

# Baseline Genetic Survey of the Threatened Pecos Bluntnose Shiner (*Notropis simus pecosensis*)



Megan Osborne and Thomas Turner  
Department of Biology

University of New Mexico

Funding provided by New Mexico Department of Game and Fish  
Share with Wildlife Program

Interim Report submitted to Chuck Hayes  
New Mexico Department of Game and Fish  
16<sup>th</sup> June 2008

---

## Executive Summary

Genetic monitoring is the analysis of genetic diversity in space and time, with emphasis on the temporal dimension to understand trends in genetic diversity in contemporary populations. In 2004, we began a genetic monitoring program of the federally threatened Pecos bluntnose shiner (*Notropis simus pecosensis*) and have continued to sample the population annually since. In addition, we have obtained samples and genetic data from 107 samples that were collected from three localities on the Pecos river in 2002 to serve as a refugial population. This data set, representing seven generations, provides unique information regarding trends in genetic diversity and genetic effective size that can be interpreted with reference to trends revealed by population monitoring. We report on the genetic status of Pecos bluntnose shiner sampled in 2007-2007 in addition to the refugial population representing the wild 2002 population. In 2008 we sampled 252 wild fish from eight localities on the Pecos river.

### Major Findings for 2008:

- (1) Genetic diversity, as measured by observed heterozygosity (microsatellites) and average gene diversity (mitochondrial DNA), was marginally lower in 2007 and 2008 than in 2006. Mitochondrial DNA allelic richness was higher in 2007 and 2008 than in 2006.
- (2) Genetic diversity of the refugial population established in 2002 was comparable to the wild population.
- (3) A significant portion of genetic variation was explained by differences among years rather than localities.
- (4) Microsatellite genetic effective size estimates corrected for overlapping generation have increased from 58 in (2005 – 2006) to about 150 (2007-2008). Mitochondrial DNA reveals the same trend between 2006-2007 ( $N_e=310$ ) and 2007-2008 ( $N_e=2648$ ).

---

## **Introduction**

The Pecos bluntnose shiner (*Notropis simus pecosensis*) is a subspecific form of the bluntnose shiner (Chernoff *et al.* 1982). The only other subspecies, the Rio Grande bluntnose shiner (*Notropis simus simus*), is presumed extinct with the last collection made in 1964. The Pecos bluntnose shiner was once relatively abundant in the Pecos river but has suffered significant declines and a reduction in its range following habitat changes caused by the installation of mainstream dams (Platania 1995; Hoagstrom 2003; Hoagstrom *et al.* 2008). As such, the Pecos bluntnose shiner was listed as threatened by the State of New Mexico in 1976 and under the Endangered Species Act in 1987 (U.S. Department of the Interior, Federal Register 1987). The species is also listed as endangered in Texas (Campbell 1995) and by the Republic of Mexico. The Pecos bluntnose shiner is now restricted to a 333 kilometer reach of the Pecos River from downstream of Sumner Reservoir to above Brantley Reservoir, New Mexico (Brooks *et al.* 1991; Platania 1995).

Since the species was federally listed there have been several dramatic population fluctuations in the Pecos bluntnose shiner population. Pecos bluntnose shiner mean annual densities and percent species composition were low in 1992 due to streamflow intermittence that occurred during between 1989 and 1991 (USFWS, 1992; Robertson 1997). Densities increased gradually between and 1991 and 2002; a time of perennial streamflow and relatively high and stable mean discharge (Hoagstrom *et al.* 2008). The core Pecos bluntnose shiner population crashed between 2002 and 2005 reaching very low densities by 2005 (Hoagstrom *et al.* 2008; Davenport *et al.* 2007). Since 2005, the population has begun to rebound. Extreme population fluctuations, like those observed in recent times for the Pecos bluntnose shiner, are expected to cause losses of genetic diversity. Allelic diversity is more sensitive than heterozygosity to contemporary impacts and will be eroded more rapidly through the process of genetic drift.

In 2004, we began genetic monitoring of the Pecos bluntnose shiner population. We follow Swartz *et al.* (2007) and define genetic monitoring as the case where two or more temporally-spaced genetic samples are taken from the same population. Temporal sampling allows changes in measures of genetic diversity including allelic richness, heterozygosity and genetic effective size to be tracked in the population. This data can then be interpreted in light of population trends apparent from routine monitoring.

In summary, the project has five objectives:

1. Obtain genetic data from Pecos bluntnose shiner population sampled annually.

- 
2. Develop genetic markers to track changes genetic diversity measures.
  3. Use genetic data to estimate the genetic effective population size.
  4. Calculate the population's allelic diversity and heterozygosity and compare this to data collected between 2002 and 2008 to establish temporal trends in these measures.
  5. Provide data to resource managers to assist in recovery efforts for the Pecos bluntnose shiner.

We present data collected from the Pecos bluntnose shiner population in 2008 and compare this data to that collected in previous years.

## Methods

### **Sampling**

Fin clips of Pecos bluntnose shiners were collected from eight sites on the Pecos river throughout the species' current that extends from Sumner to Brantley Dam, New Mexico (Table 1) between December 2007 and March 2008. In addition, samples collected between 2004 and 2008 were screened for variation using four new microsatellite primers (see below).

### **Molecular Methods**

Genomic DNA was isolated from air-dried fin clips using standard proteinase-K digestion and phenol-chloroform methods (Hillis *et al.* 1996). Genotype data from eleven microsatellite loci: *Lco1*, *Lco6*, *Lco3*, (Turner *et al.* 2004) *Ca6*, *Ca8* (Dimsoski *et al.* 2000), *Ppro118* and *Ppro126* (Bessert & Orti, 2003), *Nme208*, *Nme174*, *Nme232*, *Nme93* (Gold *et al.* 2004) and a 318 base pair region of the mitochondrial gene *ND4* were obtained for all individuals.

