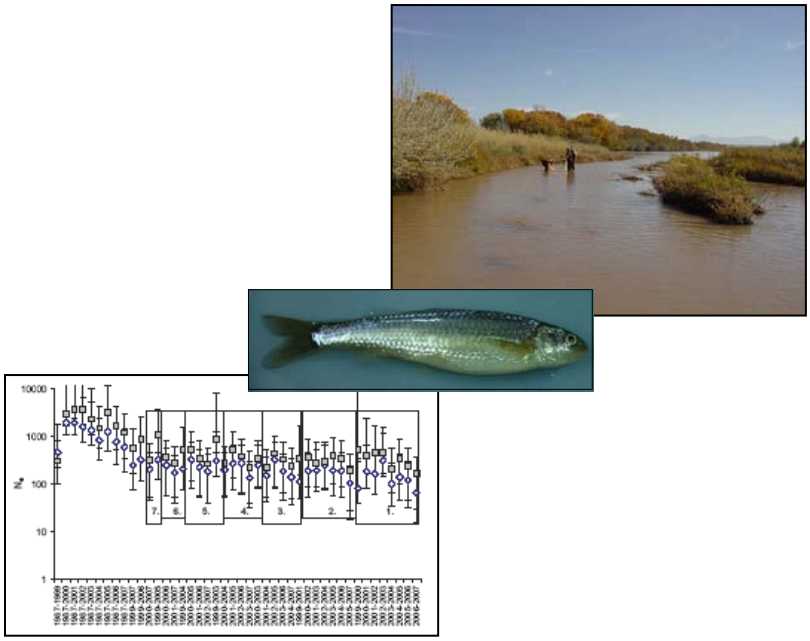
Genetic monitoring of the Rio Grande silvery minnow: Genetic status of wild and captive stocks in 2011.



Annual report FY 2011

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Executive Summary

In 2011, we continued genetic monitoring of the wild (middle Rio Grande) Rio Grande silvery minnow population and of fish bred and/or raised in captivity and subsequently released in the Middle Rio Grande, New Mexico, and in the Big Bend National Park, Texas, as part of reintroduction efforts for the species. Genetic monitoring of the Rio Grande silvery minnow commenced in 1999 and has continued annually since that time. Here we report on the genetic status of wild and captive stocks of Rio Grande silvery minnow in 2011. In 2011, we sampled 369 wild fish from the Middle Rio Grande, NM, and 396 progeny (representing 6 captive lots) of captive spawning conducted at Dexter National Fish Hatchery and Technology Center and the Albuquerque Biological Park. In addition, one captive lot released in the Middle Rio Grande derived primarily from wild-caught eggs and larvae was sampled. These were representative of the captive stocks released at Big Bend and in the Rio Grande in New Mexico. The molecular methods and data analyses were conducted as described in the attached draft manuscript and hence are not included here.

Major Findings for 2011:

(1) Microsatellite gene diversity was very similar to values recorded in previous years. Heterozygosity increased over values seen between 2008-2010. Allelic richness has remained stable since 2006 and continued to do so in 2011. Mitochondrial gene diversity was similar to values seen in 2010 whilst haplotype richness was marginally less than previous years.

(2) Genetic effective size estimates from mitochondrial DNA haploptye frequencies declined slightly for the 2010-2011 time-period to 377 (moments) from 421 for the previous temporal comparison. The estimate obtained using the pseudo-maximum likelihood method suggested an increase in the female effective size of the population.

(3) Variance effective population size (N_{eV}) calculated from microsatellite DNA allele frequencies was <u>similar</u> for the 2010-2011 temporal comparison (N_{eV} = 235-363, depending on the method used) than for the previous period 2009-2010.

(4) Captive spawned Rio Grande silvery minnow released to Big Bend National Park had comparable levels of microsatellite gene diversity and heterozygosity to the wild population. Mitochondrial gene diversity was also comparable to the wild population. Across all captive stocks, a similar number of mitochondrial DNA haplotypes were detected as seen in the wild Middle Rio Grande population.

(5) In 2011, mitochondrial DNA haplotype diversity and haplotype richness declined in the Angostura reach, increased in the Isleta reach and remained stable in the San Acacia reach. For microsatellite data, there was an increase in the number of alleles and gene diversity (values obtained using resampling approach to account for unequal sample sizes among years and reaches) but a decrease in heterozygosity for fish in the Angostura and San Acacia reaches. In the Isleta reach gene diversity increased, allelic diversity was stable and heterozygosity decreased over 2010 values.

Introduction

Genetic monitoring is defined as collection of two or more temporally spaced genetic samples from the same population. In fish, genetic monitoring to date has been confined largely to marine species and in freshwater systems, primarily involving salmonids (Table 1). Genetic monitoring studies typically employ neutral genetic markers, such as microsatellites and occasionally mitochondrial DNA, to track changes in diversity metrics across multiple contemporary time-points. The number of loci employed varies among species with between five and 14 microsatellites employed in recently published studies. The time-scale of genetic monitoring also varies considerably from a sampling over only a few years to the use of archival samples for monitoring that spans several decades. In these latter studies that encompass multiple decades sampling is rarely conducted on an annual basis so linking changes in diversity metrics to specific environmental or management actions may not be plausible. To our knowledge, the data set that we have collected for Rio Grande silvery minnow over the past 13 years represents the longest genetic monitoring time series for a non-salmonid freshwater fish. The population is sampled throughout its current range (mean annual sample size =278), using nine microsatellite

loci and a mitochondrial DNA gene to measure changes in various metrics of genetic diversity including allelic richness, heterozygosity, and genetically effective population size (N_e). The temporal component and sampling strategy provides the framework necessary to examine the impacts of changes in abundance, management actions and environmental conditions on population diversity. In species such as Rio Grande silvery minnow that are characterized by their short lifespan (the population is dominated by age-1 fish) and dramatic changes in abundance from year to year, there is the potential for negative genetic impacts to the population over relatively short time scales. For this reason, genetic monitoring is a crucial component to the management of the species. In fact, the data collected as part of the genetic monitoring program for Rio Grande silvery minnow has informed management in the following ways i) demonstrated that the genetic effective size is orders of magnitude lower that the census size, ii) there is not significant divergence of allele frequencies among fishes collected in each of the three river reaches [due to downstream movement of eggs, larvae and adult fish and stocking of the population with captive reared fish], iii) diversity of the wild population is best captured (in captive stocks) by collection of eggs produced by natural spawning events in the wild, iv) when artificial breeding is necessary, a group spawning design (with equalized sex ratio) produces fish that have levels of diversity that are comparable to that achieved with a paired mating design. These finding have helped to inform the Recovery Plan for the species and were instrumental in the development of the captive propagation and genetics management plan. Here we report on the genetic status of the population in 2011.

Results for 2011

Microsatellites- Genetic Diversity

In 2011, 359 samples were collected from the Angostura, Isleta and San Acacia reaches of the Middle Rio Grande (Table 2). A total of 6844 fish have been genotyped for nine microsatellite loci over the 13-year study (this includes samples released at Big Bend). Microsatellite locus *Ca6* was the least variable with 10 alleles detected across all populations whereas *Ppro118* was the most variable with 63 alleles. After sequential Bonferroni correction for multiple comparisons there were 198 departures from Hardy-Weinberg equilibrium (HWE) among 612 comparisons. Four loci (*Lco3, Lco6, Ca6, Ppro126*) conformed to HWE in all or nearly all comparisons. Micro-Checker suggested that null alleles probably caused departures from HWE.

