GENETIC MONITORING OF THE RIO GRANDE SILVERY MINNOW: GENETIC STATUS OF WILD AND CAPTIVE STOCKS IN 2014

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EXECUTIVE SUMMARY

We conducted genetic monitoring of the Middle Rio Grande population of Rio Grande silvery minnow (RGSM) annually from 1999-2012 and resumed monitoring in 2014. This work included monitoring stocks that were bred or reared in captivity and released to the Rio Grande in New Mexico since 2002. Monitoring in 2014 was based on genotyping 184 wild caught hatchery released fish (WCH), 288 wild caught eggs (WCE) from the middle Rio Grande, and 200 progeny of captive stocks, including 100 from the Southwestern Native Aquatic Resources and Recovery Center [SNARRC] and 100 from the Los Lunas silvery minnow refugium. Unmarked (wild-hatched) fish from the Middle Rio Grande were not included in the 2014 analyses because few were captured (n = 12), and fish from the Albuquerque Biological Park were not included because of failure of the captive stock prior to release of progeny into the wild in fall 2013. Genetic diversity of microsatellites and mtDNA for WCH and WCE were similar to levels observed in hatchery stocks. Mean estimates of variance effective size (microsatellites and mtDNA) for WCH and WCE were not significantly different but were significantly smaller than observed in wild fish in most previous years. Mean estimates of inbreeding effective size (microsatellites only) of WCH and WCE were lower than observed in the wild population in all previous years except 2004. Results indicate that replacement of the wild population by the hatchery population reduced variance and inbreeding effective size but genetic diversity remained similar to the wild population from prior years (1987, 1999-2012). Based on observed mtDNA variation and effective sizes of WCE from 2014, potential loss of mtDNA variation is of acute concern. Future genetic monitoring of all RGSM stocks will be critical to assess and mitigate further reductions in genetic diversity until a self-sustaining wild population is re-established.

Major findings for 2014

(1) The population of Rio Grande silvery minnow in the middle Rio Grande in 2014 was composed primarily of hatchery releases from Southwestern Native Aquatic Resources and Recovery Center (SNARRC). In 2014, genetic monitoring relied on assessment of genetic diversity and effective size in WCH and WCE because the rarity of wild fish (n = 12 collected) precluded population genetic analysis of wild fish.

(2) All measures of genetic diversity at microsatellite loci in WCH were similar to those observed in the hatchery source (captive spawned stock from SNARRC that were released in fall 2013). While corrected number of alleles (N_{ac}), gene diversity (H_{ec}) were similar to 2012 wild fish, observed heterozygosity (H_{oc}) was ~10% higher and F_{IS} ~50% lower than observed in 2012 wild fish. These findings support evidence from 2013-2014 from independent population surveys, which demonstrated that the population in the middle Rio Grande was composed largely of hatchery released fish. Similarly, mitochondrial gene diversity was similar to that of hatchery releases from fall 2013

SNARRC; further monitoring is required to evaluate potential loss of microsatellite and mtDNA diversity in the middle Rio Grande population.

(3) Among reaches, wild caught hatchery releases did not differ significantly in distributions of microsatellite or mtDNA variation. Small but significant differences were observed between reaches (Angostura and San Acacia; Isleta not sampled) in wild caught eggs; this result is similar to that documented in a recent study (Osborne et al. 2005).

(4) Estimates of variance effective population size (N_{eV}), calculated from microsatellite DNA allele frequencies, were significantly lower for the 2012-2014 period compared to that observed in the prior comparison from 2011-2012. This difference was observed for all three methods used to calculate N_{eV}. Reduced N_{eV} is consistent with a change in allele frequencies between generations (genetic drift) as a result of the change from a breeding population composed of a mixture of wild and hatchery fish in 2012 to a breeding population that in 2014 was composed primarily of hatchery released adults. Reduction of female effective size, N_{ef}, was consistent with this finding.

(5) TEMPOFS- and moments-based estimates of variance effective population size (N_{eV}), calculated from microsatellite DNA allele frequencies, were statistically equivalent between the 2014 WCH and WCE, whereas estimates from MLNE were statistically different between WCH (N_{eV} = 235) and WCE (N_{eV} = 362). This indicates that effective population size, based on microsatellites, remained stable or increased slightly between these generations.

(6) Estimates of inbreeding effective populations size, estimated from LDNE, were statistically equivalent between fall 2013 hatchery releases from SNARRC, 2014 WCH and 2014 WCE.

(7) Estimates of inbreeding effective populations size, estimated from LDNE, were significantly lower in 2014 WCH than in 2012 wild fish. Similarly, N_{eD} was significantly lower in wild caught eggs of 2014 than in wild fish from 2012. This change reflects a breeding population composed primarily of hatchery releases in 2014 and one composed of a mixture of wild and hatchery released fish in 2012.

INTRODUCTION

Genetic monitoring is defined as collection of two or more temporally spaced genetic samples from the same population (Schwartz et al. 2007). In fish, genetic monitoring to date has been confined largely to marine species and in freshwater systems, such studies primarily involve salmonids. 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 used 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 a monitoring program that may span 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 collected for Rio Grande silvery minnow (from 1987, 1999-2012, and 2014) represents one of the longer genetic monitoring time series for a non-salmonid freshwater fish. The population is sampled throughout its current range (mean annual sample size = 305), 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 impacts of changes in abundance, management actions and environmental conditions on genetic diversity.

In fishes that are characterized by a short lifespan (the population is dominated by age-1 fish; Horwitz et al. 2011) and in which dramatic changes in abundance occur from year to year (Dudley et al. 2011), negative genetic impacts to the population can occur over relatively short periods. Thus for species such as Rio Grande silvery minnow, genetic monitoring is a crucial component to management. For example, monitoring data for Rio Grande silvery minnow has i) demonstrated that the genetic effective size is orders of magnitude lower that the census size, ii) shown that the downstream movement of eggs, larvae, and adult fish, and the stocking of the population with captive reared fish, prevented significant divergence of allele frequencies among fishes collected in each of the three river reaches, iii) demonstrated that the diversity of the wild population was best represented in captive stocks when these stocks were derived from collections of eggs that were produced by natural spawning events in the wild (Osborne et al. 2012); and iv) shown that when artificial breeding is necessary, a group spawning design with equalized (i.e., 1:1) sex ratio produces fish that have levels of diversity that are statistically equivalent to that achieved through a paired mating design (Osborne et al. 2013). These findings have informed the Recovery Plan for the species and have been instrumental in the development of the captive propagation and genetics management plan (USFWS 2009, 2010). Here, we report on the genetic status of the population in 2014.

MATERIALS AND METHODS

Sampling- Rio Grande population

Rio Grande silvery minnow were sampled in the Rio Grande annually from 1999 to 2012 (between December and April- just prior to reproduction) and in 2014 (Tables 1 and 2). In 2014, however, adult fish hatched in the wild (i.e., unmarked by a Visible Implant Elastomer [VIE] tag) were rare, with only 12 unmarked individuals (versus 184 VIE marked fish) observed throughout the collection season (Table 1). Rarity of wild fish necessitated implementation of two backup plans in which sampling was based on collection of fin clips from wild caught hatchery releases (n = 184) and wild caught eggs (n = 288) for genetic monitoring (Table 2). In addition, 43 individuals used in a previous allozyme study of *Hybognathus* and stored in the Museum of Southwestern Biology Division of Genomic Resources (Cook et al. 1992 - referred to as 1987 sample) were genotyped. Throughout this study we use the term 'wild' to refer to unmarked fish sampled directly from the Rio Grande. 'Wild' fish may have parents that were wild or bred/reared in captivity, but were hatched in the Rio Grande. The 'Wild caught hatchery' (WCH) designation refers to fish hatched and reared in captivity and released into the wild to

supplement the Rio Grande silvery minnow population (and its reproduction) in the middle Rio Grande in 2013. Collections were made throughout the current distribution (i.e., from Cochiti reservoir to Elephant Butte reservoir in New Mexico) of Rio Grande silvery minnow, with the exception of the Cochiti reach because the species is rare or absent in that area (Bestgen and Platania 1991). Rio Grande silvery minnow were collected by seining and occasional backpack electrofishing. Fish were anesthetized with MS-222 (Tricaine methane sulfonate 200 mg/L river water) at the site of capture. Wild caught eggs (WCE) were obtained from American Southwest Ichthyological Researchers (ASIR). A biologically innocuous piece of caudal fin (1 to 3 mm^2) was removed from each individual. Fin clips were preserved in 95% ethanol. Fish were allowed to recover in untreated river water prior to release. In addition to the temporal samples collected from the Rio Grande, samples (fin clips) were included from 50 captive stocks sampled between 2000 and 2012, as well as two captive stocks sampled from SNARRC (n = 50 each) and one captive stock sampled from Los Lunas Refugium (n = 100) in 2014. Mortality from fin-clipping and VIE-tagging is negligible (SNARCC and NMFRO staff, pers. comm.).