Microsatellite loci were amplified using multiplex PCR (1x PCR buffer, 2mM MgCl<sub>2</sub>, 125µM dNTPS, 0.4µM of each primer and 0.375 units of TAQ polymerase) with the following cycling conditions: one denaturation cycle of 93°C for 3 minutes, 30 cycles of 90°C for 20 sec, 49 °C for 20 sec (*Lco1* and *Ca6*; *Lco3* and *Lco6*; and *Ca8*) or 58°C (*Nme174*; *Nme232* and *Nme93*) 60°C (*Ppro118* and *Ppro126*; *Nme208*) and 72°C for 30 sec followed by a final extension step of 72°C for 30 minutes. PCR product (1 µL) was mixed with 10 µL of formamide and 0.3 µL of size standard (ABI HD400 or ROX350) and denatured at 90°C for 5 minutes. All samples were run on an ABI3100 automated DNA sequencer and analyzed using GeneMapper software (Applied Biosystems).

Individuals were screened for variation in the mitochondrial *ND4* gene using single stranded conformational polymorphisms (SSCP) analysis following the procedures outlined in Aló & Turner (2005) and Sunnucks *et al.* (2000). A proportion of individuals from each gel were

**Table 1.** Pecos bluntnose shiner collection localities, County, UTM coordinates and number of individuals sampled per site. Sample for 2002 are a mixed refugial population that were collected at three localities.

<b>Collection Locality</b>	<b>County</b>	<b>UTM Coordinates</b>	<b>2002</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>
<b>Willow Creek Confluence</b>	Chaves	13S 0567061 3754818	20	4	17	45	5	
<b>Six-Mile Draw</b>	Chaves	13S 0567090 3746757	26	1	37	24	46	
<b>Crockett Draw</b>	Chaves	13S 0565213 3742022	24	0	0	66	56	
<b>Bosque Draw</b>	Chaves	13S 0564139 3735299	0	0	11	41	58	
<b>Cortes Gasline</b>	Chaves	13S 0563811 3737265	12	5	6	11	1	
<b>Gasline Crossing</b>	Chaves	13S 0559701 3722493	13	0	15	26	12	
<b>Hwy 70 Bridge Crossing</b>	Chaves	13S 0558355 3714591	24	0	23	19	49	
<b>Acme Gage</b>	Chaves	13S 0557699 3711107	0	7	0	58	0	
<b>Bitterlake NWR- Scout Camp</b>	Chaves	13S 0555831 3694992	3	0	7	0	27	
<b>Hwy 380 Bridge Crossing</b>	Chaves	13S 0555831 3694992	3	2	6	37	0	
<b>Sallee Ranch</b>	Chaves	13S 0559656 3686890	28	1	0	0	0	
<b>Dexter Bridge</b>	Chaves	13S 0556250 3686910	0	0	6	4	0	
<b>Lake Arthur Falls</b>	Chaves	13S 0563820 3650142	15	0	0	7	0	
<b>Brantley Inflow</b>	Eddy	13S 0560837 3606540	0	0	2	0	0	
<b>TOTAL</b>			107	168	22	138	338	252

---

sequenced using ABI Big Dye kit to confirm sequence designations. Unique haplotypes were sequenced in the forward and reverse direction. For haplotypes identified in single individuals, PCR amplification and sequencing were repeated to confirm haplotype designations. Representatives of several other Pecos river cyprinids (*Hybognathus placitus*, *Notropis girardi*, *N. jemezianus*, *N. stramineus*) were also sequenced to provide references that would allow mis-identified samples to be identified to species. DNA sequences were visualized using Sequencher Version 4.2 and aligned manually.

## **Data Analysis**

### **Genetic Diversity**

Microsatellite allele frequencies and descriptive statistics, including allelic richness ( $A_R$ ), average inbreeding co-efficients ( $F_{IS}$ ) and Nei's (1987) unbiased gene diversity ( $H_E$ ), were obtained using FSTAT Version 2.9.3.1 (Goudet 1995). Allelic richness was calculated using the methods described Petit *et al.* (1998). This method allows the number of alleles to be compared among populations independently of sample size (Leberg 2002) and is based on the smallest number of individuals typed for any locus. FSTAT was also used to conduct global tests of linkage disequilibrium among all pairs of loci and to test for departures from Hardy-Weinberg expectations (HWE). The computer program MICROCHECKER 2.2.1 (van Oosterhout *et al.* 2004) was used to investigate the possible cause of deviations from HWE, including mis-scoring due to stuttering, presence of null alleles and large allele dropout. For mitochondrial DNA, data estimates of unbiased gene diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) were obtained using ARLEQUIN Version 3.0 (Excoffier *et al.* 2005). FSTAT was used to obtain measures of allelic richness for mtDNA. Percent sequence divergence was estimated using Kimura-two parameter method implemented in PAUP. The computer program TCS (Clement *et al.* 2000) was used to construct a statistical parsimony network among mitochondrial DNA haplotypes using the method of Templeton *et al.* (1992).

### **Spatial Structure**

We tested for spatial genetic structure by calculating Weir and Cockerham's (1984)  $F$ -statistics, as implemented in the computer program ARLEQUIN (Schneider *et al.* 2000). Hierarchical analysis of variance (AMOVA) (Excoffier *et al.* 1992) partitions the total variance into covariance components due to differences among groups of populations ( $F_{CT}$ ), between populations within groups ( $F_{SC}$ ) and among all populations (irrespective of groups) ( $F_{ST}$ ).  $F_{ST}$  is the standardized variance in allele frequencies between populations and is the most commonly used measure of genetic distance between populations.  $\Phi$ -statistics were calculated from mt-DNA data (Excoffier *et al.* 1992).  $\Phi$ -statistics are equivalent to  $F$ -statistics; however, they incorporate allele frequencies and evolutionary distances between haplotypes. Significance of results was tested using a bootstrapping procedure. For Pecos bluntnose shiner, we tested whether a significant

---

proportion of genetic variance could be explained by differences among years (2002, 2004-2008) and whether a significant proportion of variance could be explained by collection locality (only for 2007 and 2008 samples).