Across all samples, there was no evidence of linkage disequilibrium among loci after sequential Bonferroni correction. In 2010 and 2011 gene diversity were similarly high (Table 4, Figure 1). In 2011, heterozygosity increased slightly over the previous year. Microsatellite gene diversity was similar across river reaches whilst heterozygosity was marginally higher in fishes from the Isleta reach. Allelic richness was lowest in the Angostura reach (Figure 2). There was not adequate statistical power to make a valid comparison of the reach specific effects of augmentation versus no-augmentation in the Angostura reach as there are only three data points (2009-2011) for which there was no augmentation compared to 6 years of augmentation.

Mt-DNA- Genetic Diversity

Across the 13-year time series, 15 mtDNA haplotypes were identified among 6634 individuals assayed. Differentiation among haplotypes was low, with one to six substitutions among them. Haplotype A was the most common in all samples except Cs-An-02 (captive spawned) which was monomorphic for haplotype D (Table 5). Three haplotypes (C, D, F) were present at moderate frequencies (>5%) and 11 haplotypes were considered rare (present at frequencies < 5%). There were no significant differences between observed gene diversity and gene diversity obtained after using resampling to adjust for differences in sample size. Across the entire time series, gene diversity was highest in the 1987 sample (h=0.743) and lowest in 2000 (h=0.364) (Table 4). In 2011, haplotype diversity (h=0.695) and number of haplotypes (9) were highest for the fishes collected from the Isleta reach. Eight haplotypes were detected in the Angostura and San Acacia reaches and haplotype diversity was similar in these reaches (h=0.5789 and h=0.5983 respectively) (Figure 3).

Microsatellites- Population Structure

Hierarchical analysis of molecular variance was conducted by grouping temporal samples by river reach. Values were not significantly different from zero, indicating that river reach did not explain a significant portion of genetic variance (F_{CT}=0.0004, P= 0.185); a result consistent with previous years.

Mt-DNA- Population Structure

Φ-statistics were calculated between wild samples collected in 1987 and from 1999-2010 partitioned by river reach. Results indicated that genetic differences among river reaches (Angostura, Isleta and San Acacia) (Φ_{CT} = -0.0008, P = 0.851) were not significantly different from zero.

Genetic Effective Size

Estimates of variance effective size were similar across the three methods used with estimates of 263 (moments), 326 (TempoFs) and 235 (MLNE) (Figure 4). The moments estimate was almost identical to the previous temporal comparison (2009-2010) whilst TempoFs suggested a slight increase (Figure X). The MLNE estimate showed a decrease from 2009-2010 values. Estimates of female effective size were 377 (95% CIs 34.1-infinity) (moments-based) and N_{ef} =43745 (95% CIs 151.9 to infinity; MLNE) (Figure 5). Effective size was also estimated using the linkage disequilibrium method. For the wild population in 2011, N_{eD} was infinity (Figure 6).

Big Bend

For fish released at Big Bend measures of microsatellite diversity (H_e, H_O and A_R) were similar to that of the wild New Mexico population. There were eight mtDNA haplotypes represented in the fish released and gene diversity across lots was 0.608 and comparable to the wild population. Pairwise Φ-statistics indicated that genetic differences among the groups of fish released at each of four localities were not significantly different from zero. For captive-bred fish released at Big Bend and the Middle Rio Grande in 2010, N_{eD} ranged from 59 to infinity.

Discussion

Genetic status of the species in 2011

Extensive demographic surveys show that the wild population of Rio Grande silvery minnow has experienced multiple, order of magnitude changes in density over the past two decades (U.S. Fish and Wildlife Service 2010). From 2000-2004 densities of Rio Grande silvery minnow were less than one fish per 100 m^2 and during this time the threat of extinction in the wild was acute. For both microsatellites and mtDNA there is considerable inter-annual variability in gene diversity metrics and effective size estimates from 1987 and 1999-2004. Following

commencement of population supplementation with fish reared in captivity, there has been a general trend was toward stabilization and marginal increases in mtDNA and microsatellite diversity and the number of alleles/haplotypes. Inter-annual variability in all of these measures decreased after 2005 with this trend continuing in 2011. Microsatellite gene diversity was very similar to values recorded in previous years. Heterozygosity increased over values seen between 2008-2010. Allelic richness has remained stable since 2006 and continued to do so in 2011. Mitochondrial gene diversity was similar to values seen in 2010 whilst haplotype richness was marginally less than previous years. The word 'haplotype' refers to the matrilineal inheritance of the mtDNA genome, which is distinct from biparental inheritance exhibited by microsatellites (i.e., both a male and female parent contributes to the 'genotype').

Estimates (from microsatellite data) of variance effective population size for 2010-2011 calculated using the temporal method remained stable from values calculated for the previous time period (2009-2010). Despite the increase in genetic effective size it is still a fraction of the estimated census size of the population. Low N_{eV} results from an important interaction of life history (e.g., pelagic eggs and larvae) and habitat fragmentation by dams that results in high variance in reproductive success among spawning pairs in the Rio Grande (Alò & Turner 2005, Osborne *et al*. 2005, Turner *et al*. 2006). It is important to note that the negative interaction of life history and fragmentation occurs even when recruitment is strong because downstream displacement of eggs and larvae is arguably expected to be greater when spring flows are higher (Dudley 2004).

Highly polymorphic loci with many rare alleles, as is typical of microsatellites, cause biased estimates of variance effective size, N_{eV}, (Hedrick 1999). Both Waples (1989) and Turner *et al*. (2001) noted that moments estimates obtained using the most commonly employed measures of allele frequency change (Nei and Tajima 1981; Pollak 1983) tended to be downward biased (resulting in overestimates of N_e) when allele frequencies are close to zero or one. To account for this potential bias, the unbiased estimator, F_S, (Jorde and Ryman 2007), as implemented in TempoFs (www.zoologi.su.se/_ryman), was also used to estimate N_{eV}. Over the past several years, estimates of effective size using the unbiased estimator and those calculated using the method of are in good agreement. There is considerably more variability in the estimates obtained using the pseudo-maximum likelihood method. However, it has been shown previously that MLNE tends to overestimate N_e when calculated from loci with highly skewed

allele frequencies (Jorde and Ryman 2007) and can provide imprecise estimates in non-equilibrium populations (Wang 2001). Estimates of effective size (moments) made from mitochondrial DNA haplotype frequency data showed similar female effective size for the 2010-2011 to the previous temporal comparisons. Estimates were also similar to those obtained from microsatellite data.

We also used the linkage disequilibrium method to estimate effective size. This method is a single sample estimator and uses different aspects of the data to estimate the effective size. From a management perspective, there are a number of theoretical and practical distinctions between N_{eI} (to which N_{eD} estimates are most closely associated) and N_{eV}. These two measures of effective size should be similar in stable populations but show predictable differences in declining (or growing) populations. For example, in declining populations N_{eI} should be larger than N_{eV} because the latter depends on the amount of genetic drift between sampled generations but the former is a measure of inbreeding in the generation prior to sampling, (Allendorf and Luikart 2007); therefore, N_{eI} is only reduced once mating between close relatives becomes more common (i.e., homozygosity increases in the population). Values of N_{eD} were uniformly higher than estimates of N_{eV}. The underlying principle of the LD method is that as N_e decreases, genetic drift increases non-random association among alleles at different loci (Hill 1981). As erosion of linkage disequilibrium can take several generations, N_{eD} may also contain information on the effective size from several generations that precede a population decline. In addition to this upward bias, single sample N_e estimators including N_{eD}, provide an estimate of the effective number of parents that produced the progeny from which the sample is drawn (Waples 2005).

Using computer simulations, Antao *et al*. (2010) evaluated the ability of the linkage disequilibrium and temporal methods to (i) detect a population decline, (ii) estimate the simulated bottleneck with low bias and high precision and (iii) to evaluate whether or not the methods were subject to a high rate of false positives (i.e. indicate a bottleneck when none had occurred). Sample sizes and loci number of our study most closely approximated the 10 loci, 50 sample scenario of Antao *et al*. (2010), and our results were in agreement with their finding that the temporal method closely estimates the size of the bottleneck in the first generation, whilst the linkage disequilibrium method always overestimates N_e and has low precision. In fact, in the first generation following a decline N_{eD} is much closer to the pre-bottleneck population size, which

supports the suggestion of Waples (2005) that this method can include information on the effective size of previous generations.