Molecular methods- microsatellites

Total nucleic acids, including genomic DNA and mitochondrial DNA (mtDNA) were extracted from air-dried fin clips using proteinase-K digestion and organic extraction methods (Hillis et al. 1996). Individuals were genotyped at nine microsatellite loci: *Lco1*, *Lco3*, *Lco6*, *Lco7*, *Lco8* (Turner et al. 2004); *Ca6* and *Ca8* (Dimsoski et al. 2000); and *Ppro118* and *Ppro126* (Bessert and Orti 2003). The following pairs of loci were amplified through multiplex PCR: *Lco1*/*Ca6* and *Lco6*/*Lco7* (1X PCR buffer, 3 mM $MgCl_2$, 125 micromol [µM] deoxyribonucleotide triphosphates [dNTPs], 0.40-0.50 µM each primer, 0.375 units *Taq* polymerase); *Lco3* and *Lco8* (1X PCR buffer, 2 mM $MgCl_2$, 125µM dNTPs, 0.40-0.50 µM each primer, 0.375 units *Taq*); and *Ppro 118*/*Ppro126* (1X PCR buffer, 3 mM $MgCl_2$, 125µM dNTPs, 0.40-0.50 µM each primer, 0.375 units *Taq*). *Ca8* was amplified alone (1X PCR buffer, 3 mM $MgCl_2$, 125µM dNTPs, 0.50µM each primer, 0.375 units *Taq* polymerase). PCR cycling conditions for all loci were as follows: one denaturation cycle of 92°C for 2 min followed by 30 cycles of 90 °C for 20s, 50°C for 20 s, 72°C for 30s. Cycling conditions for *Ppro 118*/*Ppro126* were as follows: one denaturation cycle of 92°C for 2 min followed by 30 cycles of 90 °C for 20s, 60°C for 20 s, 72°C for 30s. Primer concentrations in multiplex reactions were optimized by locus to ensure equal amplification of each microsatellite. One microliter of PCR product was mixed with 10µl of formamide + 0.4µl of HD400 size standard, denatured at 93°C for 5 minutes, and run on an ABI 3100 automated capillary sequencer. Genotype data were scored in GeneMapper Version 4.0 (Applied Biosystems).

mtDNA- ND4

A 295 base pair (bp) fragment of the mtDNA ND4 gene from each individual was amplified in a 10 µL reaction containing 1 µL template DNA, 1 µL 10× reaction buffer, 2 mM $MgCl_2$, 125 µM dNTPs, 0.5 µM forward (5'- GAC CGT CTG CAA AAC CTT AA- 3') and reverse primer (5'- GGG GAT GAG AGT GGC TTC AA – 3'), and 0.375 U *Taq*. PCR conditions were 90° C initial denaturation for 2 minutes followed by 30 cycles of 90° C for 30 seconds, 50° C for 30 seconds, and 72◦ C for 30 seconds. Nucleotide sequence variation among individual fragments was visualized with single-strand conformational polymorphism (SSCP) analysis (Sunnucks et al.

2000), and representative haplotypes from each gel (~ 20%) were verified by direct sequencing using an ABI 3100 DNA Sequencer.

Statistical analysis

MICROSATELLITE TOOLKIT (add-in for Microsoft Excel, written by S. Park, available at http://animalgenomics.ucd.ie/sdepark/ms-toolkit/) was used to check for scoring errors in microsatellites, and GENEPOP (Raymond and Rousett 1995) was used to test for departures from Hardy-Weinberg equilibrium (HWE), using the procedure of Guo and Thompson (1992). Global tests for linkage disequilibrium were conducted for all pairs of loci in each collection, using FSTAT vers. 2.9.3.1 (Goudet 1995). Sequential Bonferroni correction (Rice 1989) was applied to account for inflated Type-1 error rates associated with multiple simultaneous tests. For each microsatellite locus and population, inbreeding coefficients (F_{IS}) were obtained using FSTAT. Estimates of unbiased haplotype diversity (H_D) were obtained using ARLEQUIN vers. 3.11 (Excoffier et al. 2005) for mitochondrial DNA data. Haplotype richness (H_R) and gene diversity (Petit et al. 1998) were obtained using the program CONTRIB vers.1.02 (available at http://www.pierroton.inra.fr/genetics/labo/Software/Contrib/), which uses a rarefaction approach to correct for unequal sample sizes.

In some cases sample sizes differed between collections, particularly between some samples collected early in the study and those collected more recently. Because number of alleles and expected heterozygosity are dependent on sample size, we used resampling to examine the effect of sample size on diversity measures. For microsatellites, 1000 random subsamples (n = 43 in 1987) were drawn without replacement from each temporal sample. Diversity and 95% CIs were calculated for each locus (across subsamples) and a mean was obtained across loci for each statistic (corrected number of alleles [N_{ac}], gene diversity [H_{ec}], heterozygosity [H_{oc}]). This analysis was conducted in the R statistical package (www.r-project.org). To facilitate comparisons among collections obtained across years and river reaches, we repeated the resampling procedure for microsatellite data in *R* where diversity measures were based on n= 15 (2004, Isleta) and the smallest sample n = 6 (2004, San Acacia) was excluded.

F-statistics

Weir and Cockerham's (1984) *F*-statistics (microsatellites) and Φ-statistics (mtDNA) were calculated in Arlequin vers. 3.11 (Excoffier et al. 2005). Hierarchical analysis of molecular variance (AMOVA) was used to test whether a significant proportion of genetic variance was partitioned into components attributable to differences among wild, WCE, captive-spawned, and captive-reared stocks [i.e. wild-caught eggs [WCE] were the source] (F_{CT}, Φ_{CT}), among samples within these three groups (F_{SC}, Φ_{SC}) and among all samples (F_{ST}, Φ_{ST}). P-values for all statistics were generated using bootstrapping (1000 permutations), as implemented in Arlequin.

Estimation of genetic effective size

Variance genetic effective size (N_{eV}) and 95% confidence intervals (CIs) were estimated from temporal (annual) changes in microsatellite allele frequencies across annual samples, using the temporal method (N_{eV} and Nei and Tajima 1981; Waples 1989) implemented in

NEESTIMATOR (Do et al. 2014) and a pseudo-maximum likelihood procedure implemented in MLNE version 2.3 (Wang 2001). Highly polymorphic loci with many rare alleles, as is typical of microsatellites, can be subject to biased estimates of variance effective size, N_{eV}, (Hedrick 1999; Turner et al. 2001). 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 used to estimate N_{eV}. Rio Grande silvery minnows were sampled under Plan I (prior to reproduction, with replacement) for all methods; therefore, calculations of N_{eV} via TEMPOFS required an estimate of census size (N_c). No reliable, long-term data (i.e., spanning the entire sampling period) were available for N_c, so each pairwise comparison in TEMPOFS was run under the following two N_c scenarios: a "crashed" (N_c = 10,000) and a "large" (1,000,000 individuals) population. The former value is lower than any census size estimate to date and the latter is within the order of magnitude for which larger N_c have been recorded (Dudley et al. 2011). In all comparisons, differences in mean N_{eV} were negligible between the N_c = 10,000 and N_c = 1,000,000 scenarios, but lower and upper confidence intervals were slightly larger for the latter. Only the most conservative N_{eV} estimates (i.e., based on N_c = 1,000,000) are reported herein. Jackknife estimation over all loci was used to calculate N_{eV} and associated 95% confidence intervals.

For all methods we assumed that migration (except from hatchery stocks) from outside the study area did not affect estimates of N_e. We equated the number of years separating a pair of samples with the number of generations elapsed between samples because Rio Grande silvery minnow have essentially non-overlapping generations (based on unpublished population monitoring data of R. K. Dudley and S. P. Platania). However, to account for small but known deviation from the discrete generation model (G = 1.27), we corrected consecutive estimates of N_e and N_{ef} for overlapping generations (Turner et al. 2006; Osborne et al. 2010), using the analytical method of Jorde and Ryman (1995, 1996). In addition to consecutive pairwise estimates, we also present comparisons between the 1987 and 1999 samples to provide historical context for the contemporary estimates. As these samples (1987 and 1999) were collected more than 3-5 generations apart, the drift signal should be sufficiently large relative to sampling biases associated with age-structure such that correction for overlapping generations is unnecessary (Waples and Yokota 2007).

In addition to the estimates of N_{eV}, we used the linkage disequilibrium method (Hill 1981) to estimate N_{eD} from microsatellite DNA data for each annual sample (including wild, wild caught hatchery (2014 only), captive-spawned and wild-caught eggs), using the program LDNE (Waples and Do 2008, 2010) and methods described in Osborne et al. (2010). Single sample N_e methods (such as those provided by LDNE) yield an estimate of the effective number of parents that produced the progeny from which the sample is drawn, and most closely approximates the inbreeding effective size, N_{el} (Laurie-Ahlberg and Weir 1979; Waples 2005).

For mtDNA data, variance effective size for the female portion of the population (N_{ef}) was estimated with temporal (Turner et al. 2001) and pseudo-maximum-likelihood (MLNE) methods. TEMPOFS was not used for mtDNA data as this method assumes diploidy (Jorde and Ryman 2007).

RESULTS

Microsatellites- genetic diversity

In 2014, 484 Rio Grande silvery minnow were collected (Tables 1 and 2), 12 wild fish, 184 wild caught hatchery fish [WCH] and 288 wild caught eggs [WCE]. The 12 wild fish were not considered for because sample size was small. Few wild fish were caught despite comparable sampling effort to previous genetic monitoring years. This result is consistent with the very low numbers of wild fish caught in population monitoring surveys conducted by ASIR (Dudley et al. 2014). Within the three reaches of the middle Rio Grande, 74 WCH and 144 WCE were collected from the Angostura reach, 69 WCH from the Isleta reach, and 41 WCH and 144 WCE from the San Acacia reach; no WCE were collected from the Isleta reach.