### **Contemporary Genetic Effective Size**

The temporal method (Pollack 1983; Waples 1989; Wang & Whitlock 2003) was used to estimate the variance effective population size from microsatellite and mt-DNA data collected from samples representing 2002, 2004, 2005, 2006, 2007 and 2008. This method assesses the change in allele frequencies across generations. Estimates of  $N_{ev}$  (referred to as contemporary  $N_e$ ) accurately follow current variations in effective population size and are not greatly affected by historical events such as population bottlenecks (Avice 1994). For estimates based on Mt-DNA data, contemporary  $N_e$  is only estimated for the female portion of the population, as mtDNA is maternally inherited. Moments-based estimates of  $N_e$  and 95% confidence intervals were estimated, using the method of Waples (1989), for both Mt-DNA and microsatellites using the program NeEstimator (Peel *et al.* 2004).

Estimates of  $N_e$  were corrected for effects of overlapping generations using a model described in Jorde & Ryman (1995; 1996). The model requires a basic life table with information on age-specific survival rates ( $l_i$ ) and birth rates ( $b_i$ ). Survival rate ( $S$ ) was estimated from age-structured catch data for *N. simus pecosensis* (Hoagstrom *et al.* 2008; U.S. FWS unpublished data). Age-specific survivorship,  $l_i$ , is equal to  $S^{i-1}$  where  $l_0 = 1$ . Average reproductive contribution was estimated as modal body length at age  $i$  raised to the third power (Charnov *et al.* 1999). This value was multiplied by  $l_i$  to obtain the proportional contribution of each age class to the offspring pool ( $p_i$ ) and then  $p_i$  values were summed over  $k$  age classes. Birth rates at each age class were divided by  $\sum_{i=1}^k p_i$  to produce a standardized birth rate ( $b_i$ ), corrected to reflect a non-growing population with stable age structure, i.e.,  $\sum_{i=1}^k l_i b_i = 1 = R_0$ . We assumed that males and females had identical mortality and reproduction schedules. Resulting life tables were used to calculate a correction factor ( $C$ ) for overlapping generations by using 100 iterations of Equation 5 in Jorde & Ryman (1996). The value  $C$  accounts for variance due to mortality as a cohort passes from one year class to the next and for genetic covariance among cohorts (because individuals from multiple age classes are the parents of a given cohort). The mean generation length in years ( $G$ ) was calculated using Equation 10 in Jorde & Ryman (1996). The correction can be applied to effective size estimates for adjacent cohorts obtained from both mitochondrial and microsatellite data.

---

## Results and Discussion

### ***Genetic Diversity- Microsatellites***

In 2008 we collected 252 Pecos bluntnose shiner from eight localities on the Pecos river, New Mexico. Genetic variation was assessed using eleven microsatellite loci. These markers were moderately to highly polymorphic. Lco1 was the most polymorphic marker with 46 alleles detected across all samples (Appendix 1). Allelic richness was slightly lower in 2007 and 2008 than in 2006 (Table 2) (2005 sample was not included in calculations of  $A_R$  because of the small sample size). The lowest allelic richness was seen in 2002 but this sample is the refugial population that was originally derived from only three collection localities. Nei's (1987) unbiased gene diversity ( $H_E$ ) and observed heterozygosity ( $H_O$ ) also declined slightly during the same period. There were 44 deviations (from a total of 66 tests) from Hardy-Weinberg equilibrium after Bonferroni correction for multiple comparisons. These were explained by heterozygote deficiency and are reflected in the values of the average inbreeding co-efficients ( $F_{IS} = 0.192$ - $0.241$ ). MICROCHECKER showed that there was no evidence of large allele dropout or of scoring errors. Null alleles were the most likely cause of homozygote excess. There were seven instances of linkage disequilibrium that were significant after Bonferroni correction.

### ***Genetic Diversity- Mitochondrial DNA***

A 318 base pair fragment of ND4 was sequenced. Across all years, 49 unique haplotypes were identified. In both 2007 and 2008 29 haplotypes were observed. Four haplotypes were identified in the 2002 sample that have not been seen subsequently. ND4 haplotypes were separated by one to eight base pairs (0.315 to 2.25 % sequence divergence, Kimura-two parameter distance). The majority of substitutions were transitions, but several transversions were also observed. Two haplotypes (B and A) were present at moderate to high frequencies (A- 12% - 44% and B- 38.9% and 69.2%). The remainder of the haplotypes were considered rare, i.e. present in fewer than 5.0% of individuals (Table 3). In 2008 four new haplotypes were identified. Haplotype diversities in 2007 and 2008 were marginally lower than in 2006.