Genetic diversity of captive stocks released to the middle Rio Grande, New Mexico

In 2010, one stock released in the middle Rio Grande New Mexico was derived primarily from wild-caught eggs (collected in 2006 and 2007) and three stocks were derived from captive spawning. The stock reared from wild-caught eggs had higher gene diversity and allelic richness than the captive spawned stocks. This highlights the importance of using wild-caught eggs when possible. Collection of wild produced eggs helps to preserve rare alleles that may otherwise be lost when captive stocks are derived from relatively few breeders. Eggs collected from natural spawning events should encompass the genetic variation of the wild population.

Genetic diversity of captive stocks released to Big Bend National Park, Texas

In 2011 we characterized genetic variation in four lots of captive spawned fish that were released at four localities in the Big Bend National Park in the fall of 2010. These fish were bred at Dexter National Fish Hatchery and Technology Center. The fish released from Dexter in 2010 were a mixed lot (DXCs09 and DXCs10) and were produced using a group spawning design (10 males and 10 females per tank; broodstock DxCs09- 380 males: 380 females, DxCs10- 230 males:230 females). With the exception of allelic richness, diversity measures for these stocks were comparable to the wild population. This baseline data will allow us to track the genetic fate of the reintroduced population. Having a genetically diverse population initially, will help to reduce the chances of a genetic bottleneck and hence to maximize the long-term viability of this population. We also estimated the effective number of breeders for these captive lots, in all cases values of N_{eD} were high

Our results for 2010 are consistent with our previous studies of captive Rio Grande silvery minnow stocks, and suggest that, when possible, wild caught eggs should be salvaged and reared for repatriation to the river and for refreshing captive stocks. Using stocks reared from wild-caught eggs would be particularly beneficial for the Big Bend reintroduction program, as these stocks tend to contain more of the rare alleles present in the wild population. It is also important to maintain as many groups of captive fishes at different rearing and grow-out facilities as practical, as mixed-lot repatriates appear to represent more genetic diversity than

single lots, perhaps due to slight variation among rearing conditions and increased numbers of broodstock for mixed lots.

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Table 1. Published genetic monitoring studies in other species of fish including the time span of the study, the number of time points sampled (and number of individuals sampled at each time-point), genetic metrics measured and the type of genetic markers employed in the study.

Species	Time Span	Time Points	Inbreeding effective size	Variance effective size	Temporal Sample Size	# microsatellite loci	MtDNA	Diversity Metrics (H_e, H_o, A_R)
Carp[1]	1996-2008	2	-	Yes	31-59	7	-	Yes
Steelhead[2]	1949-2005	~decadal	Yes	sibship	25-78	5-9	-	Yes
Chinook[3]	1985-2001	4	-	Yes	22-111	14	-	Yes
Atlantic[4]	1944-1998	2-4	Yes	Yes	~40	7	-	Yes
Brook[4]	1944-1998	1-4	Yes	Yes	~40	7	-	Yes
Razorbacks[5]	1997-2003	7	-	Yes	3-54	-	Yes	Yes
Perch[6]	1977-2000	5	-	Yes	~30	5	-	Yes
Roach[6]	1977-2000	5	-	Yes	~30	5	-	Yes
Sandbar shark[7]	2002-2006	5	-	Yes	53-201	8	-	Yes
Thornback ray[8]	1965-2003	4	-	Yes	10-35	11	-	Yes
Red drum[9]	1986-1989	4	-	Yes	301-392	-	Yes	Yes
Snapper[10]	1950-1998	5	-	Yes	30-50	7	-	Yes
Houting[11]	1980-2002	3	-	Yes	39-50	12	-	Yes
Herring[12]	1979-2003	3	-	Yes	80-277	9	-	Yes
Pecos bluntnose shiner[13]	2002-2009	7	Yes	Yes	22-338	7	-	Yes
RGSM	**1987-2011**	**14**	Yes	Yes	**43-476**	**9**	**Yes**	**Yes**

[1]Karaiskou *et al*. 2011; [2]Gow et al. 2011; [3]Eldridge & Killibrew 2008; [4]Fraser *et al*. 2007; [5]Dowling *et al*. 2005; [6]Demandt *et al*. 2010; [7]Portnoy *et al*. 2009; [8]Chevolet *et al*. 2008; [9]Turner *et al*. 1999; [10]Hauser *et al*. 2002; [11] Hansen et al. 2006; [12]Larsson et al. 2010; [13]Osborne et al. 2010

Table 2. Sample sizes, collection localities on the Rio Grande, river reaches for wild Rio Grande silvery minnow samples collected in 2011.

River	Locality	Sample Size
Angostura	Angostura DD	1
Angostura	Alameda	14
Angostura	Sandia Line 14	14
Angostura	AMAFCA Channel	2
Angostura	Dixon Rd	2
Angostura	Central Ave Bridge	38
Isleta	Below Isleta DD	13
Isleta	Alejandro Drain	2
Isleta	Los Lunas	49
Isleta	Peralta	50
Isleta	Bernardo	34
San Acacia	2 mi downstream San	44
San Acacia	San Antonio	49
San Acacia	San Marcial	47

Table 3. Number of wild samples collected by year and river reach (Angostura, Isleta and San Acacia).

	Angostura	Isleta	San Acacia
1987	15	-	28
1999	-	-	46
2000	-	-	194
2001	-	65	63
2002	67	121	201
2003	71	65	33
2004	141	15	6
2005	190	109	95
2006	95	143	145
2007	48	128	42
2008	165	191	123
2009	175	153	150
2010	149	146	151
2011	71	148	140

Table 4. Summary statistics for microsatellite and mtDNA – ND4 loci for wild, hatchery reared wild-caught eggs (WcE), captively spawned (Cs) Rio Grande silvery minnow. Grey shading indicates lots that were released at Big Bend National Park.