Over the 16-year study, a total of 7340 fish from the middle Rio Grande and hatchery stocks have been genotyped at nine microsatellite loci. 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 20 departures from Hardy-Weinberg equilibrium (HWE) among 45 comparisons versus 25 departures before correction for multiple tests. Four loci (*Lco3*, *Lco6*, *Ca6*, *Ppro126*) conformed to HWE nearly all comparisons. MICRO-CHECKER (Van Oosterhout et al. 2004) suggested that null alleles probably caused departures from HWE. These results were essentially identical to by-locus departures typically observed throughout the 16-year study. For tests of gametic disequilibrium, 40 of 180 comparisons were significant after sequential Bonferroni correction. Twenty three of significant tests occurred among wild caught eggs from the Rio Grande Nature Center, which is a small, contained backwater represented by relatively few individuals for which non-random associations among loci might be expected in offspring; this also is supported by the low effective number of breeders (N_{eD} = 46; Table 3) that contributed to WCE collected at the Rio Grande Nature Center. For the WCH, all measures of genetic diversity (e.g., corrected number of alleles, N_{ac}; observed heterozygosity, H_{oc}; and gene diversity, H_e) at microsatellite loci were most similar to values observed in the hatchery source (captive spawned stock from SNARRC released in fall 2013; Table 3). Similarly, for WCE, N_{ac}, H_{oc}, and H_{ec} were most similar to those observed in the WCH (Table 3), which would have dominated reproduction in the Rio Grande in 2014. In contrast, while values for N_{ac} and H_{ec} in 2014 WCH were similar to those observed in wild fish in 2012 (Figure 1a), H_{oc} was ~10% higher (Figure 1a;Table 3) and F_{IS} ~50% lower (Table 3) than that observed in the wild fish from 2012.

mtDNA- genetic diversity

Across the 16-year time series, 17 mtDNA haplotypes were identified among 7340 individuals assayed (Table 4; Osborne and Turner 2012). 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 in 2002), which was monomorphic for Haplotype D. Three haplotypes (C, D, F) were present at moderate frequencies (>5%) and 11 haplotypes were uncommon (<5%) or rare <1%). Across the time series, haplotype diversity was highest in the 1987 sample (h = 0.743) and lowest in 2000 (h = 0.364). In 2014, eight haplotypes (A, C, D, E, F, K, O and V; Table 4) were observed among all collections, and haplotype diversity ranged from 0.53 (SNARRC) to 0.64 (WCE from Rio Grande in the Isleta reach), with a value of 0.61 for WCH; numbers of haplotypes and haplotype diversities observed in 2014 samples were typical of those from previous years (Figure 1b; Tables 3 and 4).

Microsatellites- population structure

Hierarchical analysis of molecular variance was conducted by grouping temporal samples of 2014 WCH (by river reach). Values were not significantly different from zero. This was consistent with results from prior years and indicated that river reach did not explain a significant portion of genetic variance (F_{ST} = 0.004, P = 0.501). For 2014 WCE, there was a significant difference between the Angostura and San Acacia reaches (Φ_{ST} = 0.001, P <0.01).

mtDNA- population structure

Φ-statistics, among river reaches, was calculated for 2014 WCH. Genetic differences among the Angostura, Isleta, and San Acacia reaches were not significant (Φ_{ST} = 0.01, P = 0.156). Similarly, no significant difference was observed among 2014 WCE from the Angostura and San Acacia reaches (Φ_{ST} = 0.0009, P = 0.320); eggs were unavailable from the Isleta reach in 2014.

Genetic effective size- microsatellites

Estimates of variance effective size, N_{eV}, from microsatellites, are shown in Figures 2a (TEMPOFS), 2b (MLNE), and 2c (moments). For the 2012-2014 period, estimates were N_{eV} = 160 (moments), N_{eV} = 142 (TEMPOFS, 95% CI 105-221), and N_{eV} = 235 (MLNE, 95% CI 198-288). For wild caught eggs collected in spring 2014, estimates were N_{eV} = 156 (moments), N_{eV} = 191 (TEMPOFS, 95% CI 121-450), and N_{eV} =362 (MLNE, 95% CI 252- 605). All estimates significantly lower than those reported by Osborne and Turner (2012) for the 2011-2012 period (moments, N_{eV} = 462 (95% CI, 286-855); TempoFS, ∞ (95% CI, 9829-∞); and MLNE, N_{eV} = 803 (95% CI, 527-1523). Estimates of female variance effective size, N_{ef}, are shown in Figures 3a (MLNE) and 3b (moments). From 2012-2014, estimates were N_{ef} = 66 (moments) and N_{ef} = 92 (MLNE, 95% CI 49-271) from 2012 to 2014. From the 2014 WCH to 2014 WCE generation, estimates were N_{ef} = 6 (moments) and N_{ef} = 14 (MLNE, 95% CI 10-33).

Estimates of inbreeding effective size (Figure 4; Table 3) were N_{eD} = 133 (95% CI 101-184) for 2014 WCH and N_{eD} = 117 (95% CI 88-162) for WCE. For captive stocks released in the middle Rio Grande in fall 2013, estimates were N_{eD} = 74 (95% CI 62-90) for Los Lunas Refugium and N_{eD} = 112 (95% CI 87-152) for SNARRC. Estimates of N_{eD} for WCH and WCE in 2014 were statistically equivalent to each other and to hatchery releases from captive spawned individuals from SNARRC, the primary source of hatchery releases to the middle Rio Grande in fall 2013; Estimates of N_{eD} for WCH and WCE in 2014, however, were significantly lower than that observed in wild fish from 2012 (N_{eD} = 10,064; Figure 4 and Table 3), and, further, were the lowest estimates observed over the entire 16 year study.

DISCUSSION

Rio Grande silvery minnow in the middle Rio Grande in 2014

Wild Rio Grande silvery minnow in the middle Rio Grande in 2014 were rare and we recorded the lowest number of fin clips obtained from wild fish (n = 12) throughout 16 years of genetic monitoring, despite similar sampling effort. As a result of this rarity, genetic monitoring was implemented using backup plans for evaluation of wild caught hatchery releases (n = 184)

and wild caught eggs (n = 288) in order to obtain a sufficient number of individuals for genetic analysis that also represented the breeding population in the wild in 2014 and its offspring. Thus, the breeding population in the middle Rio Grande in 2014 was composed primarily of hatchery released fish, and the generation of Rio Grande silvery minnow produced in the wild, as represented by wild caught eggs, was primarily derived from hatchery released adults.

Genetic status of the species in 2014

Measures of genetic diversity of wild caught hatchery releases were reflective of the hatchery stock from which this population was sourced. For microsatellites, corrected number of alleles (N_{ac}), observed heterozygosity (H_{oc}), gene diversity (H_{ec}), and the inbreeding coefficient F_{IS} were essentially identical to that observed in the hatchery stock released by SNARRC in fall 2013, which accounted for the majority of hatchery releases in fall 2013 because Los Lunas Refugium released few fish (77) into the middle Rio Grande in 2013, and a die off of fish slated for release from the Albuquerque Biopark meant no fish were released from that facility. Similarly, estimates of genetic diversity in wild caught eggs from 2014 were most similar to that of fall 2013 hatchery releases from SNARRC and to the wild caught hatchery releases from 2014 (i.e., fish derived primarily from the fall 2013 release of fish from SNARRC). These findings are as expected for a population in which the hatchery fish and their wild produced eggs largely represent the present population in the river and in which few wild fish from 2013 (i.e., hatched in the wild) contributed to reproduction. Similarly, no differences in genetic diversity were observed among reaches because wild caught hatchery releases were derived primarily from a single release from SNARRC (i.e., a single admixed population from the hatchery).

Mitochondrial DNA genetic diversity of wild caught eggs also was similar to that observed in the hatchery stocks, particularly those from SNARRC, which was the direct source of the vast majority of hatchery fish released to the wild in 2014 and the source of all wild caught hatchery releases. Although a significant difference was observed in the distribution of mtDNA variation between wild caught eggs from the Angostura and San Acacia reaches (Isleta not sampled), this is consistent with previous documentation of ephemeral spatial structure in wild caught eggs (Osborne et al. 2005). Apparent reduction of mtDNA variation to the five most common haplotypes in the wild presents a concern that mtDNA variation may be lost as a result of genetic drift in the wild, where the majority of breeding individuals in 2014 were of hatchery origin; variation in the hatcheries is less (by haplotype number) than occurs regularly in the wild in previous years. We cannot dismiss the possibility that sampling effects led to apparent reduction in haplotype number compared to that observed in most previous years because fewer fish (n = 100 each from SNARRC and Los Lunas Refugium), and collections in the wild were relatively small (n = 184) compared to most other years. Continuation of annual genetic monitoring will be critical for determining the role of sampling and genetic drift in observed numbers of haplotypes in 2014 and if changes related to genetic drift occur in the future.

Similar to estimates of genetic diversity, the estimate of inbreeding effective population size, N_{eD}, of wild caught hatchery releases (N_{eD} = 133) was statistically equivalent to their primary source population- fall 2013 hatchery releases from SNARRC- for which N_{eD} = 112. Similarly, for wild caught eggs in 2014, N_{eD} = 117 was statistically equivalent to that observed in the 2014 wild caught hatchery releases (N_{eD} = 133) and hatchery stocks from SNARRC (N_{eD} =

112). The latter result is consistent with hatchery released fish having largely represented the breeding population in the wild in 2014, as is also consistent with the rarity of wild fish in the same year (n = 12), and suggests that wild fish did not contribute substantially to WCE in 2014.

The observed N_{eD} estimates for WCH releases and of WCE in 2014 were significantly lower- by one to two orders of magnitude- than that observed in most prior years for the wild population (~1,000- >10,000). This difference is essentially identical to the difference observed between N_{eD} of captive stocks and wild fish in previous years of genetic monitoring. In prior years of genetic monitoring (following implementation of the hatchery supplementation program), fish hatched in the river were derived from a mixture of wild fish and fish released from the hatchery, whereas in 2014 the breeding population was composed almost exclusively of hatchery released fish.