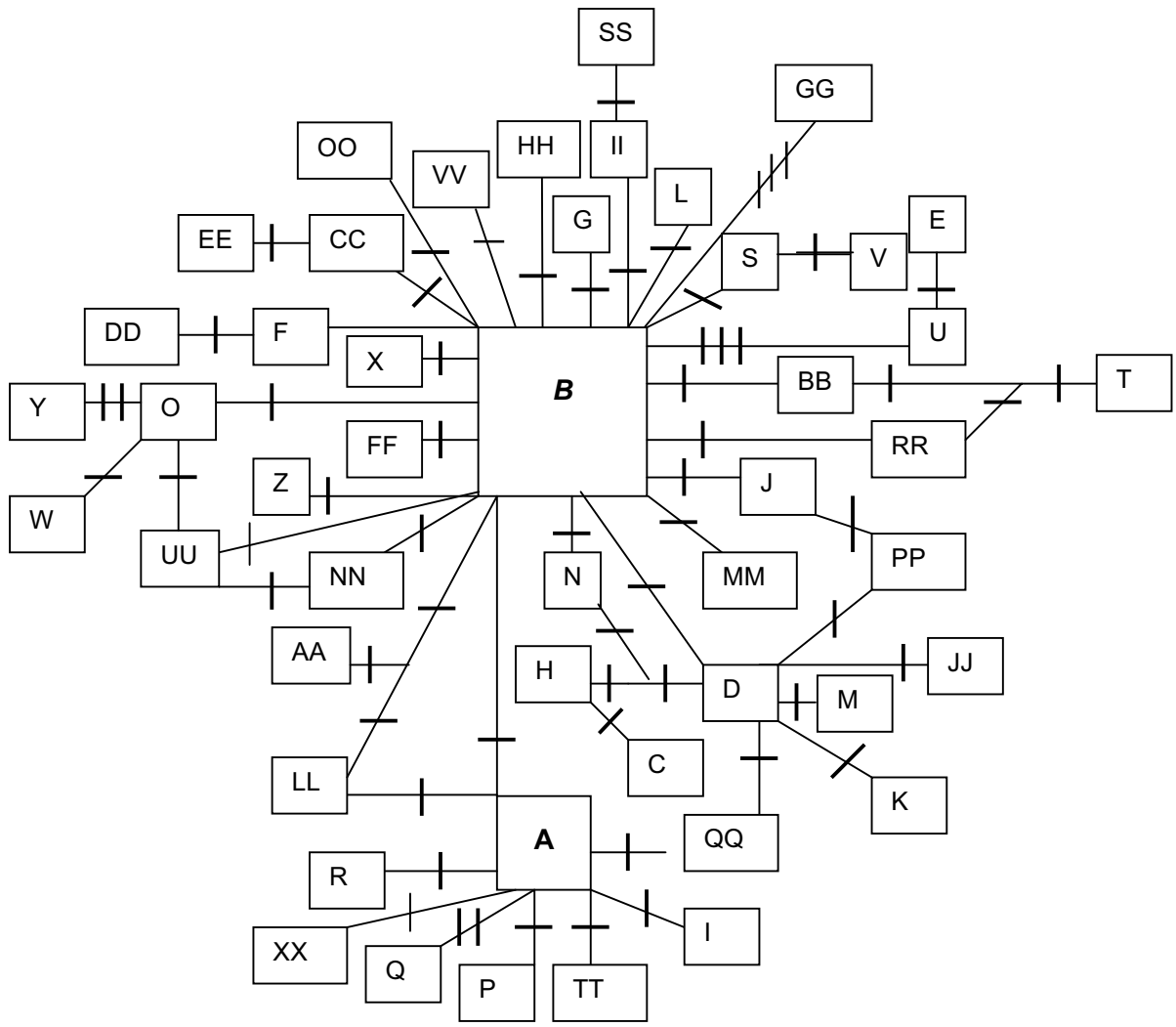
The statistical phylogenetic network was star-like and revealed that all sequences were closely related to the most frequently encountered and presumably ancestral haplotypes A and B (Figure 1). Past rapid population growth following a bottleneck leaves a characteristic signature in which DNA sequences form the star-like pattern and there is an excess of rare sequence variants that are closely related to the most common allele (ancestral).

### ***Population comparisons- Microsatellites***

Pairwise F-statistics were calculated to determine whether there were significant differences in allele frequencies among samples collected between 2002 and 2008 (Table 4). There were

**Table 2.** Summary of diversity statistics for microsatellite and Mt-DNA data. Abbreviations are N- number of individuals,  $A_R$ - allelic richness,  $H_E$ - Nei's unbiased gene diversity,  $H_O$  - observed heterozygosity,  $h$ - gene diversity.

	<b>2002</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>
<b><i>Microsatellites</i></b>						
<b>N</b>	107	172	22	139	338	252
<b><math>H_E</math></b>	0.840	0.849	0.849	0.859	0.843	0.843
<b><math>H_O</math></b>	0.670	0.645	0.657	0.694	0.679	0.664
<b><math>A_R</math></b>	20.699	21.313	-	22.557	21.753	21.312
<b><math>F_{IS}</math></b>	0.204	0.241	0.231	0.192	0.195	0.212
<b><i>Mt-ND4</i></b>						
<b>N</b>	105	168	18	132	332	248
<b>Number of Haplotypes</b>	16	20	4	20	29	29
<b><math>h</math></b>	0.544	0.614	0.673	0.658	0.509	0.554
<b><math>A_R</math></b>	16.000	18.596	-	19.540	21.278	23.855
<b>Nucleotide Diversity</b>	0.003	0.003	0.003	0.004	0.002	0.003



**Figure 1.** Statistical phylogenetic network of mitochondrial ND4 sequences for Pecos bluntnose shiner. Bars indicate a nucleotide change.

**Table 3.** ND4 haplotype frequencies by year.

	<b>2002</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>
<b>A</b>	0.162	0.218	0.444	0.250	0.120	0.117
<b>B</b>	0.657	0.582	0.389	0.530	0.690	0.657
<b>C</b>	0	0.006	0	0	0.003	0.004
<b>D</b>	0.010	0.006	0	0.008	0.006	0
<b>E</b>	0	0.012	0	0	0	0
<b>F</b>	0.019	0.018	0	0.008	0.009	0.012
<b>G</b>	0	0.006	0	0	0.015	0.004
<b>H</b>	0	0.012	0	0.008	0.003	0.004
<b>I</b>	0	0.018	0	0	0.006	0.008
<b>J</b>	0	0.006	0	0.015	0.012	0.008
<b>K</b>	0	0.006	0	0.008	0.003	0
<b>L</b>	0	0.018	0	0	0.003	0.016
<b>M</b>	0.019	0.018	0	0.030	0.027	0.024
<b>N</b>	0.010	0.029	0.111	0.008	0.024	0.020
<b>O</b>	0.029	0.012	0.056	0.015	0.006	0.004
<b>P</b>	0	0.006	0	0	0.003	0
<b>Q</b>	0	0.012	0	0	0	0.004
<b>R</b>	0	0.006	0	0.008	0	0
<b>S</b>	0	0.006	0	0.008	0.006	0.004
<b>T</b>	0.010	0.006	0	0.008	0	0.008
<b>U</b>	0.010	0	0	0.008	0.003	0.016
<b>V</b>	0.010	0	0	0.023	0	0
<b>W</b>	0	0	0	0.015	0	0.004
<b>Y</b>	0	0	0	0.015	0	0
<b>X</b>	0	0	0	0.023	0	0
<b>Z</b>	0	0	0	0.008	0	0
<b>AA</b>	0	0	0	0.008	0	0.008
<b>BB</b>	0.010	0	0	0	0.006	0.012
<b>CC</b>	0	0	0	0	0.009	0.012
<b>DD</b>	0	0	0	0	0.003	0
<b>EE</b>	0.010	0	0	0	0	0
<b>FF</b>	0.019	0	0	0	0	0.008
<b>GG</b>	0	0	0	0	0	0.004
<b>HH</b>	0.010	0	0	0	0	0
<b>II</b>	0.010	0	0	0	0	0
<b>JJ</b>	0	0	0	0	0.006	0
<b>KK</b>	0	0	0	0	0.003	0.004
<b>LL</b>	0.010	0	0	0	0	0
<b>MM</b>	0	0	0	0	0	0.008
<b>NN</b>	0	0	0	0	0.003	0.004
<b>OO</b>	0	0	0	0	0.003	0
<b>PP</b>	0	0	0	0	0.003	0.012
<b>QQ</b>	0	0	0	0	0.003	0
<b>RR</b>	0	0	0	0	0.009	0
<b>SS</b>	0	0	0	0	0.006	0
<b>TT</b>	0	0	0	0	0.006	0
<b>UU</b>	0	0	0	0	0	0.004
<b>VV</b>	0	0	0	0	0	0.004
<b>XX</b>	0	0	0	0	0	0.004

