Genetic Monitoring of Rio Grande Silvery Minnow

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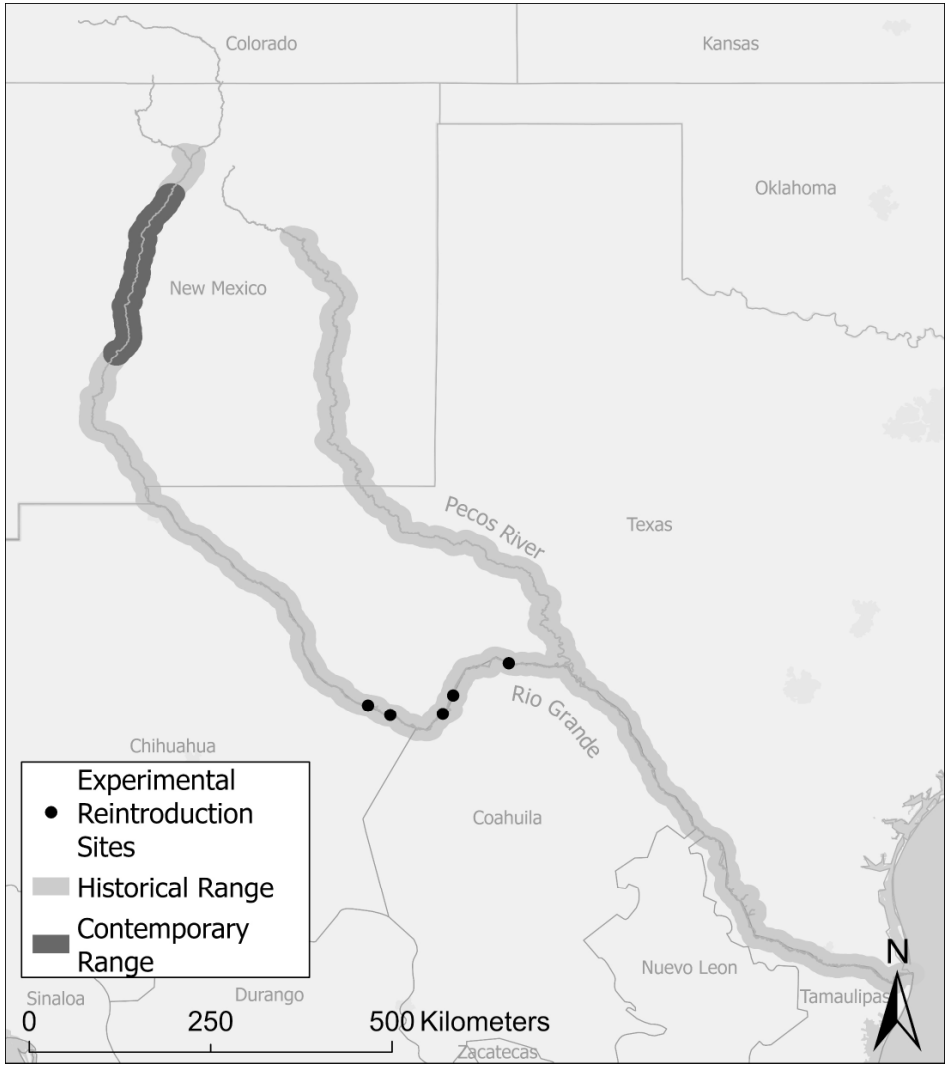
THE UNIVERSITY of NEW MEXICO

Museum of Southwestern Biology



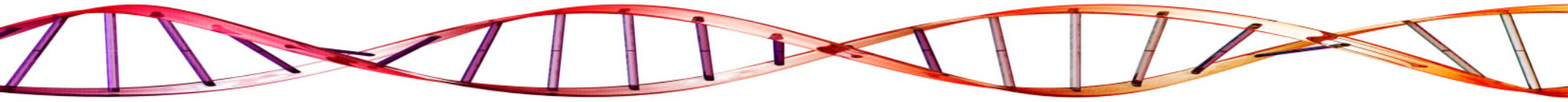
Map credit: T. Archdeacon

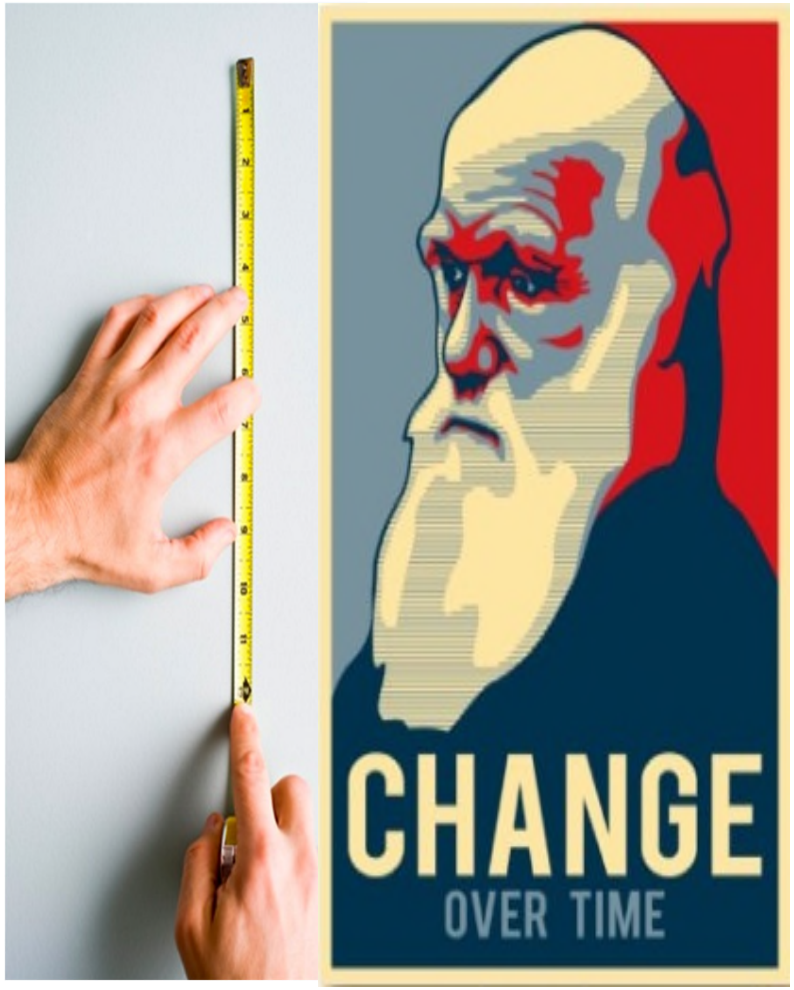
Rio Grande Silvery Minnow
Hybognathus amarus

- Distribution: Rio Grande and Pecos River
- Small-bodied, short-lived cyprinid
- Pelagic broadcast spawner
- Spawning associated with increases in flows/temperature in the spring

Genetic Diversity

- Populations with higher genetic diversity and larger effective population sizes have greater evolutionary/adaptive capacity to respond to ecological stressors.
- Amount of standing genetic diversity determined by the demography/history, effective population size (N_e), aspects of life-history & selection.
- Provides a way to assess genetic health of the population.