		Microsatellites							Mt-DNA			
Population	**N**	H_E	H_O	F_{IS}	A_R	N_{eD}	**-95%**	**95%**	**N**	***h***	H_R	**No. Haps**
1987	43	0.797	0.71	0.111	-	∞	139.3	∞	37	0.743	6.000	7
1999	46	0.815	0.647	0.210	-	∞	∞	∞	44	0.427	3.816	5
2000	194	0.815	0.697	0.145	13.298	∞	∞	∞	124	0.364	3.359	6
2001	128	0.808	0.721	0.107	13.729	2007.7	495.1	∞	122	0.609	6.063	10
2002	389	0.794	0.68	0.143	13.676	1950.6	701.7	∞	387	0.63	4.163	8
2003	169	0.818	0.709	0.134	13.902	2997.7	563.8	∞	167	0.524	4.890	9
2004	162	0.82	0.738	0.100	13.792	595.5	357.2	1558.7	161	0.62	6.277	10
2005	394	0.817	0.725	0.113	13.947	2724.3	1013.5	∞	396	0.61	5.633	10
2006	383	0.826	0.726	0.122	14.04	2561.7	1291.4	34063.9	378	0.622	5.670	10
2007	218	0.829	0.727	0.123	13.821	∞	1210.7	∞	218	0.579	5.363	10
2008	474	0.824	0.713	0.135	14.043	4458.5	1478.5	∞	466	0.569	5.301	11
2009	476	0.832	0.689	0.172	14.049	3607.6	1676.9	∞	472	0.592	5.649	12
2010	440	0.837	0.693	0.172	14.155	∞	2022.8	∞	433	0.649	6.087	9
2011	368	0.8341	0.7221	0.134	14.097	∞	2950	∞	359	0.634	5.741	11
WILD-CAUGHT EGGS												
WcE-01*	178	0.82	0.651	0.206	13.766	1379.6	655.6	∞	157	0.627	6.999	8
WcE-SA-01	50	0.831	0.727	0.126	13.038	∞	238.3	∞	51	0.624	6.000	6
WcE-An-02	50	0.784	0.73	0.070	11.065	85.6	54.1	173.4	49	0.481	2.949	3
WcE-SA-02	81	0.819	0.68	0.171	13.907	∞	461.7	∞	80	0.702	7.376	8
WcE-SA-03	51	0.83	0.696	0.164	13.868	5008.5	307.6	∞	51	0.714	7.848	8
MJO-07-005	54	0.827	0.739	0.108	13.801	1065	195.9	∞	53	0.602	6.733	7
MJO-07-006	49	0.814	0.723	0.114	14.171	∞	520.6	∞	48	0.581	5.962	6
*MJO-10-005	49	0.839	0.701	0.167	13.306	259.7	86.9	∞	49	0.710	4.682	6
CAPTIVE SPAWNED												
MJO-06-29	50	0.804	0.745	0.074	10.394	42.2	28.7	68.7	50	0.517	5.000	5
Cs-01	64	0.794	0.659	0.172	11.931	43.7	35.6	55	58	0.46	4.982	5
Cs-An-02	51	0.686	0.675	0.015	7.507	21.6	14.9	32.5	51	0	1.000	1
Cs-SA-02	53	0.803	0.673	0.163	12.034	72.7	52.5	110.9	53	0.751	5.919	6
TFT039	51	0.806	0.7	0.133	11.691	106.3	56	433.5	52	0.558	3.995	4
Cs-04	50	0.824	0.691	0.163	13.247	65.5	45.7	105.7	47	0.586	5.911	6
TFT-04-23	50	0.779	0.683	0.124	11.071	20.4	16.5	25.4	47	0.593	4.996	5
TFT-04-24	48	0.828	0.717	0.135	11.087	40.2	29.7	57.8	48	0.609	4.949	5

TFT-04-25	50	0.81	0.768	0.053	10.661	24.9	20	31.5	53	0.702	5.934	6
TFT-04-29	54	0.839	0.763	0.092	13.028	∞	532.2	∞	53	0.609	4.903	5
TFT-04-30	56	0.826	0.727	0.121	13.524	323.1	134	∞	45	0.656	4.790	5
TFT-04-31	50	0.805	0.701	0.13	11.998	83.2	54.7	154.7	50	0.706	6.865	7
TFT-05-006	50	0.792	0.649	0.183	9.768	49.4	38.8	65.7	50	0.625	5.803	6
TFT-05-007	49	0.797	0.705	0.117	11.305	86.6	53.2	191.3	48	0.55	4.884	5
TFT-05-008	50	0.804	0.663	0.178	10.584	32.2	26.7	39.5	49	0.611	4.934	5
TFT-05-009	50	0.804	0.717	0.109	11.899	219.9	98.8	∞	50	0.506	3.996	4
TFT-05-011	51	0.808	0.693	0.144	11.447	136.6	81	354	53	0.573	5.853	6
MJO-06-25	50	0.814	0.721	0.115	13.282	184.5	110.1	487.9	49	0.635	4.934	5
MJO-06-028	50	0.805	0.705	0.125	11.295	87.6	57.2	164.3	50	0.738	4.996	5
MJO-07-007	50	0.813	0.739	0.091	11.993	60.4	48.3	78.5	50	0.605	4.869	5
MJO08_006	50	0.827	0.669	0.192	13.493	304.3	173.4	1047.5	47	0.662	6.911	7
MJO08_007	50	0.841	0.721	0.144	13.105	392.8	160.8	∞	50	0.625	6.803	7
MJO08_008	50	0.834	0.711	0.149	13.377	614.7	194.9	∞	49	0.706	5.997	6
MJO08_009	51	0.843	0.715	0.153	13.798	174.6	106.1	425.8	51	0.658	5.995	6
MJO-09-001	68	0.819	0.706	0.138	13.606	217.6	134.7	498.1	67	0.612	8.326	9
MJO-09-002	72	0.7985	0.600	0.162	13.322	642.9	237.8	∞	72	0.555	7.189	8
MJO-09-003	71	0.8112	0.719	0.115	13.12	257.8	131.5	1736.7	71	0.64	5.76	6
MJO-09-004	69	0.8171	0.713	0.128	13.228	425.1	186.2	∞	66	0.442	4.768	6
MJO-09-005	50	0.8267	0.691	0.166	13.888	430.6	217.4	5708.1	49	0.735	5.000	5
MJO-09-006	50	0.8209	0.7061	0.141	12.987	109.7	76.4	182.3	50	0.53	4.000	4
MJO-09-007	50	0.8228	0.7034	0.146	13.347	207.9	135.3	418.9	51	0.675	5.680	6
MJO-09-008	50	0.8197	0.7119	0.133	13.731	176.3	101.1	537.4	50	0.776	6.803	7
MJO-09-009	50	0.8203	0.6975	0.151	13.347	187.2	112.9	472.6	50	0.504	7.799	8
MJO-09-010	48	0.8158	0.6975	0.146	12.716	408.8	177.9	∞	43	0.681	8.928	9
MJO-09-011	50	0.7926	0.6566	0.173	11.258	78.8	59.2	112.6	49	0.767	5.000	5
MJO-09-012	49	0.8027	0.6747	0.161	11.532	43.3	33.3	59	43	0.666	7.000	7
MJO-09-013	50	0.811	0.6698	0.176	12.228	1032	274	∞	50	0.563	7.792	8
MJO-09-014	50	0.8037	0.6814	0.154	12.779	122	86.9	195.2	47	0.742	7.955	8
LL11	50	0.829	0.738	0.110	12.842	301.5	123	∞	49	0.681	0.370	5
MJO-10-001	51	0.841	0.749	0.111	13.131	997	226.5	∞	51	0.630	5.830	8
MJO-10-002	49	0.831	0.754	0.094	13.799	914	182.8	∞	49	0.659	3.932	5
MJO-10-003	48	0.822	0.745	0.094	13.569	∞	409.1	∞	48	0.504	4.312	6
MJO-10-004	50	0.805	0.769	0.046	13.775	496.9	189.7	∞	50	0.639	3.510	5
MJO-10-006	49	0.783	0.699	0.108	11.350	58.8	32.3	163.4	49	0.664	4.875	6
MJO-10-007	48	0.825	0.747	0.101	12.994	106.1	60.2	312.1	48	0.518	5.480	7
MJO-11-005	48	0.810	0.729	0.100	12.838	118.2	81.6	201.2	47	0.594	2.999	4

Sample size (N), expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness (A_R) and average weighted inbreeding co-efficient (F_{IS}) are given over all loci. N_{eD} estimates (based on nine microsatellite loci) and associated 95% confidence intervals (obtained using jack-knifing) are given. For ND4 sample size (N), gene diversity (h), haplotype richness (H_R) and observed number of haplotypes are given. *WcE-01 sample was also collected from San Acacia but reared at Dexter (WcE-SA-01 was reared at the Albuquerque Biopark). (An- Angostura, SA- San Acacia, numerals following refer to the years eggs were collected, for example WcE-SA-01 were wild-caught eggs collected from the San Acacia reach in 2001). * Mixed lot- including wild-caught eggs and progeny of communal spawning.

Table 5. Mt-DNA haplotype frequencies across all wild and captive (wild-caught eggs and captive spawned) stocks.