**Table 4.** Pairwise  $F_{STs}$  calculated from microsatellite allele frequencies among samples collected between 2002 and 2008 (below diagonal). Associated P-values are given above the diagonal. Significant values are shaded.

	2002	2004	2005	2006	2007	2008
2002	*	0.0000	0.0000	0.0000	0.0000	0.0000
2004	0.0162	*	0.0000	0.0068	0.5918	0.9951
2005	0.0414	0.0191		0.0000	0.0000	0.0000
2006	0.0145	0.0023	0.0212	*	0.0088	0.5088
2007	0.0138	0.0003	0.0231	0.0015	*	0.0000
2008	0.0200	-0.0006	0.0182	0.0005	0.0022	*

**Table 5.** Pairwise values of  $F_{ST}$  (below diagonal) calculated from microsatellite data among 2007 and 2008 samples from collection localities on the Pecos River. Associated P-values are given above the diagonal.

	Acme	Bosque	Cortez	Crocket	Gasline	Hwy 70	Hwy 380	Six-mile	Willow	Bosque	Crocket	Gasline	Hwy 70	Bitterlake	Six-mile
	2007	2007	2007	2007	2007	2007	2007	2007	2007	2008	2008	2008	2008	2008	2008
<b>Acme 2007</b>	*	0.001	0.061	0.000	0.688	0.005	0.000	0.948	0.000	0.003	0.000	0.122	0.000	0.027	0.000
<b>Bosque 2007</b>	0.009	*	0.064	0.306	0.687	0.067	0.000	0.087	0.022	0.002	0.414	0.218	0.013	0.486	0.006
<b>Cortez 2007</b>	0.014	0.012	*	0.029	0.800	0.002	0.000	0.719	0.134	0.057	0.002	0.019	0.013	0.113	0.002
<b>Crocket 2007</b>	0.008	0.002	0.014	*	0.993	0.146	0.000	0.563	0.142	0.045	0.060	0.286	0.012	0.052	0.032
<b>Gasline 2007</b>	0.001	0.001	-0.001	-0.004	*	0.216	0.007	0.925	0.659	0.918	0.698	0.242	0.995	0.956	0.901
<b>Hwy 70 2007</b>	0.013	0.008	0.028	0.006	0.006	*	0.000	0.032	0.016	0.000	0.010	0.226	0.010	0.030	0.001
<b>Hwy 380 2007</b>	0.017	0.016	0.024	0.013	0.011	0.021	*	0.000	0.001	0.000	0.000	0.002	0.000	0.000	0.000
<b>Six-mile 2007</b>	-0.001	0.006	-0.001	0.001	-0.004	0.011	0.015	*	0.466	0.922	0.332	0.008	0.039	0.661	0.006
<b>Willow 2007</b>	0.008	0.006	0.009	0.003	0.001	0.010	0.009	0.002	0.000	0.039	0.065	0.017	0.002	0.023	0.000
<b>Bosque 2008</b>	0.007	0.007	0.013	0.004	0.000	0.015	0.013	-0.001	0.005	*	0.463	0.169	0.053	0.029	0.022
<b>Crocket 2008</b>	0.011	0.002	0.019	0.004	0.001	0.011	0.014	0.003	0.004	0.002	0.000	0.250	0.187	0.063	0.045
<b>Gasline 2008</b>	0.010	0.007	0.024	0.007	0.008	0.010	0.023	0.020	0.014	0.009	0.008	*	0.543	0.514	0.252
<b>Hwy 70 2008</b>	0.009	0.006	0.018	0.006	-0.003	0.012	0.021	0.008	0.008	0.005	0.003	0.005	0.000	0.526	0.221
<b>Bitterlake 2008</b>	0.008	0.002	0.014	0.006	-0.002	0.013	0.028	0.002	0.008	0.007	0.007	0.006	0.003	*	0.285
<b>Six-mile 2008</b>	0.012	0.007	0.021	0.005	-0.001	0.015	0.022	0.011	0.011	0.005	0.005	0.008	0.003	0.004	*

---

eleven significant differences from 15 total comparisons after Bonferroni correction. The 2002 sample differed significantly from all subsequent samples, as did the 2005 sample. In 2005 the sample size was very small. Pairwise F-statistics were calculated among samples collected at different localities in 2007 and 2008. Significant values of  $F_{ST}$  were observed in 18 of a total of 105 comparisons after Bonferroni correction (Rice 1989) was applied. Eleven of these involved the collection made at Hwy 380 in 2007 (Table 5). For AMOVA analysis, samples were grouped by populations across years.  $F_{SC}$  was significant ( $F_{SC}=0.0085$ ,  $P=0.001$ ), indicating that some variation could be attributed to differences within localities between years. Significant variation could not be ascribed to differences among localities across years ( $F_{CT}=-0.00046$ ,  $P=0.3431$ ).