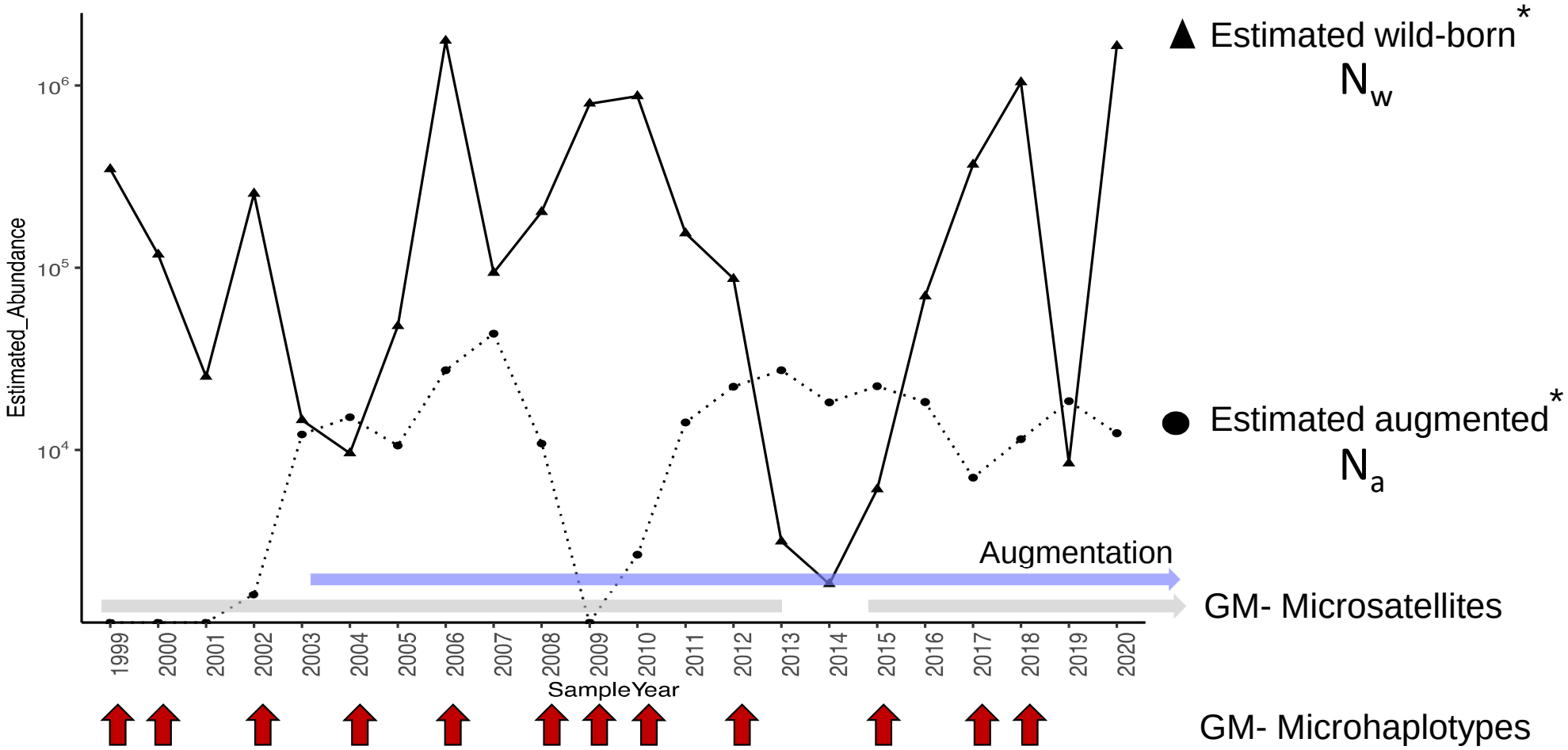


What is *Genetic Monitoring*?

- Tracking genetic diversity over contemporary time scales
- Multiple time points
- Consistent methodology across time series
- Rio Grande Silvery Minnow: 1999-2022

Why conduct genetic monitoring?

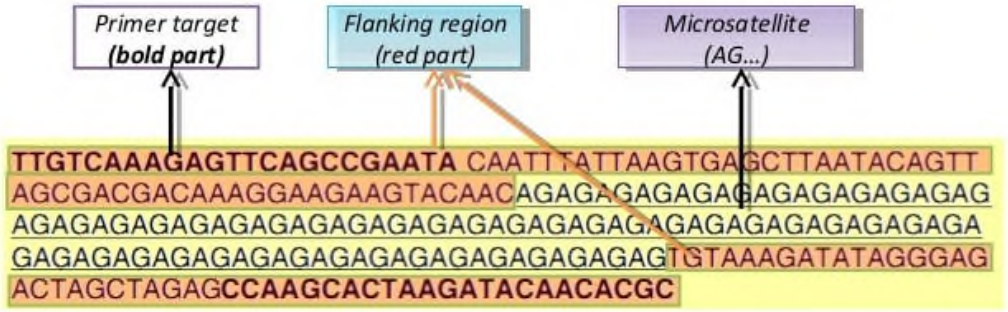
- Environmental conditions are highly variable-- Dramatic population fluctuations from year to year
- Short-lived species
- Captive breeding/rearing and augmentation (2003-2022)
- Genetic diversity affected over contemporary time scales
- Can be paired with demographic data



* Yackulic et al. 2022. Quantifying flow and nonflow management impacts on an endangered fish by integrating data, research, and expert opinion. *Ecosphere*, *13*(9).

Microsatellites

- DNA nucleotide repeats (AGAGAG)
- Non-coding sequences, frequent
- Assay variation at 10's of loci
- Highly polymorphic (i.e., lots of alleles)
- Ascertainment bias/scoring errors
- RGSM-- 9 microsatellites (9-60 alleles/locus)
- 1987, 1999-2022



Single Nucleotide Polymorphisms

- Substitutions of single nucleotide (ACGT)
- Microhaplotypes- multiple SNPs at a locus
- Found in coding and non-coding sequences
- Very frequent in the genome
- Can assay variation at 100-1000's of loci
- Reflect genome-wide variation
- RGSM– 5,549 SNPs from 3,151 loci (2-10 alleles/loci)
- 1999-2018

Definitions

Heterozygous- The presence of different alleles at one or more loci on homologous chromosomes (Aa).

Heterozygosity (H_e) –Proportion of heterozygous individuals for a locus in a population.

Homozygous- The presence of the same alleles at one or more loci on homologous chromosomes (AA).

Genetic effective size (N_e) – The effective size of a breeding population under idealized conditions, meeting the assumptions of Hardy-Weinberg (i.e., equal sex ratio, random mating).

Genetic drift – is the random change in allele frequencies from generation to generation because of sampling error.

The finite number of genes passed on to progeny will be an imperfect sample of the parental allele frequencies.

The effects of genetic drift are (i) allele frequencies will change and (ii) genetic variation will be lost. The smaller the population, the greater the change in allele frequencies due to drift.

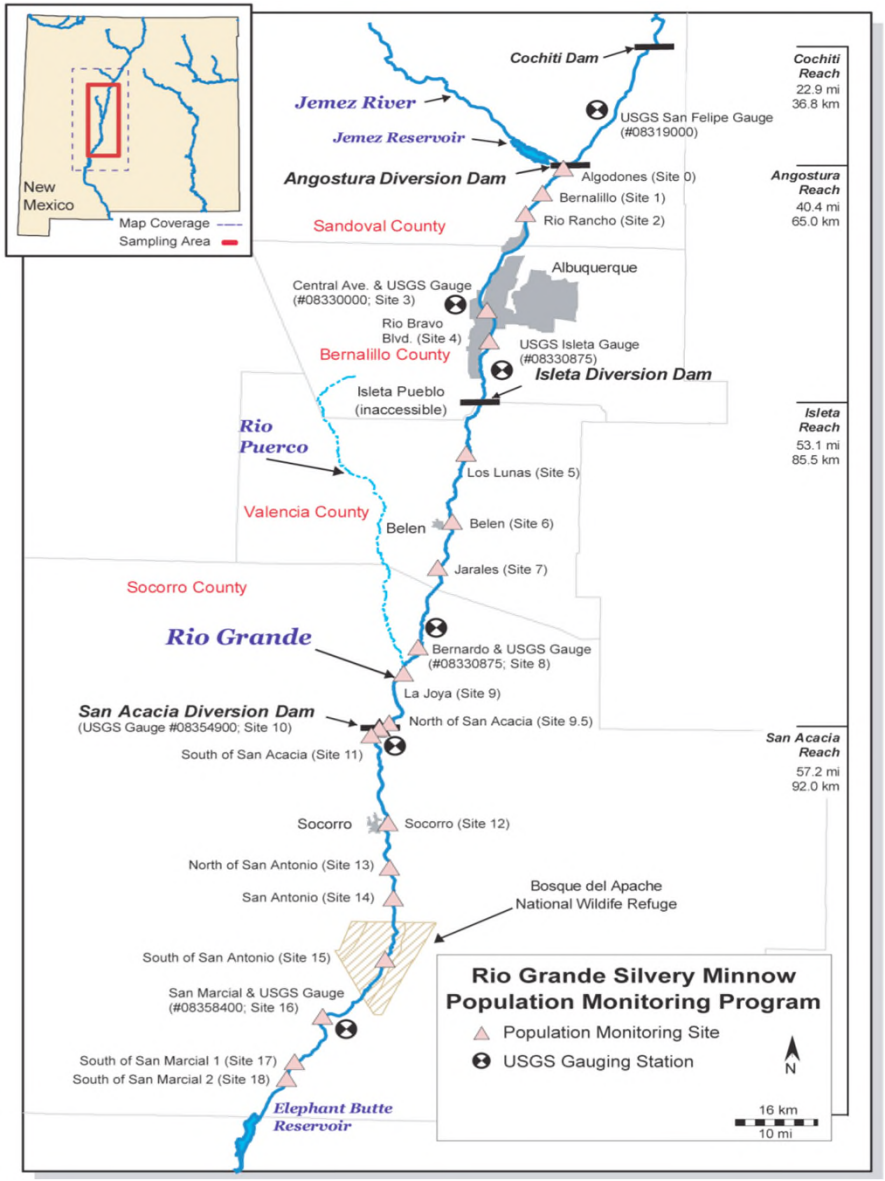
What are we measuring?