	A	C	D	E	F	K	I	J	M	N	P	O	Q	S	T	W
Wild																
1987	0.459	0.162	0.162	0.054	0.081	0.027	-	-	0.054	-	-	-	-	-	-	-
1999	0.75	-	0.114	0.068	0.045	0.023	-	-	-	-	-	-	-	-	-	-
2000	0.79	0.008	0.048	0.048	0.097	0.008	-	-	-	-	-	-	-	-	-	-
2001	0.607	0.09	0.057	0.033	0.098	0.074	0.008	0.016	0.008	-	-	0.008	-	-	-	-
2002	0.556	0.199	0.137	0.01	0.059	0.034	-	0.003	-	-	-	0.003	-	-	-	-
2003	0.671	0.054	0.15	0.03	0.054	0.012	-	0.006	0.006	-	-	0.018	-	-	-	-
2004	0.596	0.087	0.106	0.019	0.075	0.05	0.012	-	0.019	-	0.006	0.031	-	-	-	-
2005	0.598	0.126	0.088	0.028	0.086	0.018	0.015	0.003	0.028	-	-	0.01	-	-	-	-
2006	0.587	0.135	0.093	0.048	0.048	0.048	0.003	-	0.029	-	-	0.008	-	-	0.003	-
2007	0.628	0.11	0.083	0.023	0.087	0.037	0.005	-	0.005	-	-	0.018	0.005	-	-	-
2008	0.635	0.12	0.079	0.026	0.067	0.045	0.004	-	0.009	-	0.002	0.006	-	0.006	-	-
2009	0.614	0.14	0.076	0.028	0.064	0.034	0.006	0.004	0.019	-	0.002	0.011	-	0.002	-	-
2010	0.562	0.124	0.097	0.032	0.069	0.053	0.014	-	0.016	-	-	0.032	-	-	-	-
2011	0.574	0.142	0.109	0.028	0.064	0.031	0.006	-	0.031	-	-	0.011	0.003	0.003	-	-
Wild-caught eggs																
WcE-01	0.573	0.197	0.051	0.064	0.064	0.032	-	-	0.013	0.006	-	-	-	-	-	-
WcE-SA-01	0.569	0.137	0.059	0.059	0.098	0.078	-	-	-	-	-	-	-	-	-	-
WcE-An-02	0.653	0.02	0.327	-	-	-	-	-	-	-	-	-	-	-	-	-
WcE-SA02	0.488	0.225	0.05	0.013	0.138	0.05	-	-	0.038	-	-	-	-	-	-	-
WcE-SA-03	0.49	0.078	0.196	0.059	0.098	0.039	-	-	0.02	-	-	0.02	-	-	-	-
MJO07-005	0.604	0.094	0.019	0.019	0.17	0.075	-	0.019	-	-	-	-	-	-	-	-
MJO07-006	0.604	0.083	0.125	0.021	0.083	0.042	-	-	-	-	-	0.042	-	-	-	-
MJO-10-005	0.477	0.182	0.159	-	0.136	0.023	-	-	-	-	-	0.023	-	-	-	-

Captive spawned	**A**	**C**	**D**	**E**	**F**	**K**	**I**	**J**	**M**	**N**	**P**	**O**	**Q**	**S**	**T**	W
MJO06-29	0.68	0.14	0.08	-	0.060	-	-	-	0.040	-	-	-	-	-	-	
Cs-01	0.724	0.052	-	0.034	0.069	0.121	-	-	-	-	-	-	-	-	-	
Cs-An-02	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
Cs-SA-02	0.434	0.075	0.17	0.132	0.17	-	-	-	-	-	0.019	-	-	-	-	
Cs-04	0.596	0.255	0.021	-	0.043	0.064	-	-	-	-	-	0.021	-	-	-	
TFT039	0.596	0.269	0.038	-	0	0.096	-	-	-	-	-	-	-	-	-	
TFT04-23	0.617	0.043	0.191	-	0	0.043	-	-	-	-	-	0.106	-	-	-	-
TFT04-24	0.583	0.125	0.208	-	0.021	0.063	-	-	-	-	-	-	-	-	-	-
TFT04-25	0.434	0.057	0.113	0.057	0.283	0.057	-	-	-	-	-	-	-	-	-	-
TFT04-29	0.566	0.245	-	0.075	-	0.094	-	-	0.019	-	-	-	-	-	-	-
TFT04-30	0.400	0.333	-	-	-	0.244	-	-	-	-	0.022	-	-	-	-	-
TFT04-31	0.420	0.340	0.02	-	0.060	0.04	-	-	0.100	-	-	0.02	-	-	-	-
TFT05-06	0.500	0.360	0.02	-	0.020	0.08	-	-	0.020	-	-	-	-	-	-	-
TFT05-07	0.625	0.292	0.021	0.063	-	0	-	-	-	-	-	-	-	-	-	-
TFT05-08	0.592	0.082	-	0.102	-	0.224	-	-	-	-	-	-	-	-	-	-
TFT05-09	0.68	0.160	-	-	-	0.12	-	-	0.040	-	-	-	-	-	-	-
TFT05-11	0.623	0.057	0.113	0.019	0.17	-	-	-	0.019	-	-	-	-	-	-	-
MJO06-25	0.551	0.245	0.061	-	0.061	0.082	-	-	-	-	-	-	-	-	-	-
MJO06-28	0.400	0.140	0.220	-	0.22	0.02	-	-	-	-	-	-	-	-	-	-
MJO07-007	0.560	0.020	0.120	0.280	0.020	-	-	-	-	-	-	-	-	-	-	-
MJO08_06	0.533	0.222	0.044	0.044	0.111	0.022	-	-	-	-	-	0.022	-	-	-	-
MJO08_07	0.580	0.180	0.02	0.06	0.12	0.02	-	-	0.020	-	-	-	-	-	-	-
MJO08_08	0.49	0.204	0.061	0.082	0.122	0.041	-	-	-	-	-	-	-	-	-	-
MJO08_09	0.549	0.176	0.059	0.039	0.118	0.059	-	-	-	-	-	-	-	-	-	-
MJO09_01	0.613	0.177	0.048	0.016	0.065	0.032	-	-	0.016	-	-	0.016	-	-	-	0.016
MJO09_02	0.647	0.206	0.074	0.015	0.015	0.015	0.029	-	-	-	-	-	-	-	-	-
MJO09_03	0.578	0.203	0.063	-	0.094	0.016	-	-	0.047	-	-	-	-	-	-	-

Captive spawned	**A**	**C**	**D**	**E**	**F**	**K**	**I**	**J**	**M**	**N**	**P**	**O**	**Q**	**S**	**T**	W
MJO09_04	0.734	0.172	0.031	-	0.031	0.016	-	-	0.016	-	-	-	-	-	-	-
MJO09_05	0.449	0.163	0.163	-	0.122	0.102	-	-	-	-	-	-	-	-	-	-
MJO09_06	0.660	0.080	0.080	-	0.180	-	-	-	-	-	-	-	-	-	-	-
MJO09_07	0.510	0.216	0.157	-	0.078	-	-	-	0.020	-	-	0.020	-	-	-	-
MJO09_08	0.300	0.300	0.160	0.020	0.180	-	-	-	0.020	-	-	0.020	-	-	-	-
MJO09_09	0.700	0.080	0.060	0.020	0.040	0.060	-	-	0.020	-	-	-	0.020	-	-	-
MJO09_10	0.535	0.186	0.070	0.023	0.070	0.023	0.047	-	0.023	-	0.023	-	-	-	-	-
MJO09_11	0.327	0.306	0.163	-	0.082	0.122	-	-	-	-	-	-	-	-	-	-
MJO09_12	0.558	0.070	0.070	-	0.070	0.047	-	-	0.140	-	0.047	-	-	-	-	-
MJO09_13	0.640	0.040	0.040	0.020	0.180	0.040	0.020	-	0.020	-	-	-	-	-	-	-
MJO09_14	0.440	0.100	0.060	0.060	0.160	0.040	-	-	-	-	0.020	0.060	-	-	-	-
LL11	0.469	0.224	0.245	-	0.041	0.000	-	-	0.020	-	-	-	-	-	-	-
MJO-10-001	0.569	0.098	0.098	0.020	0.020	0.039	-	-	0.020	-	-	0.020	-	-	-	-
MJO-10-002	0.531	0.184	0.184	-	0.041	-	-	-	0.061	-	-	-	-	-	-	-
MJO-10-003	0.688	0.104	0.146	0.021	-	0.021	-	-	0.021	-	-	-	-	-	-	-
MJO-10-004	0.531	0.224	0.204	-	0.020	-	-	-	0.020	-	-	-	-	-	-	-
MJO-10-006	0.531	0.224	0.041	0.061	-	-	0.102	-	0.041	-	-	-	-	-	-	-
MJO-10-007	0.688	0.063	0.042	0.021	0.083	0.083	-	-	-	-	-	-	0.021	-	-	-
MJO-11-005	0.596	0.213	0.106	-	0.085		-	-	-	-	-	-	-	-	-	-