The change in composition of the breeding population in the wild also is reflected in estimates of variance effective population size, N_{eV}, which is a measure of the degree of genetic drift between generations. Each of the three methods used to estimate variance effective size between 2012 and 2014 revealed that N_{eV} was significantly lower than was observed in the prior period for which data is available (2011-2012). This difference is indicative of a shift from a wild population derived from a mixture of wild and hatchery released parents. However, the same estimates for N_{eV} between the 2014 wild caught hatchery releases and 2014 wild caught eggs was statistically equivalent for two methods and significantly higher for the remaining method. This indicated that effective population size remained stable or slightly increased in the population in the middle Rio Grande. However, dramatic declines in female (mtDNA) variance effective size were observed from 2012 to 2014, and again from 2014 wild caught hatchery releases (the great majority of the breeding population in the middle Rio Grande in 2014) and 2014 wild caught eggs. Because effective size of a haploid, maternally inherited locus (i.e., mtDNA) is expected to be ¼ that of a diploid, bi-parentally inherited locus (i.e., microsatellite loci), loss of mtDNA variation through genetic drift is of acute concern.

Conclusions

Sixteen years of genetic monitoring of the wild middle Rio Grande population and of released captive reared/bred silvery minnow provides a rare opportunity to track the genetic effects of population fluctuations associated with inter-annual variability in flows and of various management activities. Through at least 2012, the trajectory of genetic change in the wild Rio Grande silvery minnow population was determined largely by supplementation with captive reared stocks and not by changes in population size in the wild (Osborne et al. 2012). By 2014, however, rarity of wild Rio Grande silvery minnow (i.e., hatched in the Rio Grande) meant that the middle Rio Grande population was composed primarily of hatchery released adults and, following reproduction, the addition of offspring from these released fish. While levels of genetic diversity, including heterozygosity and average number of alleles, were largely maintained relative to levels observed in previous years (1987, 1999-2012), the distribution of genetic variation in the Rio Grande in 2014 was most similar to the source population of hatchery releases from SNARRC, which contributed most of the hatchery fish to the river in 2013. As a consequence, inbreeding effective size of wild caught hatchery fish was statistically identical to that of the hatchery population from SNARRC, and the inbreeding effective size of wild caught eggs was statistically equivalent to that of the wild caught hatchery releases. Thus,

the low inbreeding effective population size of the Rio Grande population in 2014 was significantly less than that observed in most previous years by one to two orders of magnitude because the population is past years was derived from a mixture of wild and hatchery fish as opposed to almost exclusively from hatchery fish in 2014. In short, the inbreeding effective size of wild caught hatchery fish was statistically equivalent to hatchery fish from SNARRC (the primary source of releases to the middle Rio Grande), and, because hatchery fish overwhelmingly accounted for reproduction in the wild in 2014, wild caught eggs also had an inbreeding effective size that reflected the effective number of breeders that year (i.e., fish from SNARRC). Thus inbreeding and variance effective sizes in the Rio Grande population in 2014 converged, as expected. This is further evidence that diversity in the population is being largely maintained by release of captive reared fish. These results also highlight the importance of continued monitoring the captive stocks and of the wild population as any detrimental effects (such as losses of diversity) in the captive stocks will ultimately be transferred to the population in the middle Rio Grande, as was demonstrated in 2014 following the recent catastrophic decline of the wild population and heavy stocking with captive bred fish. Monitoring of mtDNA variation in the middle Rio Grande population and in hatchery stocks is of high priority because of the potential of loss of haplotypes as a result of genetic drift and the essential replacement of the wild population by fall 2013.

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Table 1. Number of wild samples collected from the Rio Grande by year, site and river reach (Angostura, Isleta and San Acacia).

Year	Angostura	Isleta	San Acacia	Total
1987	15	-	28	43
1999	-	-	46	46
2000	-	-	194	194
2001	-	65	63	128
2002	67	121	201	389
2003	71	65	33	169
2004	141	15	6	162
2005	190	109	95	394
2006	95	143	145	383
2007	48	128	42	218
2008	165	191	123	479
2009	175	153	150	478
2010	149	146	151	446
2011	71	148	140	359
2012	147	215	154	516
2013	-	-	-	-
2014	5	3	4	12
Total	1339	1502	1575	4416

Table 2. Sample sizes, collection localities on the Rio Grande, river reaches for wild Rio Grande silvery minnow samples collected for 2014 genetic monitoring.

A. *Wild caught hatchery releases*	Angostura	Isleta	San Acacia
Alameda Bridge	2	-	-
Rio Grande Nature Center	54	-	-
Central Ave Bridge	13	-	-
Cesar Chavez Bridge	5	-	-
Isleta DD	-	10	-
Alejandro Gate	-	2	-
HWY6 Bridge Los Lunas	-	53	-
Peralta Drain	-	3	-
Bernardo	-	1	-
SADD	-	-	35
1.5 mi downstream of SADD	-	-	3
San Antonio at 380 Bridge	-	-	1
San Marcial RR Bridge	-	-	2
Total fin clips	74	69	41

B. *Wild caught eggs*	Angostura	Isleta	San Acacia
Rio Grande Nature Center on 13 May 2014	144	-	-
RM 58.8 on 06 May 2014	-	-	24
RM 58.8 on 12 May 2014	-	-	24
RM 58.8 on 13 May 2014	-	-	24
RM 58.8 on 14 May 2014	-	-	24
RM 58.8 on 19 May 2014	-	-	24
RM 58.8 on 20 May 2014	-	-	24
Total eggs	144	-	144

C. *All collections by reach*	Angostura	Isleta	San Acacia
Total	218	69	185

Table 3. Diversity statistics for microsatellites and mtDNA. N is sample size, N_{ac} is average number of alleles across loci, H_{ec} is Nei's gene diversity, H_{oc} is observed heterozygosity, F_{IS} is inbreeding co-efficient, N_h is number of haplotypes, h is haplotype diversity, and H_R is haplotype richness. Linkage disequilibrium estimates of effective size, N_{eD}, are also given. No genetic monitoring was conducted in 2013. * indicates N_{el} observed in 2014 WCH was the lowest value recorded in the history of genetic monitoring of RGSM.

	Msats								mtDNA			
Wild-MRG	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	N_h	h	H_R
1987	43	14.00	0.797	0.710	0.111	∞	139	∞	37	7	0.74	6.00
1999	46	12.23	0.814	0.647	0.210	∞	∞	∞	44	5	0.43	3.82
2000	194	14.33	0.814	0.697	0.145	∞	∞	∞	124	6	0.36	3.36
2001	128	15.01	0.807	0.721	0.107	2008	495	∞	122	10	0.61	6.06
2002	389	14.75	0.793	0.681	0.143	1951	702	∞	387	8	0.63	4.16
2003	169	14.95	0.817	0.709	0.134	2998	564	∞	167	9	0.52	4.89
2004	162	14.85	0.819	0.737	0.100	596	357	1559	161	10	0.62	6.28
2005	394	14.90	0.816	0.724	0.113	2724	1014	∞	396	10	0.61	5.63
2006	383	15.26	0.826	0.727	0.122	2562	1291	34064	378	10	0.62	5.67
2007	218	15.08	0.828	0.726	0.123	∞	1211	∞	218	10	0.58	5.36
2008	474	15.16	0.823	0.713	0.135	4459	1479	∞	466	11	0.57	5.30
2009	476	15.11	0.830	0.689	0.172	3608	1677	∞	472	12	0.59	5.65
2010	440	15.19	0.834	0.692	0.172	∞	2023	∞	433	9	0.65	6.09
2011	362	15.32	0.830	0.724	0.130	∞	3117	∞	359	11	0.63	5.74
2012	517	15.42	0.827	0.728	0.123	10064	1782	∞	522	11	0.66	5.68
2013	-	-	-	-	-	-	-	-	-	-	-	-
2014_WCH	184	14.80	0.831	0.774	0.069	133*	101	184	182	6	0.61	3.87

	Msats								mtDNA			
Wild caught eggs	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	N_h	h	H_R
WCE_01*	178	14.76	0.819	0.651	0.206	1380	656	∞	157	8	0.63	7.00
WCE SA 01	50	13.95	0.830	0.727	0.070	86	54	173	51	6	0.62	6.00

WCE_SA_02	81	14.95	0.818	0.680	0.171	∞	462	∞	80	8	0.70	7.38
WCE_SA_03	51	14.99	0.830	0.696	0.164	5009	308	∞	51	8	0.71	7.85
MJO_07_005	54	15.31	0.827	0.738	0.091	60	48	79	53	7	0.60	6.73
MJO_07_006	49	15.64	0.814	0.723	0.108	1065	196	∞	48	6	0.58	5.96
MJO_07_015	49	15.42	0.818	0.694	0.154	871	270	∞	49	7	0.63	5.40
MJO_07_016	50	15.29	0.837	0.756	0.097	2425	359	∞	50	7	0.60	5.79
MJO_07_017	50	14.49	0.813	0.720	0.115	277	143	2070	46	8	0.76	6.57
2013	-	-	-	-	-	-	-	-	-	-	-	-
2014_WCE_RG	144	14.23	0.818	0.721	0.118	173	123	269	143	5	0.64	3.84
2014_WCE_RGNC	144	13.54	0.817	0.721	0.118	46	39	54	139	5	0.58	3.32
2014_WCE_ALL	288	14.25	0.821	0.722	0.122	117	88	162	281	5	0.61	3.68