Microsats/SNPs	MtDNA	Bottleneck Effect
Gene diversity/ Heterozygosity H_{EC}/H_{OC}	Haplotype diversity	• Rate of loss– determined by N_e • Rare alleles contribute little to heterozygosity so loss of alleles may not be accompanied by loss of heterozygosity
Allelic richness A_R	Haplotype richness	• Sensitive metric (microsatellites) • Depends on number of alleles & frequencies • Rare alleles more likely to be lost
F_{ST}/ DAPC		• Temporal divergence of allele frequencies. F_{ST} ranges 0 (no difference) to 1 (complete divergence)
IBD F		• Relatedness metric, higher values indicate more related individuals
*g*2		• Population parameter that summarizes the variance in inbreeding in the population • Dependent on demographic history (bottlenecks admixture)– higher values when there is inbreeding

Genetic Effective Population Size

- The number of putative parents and the mean and variance in the number of offspring
- Tracked in genetic monitoring because N_e can be a sensitive indicator of population decline and provides information about loss of adaptive potential and population viability
- Effective population size is LESS than CENSUS SIZE

	VARIANCE EFFECTIVE SIZE, N_{eV}	INBREEDING EFFECTIVE SIZE, N_{eD}
How it is measured	Allele frequency changes	Linkage disequilibrium (non-random association of alleles at different loci)
What it measures	Loss of genetic variation from GENETIC DRIFT	Number of parents for the sampled generation
# of temporal samples	2	1
Stable populations	=	=
Population fluctuation Supplementation	≠	≠



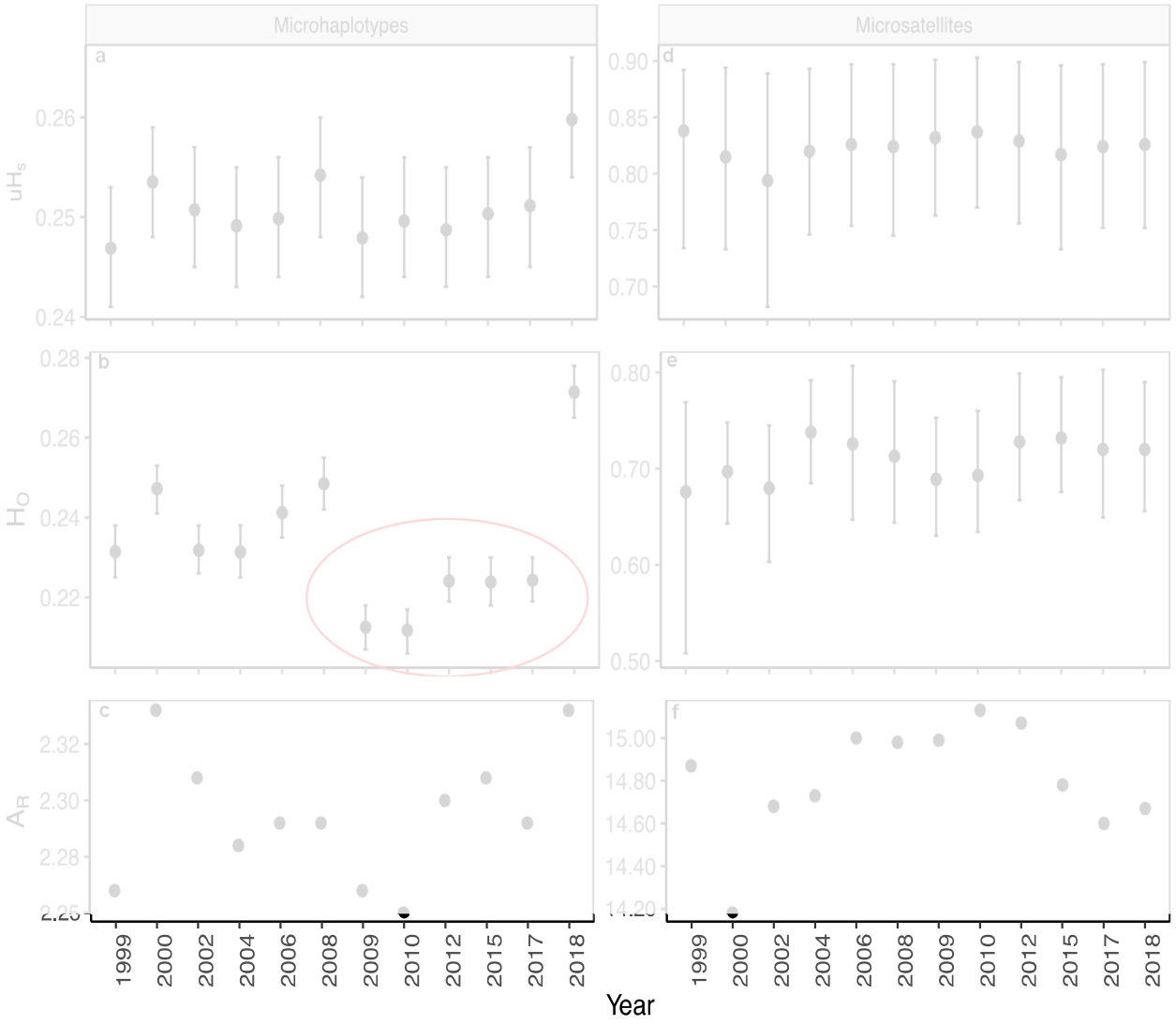
Genetic Sampling

- Unmarked fish sampled from three localities within each river reach
  - Angostura
  - Isleta
  - San Acacia
- Non-destructive sampling
- N = 7,034 unmarked fish from the MRG
- N = 5,291 captive bred/reared individuals released to the MRG
- Annual samples from refugial broodstocks

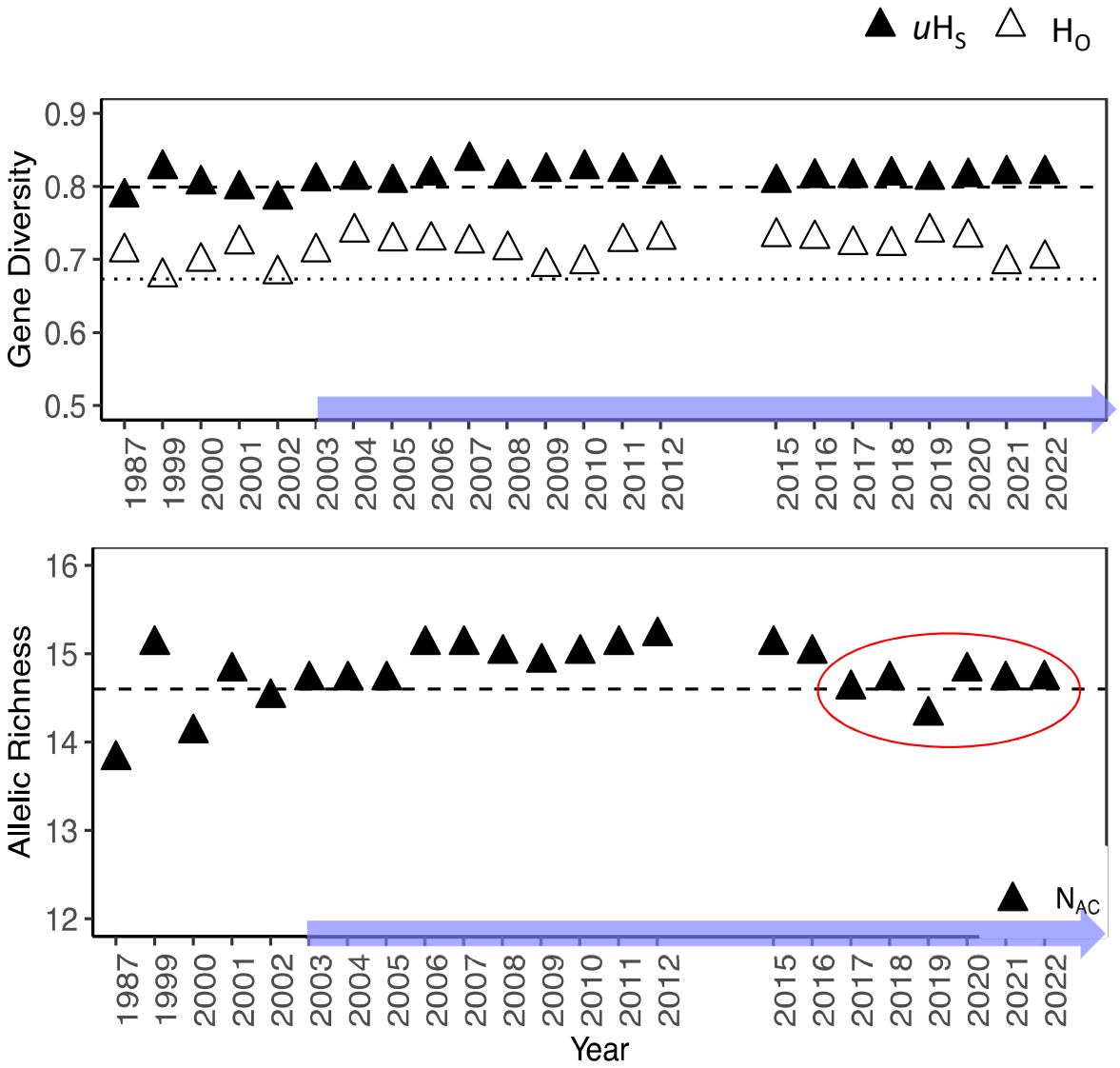
SNP development samples in parentheses

Year	Angostura	Isleta	San Acacia	Total
1987	15	-	28	**43**
1999	-	-	46 (30)	**46**
2000	-	-	194 (42)	**194**
2001	-	65	63	**128**
2002	67 (10)	121 (10)	201 (10)	**389**
2003	71	65	33	**169**
2004	141 (1)	15 (10)	6 (15)	**162**
2005	190	109	95	**394**
2006	95 (10)	143 (10)	145 (10)	**383**
2007	48	128	42	**218**
2008	165(10)	191 (10)	123 (10)	**479**
2009	175 (10)	153	150 (20)	**478**
2010	149 (9)	146 (10)	151 (9)	**446**
2011	71	148	140	**359**
2012	147 (10)	215 (11)	154 (9)	**516**
2015	75 (11)	33 (11)	35 (9)	**143**
2016	171	121	128	**420**
2017	159 (10)	156 (10)	154 (10)	**469**
2018	152 (10)	148 (10)	143 (21)	**443**
2019	73	10	54	**137**
2020	148	127	151	**426**
2021	118	60	61	**239**
2022	159	51	131	**339**