### ***Population comparisons- Mitochondrial DNA***

Pairwise  $\Phi_{ST}$ 's were calculated to determine if there was significant divergence among samples collected in different years. From fifteen pairwise comparisons five were significant after Bonferroni correction for multiple comparisons. The other significant comparisons, were between both 2007 and 2004-2006 collections and between the 2008 and 2004, and 2008 and 2006 collections (Table 6). For AMOVA analysis, samples were grouped by populations across years.  $F_{SC}$  was significant ( $F_{SC}=0.0033$ ,  $P<0.0001$ ), indicating that some variation could be attributed to differences within localities between years. The large difference in sample sizes between some localities between years probably explains this significant result. Significant variation could not be ascribed to differences among localities across years ( $F_{CT}=-0.0094$ ,  $P=0.7321$ ).

### ***Contemporary Effective Population size- Microsatellites***

Using microsatellite data, fifteen temporal comparisons were used to estimate the contemporary effective population size. Contemporary effective size estimates ranged from a low of 57.3 (95% confidence intervals 39.4 to 85.6) to a high of 467.2 for the 2004 to 2008 comparison (95% CI 330.8 - 687.9) (Table 7). To account for overlapping generations in Pecos bluntnose shiner, the equations of Jorde and Ryman (1995) and life table data were used to estimate a correction factor (C) and generation time (G). The model accounts for the effects of genetic drift as a cohort passes from one age class to the next and for the contributions of parents in multiple age classes to progeny in a non-growing population. Estimates of  $N_e$  obtained using NeEstimator were multiplied by the ratio C/G. For Pecos bluntnose shiner, the generation time was estimated to be 1.36 and a correction factor of 2.145 was obtained, hence C/G was 1.5772. Corrected  $N_e$  estimates from adjacent cohorts reveal an increase in effective size from 70.82 (2004-2005) to 151.57 (2007-2008).

### ***Contemporary Effective Population size- Mt-DNA***

The effective size estimate ( $N_{ef}$ ), based on mitochondrial DNA data for 2007 and 2008 was

**Table 6.** Pairwise  $F_{STs}$  calculated from Mt-DNA haplotype frequencies among samples collected between 2002 and 2008 (below diagonal). Associated P-values are given above the diagonal. Significant values are shaded.

	<b>2002</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>
<b>2002</b>	*	0.1133	0.0088	0.1572	0.4014	0.7578
<b>2004</b>	0.0051	*	0.2090	0.4971	0.0000	0.0029
<b>2005</b>	0.0724	0.0132	*	0.1699	0.0010	0.0039
<b>2006</b>	0.0035	-0.0009	0.0143	*	0.0010	0.0029
<b>2007</b>	-0.0002	0.0146	0.0967	0.0162	*	0.7393
<b>2008</b>	-0.0022	0.0130	0.0810	0.0141	-0.0012	*

**Table 7.** Temporal estimates of  $N_e$  for microsatellite and mitochondrial DNA data ( $N_{ef}$ ) and associated 95% confidence limits. The number of generations separating the sampling periods are given.

<b>Generations</b>	<b>Temporal Comparison</b>	<b><math>N_e</math></b>	<b>-95%</b>	<b>95%</b>	<b><math>N_{ef}</math></b>	<b>-95%</b>	<b>95%</b>
1	2004-2005	70.8	26.5	96.5	infinity	89.0	Infinity
1	2005-2006	58.8	23.2	70.3	infinity	55.5	Infinity
1	2006-2007	152.2	70	137	310.4	99.68	Infinity
1	2007-2008	151.6	73.22	12702	2648	279.16	Infinity
2	2002-2004	81.1	61.6	107.3	637.3	62.3	Infinity
2	2004-2006	286.5	182.7	513.1	413.9	61.1	Infinity
2	2005-2007	90.2	55.1	178.7	infinity	82.1	Infinity
2	2006-2008	152.6	55.1	211.4	193.9	59.1	Infinity
3	2002-2005	57.3	39.7	86.4	infinity	86.3	Infinity
3	2004-2007	381.2	271.7	556.4	785.1	149.9	infinity
3	2005-2008	114.3	71.9	209.4	infinity	101.6	Infinity
4	2002-2006	166.2	124.8	223.8	1481.9	110.8	Infinity
4	2004-2008	467.2	330.8	687.9	776.3	141.4	Infinity
5	2002-2007	381.2	271.7	556.4	14302.2	275.0	Infinity
6	2002-2008	223.9	173.4	289.1	infinity	361.7	infinity

---

2648 (Table 7). This is a substantial increase from the estimate based on the preceding cohort (2006-2007  $N_{ef} = 310.4$ ). The harmonic mean across temporal comparisons (excluding estimates of infinity) was 560.7.

## Conclusions

The low densities of Pecos bluntnose shiner population that occurred between 2002 and 2005 were accompanied by a decrease in the genetic effective size in 2005 and 2006. Recent population monitoring data for the Pecos bluntnose shiner indicates that the population is gradually rebounding (USFWS unpublished data) and this is reflected in the large increases in the genetic effective population size. Population fluctuations are predicted to erode allelic diversity, however high levels of genetic variation particularly at the mtDNA ND4 gene persist in Pecos bluntnose shiner. To date we have identified 49 distinct mitochondrial DNA haplotypes in around 1000 individuals. This can be compared to Rio Grande silvery minnow, in which we have detected only 14 haplotypes in the same fragment of DNA in roughly 5000 individuals screened over the past decade. The genetic effective size of the remaining Rio Grande silvery minnow population also appears to hover around 100, even in years when there are huge increases in density. In Rio Grande silvery minnow early life-history and river fragmentation and drying of substantial regions act to erode genetic variation and to depress the genetic effective size of the population (Turner et al. 2006). In the Pecos bluntnose shiner, high levels of diversity may persist because the river reach where the species persists is not fragmented, allowing at least some individuals to move to refugia when conditions are adverse. These individuals are then able to recolonize the formerly desiccated regions. However Hoagstrom *et al.* (2008) found that fluvial species like Pecos bluntnose shiner do poorly during river intermittence, possibly due to declining water quality (Ostrand & Marks 2000; Ostrand & Wilde 2004) or poor reproductive success (Durham and Wilde 2006).