	Msats								mtDNA			
Captive spawned	N	N_{ac}	H_{ec}	H_{oc}	F_{IS}	N_{eD}	-95%	95%	N	N_h	*h*	H_R
MJO_06_29	50	11.37	0.804	0.745	0.074	42	29	69	50	5	0.52	5.00
CS_01	64	12.81	0.794	0.658	0.172	44	36	55	58	5	0.46	4.98
CS_AN_02	51	8.48	0.685	0.675	0.015	22	15	33	51	1	0.00	1.00
CS_SA_02	53	13.15	0.802	0.673	0.163	73	53	111	53	6	0.75	5.92
TFT_03_09	51	12.77	0.806	0.7	0.133	106	56	434	52	4	0.56	4.00
CS_04	50	14.09	0.823	0.69	0.163	66	46	106	47	6	0.59	5.91
TFT_04_23	50	11.65	0.779	0.683	0.124	20	17	25	47	5	0.59	5.00
TFT_04_24	48	11.76	0.828	0.717	0.135	40	30	58	48	5	0.61	4.95
TFT_04_25	50	11.66	0.81	0.768	0.053	25	20	32	53	6	0.70	5.93
TFT_04_29	54	14.01	0.839	0.762	0.092	∞	532	∞	53	5	0.61	4.90
TFT_04_30	56	14.70	0.825	0.727	0.121	323	134	∞	45	5	0.66	4.79
TFT_04_31	50	12.80	0.805	0.701	0.13	83	55	155	50	7	0.71	6.87
TFT_05_06	50	10.31	0.792	0.649	0.183	49	39	66	50	6	0.63	5.80
TFT_05_07	49	12.15	0.797	0.704	0.117	87	53	191	48	5	0.55	4.88
TFT_05_08	50	11.15	0.804	0.663	0.178	32	27	40	49	5	0.61	4.93
TFT_05_09	50	12.90	0.804	0.717	0.109	220	99	∞	50	4	0.51	4.00
TFT_05_11	51	12.56	0.808	0.693	0.144	137	81	354	53	6	0.57	5.85

MJO_06_25	50	14.85	0.813	0.721	0.115	185	110	488	49	5	0.64	4.93
MJO_06_28	50	12.41	0.805	0.705	0.125	88	57	164	50	5	0.74	5.00
MJO_07_07	50	13.16	0.813	0.739	0.114	∞	521	∞	50	5	0.61	4.87
LL_11	50	14.18	0.829	0.738	0.11	302	123	∞	49	5	0.68	0.37
MJO_10_05	49	14.04	0.839	0.7	0.167	260	87	∞	44	6	0.71	3.00
MJO_10_06	49	12.36	0.782	0.698	0.108	59	32	163	49	6	0.66	4.88
MJO_10_07	48	14.06	0.825	0.742	0.101	106	60	312	48	7	0.52	5.48
MJO_11_05	48	13.97	0.81	0.73	0.1	118	82	201	47	4	0.59	3.00
MJO_11_11	50	11.87	0.769	0.693	0.101	37	30	45	51	8	0.69	6.73
MJO_11_12	50	11.61	0.785	0.712	0.094	27	21	35	50	5	0.56	3.92
MJO_11_13	48	13.35	0.806	0.715	0.115	46	34	68	48	5	0.34	3.70
MJO_11_14	50	13.77	0.829	0.754	0.092	68	52	97	50	6	0.47	4.60
LL_12	49	12.48	0.794	0.684	0.141	41	33	52	48	6	0.63	4.49
MJO_12_09	50	14.03	0.829	0.721	0.133	62	46	88	49	4	0.60	3.00
MJO_12_10	50	14.16	0.81	0.719	0.113	121	69	371	50	7	0.64	5.71
2013_LLR	100	14.51	0.825	0.765	0.075	74	62	90	100	6	0.63	4.56
2013_DEX	100	14.70	0.818	0.765	0.066	112	87	152	99	6	0.53	4.23

Table 4. MtDNA haplotype frequencies (%) for the wild middle Rio Grande population, wild caught hatchery fish, fish reared from wild-caught eggs, and fish reared from captive spawning.

	mtDNA haplotypes																
Wild-MRG	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	W	V
1987	45.9	16.2	16.2	5.4	8.1	2.7	-	-	5.4	-	-	-	-	-	-	-	-
1999	75.0	-	11.4	6.8	4.5	2.3	-	-	-	-	-	-	-	-	-	-	-
2000	79.0	0.8	4.8	4.8	9.7	0.8	-	-	-	-	-	-	-	-	-	-	-
2001	60.7	9.0	5.7	3.3	9.8	7.4	0.8	1.6	0.8	-	0.8	-	-	-	-	-	-
2002	55.6	19.9	13.7	1.0	5.9	3.4	-	0.3	-	-	0.3	-	-	-	-	-	-
2003	67.1	5.4	15.0	3.0	5.4	1.2	-	0.6	0.6	-	1.8	-	-	-	-	-	-
2004	59.6	8.7	10.6	1.9	7.5	5.0	1.2	-	1.9	-	3.1	0.6	-	-	-	-	-
2005	59.8	12.6	8.8	2.8	8.6	1.8	1.5	0.3	2.8	-	1.0	-	-	-	-	-	-
2006	58.7	13.5	9.3	4.8	4.8	4.8	0.3	-	2.9	-	0.8	-	-	-	0.3	-	-
2007	62.8	11.0	8.3	2.3	8.7	3.7	0.5	-	0.5	-	1.8	-	0.5	-	-	-	-
2008	63.5	12.0	7.9	2.6	6.7	4.5	0.4	-	0.9	-	0.6	0.2	-	0.6	-	-	-
2009	61.4	14.0	7.6	2.8	6.4	3.4	0.6	0.4	1.9	-	1.1	0.2	-	0.2	-	-	-
2010	56.2	12.4	9.7	3.2	6.9	5.3	1.4	-	1.6	-	3.2	-	-	-	-	-	-
2011	57.4	14.2	10.9	2.8	6.4	3.1	0.6	-	3.1	-	1.1	-	0.3	0.3	-	-	-
2012	53.8	16.5	11.6	3.4	7.2	3.0	0.4	0.4	1.7	-	1.7	-	0.2	-	-	-	-
2013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2014_WCH	57.7	20.9	6.0	-	11.5	0.1	-	-	-	-	3.3	-	-	-	-	-	-

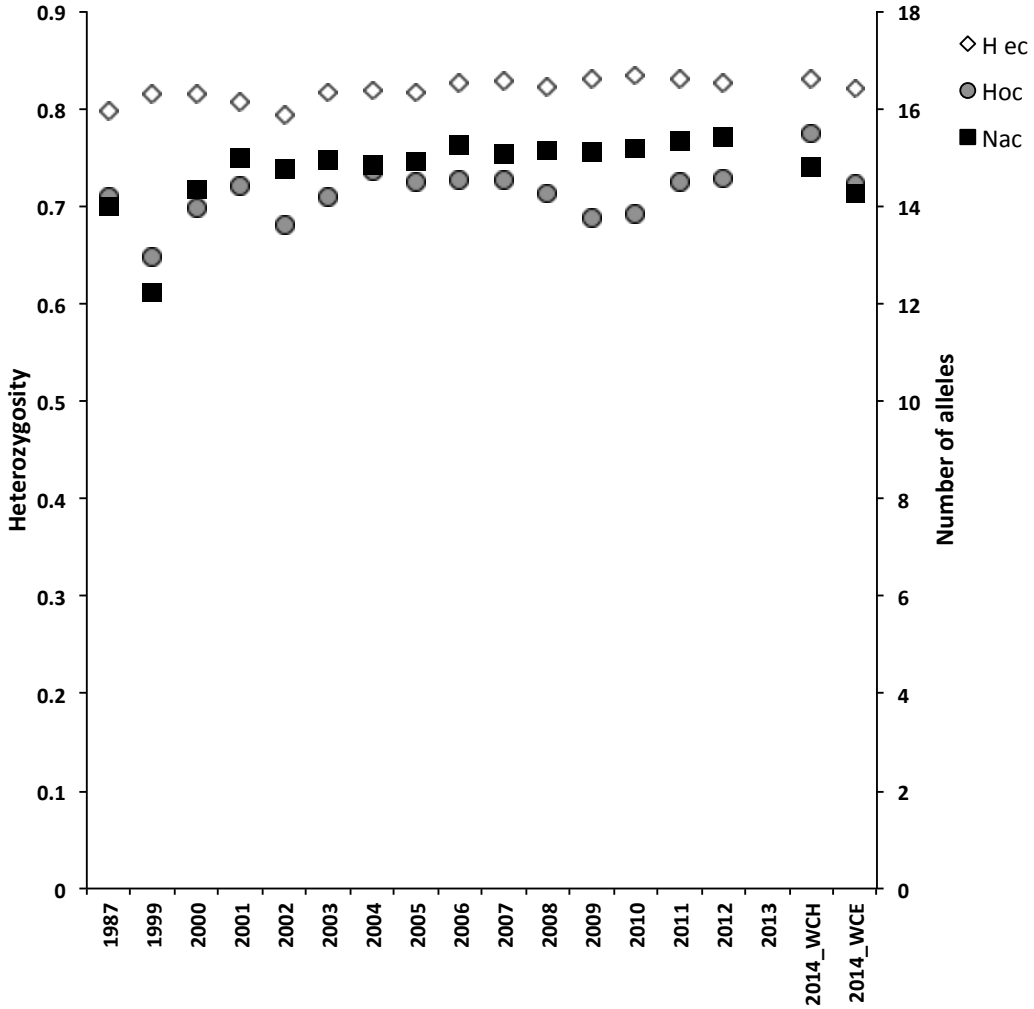
	mtDNA haplotypes																
Wild caught eggs	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	U	V
WCE_01*	57.3	19.7	5.1	6.4	6.4	3.2	-	-	1.3	0.6	-	-	-	-	-	-	-
WCE_SA_01	56.9	13.7	5.9	5.9	9.8	7.8	-	-	-	-	-	-	-	-	-	-	-
WCE_AN_02	65.3	2.0	32.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WCE_SA_02	48.8	22.5	5.0	1.3	13.8	5.0	-	-	3.8	-	-	-	-	-	-	-	-
WCE_SA_03	49.0	7.8	19.6	5.9	9.8	3.9	-	-	2.0	-	2.0	-	-	-	-	-	-
MJO_07_005	60.4	9.4	1.9	1.9	17.0	7.5	-	1.9	-	-	-	-	-	-	-	-	-