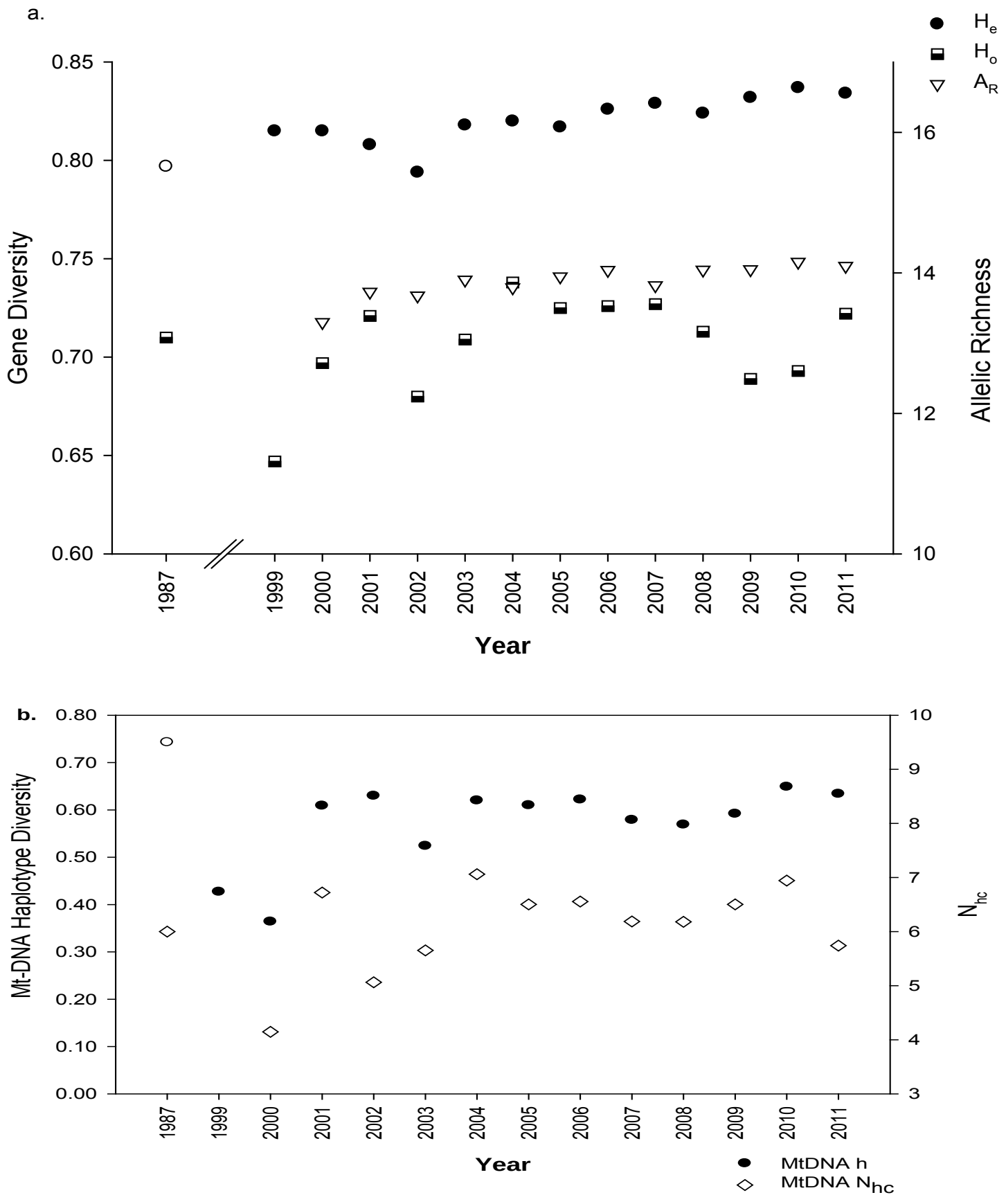

Figure 1. Genetic diversity metrics calculated from (a) microsatellite DNA data and (b) mtDNA for wild samples by year.