Genetic Diversity

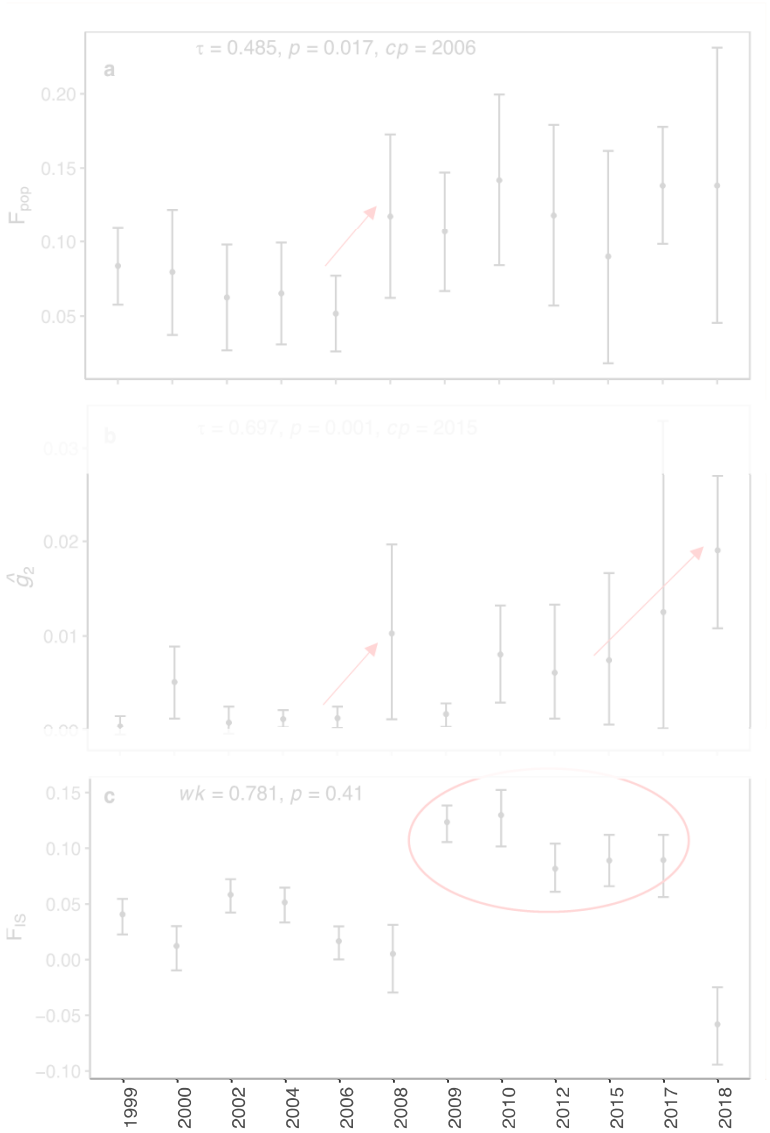


- Microhaplotypes- improved sensitivity compared to microsatellites
- Gene diversity (uH_S)– little change across the time series
- Decreases in H_o detected by microhaplotypes
- Decline in A_R 2015-2018 detected by microsatellites



Genetic Diversity

- Augmentation stabilized genetic diversity (uH_S, H_O, A_R)
- Decreases in H_O detected by microhaplotypes
- Decline in A_R 2015-2018 detected by microsatellites



Microhaplotype-based inbreeding metrics

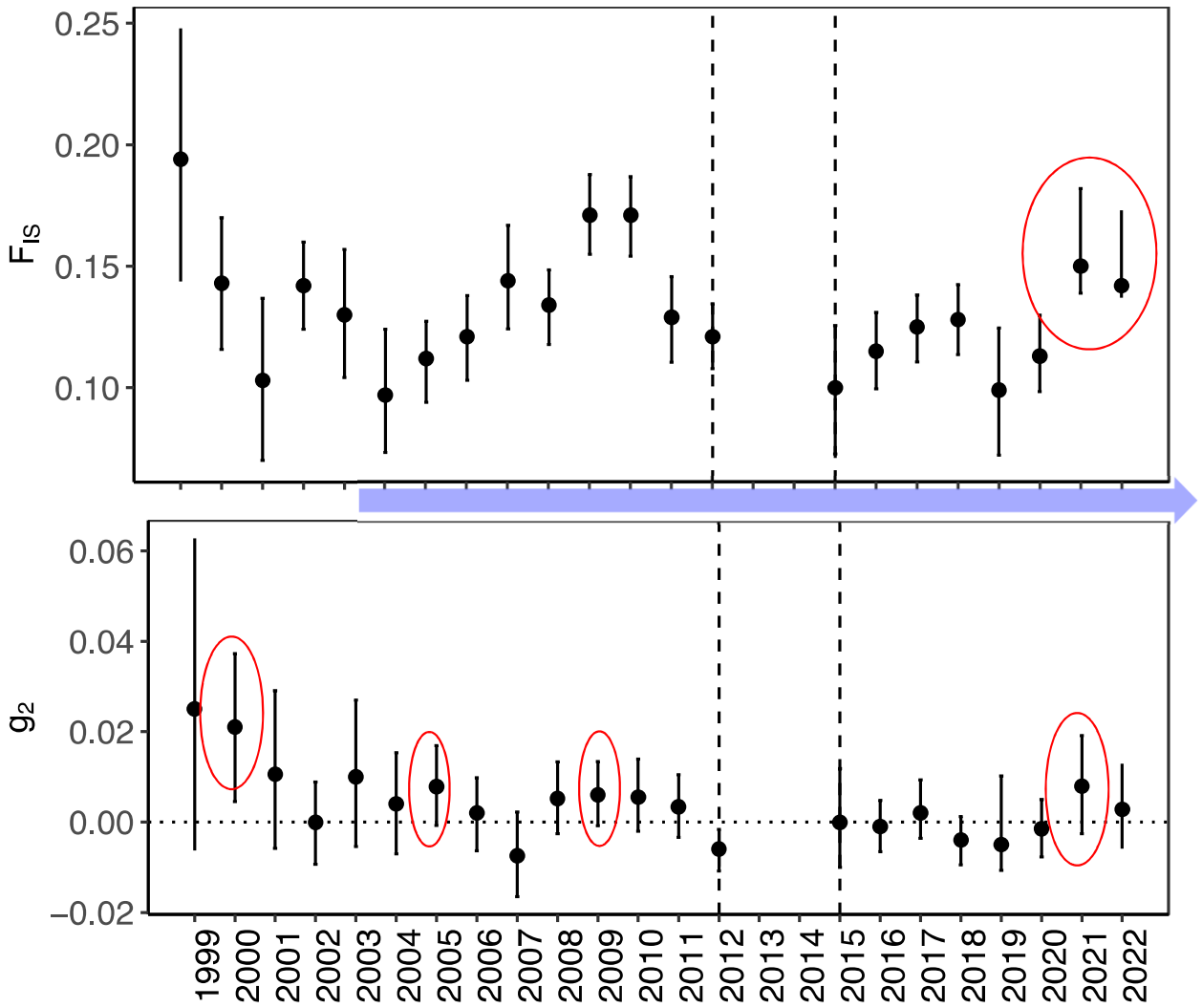
- Mean individual inbreeding coefficient per population (F_{POP}). Significant increase between 2006-2008.

- g_2 increases from 2015.
- Higher variance in inbreeding.

- F_{IS}: Measures heterozygote deficiency/excess per locus. 2009-2017- deficiency of heterozygotes