MJO_07_006	60.4	8.3	12.5	2.1	8.3	4.2	-	-	-	-	4.2	-	-	-	-	-	-
MJO_07_015	57.1	22.4	4.1	2.0	4.1	8.2	-	-	2.0	-	-	-	-	-	-	-	-
MJO_07_016	62.0	12.0	6.0	-	8.0	4.0	-	-	4.0	-	4.0	-	-	-	-	-	-
MJO_07_017	43.5	19.6	6.5	4.3	13.0	8.7	-	-	2.2	-	-	-	2.2	-	-	-	-
2013	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2014_WCE_RG	54.2	7.0	22.5	-	4.9	-	-	-	-	-	11.3	-	-	-	-	-	-
2014_WCE_RGNC	58.3	7.2	28.1	-	1.4	-	-	-	-	-	5.0	-	-	-	-	-	-
2014_WCE_ALL	56.2	7.1	25.3	-	3.2	-	-	-	-	-	8.2	-	-	-	-	-	-

	mtDNA haplotypes																
Captive spawned	A	C	D	E	F	K	I	J	M	N	O	P	Q	S	T	U	V
MJO_06_29	68.0	14.0	8.0	-	6.0	-	-	-	4.0	-	-	-	-	-	-	-	-
CS_01	72.4	5.2	-	3.4	6.9	12.1	-	-	-	-	-	-	-	-	-	-	-
CS_AN_02	-	-	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CS_SA_02	43.4	7.5	17.0	13.2	17.0	-	-	-	-	-	-	1.9	-	-	-	-	-
TFT_03_09	59.6	26.9	3.8	-	-	9.6	-	-	-	-	-	-	-	-	-	-	-
CS_04	59.6	25.5	2.1	-	4.3	6.4	-	-	-	-	2.1	-	-	-	-	-	-
TFT_04_23	61.7	4.3	19.1	-	-	4.3	-	-	-	-	10.6	-	-	-	-	-	-
TFT_04_24	58.3	12.5	20.8	-	2.1	6.3	-	-	-	-	-	-	-	-	-	-	-
TFT_04_25	43.4	5.7	11.3	5.7	28.3	5.7	-	-	-	-	-	-	-	-	-	-	-
TFT_04_29	56.6	24.5	-	7.5	-	9.4	-	-	1.9	-	-	-	-	-	-	-	-
TFT_04_30	40.0	33.3	-	-	-	24.4	-	-	-	-	-	2.2	-	-	-	-	-
TFT_04_31	42.0	34.0	2.0	-	6.0	4.0	-	-	10.0	-	2.0	-	-	-	-	-	-
TFT_05_06	50.0	36.0	2.0	-	2.0	8.0	-	-	2.0	-	-	-	-	-	-	-	-
TFT_05_07	62.5	29.2	2.1	6.3	-	0.0	-	-	-	-	-	-	-	-	-	-	-
TFT_05_08	59.2	8.2	-	10.2	-	22.4	-	-	-	-	-	-	-	-	-	-	-
TFT_05_09	68.0	16.0	-	-	-	12.0	-	-	4.0	-	-	-	-	-	-	-	-
TFT_05_11	62.3	5.7	11.3	1.9	17.0	-	-	-	1.9	-	-	-	-	-	-	-	-
MJO_06_25	55.1	24.5	6.1	-	6.1	8.2	-	-	-	-	-	-	-	-	-	-	-
MJO_06_28	40.0	14.0	22.0	-	22.0	2.0	-	-	-	-	-	-	-	-	-	-	-
MJO_07_07	56.0	2.0	12.0	28.0	2.0	-	-	-	-	-	-	-	-	-	-	-	-

LL_11	46.9	22.4	24.5	-	4.1	-	-	-	2.0	-	-	-	-	-	-	-	-
MJO_10_05	47.7	18.2	15.9	-	13.6	2.3	-	-	-	-	2.3	-	-	-	-	-	-
MJO_10_06	53.1	22.4	4.1	6.1	-	-	10.2	-	4.1	-	-	-	-	-	-	-	-
MJO_10_07	68.8	6.3	4.2	2.1	8.3	8.3	-	-	-	-	-	-	2.1	-	-	-	-
MJO_11_05	59.6	21.3	10.6	-	8.5	-	-	-	-	-	-	-	-	-	-	-	-
MJO_11_11	52.9	5.9	3.9	3.9	3.9	17.6	-	-	5.9	-	-	-	-	-	-	-	5.9
MJO_11_12	64.0	12.0	4.0	-	-	-	-	-	14.0	-	6.0	-	-	-	-	-	-
MJO_11_13	81.3	6.3	6.3	4.2	-	-	-	-	2.1	-	-	-	-	-	-	-	-
MJO_11_14	72.0	4.0	6.0	4.0	-	12.0	-	-	-	-	2.0	-	-	-	-	-	-
LL_12	56.3	4.2	12.5	-	22.9	2.1	-	-	-	-	-	-	2.1	-	-	-	-
MJO_12_09	59.2	18.4	8.2	-	14.3	-	-	-	-	-	-	-	-	-	-	-	-
MJO_12_10	58.0	8.0	10.0	-	10.0	6.0	-	-	-	-	2.0	-	6.0	-	-	-	-
2013_LLR	57.0	20.0	4.0	9.0	3.0	-	-	-	-	-	7.0	-	-	-	-	-	-
2013_DEX	66.7	11.1	9.1	-	5.1	-	-	-	-	-	7.1	-	-	-	-	-	1.0

Figure 1. Diversity metrics (annual) from microsatellites and mtDNA. Estimates obtained from resampling of microsatellites (H_{ec}, H_{oc}, N_{ac}) are shown in the upper panel, and estimates from mtDNA (h and H_R) are shown in the lower panel.