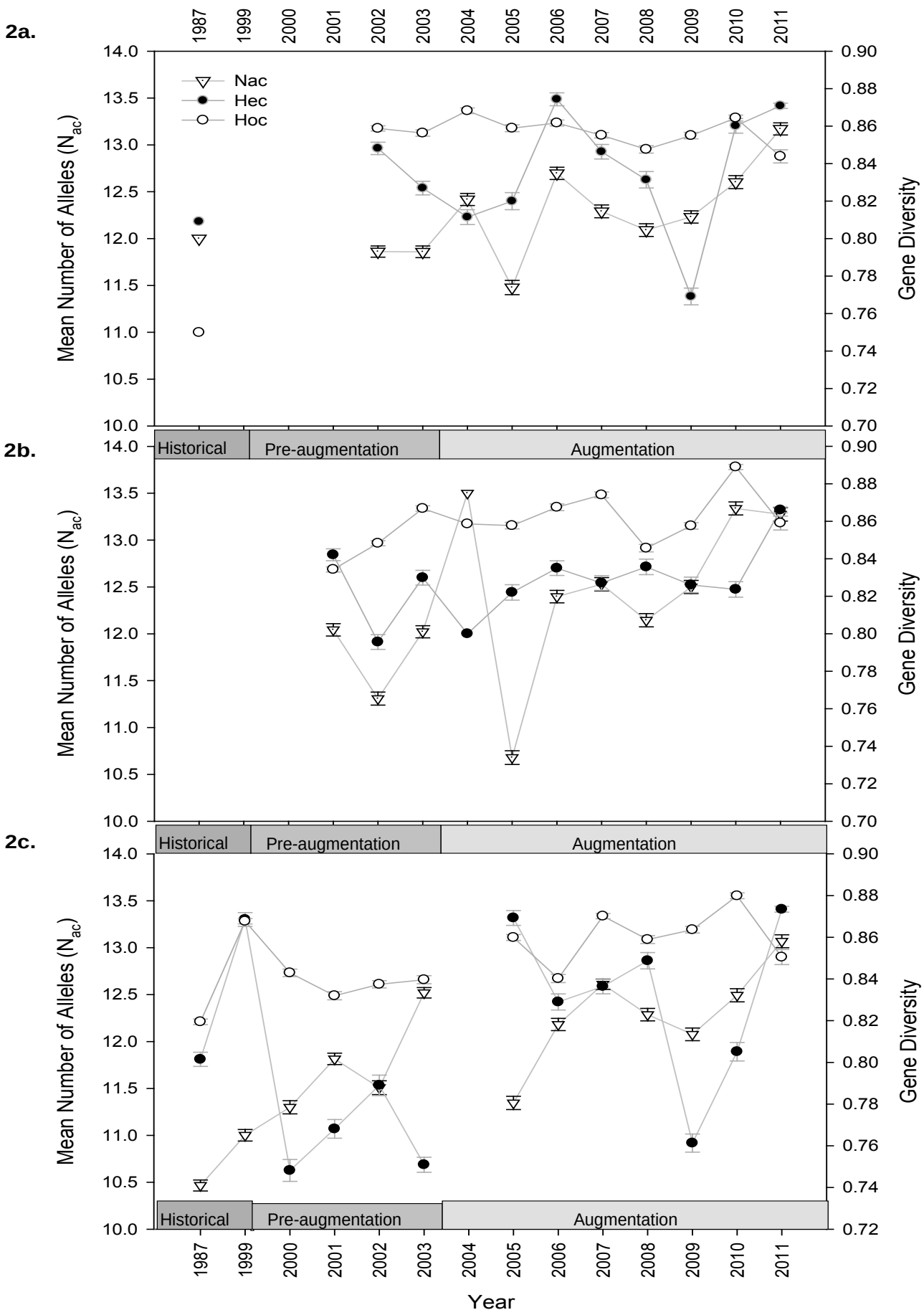

Figure 2. Gene diversity (observed and expected heterozygosity) and mean number of alleles (obtained using resampling to account for differences in sample size among years) by river reach a) Angostura, b) Isleta, c) San Acacia by year. 95% CIs are given.

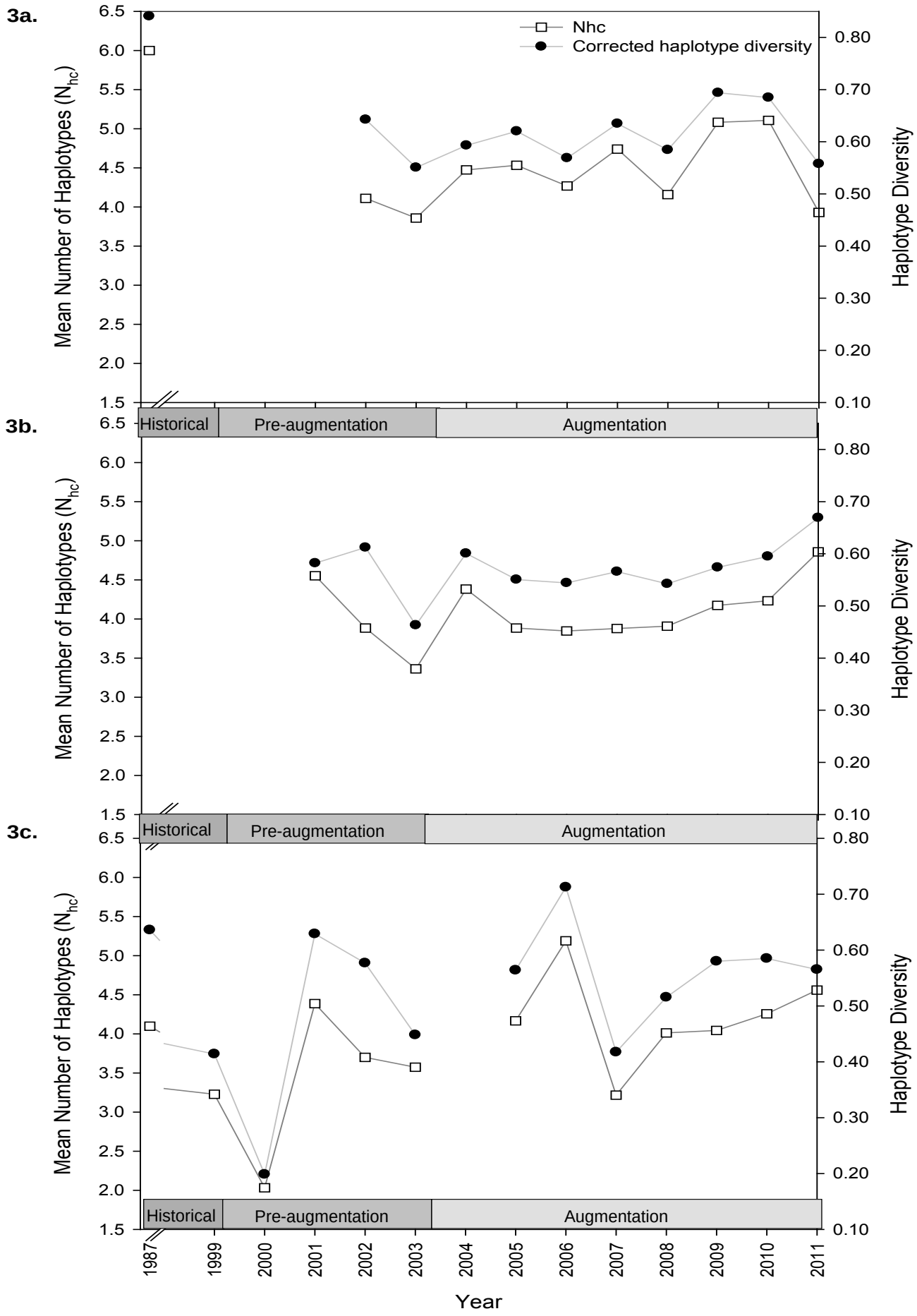

Figure 3. Haplotype diversity and mean number of haplotypes (obtained using resampling to account for differences in sample size among years) by river reach a) Angostura, b) Isleta, c) San Acacia by year. 95% CIs are given.