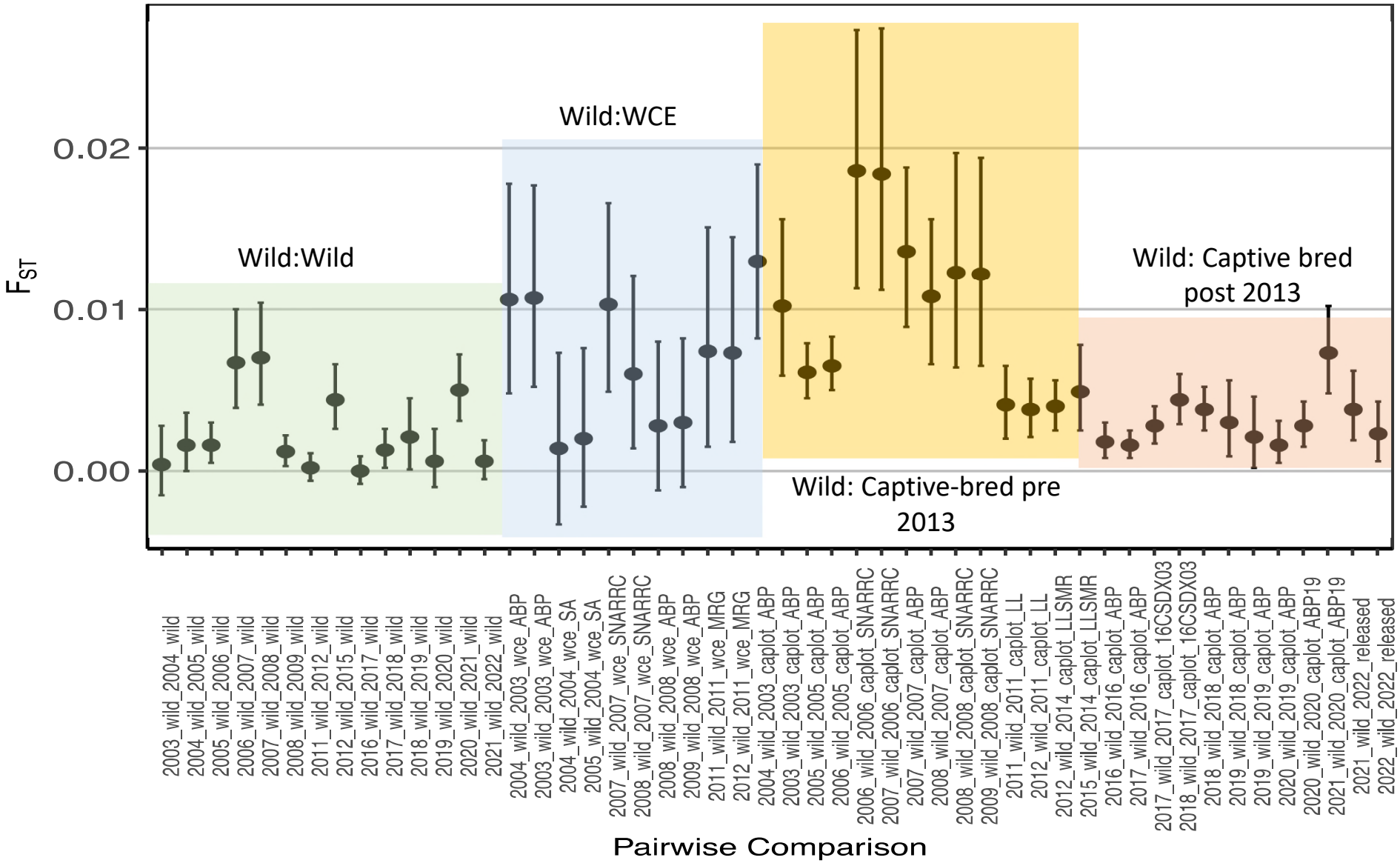
Osborne, Caeiro-Dias and Turner (In Press). Transitioning from microsatellites to SNP-based microhaplotypes in genetic monitoring programs: lessons from a 20-year time series of paired data. Molecular Ecology.

Microsatellite-based inbreeding statistics

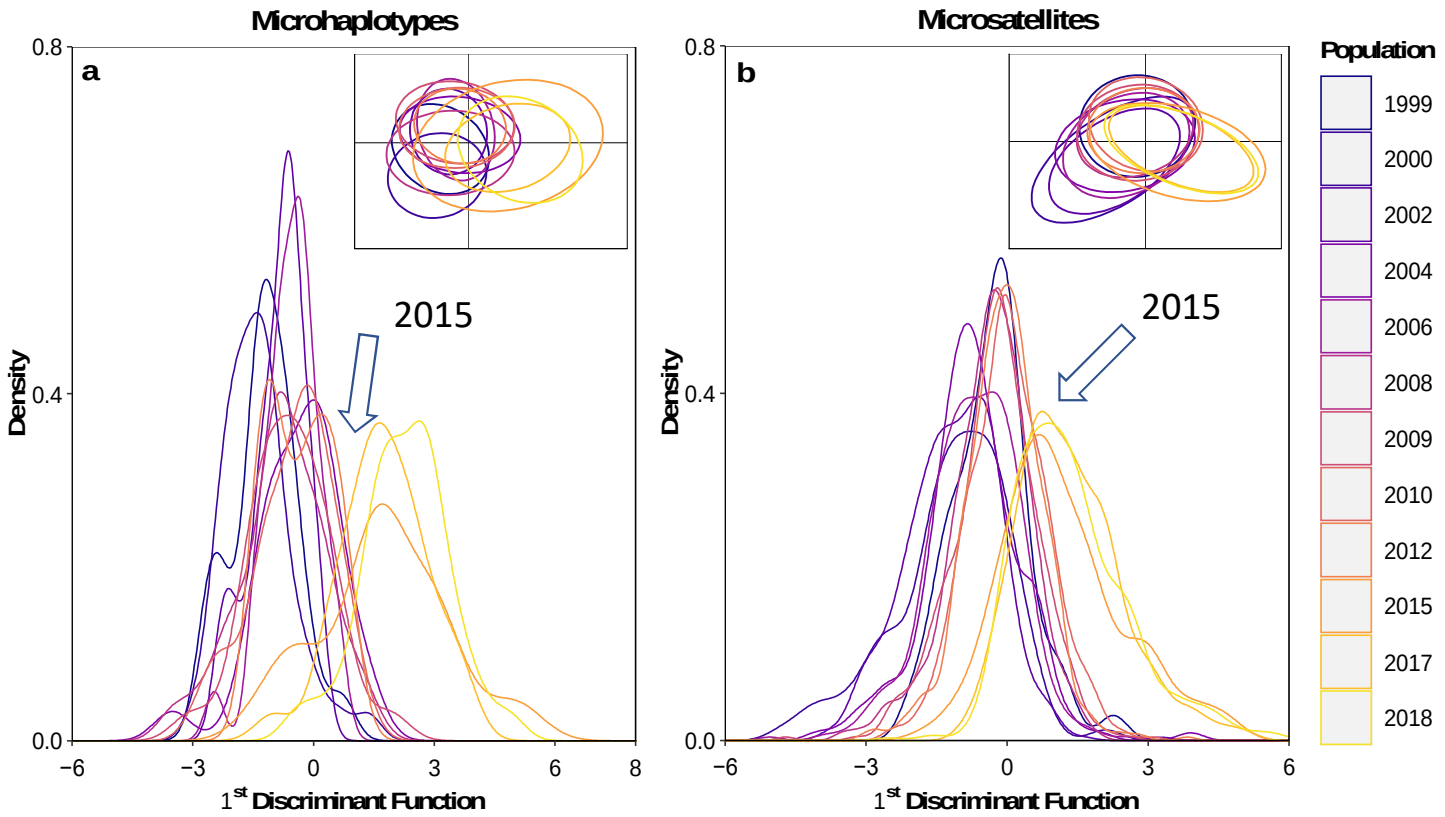


- Increase in F_{IS}- in 2021-2022
- Deficiency in heterozygotes in the population

- Microsatellites- less sensitive to changes in g_2 because fewer loci are assayed
- 2021- g_2 increases.
- Increases in g_2 associated with year following bottleneck (small wild population, more fish released from hatchery)


Wild:Wild
Wild:WCE
Wild: Captive-bred pre 2013
Wild: Captive bred post 2013
F_{ST}
0.02
0.01
0.00
2003_wild_2004_wild
2004_wild_2005_wild
2005_wild_2006_wild
2006_wild_2007_wild
2007_wild_2008_wild
2008_wild_2009_wild
2011_wild_2012_wild
2012_wild_2015_wild
2016_wild_2017_wild
2017_wild_2018_wild
2018_wild_2019_wild
2019_wild_2020_wild
2020_wild_2021_wild
2021_wild_2022_wild
2004_wild_2003_wce_ABP
2003_wild_2003_wce_ABP
2004_wild_2004_wce_SA
2005_wild_2004_wce_SA
2007_wild_2007_wce_SNARRC
2008_wild_2007_wce_SNARRC
2008_wild_2008_wce_ABP
2009_wild_2008_wce_ABP
2011_wild_2011_wce_MRG
2012_wild_2011_wce_MRG
2004_wild_2003_caplot_ABP
2003_wild_2003_caplot_ABP
2005_wild_2005_caplot_ABP
2006_wild_2005_caplot_ABP
2006_wild_2006_caplot_SNARRC
2007_wild_2006_caplot_SNARRC
2007_wild_2007_caplot_ABP
2008_wild_2007_caplot_ABP
2008_wild_2008_caplot_SNARRC
2009_wild_2008_caplot_SNARRC
2011_wild_2011_caplot_LL
2012_wild_2011_caplot_LL
2012_wild_2014_caplot_LLSMR
2015_wild_2014_caplot_LLSMR
2016_wild_2016_caplot_ABP
2017_wild_2016_caplot_ABP
2017_wild_2017_caplot_16CSDX03
2018_wild_2017_caplot_16CSDX03
2018_wild_2018_caplot_ABP
2019_wild_2018_caplot_ABP
2019_wild_2019_caplot_ABP
2020_wild_2019_caplot_ABP
2020_wild_2020_caplot_ABP19
2021_wild_2020_caplot_ABP19
2021_wild_2022_released
2022_wild_2022_released
Pairwise Comparison

Microhaplotypes/microsatellites record shifts in allele frequencies associated with population bottlenecks.