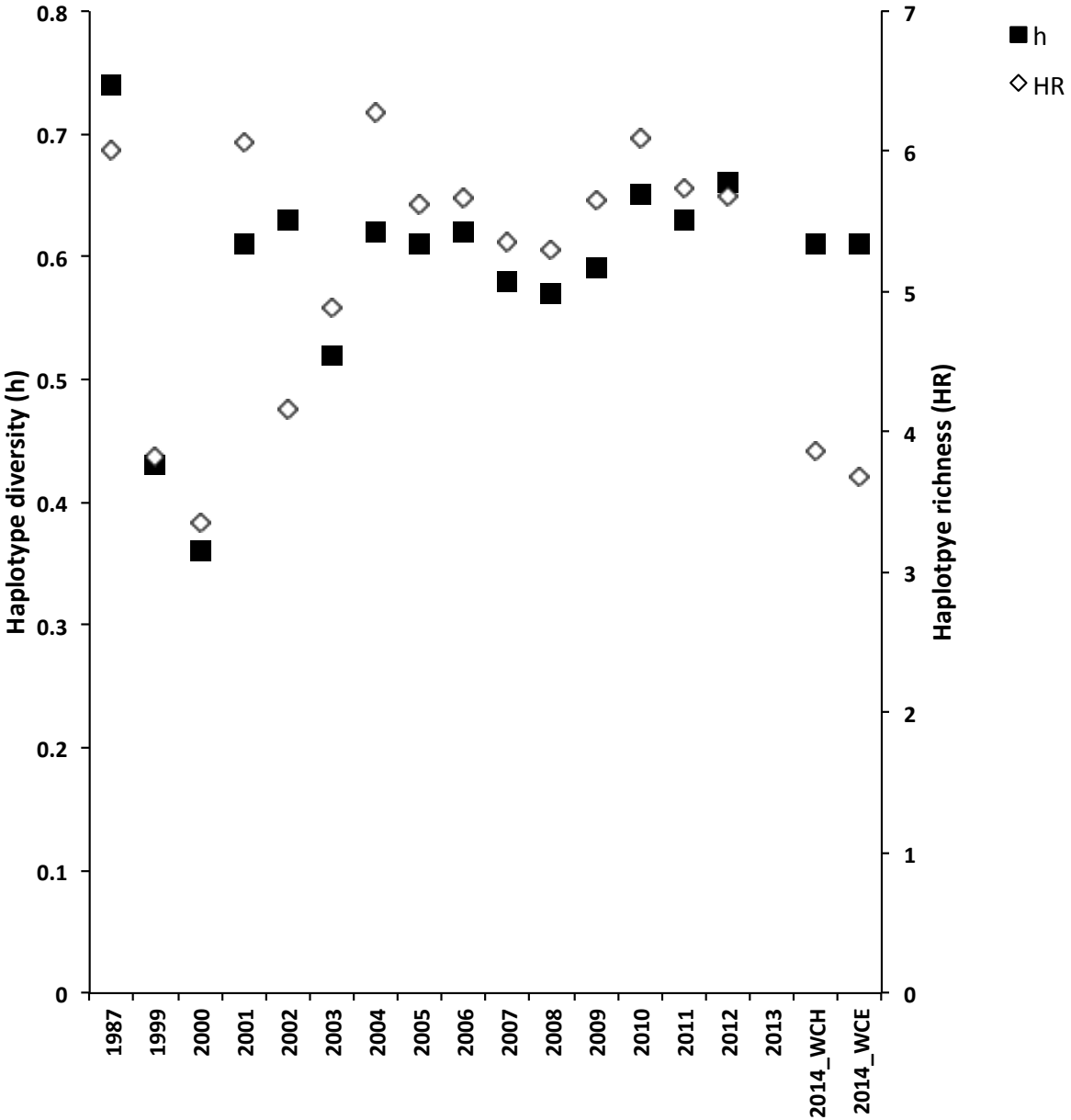
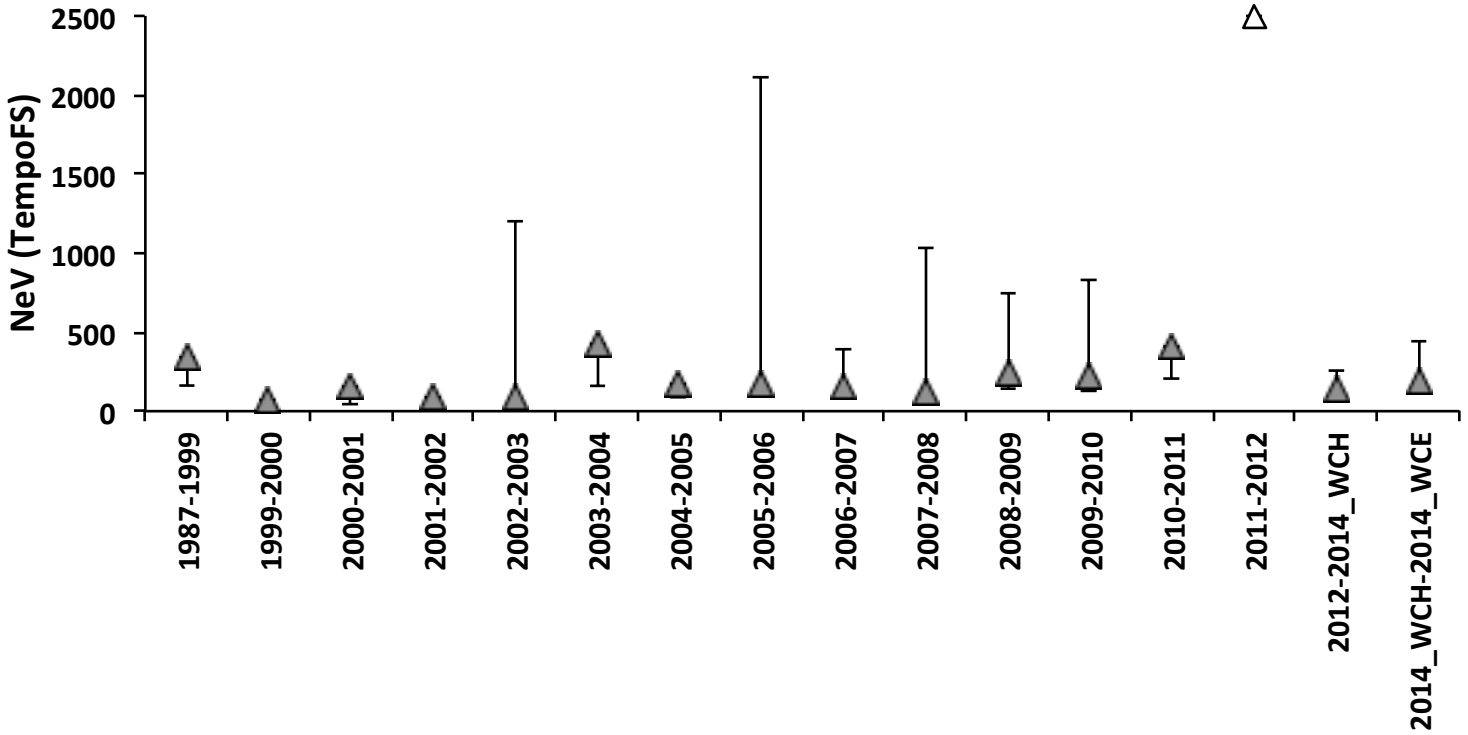
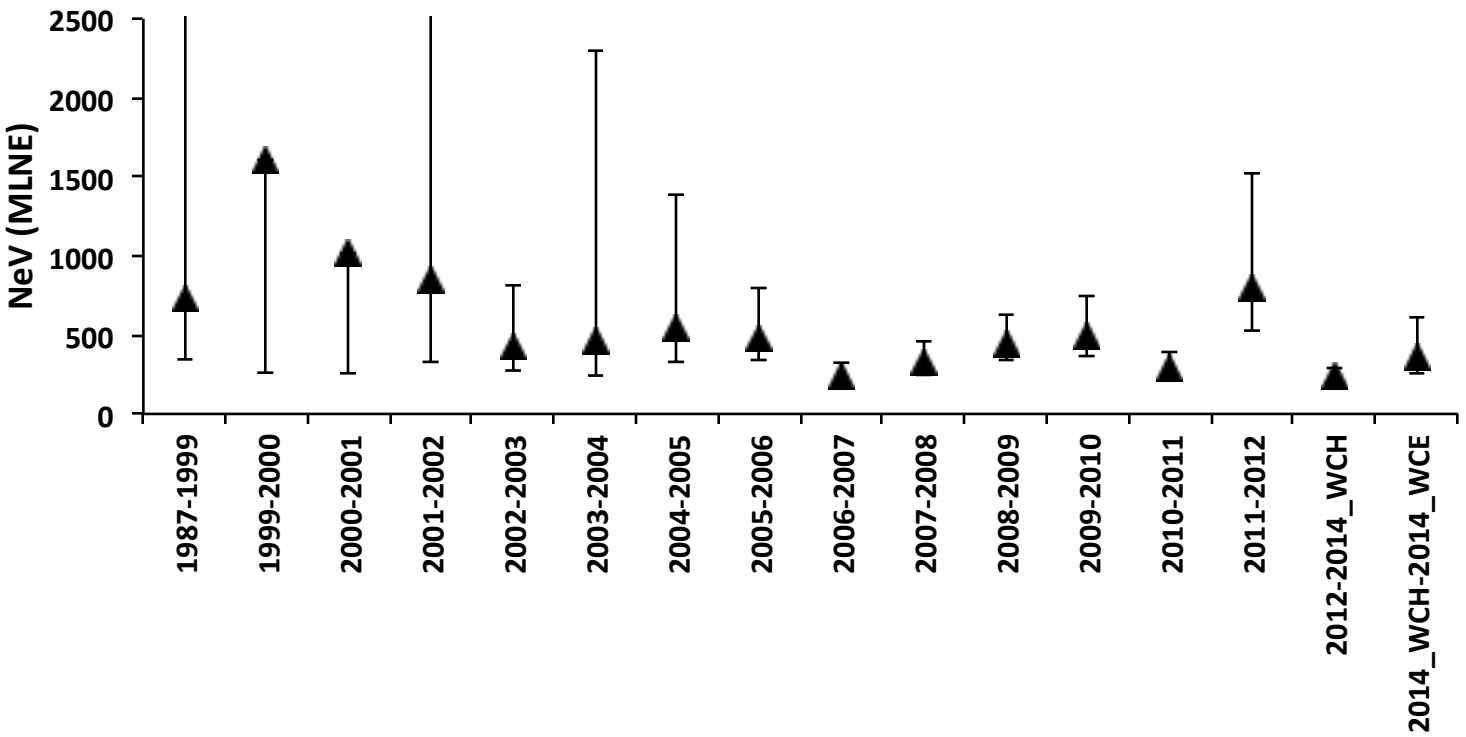

Haplotype diversity (h)
Haplotpye richness (HR)
h
HR
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
1
2
3
4
5
6
7
1987
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014_WCH
2014_WCE

Figure 2. Variance effective size calculated from microsatellite data, as based on TEMPOFS (upper) MLNE (middle), and moments (lower) estimates and their associates 95% CIs. Infinite mean estimates are indicated by open triangles; upper error bars are not shown cases where for upper bounds are infinite.




NeV (MLNE)
2500
2000
1500
1000
500
0
1987-1999
1999-2000
2000-2001
2001-2002
2002-2003
2003-2004
2004-2005
2005-2006
2006-2007
2007-2008
2008-2009
2009-2010
2010-2011
2011-2012
2012-2014_WCH
2014_WCH-2014_WCE