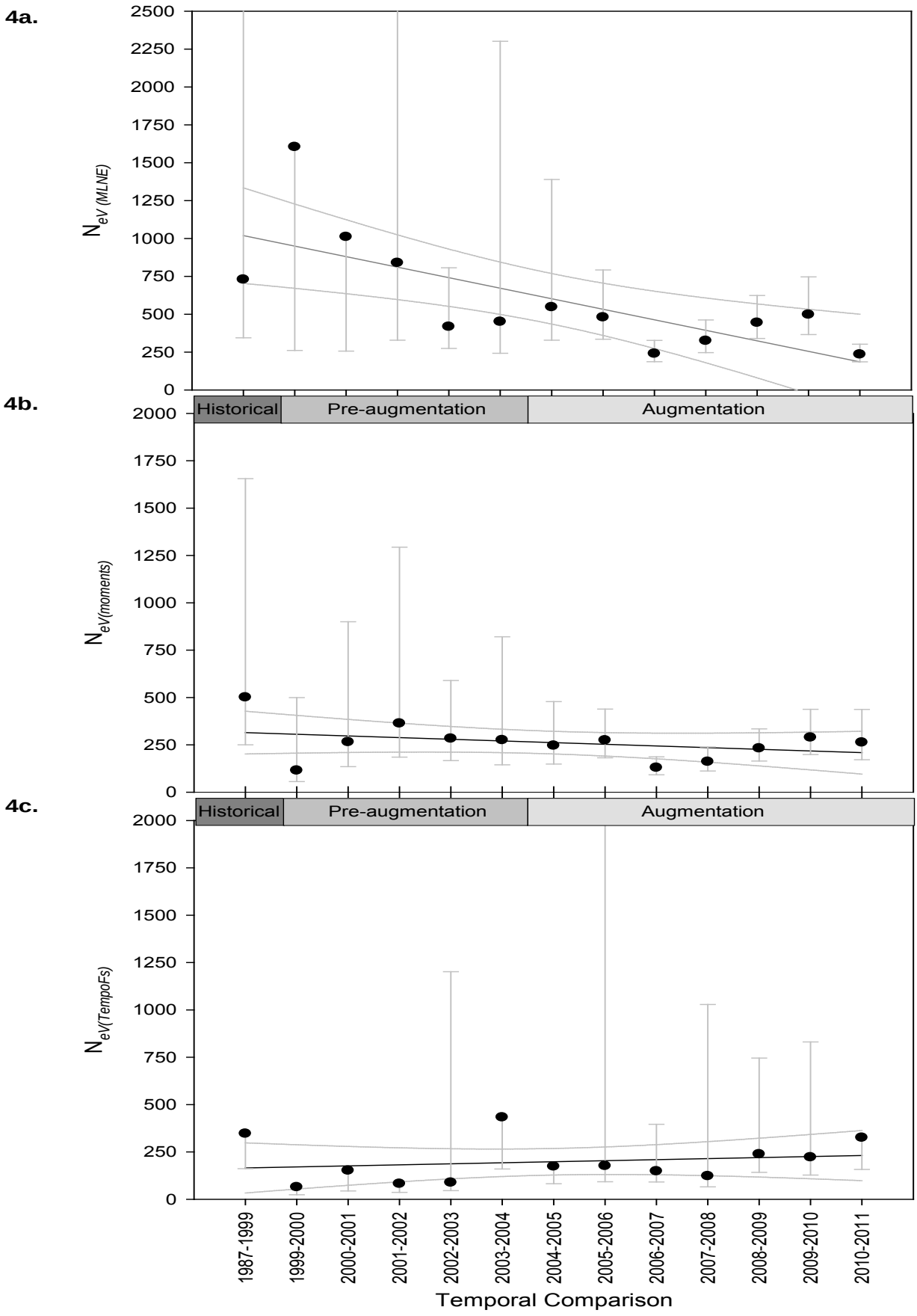

Figure 4. Variance effective size calculated from microsatellite DNA data using (a) MLNE, (b) Moments-based, and (c) TempoFs methods and associated 95% CIs. Linear regressions are shown with associated 95% CIs.

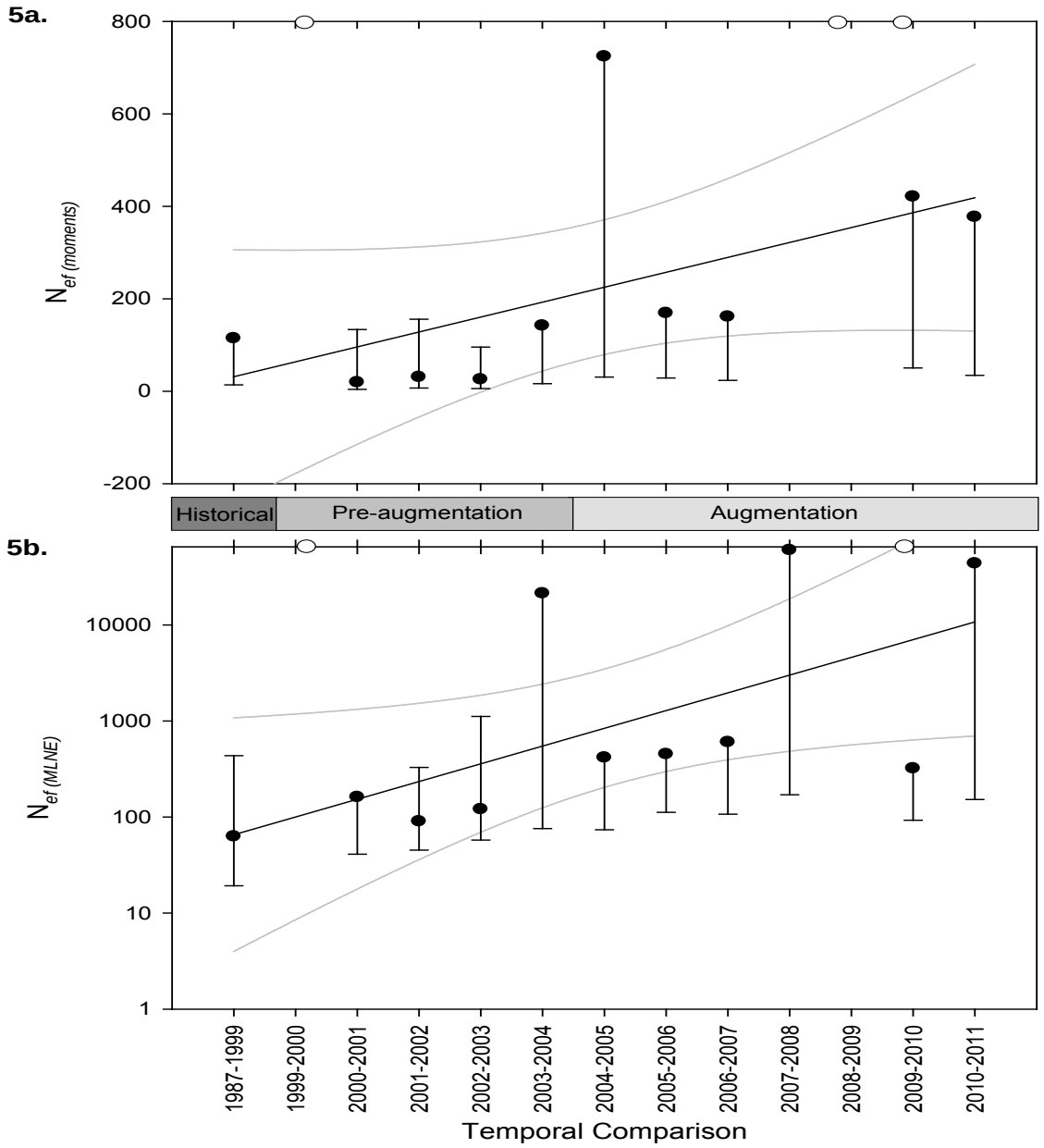

Figure 5. Variance effective size calculated from mitochondrial DNA-ND4 using (a) MLNE, (b) Moment -based methods and 95% CIs.

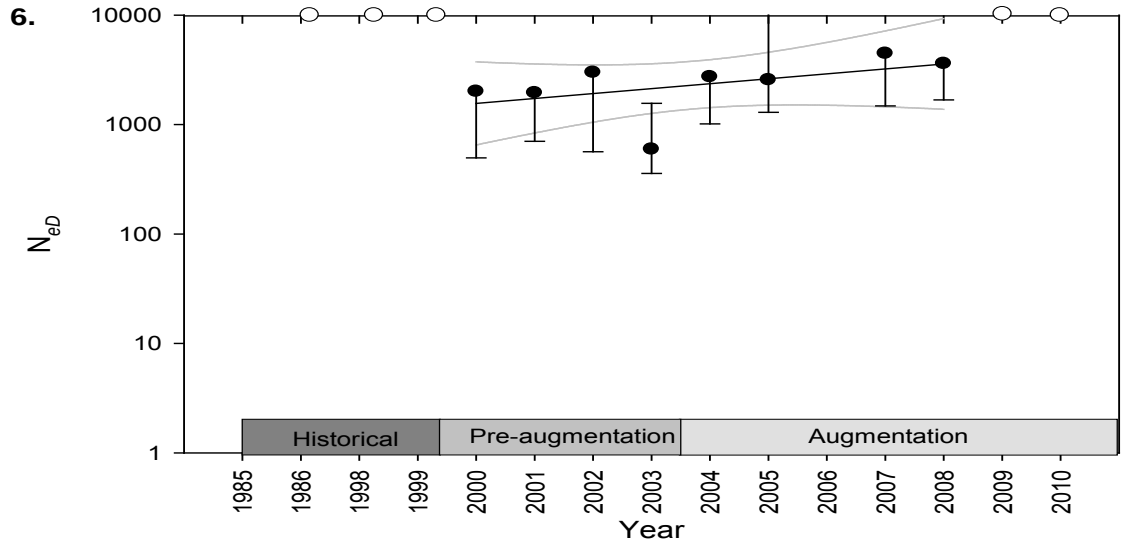

Figure 6. Estimates of NeD and 95% CIs by year. Linear regressions are shown with associated 95% CIs. Estimates of infinity are shown by open circles.