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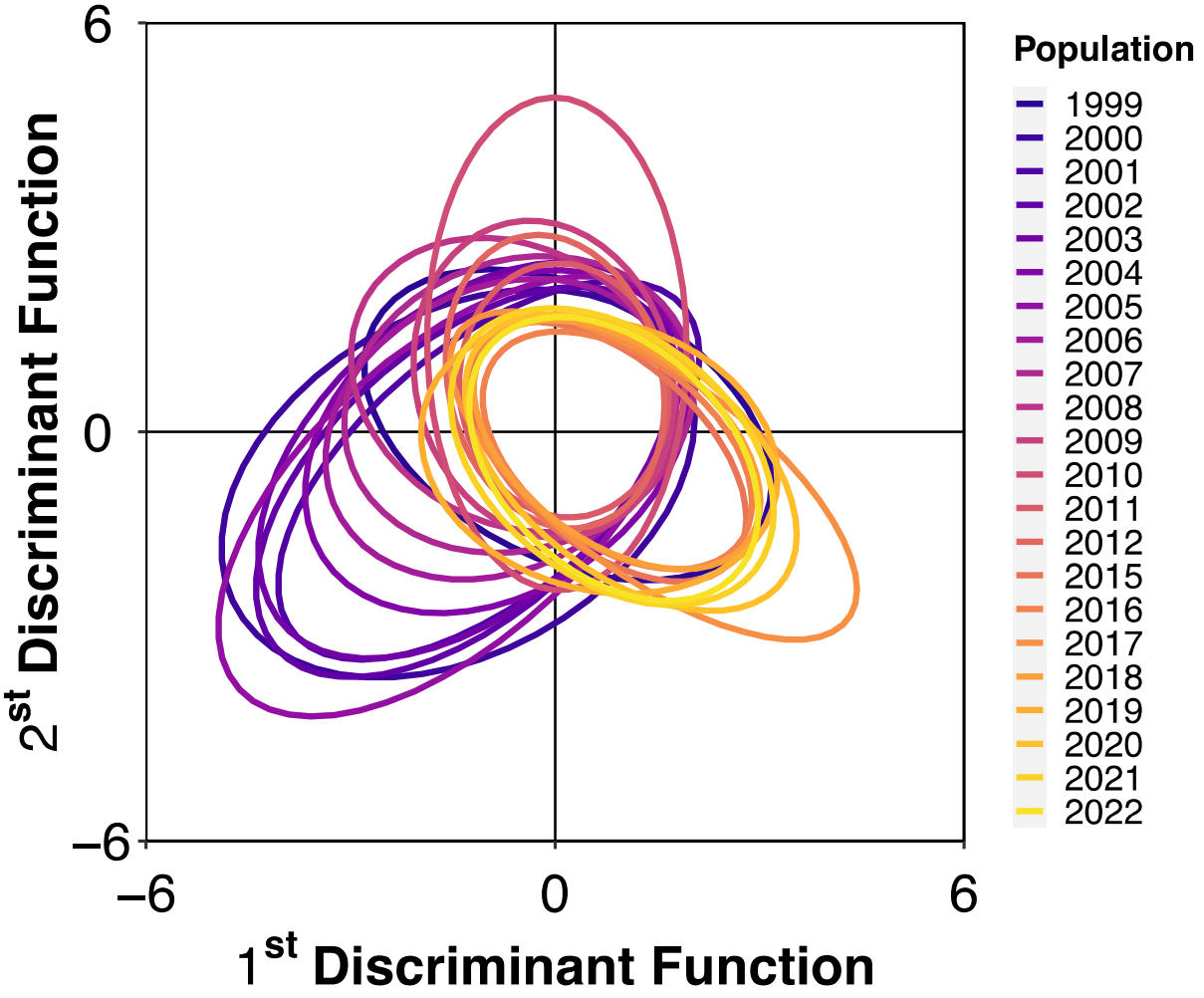
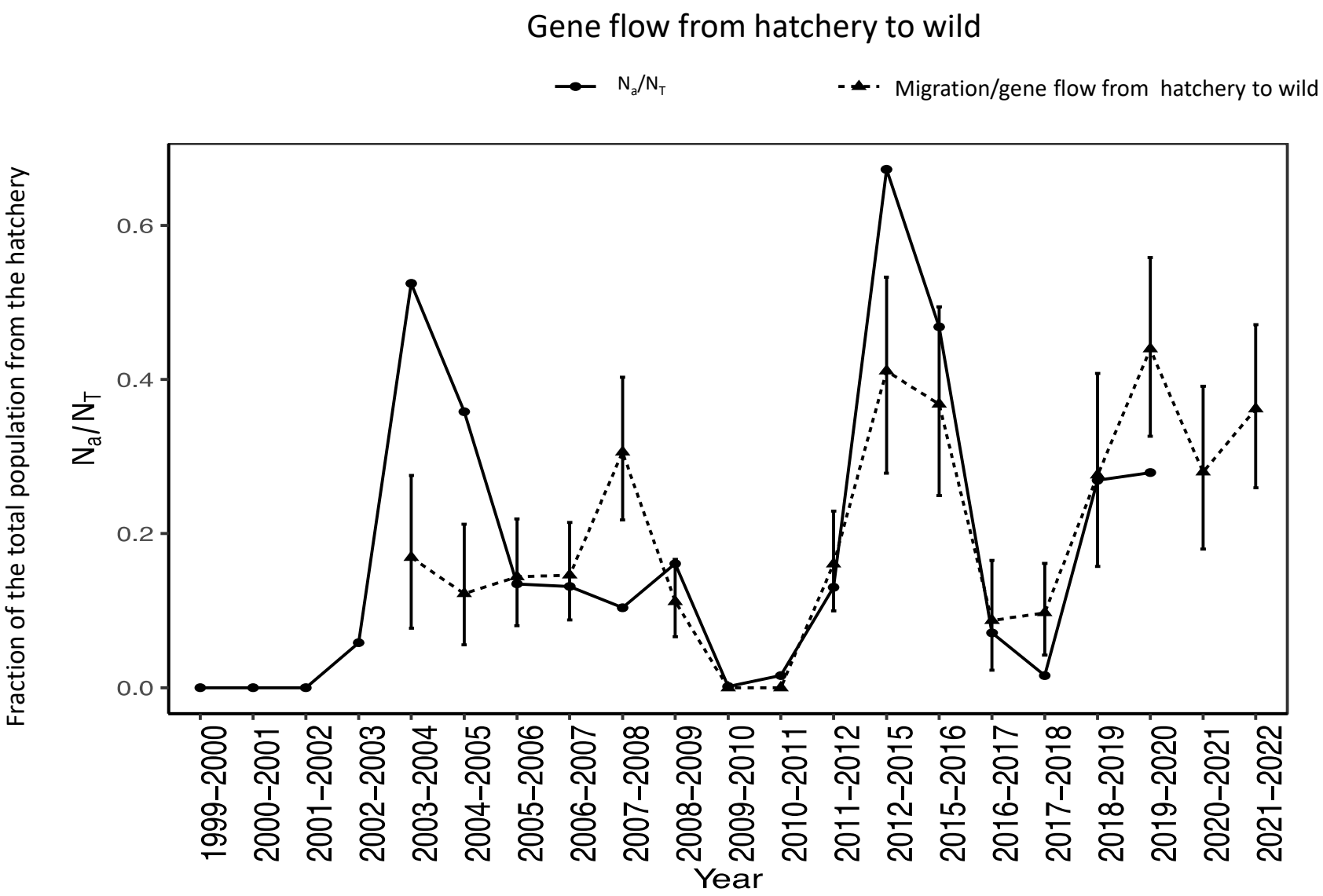
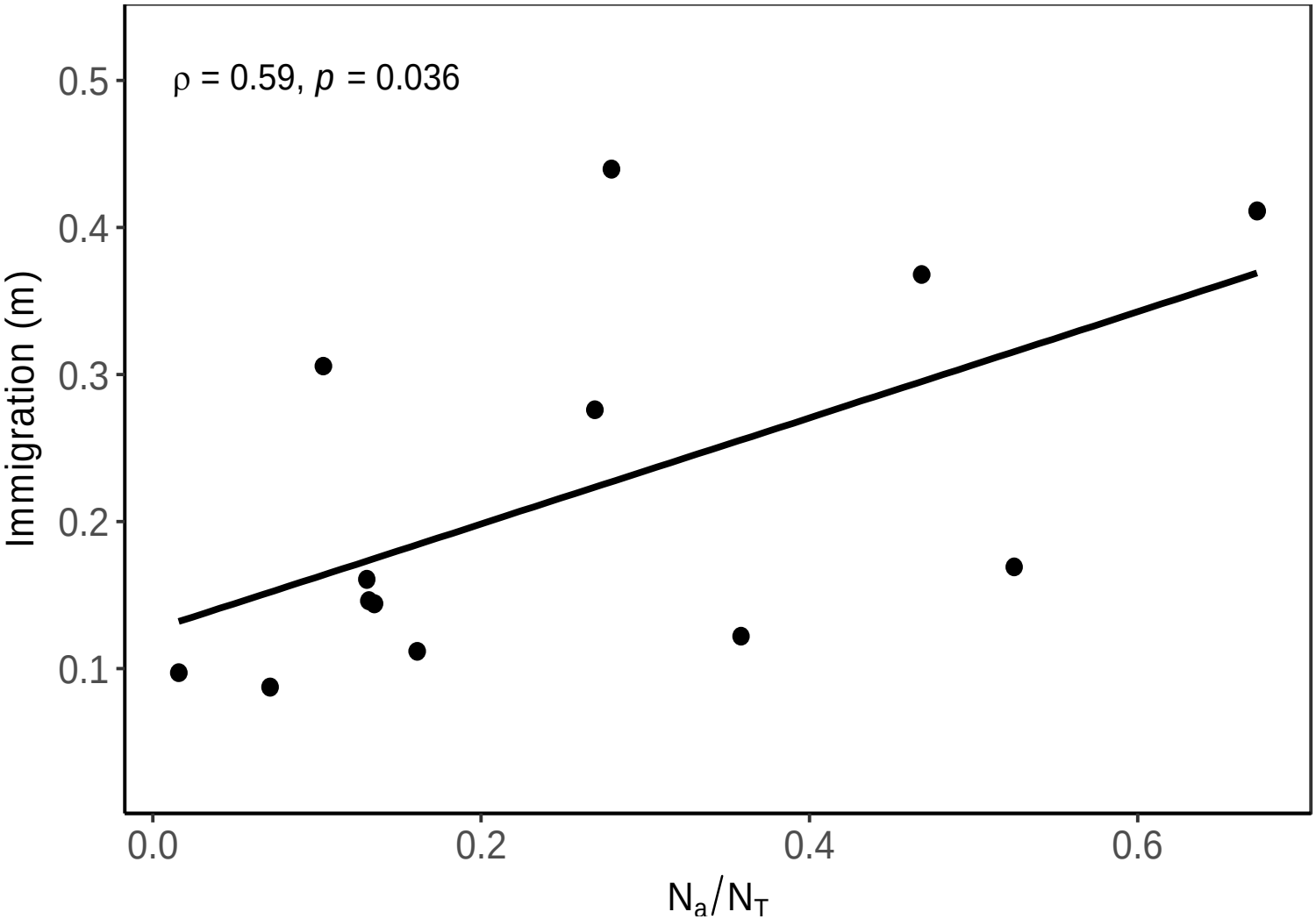
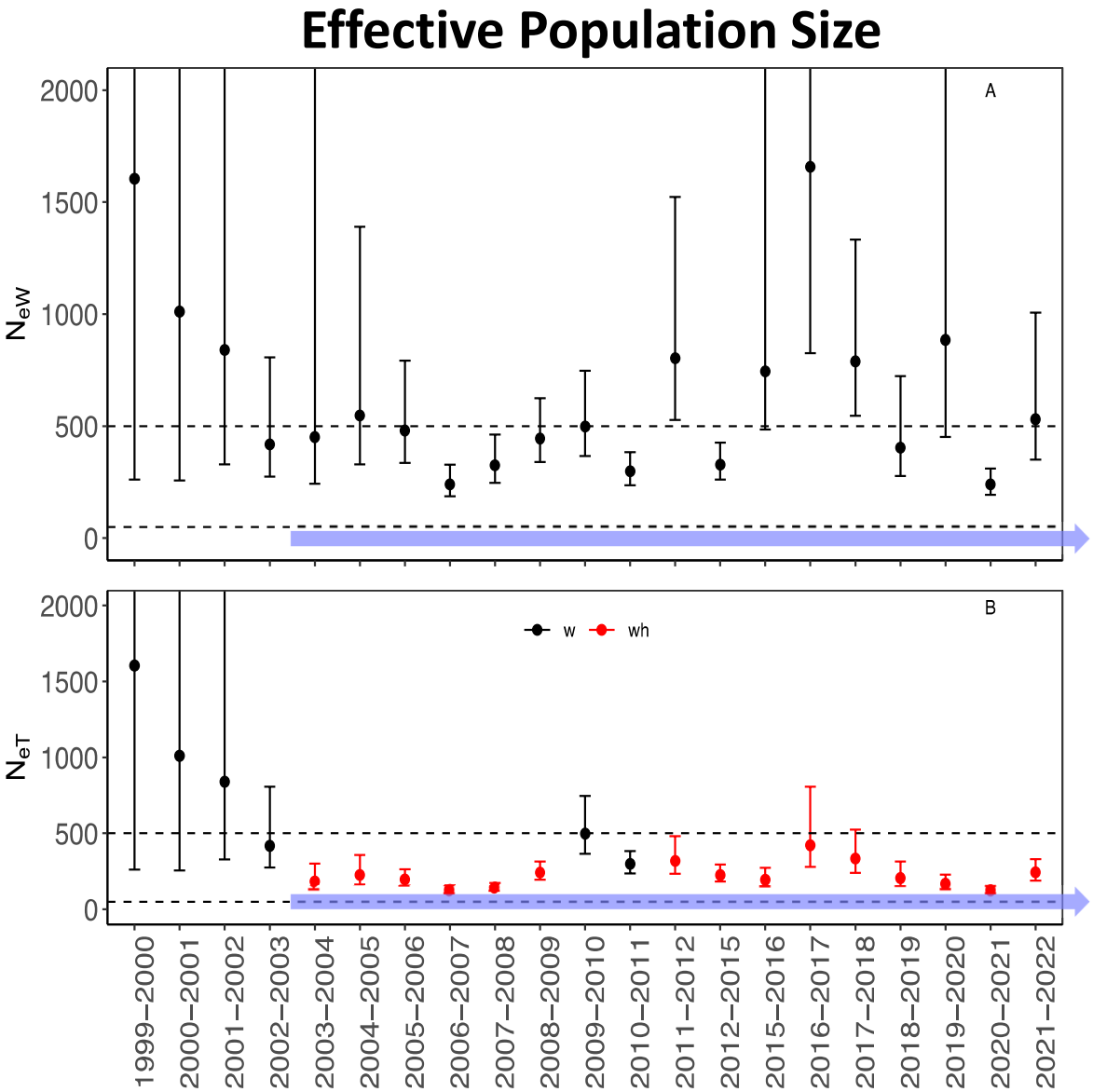
Microhaplotypes/microsatellites record shifts in allele frequencies associated with population bottlenecks.