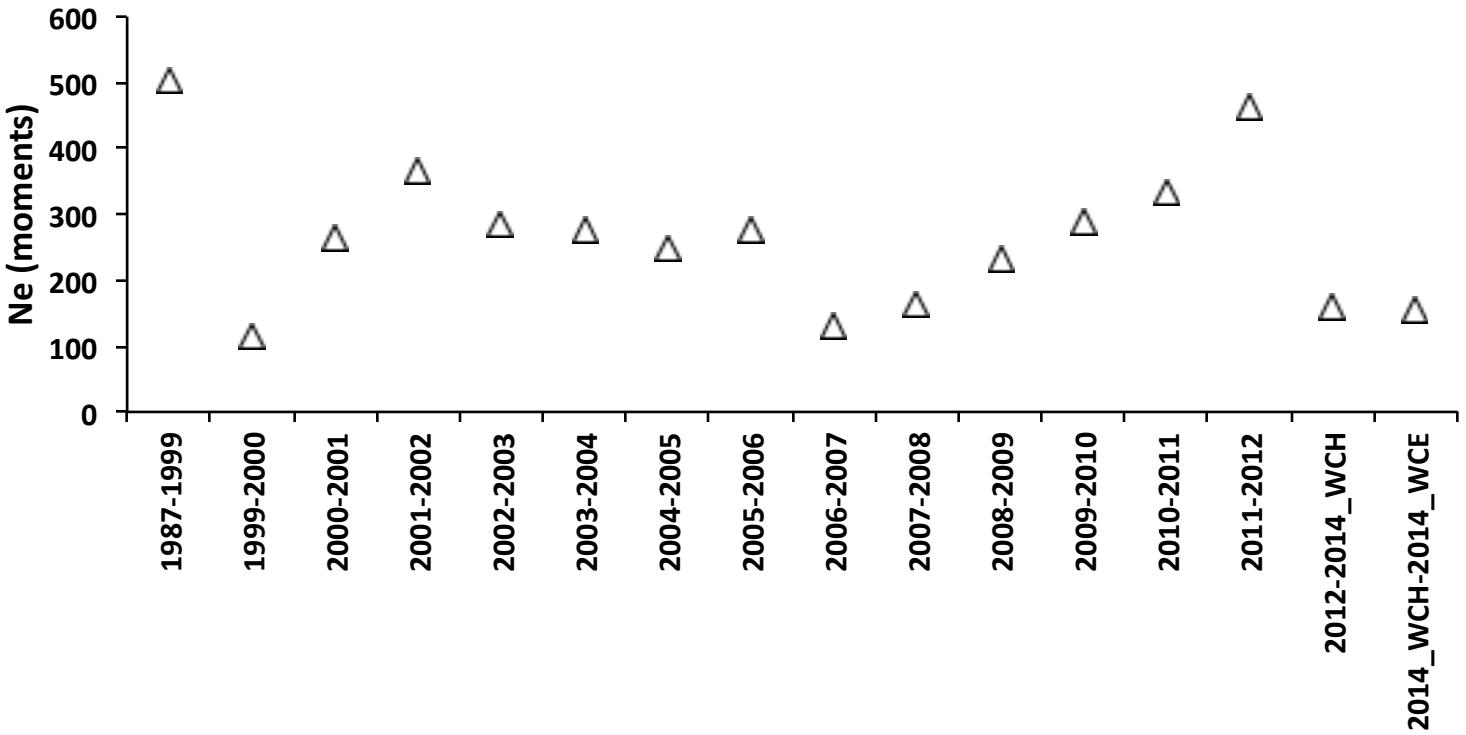
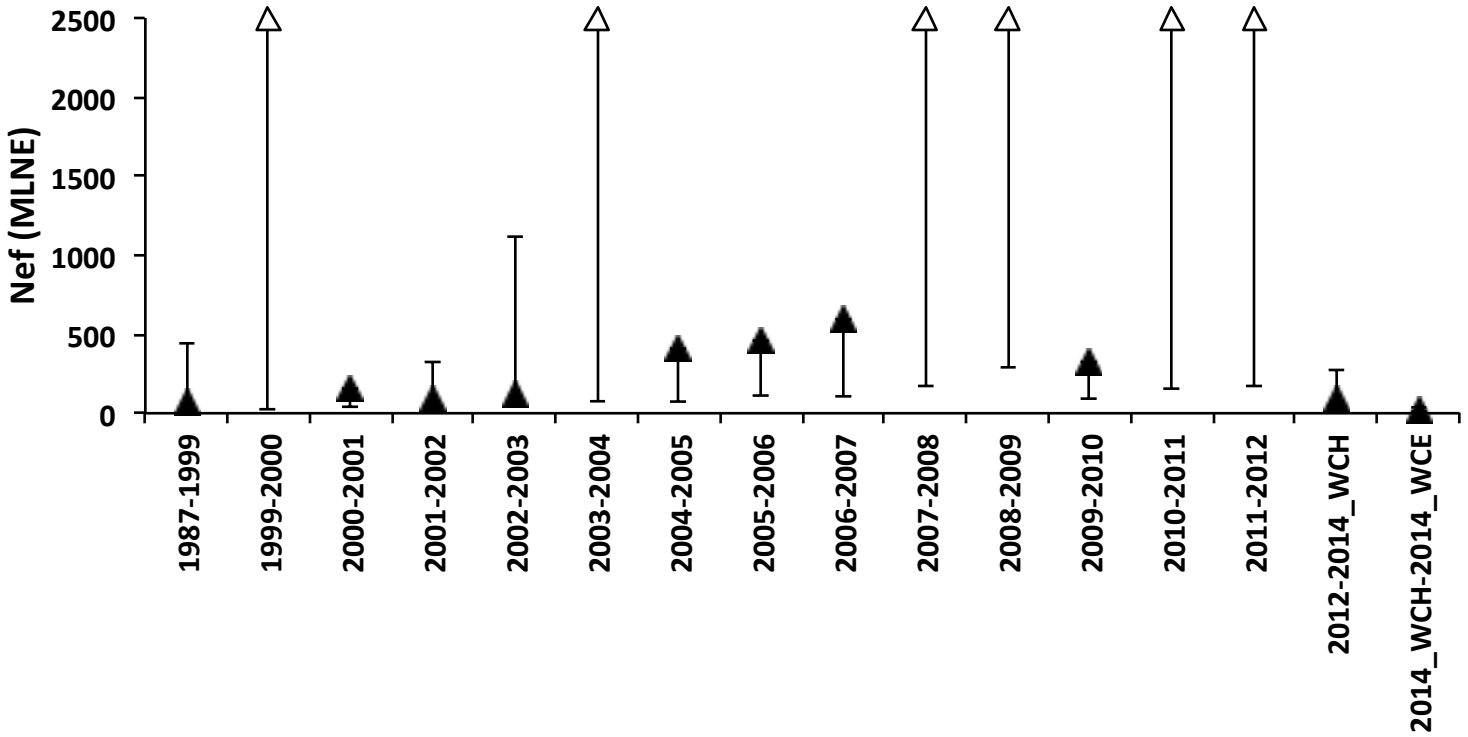

Ne (moments)
600
500
400
300
200
100
0
1987-1999
1999-2000
2000-2001
2001-2002
2002-2003
2003-2004
2004-2005
2005-2006
2006-2007
2007-2008
2008-2009
2009-2010
2010-2011
2011-2012
2012-2014_WCH
2014_WCH-2014_WCE

Figure 3. Female variance effective size estimates and their associated 95% CIs, based on mtDNA data and calculated using MLNE (upper) and moments (lower) methods. Infinite mean estimates are indicated by open triangles; upper error bars are not shown cases where for upper bounds are infinite.



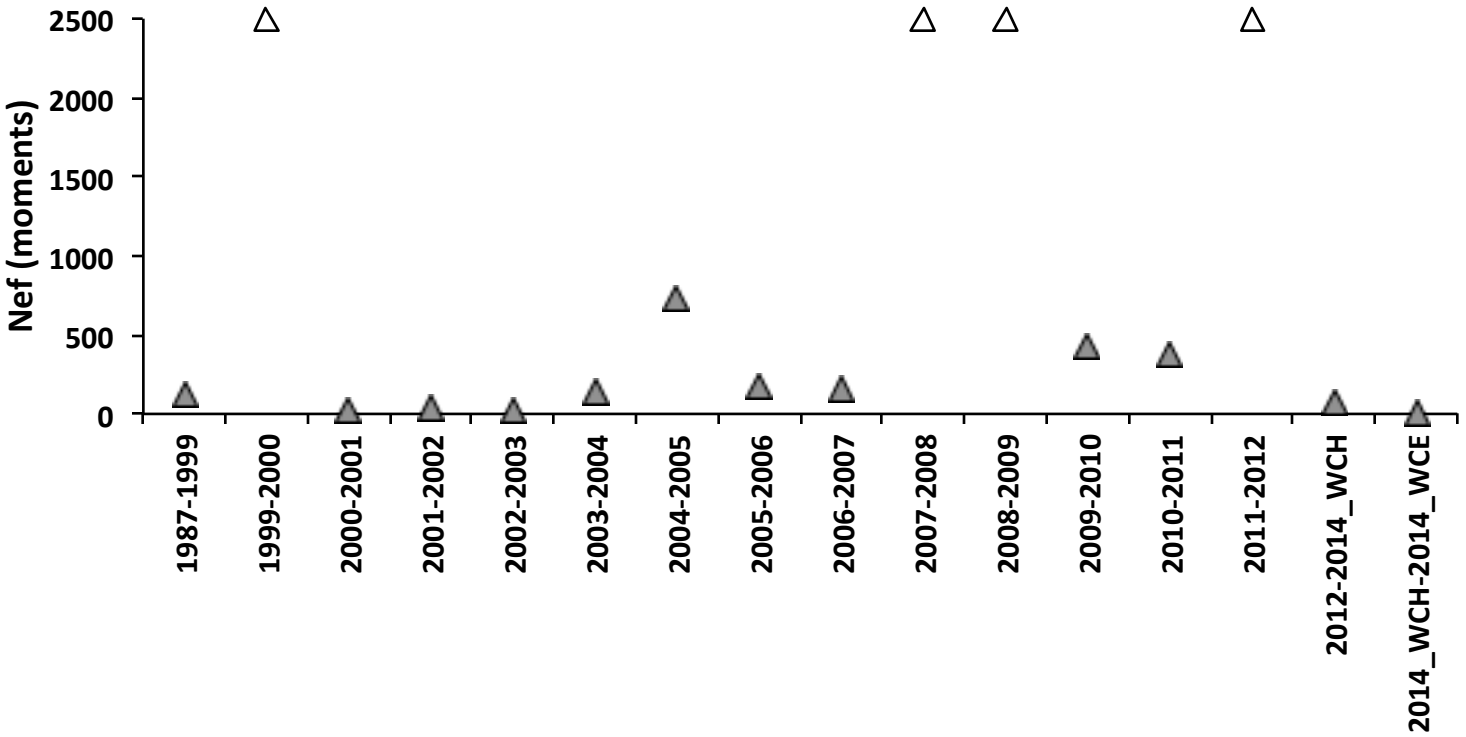

Nef (moments)
2500
2000
1500
1000
500
0
1987-1999
1999-2000
2000-2001
2001-2002
2002-2003
2003-2004
2004-2005
2005-2006
2006-2007
2007-2008
2008-2009
2009-2010
2010-2011
2011-2012
2012-2014_WCH
2014_WCH-2014_WCE

Figure 4. Estimates of inbreeding effective size (N_{eD}) their associated 95% confidence intervals. Infinite estimates are indicated by open circles.