E	0.900	0.894	0.909	0.922	0.761	0.860	0.849
	F_{IS}	-0.068	-0.003	-0.062	-0.004	-0.019	0.024	0.024
	Size Range	149-249						
*Dlu*409								
	N_A	14	15	17	22	13	10	14

*Dlu*409	A_R	13.8	14.0	15.1	15.5	10.5	9.5	10.6
	H_O	0.919	0.930	0.944	0.948	0.809	0.934	0.844
	H_E	0.909	0.879	0.920	0.921	0.813	0.853	0.843
	F_{IS}	-0.002	-0.046	-0.018	-0.027	0.011	-0.091	0.002
	Size Range					191-279		
*Dlu*4184								
	N_A	19	13	22	25	12	12	14
	A_R	18.1	12.6	17.1	16.4	11.7	10.9	12.2
	H_O	1.00	0.860	0.944	0.911	0.884	0.860	0.896
	H_E	0.925	0.890	0.923	0.921	0.878	0.866	0.869
	F_{IS}	-0.067	0.045	-0.015	0.012	0.001	0.020	-0.008
	Size Range					188-296		
*Dlu*439								
	N_A	23	17	25	28	23	19	25
	A_R	21.3	15.8	19.7	19.5	18.8	15.8	18.7
	H_O	0.946	0.953	0.915	0.931	0.928	0.978	0.947
	H_E	0.939	09.05	0.936	0.941	0.922	0.916	0.928
	F_{IS}	0.007	-0.042	0.029	0.012	-0.002	-0.062	-0.017
	Size Range					166-334		
*Dlu*4201								
	N_A	14	12	17	16	13	15	15
	A_R	13.5	11.1	14.7	14.2	12.2	14.0	13.4
	H_O	0.838	0.953	0.915	0.919	0.913	0.957	0.896
	H_E	0.902	0.857	0.902	0.911	0.889	0.903	0.910
	F_{IS}	0.084	-0.100	-0.007	-0.007	-0.015	-0.055	0.029
	Size Range					138-224		
*Dlu*4300								
	N_A	21	17	26	33	18	17	19
	A_R	20.1	16.1	22.5	21.4	15.2	12.8	14.9
	H_O	1.00	0.907	0.958	0.948	0.870	0.957	0.875
	H_E	0.939	0.920	0.951	0.948	0.914	0.889	0.900
	F_{IS}	-0.051	0.026	-0.001	0.003	0.059	-0.069	0.027
	Size Range					201-345		
*Dlu*4296								
	N_A	19	14	19	22	14	13	15
	A_R	17.8	13.0	16.1	16.6	13.4	11.7	11.9
	H_O	0.946	0.837	0.944	0.948	0.899	0.967	0.833

*Dlu*4296	H_E	0.926	0.878	0.923	0.927	0.852	0.888	0.857
	F_{IS}	-0.008	0.058	-0.015	-0.020	-0.013	-0.084	0.030
	Size Range					124-218		
US6								
	N_A	9	12	12	16	8	9	10
	A_R	8.9	10.6	9.4	10.8	8.0	8.1	8.4
	H_O	0.811	0.860	0.803	0.871	0.913	0.846	0.865
	H_E	0.841	0.829	0.814	0.847	0.829	0.849	0.812
	F_{IS}	0.049	-0.026	0.021	-0.026	-0.093	0.004	-0.056
	Size Range					154-214		
*Dlu*4283								
	N_A	20	19	23	29	18	18	19
	A_R	18.9	17.4	18.0	19.3	15.8	14.3	15.8
	H_O	0.865	0.884	0.972	0.940	1.00	0.957	0.927
	H_E	0.924	0.921	0.926	0.938	0.919	0.901	0.915
	F_{IS}	0.077	0.052	-0.043	0.001	-0.064	-0.057	-0.001
	Size Range					194-318		
*Xte*27								
	N_A	7	6	7	9	4	4	5
	A_R	7.0	5.5	6.7	6.2	4.0	4.0	3.6
	H_O	0.865	0.744	0.586	0.644	0.477	0.418	0.490
	H_E	0.924	0.652	0.581	0.642	0.400	0.401	0.486
	F_{IS}	-0.018	-0.129	-0.000	-0.000	-0.181	-0.037	0.000
	Size Range					182-218		
Mean								
	N_A	17.0	14.5	19.3	22.9	13.9	13.4	15.5
	A_R	16.1	13.4	16.1	16.2	12.4	11.6	12.5
	H_O	0.898	0.884	0.910	0.905	0.864	0.884	0.867
	H_E	0.891	0.868	0.890	0.902	0.835	0.846	0.850
	F_{IS}	0.006	-0.006	-0.014	-0.002	-0.021	-0.039	-0.010
	P_{HW} H_{excess}	ns	ns	ns	ns	ns	0.0000	ns
	P_{HW} $H_{deficiency}$	ns	ns	ns	ns	ns	ns	ns