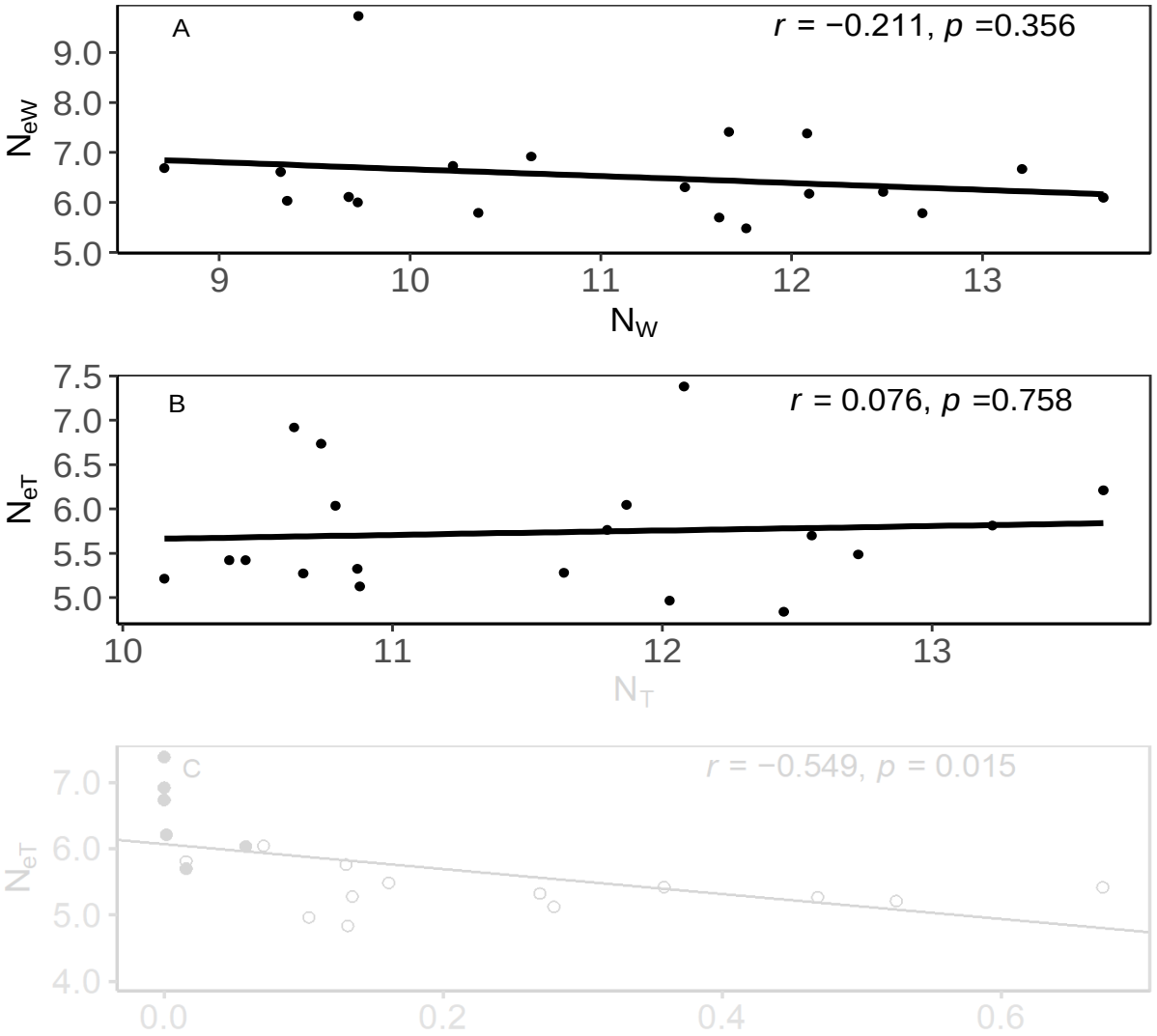

Gene flow from hatchery to wild
N_a/N_T
Migration/gene flow from hatchery to wild
Fraction of the total population from the hatchery
N_a/N_T
0.0
0.2
0.4
0.6
1999–2000
2000–2001
2001–2002
2002–2003
2003–2004
2004–2005
2005–2006
2006–2007
2007–2008
2008–2009
2009–2010
2010–2011
2011–2012
2012–2015
2015–2016
2016–2017
2017–2018
2018–2019
2019–2020
2020–2021
2021–2022
Year