The genetic effective size, estimated from eleven microsatellite DNA loci, shows the same trend as mitochondrial DNA data with increasing values from 2004-2005 to 2006-2008. However, effective size estimates are considerably lower for microsatellite DNA. These estimates are all below the theoretical benchmarks set out in the conservation genetics literature that are required to maintain both neutral and adaptive genetic variation. It has been proposed that  $N_e$  should exceed 500 to preserve variation at selectively neutral loci (Frankham 1995) while  $N_e$  should exceed 5000 if a species is to maintain sufficient variation for quantitative traits such as fecundity, spawning time and body size (Lande 1995).

---

## Acknowledgments

Stephen Davenport (U.S. Fish and Wildlife Service) and Nik Zymonas (New Mexico Department of Game and Fish) provided vital assistance with sample collection. Funding was provided by the New Mexico Department of Game in Fish, Share with Wildlife Program. Tracy Diver, Alana Sharp & Sierra Netz provided invaluable laboratory and/or field assistance.

## Literature Cited

- Alò, D., and T. F. Turner. 2005. Effects of habitat fragmentation on effective population size in the endangered Rio Grande silvery minnow. *Conservation Biology* 19, 1138 – 1148.
- Avise, J.C. (1994) *Molecular Markers, Natural History and Evolution*, Chapman and Hall, New York, NY. 511 pp.
- Bessert, M.L., & G. Ortí. 2003. Microsatellite loci for paternity analysis in the fathead minnow, *Pimephales promelas* (Teleostei: Cyprinidae). *Molecular Ecology Notes* 3, 532-534.
- Brooks, J. E., Platania, S. P. & D.L. Propst. 1991. Effects of Pecos River Reservoir Operation on the Distribution and Status of Pecos Bluntnose Shiner (*Notropis simus pecosensis*): Preliminary Findings: Report submitted to U.S. Bureau of Reclamation, Salt Lake City, UT, and Albuquerque, NM, 18 p.
- Campbell, L. 1995. Endangered and threatened animals of Texas; their life history and management. Texas Parks and Wildlife Department. Austin.
- Charnov, E. L., Turner, T. F., & Winemiller, K. O. 1999 Reproductive constraints and the evolution of life histories with indeterminate growth. *Proceedings National Academy of Science*. USA 98, 9460-9464.
- Chernoff, B., Miller, R. R. and C. R. Gilbert. 1982. *Notropis orca* and *Notropis simus*, cyprinid fishes from the American southwest with description of a new subspecies. *Occasional Papers Museum of Zoology*, University of Michigan 689, 1-49.
- Clement M., D. Posada & K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9 (10), 1657-1659
- Dimoski, P., G. Toth & M. Bagley. 2000. Microsatellite characterization in central stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). *Molecular Ecology* 9, 2187 - 2189.
- Durham, B. W. and G. R. Wilde. 2006. Influence of stream discharge on reproductive success of a prairie stream fish assemblage. *Transactions of the American Fisheries Society* 135, 1644-1653.
- Excoffier, L., P. E. Smouse & J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131, 479-491.
- Gold, J.R., Saillant E., Burrige C.P., Blanchard A. & J.C. Patton. 2004. Population structure and effective size reduction in critically endangered Cape Fear shiners *Notropis mekistocholas*. *Southeastern Naturalist* 3, 89–102.
- Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* 86, 485-486.

- 
- Hillis, D., Moritz, C. & B. Mable. 1996. *Molecular Systematics*, Sinauer. USA
- Hoagstrom, C.W., J.E. Brooks & S.R. Davenport. 2008. Spatiotemporal population trends of *Notropis simus pecosensis* in relation to habitat conditions and the annual flow regime of the Pecos River, 1992-2005. *Copeia*, 1, 5-15.
- Hoagstrom, C. W. 2003. Pecos bluntnose shiner population dynamics, Pecos River, New Mexico, February 1992 through August 2002. U.S Fish and Wildlife Service Final Research Report submitted to the U.S. Bureau of Reclamation, Albuquerque Area Office, NM. 148 pp.
- Jorde, P. E., & Ryman, N. 1996 Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics* **143**, 1369-1381.
- Jorde P.E, Ryman N. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics* 139, 1077-1090.
- Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* 46 (2), 477-494.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press: New York, USA.
- Ostrand, K.G. and D. E. Marks. 2000. Mortality of prairie stream fishes confined to an isolated pool. *Texas Journal of Science* 52, 255-258.
- Ostrand, K. G. and G. R. Wilde. 2004. Changes in prairie stream fish assemblages restricted to isolated streambed pools. *Transactions of the American Fisheries Society* 133, 1329-1338.
- Peel, D., Ovenden, J. R. & S.L. Peel 2004. NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries. <http://www.dpi.qld.gov.au/fishweb/11637.html>.
- Pollack, E. 1983. A new method for estimating the effective population size from allele frequency changes. *Genetics* 104, 531-548.
- Propst, D. L. 1999. Threatened and Endangered Fishes of New Mexico. Technical Report No. 1. New Mexico Department of Game and Fish, NM. 84 pp.
- Petit, R. J., El Mousadik, A. & O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* 12, 844 – 855.
- Platania, S. P. 1995. Distribution, relative abundance, and conservation status of Pecos bluntnose shiner, *Notropis simus pecosensis*. Report to the New Mexico Department of Game and Fish, Santa Fe New Mexico, USA. 32 pp.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43, 223-225.
- Robertson, L. 1997. Water operations on the Pecos river, New Mexico and the Pecos bluntnose shiner, a federally listed minnow, p407-421. In: *Competing Interests in Water Resources: Searching for Consensus*. H.W. Greydanus and S.S. Anderson (eds.). U.S. Committee on Irrigation and Drainage, Washington, D.C.
- Schneider, S., D. Roessli & L. Excoffier. 2000. Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva. 111 pp.
- Schwartz, M.K., G. Luikart & R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution* 22(1), 11-16.
-