1. N_A = number of alleles; A_R = allelic richness corrected for minimum sample size; H_O = observed heterozygosity; H_E = expected heterogosity; F_{IS} = inbreeding coeffieicent; P_{HW} H_{excess} = Probability of Global Hardy-Weinberg test, test of heterozygote excess; P_{HW} $H_{deficiency}$ = Probability of Global Hardy-Weinberg test, test of heterozygote deficiency; ns = non significant

Appendix 2. Sequences of the 18 cytochrome b haplotypes found in the Dexter captive stocks

Haplotype A
ATCCATTCCCAACAAACTAGGTGGTGTTCTAGCATTATTGTCCTCCATTCTTGTATTGATAGTGG
TACCCATCCTTCACACCTCCAAGCAACGGGGACTAACATTTCGCCCGGCCACCCAATTCCTATTC
TGAACCTTAGTTGCTGATATGATTATTCTAACATGAATTGGAGGAATGCCAGTAGAACATCCGT
TTATTGTTATTGGACAAGTTGCGTCCGCCCTATACTTCGCCCTATTCCTAATCTTAACCCCGCTAG
CCGGGTGATTAGAAAACAAGGCACTAGAATGAGCTTGCTCTAGTA
Haplotype B
ATCCATTCCCAACAAACTAGGTGGTGTTCTAGCATTATTGTCCTCCATTCTTGTATTGATAGTGG
TACCCATCCTTCACACCTCCAAGCAACGAGGACTAACATTTCGCCCGGCCACCCAATTCCTATTC
TGAACCTTAGTTGCTGATATGATTATCCTAACATGAATTGGAGGAATGCCAGTAGAACATCCGT
TTATTGTTATTGGACAAGTTGCGTCCGCCCTATACTTCGCCCTATTCCTAATCTTAACCCCGCTAG
CCGGGTGATTAGAAAACAAGGCACTAGAATGAGCTTGCTCTAGTA
Haplotype C
ATCCATTCCCAACAAACTAGGTGGTGTTCTAGCATTATTGTCCTCCATTCTTGTATTGATAGTGG
TACCCATCCTTCACACCTCCAAGCAACGAGGACTAACATTTCGCCCGGCCACCCAATTCCTATTC
TGAACCTTAGTTGCTGATATGATTATCCTAACATGAATTGGAGGGATGCCAGTAGAACATCCGT
TTATTGTTATTGGACAAGTTGCGTCCGCCCTATACTTCGCCCTATTCCTAATCTTAACCCCGCTAG
CCGGGTGATTAGAAAATAAGGCACTAGAATGAGCTTGCTCTAGTA
Haplotype E
ATCCATTCCCAACAAACTAGGTGGTGTTCTAGCATTATTGTCCTCCATTCTTGTATTGATAGTGG
TACCCGTCCTTCACACCTCCAAGCAACGAGGACTAACATTTCGCCCGGCCACCCAATTCCTATTC
TGAACCTTAGTTGCTGATATGATTATCCTAACATGAATTGGAGGAATGCCAGTAGAACATCCGT
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Haplotype I
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