Genetic estimates of gene-flow (m) are positively associated with fraction of the population comprised of augmented individuals

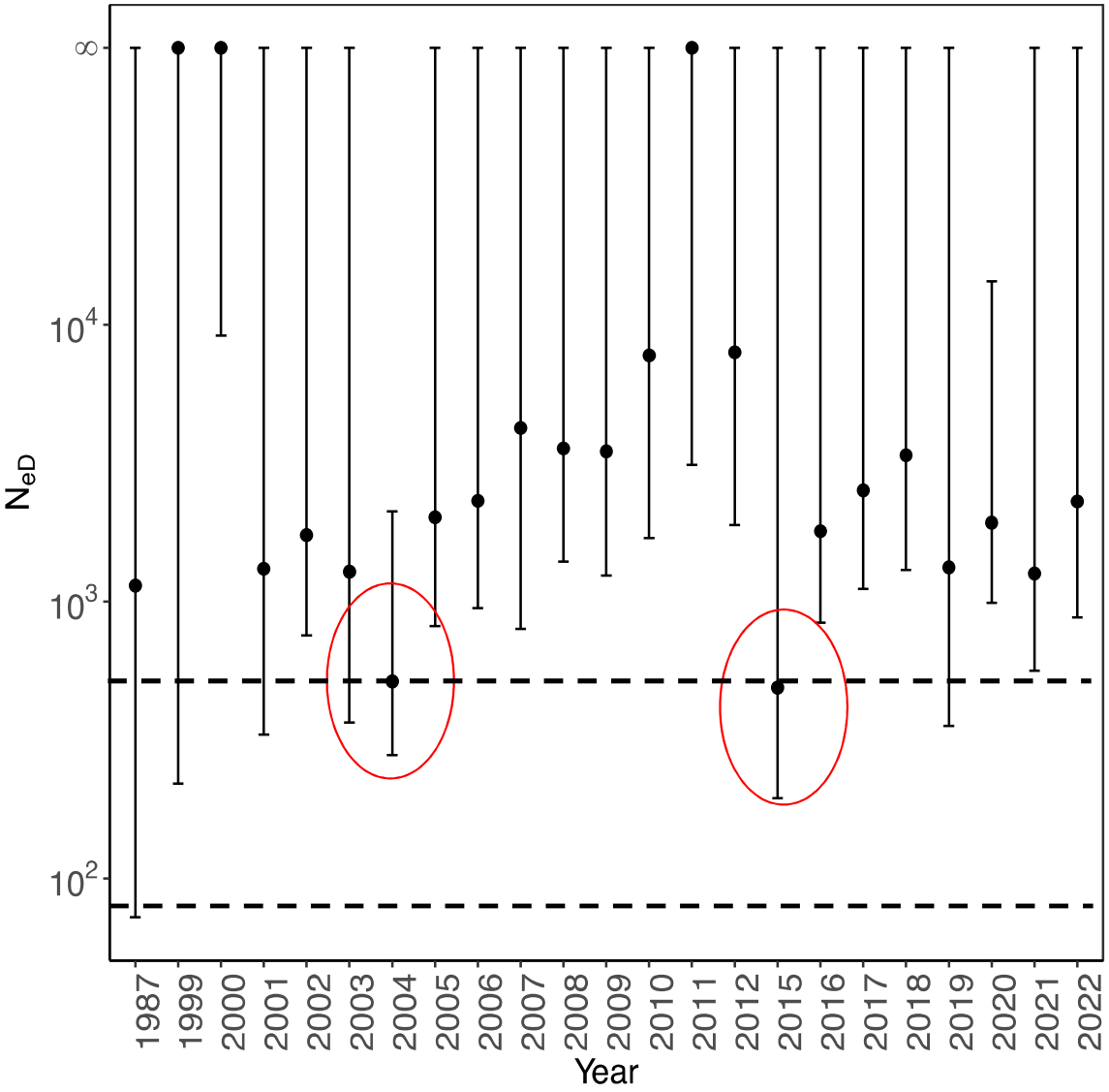


- **What it measures:** change in allele frequencies between temporal samples.
- Dashed lines N_e= 50/500
  - N_{eV}<500 long-term risk (loss of genetic variation from genetic drift)
- Small N_e– more rapid loss of diversity
- Augmentation with hatchery fish act as additional source of genetic drift– lowering N_e



N_{eW} /N_{eT} are decoupled from demographic abundance estimates. Increases in abundance are not accompanied by increases in $N_{e.}$

When population is largely derived from more limited pool of captive breeders N_{eT} is reduced– increased genetic drift



Linkage Disequilibrium Effective Size

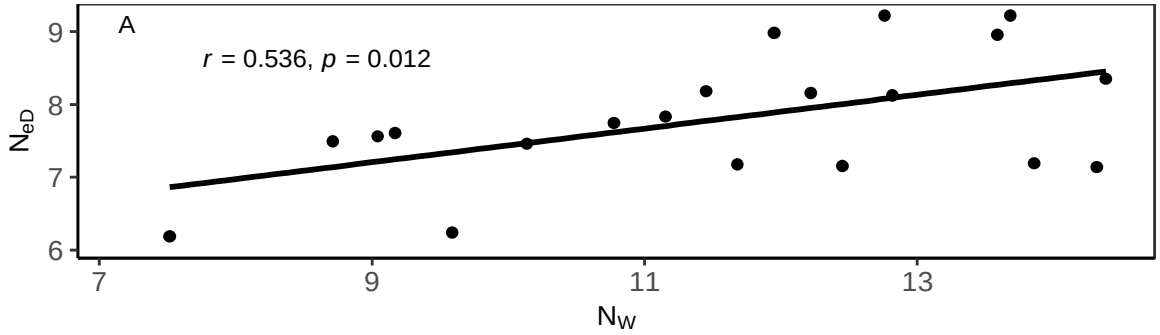
What it measures: Number of parents for the current generation

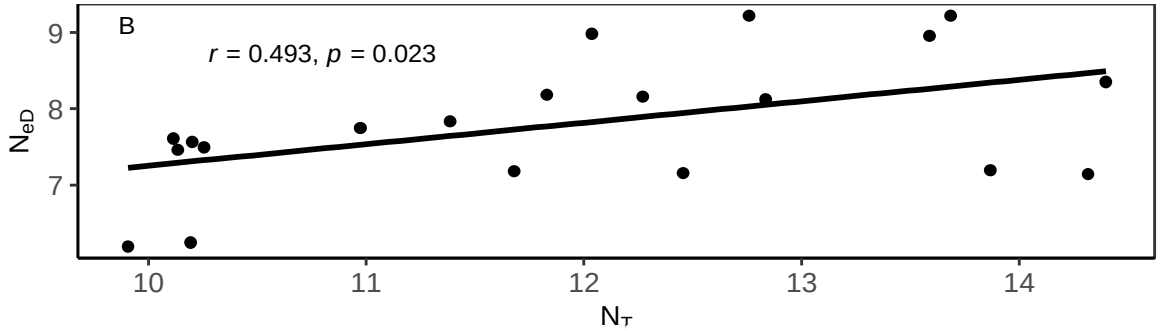
- Dashed lines N_e= 50/500
  - N_{el}<50 imminent risk

Number of temporal samples: 1

Both SNPs and microsatellites detect declines

N_{eD} estimates are always higher than N_{eV}





Positive association between N_{eD} and estimated abundance (wild-only [N_W] and wild+augmented [N_T]).

Fraction of the total population from the hatchery

Negative relationship between the ratio of augmented to wild individuals in the population.

When population is largely derived from more limited pool of captive breeders N_{eD} is less

- Genetic diversity– maintained despite repeated population declines but possible declines in A_R and H_O, increases in inbreeding metrics (F_{IS}, g_2)
- Shift in allelic composition of the population when comparing pre-bottleneck and post-bottleneck populations
- Effective population size (N_{eD})– positively associated with abundance
- Genetic estimates of gene flow are positively associated with the fraction of the population derived from the hatchery
- When riverine population is dominated by augmented fish- N_e is reduced.

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