- 
- Sunnucks, P., A. C. C. Wilson, L. B. Beheregaray, K. Zenger, J. French & A. C. Taylor. 2000. SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Molecular Ecology* 9, 1699 - 1710.
- Templeton, A. R. 1992 Human origins and analysis of mitochondrial DNA sequences. *Science* 255, 737.
- Templeton, A. R., K. A. Crandall and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. 111. Cladogram estimation. *Genetics* 132, 619-633.
- Turner, T. F., T. E. Dowling, R. E. Broughton & J. R. Gold. 2004. Variable microsatellite markers amplify across divergent lineages of cyprinid fishes (subfamily Leuciscinae). *Conservation Genetics* 5, 273 - 281.
- U.S. Department of the Interior. 1987. Endangered and threatened wildlife and plants: determination of threatened status for the Pecos bluntnose shiner and designation of its critical habitat. *Federal Register* 52, 5295-5303.
- U.S. Fish and Wildlife Service. 1992. Pecos bluntnose shiner recovery plan. U. S. Fish and Wildlife Service, Region 2, Albuquerque, New Mexico.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D. P. M. & P. Shipley. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4, 535-538.
- Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121, 379–391.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D. P. M. & P. Shipley. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4, 535-538.
- Wang, J. & Whitlock. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 163, 429-446.

**Appendix 1.** Summary statistics for microsatellites tabulated by locus and year. Abbreviations are N- number of individuals, total number of alleles,  $A_R$ - allelic richness,  $H_E$ - Nei's unbiased gene diversity,  $H_O$  - observed heterozygosity.

Year	Locus	N	Total Alleles	$A_R$ (n=18)	$H_E$	$H_O$	$F_{IS}$
2002	118	107	32	18.335	0.949	0.738	0.223
	126	107	9	4.544	0.289	0.262	0.091
	Lco1	103	34	19.667	0.957	0.709	0.260
	Ca6	107	9	7.824	0.846	0.925	-0.094
	Lco3	104	13	8.511	0.798	0.644	0.193
	Lco6	95	24	12.521	0.861	0.600	0.304
	Ca8	98	26	17.648	0.949	0.582	0.388
	Nme232	103	18	12.846	0.899	0.893	0.007
	Nme93	105	24	15.771	0.939	0.781	0.169
	Nme208	107	24	12.973	0.864	0.664	0.234
	Nme174	103	21	13.471	0.892	0.573	0.359
2004	118	172	30	17.238	0.943	0.802	0.150
	126	172	12	5.009	0.375	0.326	0.123
	Lco1	169	39	20.413	0.959	0.769	0.199
	Ca6	172	10	7.930	0.855	0.814	0.048
	Lco3	168	13	8.436	0.726	0.714	0.016
	Lco6	169	25	13.358	0.890	0.550	0.382
	Ca8	158	29	17.537	0.948	0.475	0.500
	Nme232	146	20	12.546	0.879	0.808	0.081
	Nme93	148	24	14.850	0.925	0.750	0.190
	Nme208	172	32	15.425	0.917	0.576	0.373
	Nme174	163	23	14.323	0.916	0.509	0.445
2005	118	21	17	16.318	0.950	0.810	0.151
	126	22	10	9.027	0.661	0.636	0.078
	Lco1	20	20	18.680	0.960	0.727	0.247
	Ca6	22	9	8.599	0.769	0.750	0.026
	Lco3	21	9	8.533	0.714	0.619	0.136
	Lco6	21	13	12.120	0.870	0.333	0.623
	Ca8	18	15	15.000	0.895	0.500	0.449
	Nme232	18	14	14.000	0.895	0.833	0.071
	Nme93	21	16	14.960	0.927	0.810	0.129
	Nme208	21	11	10.876	0.854	0.524	0.392
	Nme174	22	12	10.998	0.848	0.682	0.199
2006	118	135	32	16.694	0.932	0.711	0.237
	126	138	12	5.708	0.425	0.391	0.105
	Lco1	132	38	20.438	0.961	0.788	0.181
	Ca6	136	13	8.170	0.851	0.897	-0.055
	Lco3	137	12	8.480	0.743	0.708	0.048
	Lco6	132	26	14.657	0.912	0.636	0.303
	Ca8	132	31	19.138	0.955	0.568	0.406
	Nme232	108	22	13.362	0.907	0.880	0.031
	Nme93	114	25	15.436	0.936	0.754	0.194
	Nme208	133	30	15.878	0.927	0.617	0.336
	Nme174	122	22	13.757	0.903	0.689	0.239

<b>Year</b>	<b>Locus</b>	<b>N</b>	<b>Total Alleles</b>	<b>A<sub>R</sub> (n=18)</b>	<b>H<sub>E</sub></b>	<b>H<sub>O</sub></b>	<b>F<sub>IS</sub></b>	
<b>2007</b>	118	331	35	18.934	0.954	0.767	0.196	
	126	334	13	5.173	0.347	0.320	0.080	
	Lco1	301	39	20.325	0.961	0.761	0.208	
	Ca6	312	13	8.236	0.841	0.840	0.001	
	Lco3	314	12	8.040	0.693	0.654	0.056	
	Lco6	291	30	13.999	0.892	0.577	0.353	
	Ca8	314	31	18.620	0.955	0.557	0.417	
	Nme232	311	25	12.893	0.898	0.875	0.026	
	Nme93	326	29	14.840	0.929	0.779	0.162	
	Nme208	335	33	15.049	0.916	0.737	0.195	
	Nme174	320	25	13.800	0.893	0.606	0.321	
	<b>2008</b>	118	253	34	17.841	0.944	0.787	0.167
		126	252	8	4.233	0.319	0.298	0.040
		Lco1	248	39	20.457	0.961	0.722	0.249
Ca6		251	12	7.779	0.844	0.869	-0.030	
Lco3		249	11	7.607	0.682	0.671	0.017	
Lco6		251	30	13.585	0.887	0.542	0.390	
Ca8		243	28	18.429	0.954	0.547	0.427	
Nme232		253	25	13.190	0.899	0.830	0.076	
Nme93		252	25	14.691	0.928	0.762	0.179	
Nme208		252	30	15.797	0.928	0.667	0.278	
Nme174		249	24	15.159	0.926	0.614	0.337	