## Patterns of genetic variation in razorback sucker from Lakes Mohave and Mead



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## Background

- Endemic to Colorado River system
- Live 40+ years
- Feed on algae, insect larvae, plankton, and detritus

- Grows up to 1 meter long and 5kg
- Abrupt, bony hump
- Iteroparous
- Highly fecund
- ca. 200,000 eggs/large adult female

- Federally listed as endangered in 1991
- 50+ yrs no natural recruitment in Lake Mohave
- Water development
- Non-native fish predation and competition



## History of population declines

- Reservoirs fill
- Populations expand
- Introduction of nonative species
- Failure to recruit
- Populations senesce and disappear
- Demise hastened by large predators


## Impact on genetic diversity

- Genetic diversity decreases with population size
- Can have negative effects on health of population (e.g., inbreeding depression)
- Can be used to monitor population size



## Objective

- Use molecular markers to inform management of razorback sucker by monitoring patterns of genetic diversity



## Mitochondrial DNA

- Small, maternally inherited
- Extremely variable -SSCP
- >33 haplotypes
- Parsimony analysis
- Differ by 1-2 bp



## Nuclear DNA

- Microsatellites
- Tandem repeats (ex CACACACACACAC)

$80 \quad 190 \quad 200 \quad 210 \quad 220 \quad 230 \quad 240 \quad 250 \quad 260$


## Microsatellites

- Biparental inheritance
- Also highly variable

Individual 1: 7276 bps Individual 2: 7476 bps Individual 3: 7676 bps


## Statistics

- Allelic Richness: The number of alleles per locus corrected for sample size (not comparable across studies)
- Gene Diversity: A measure of genetic variation in a population (expected frequency of heterozygous individuals)
- F-statistics: measure distribution of genetic variation within and among groups
- $F_{\text {IS }}$ (within samples)
- $\mathrm{F}_{\text {ST }}$ (among samples)
- $\mathrm{F}_{\mathrm{sc}}$ (among samples with regions/years)
- $\mathrm{F}_{\mathrm{CT}}$ (among regions or years)


## Levels of focus/interest

- Geographic structure among extant populations (for context)
- Lake Mohave
- Lake Mead


## Geographic samples

| LOCATION | N |
| :--- | :---: |
| 1. Lake Mohave | 49 |
| 2. Lake Mead | 16 |
| 3. Grand Canyon | NA |
| 4. Lake Powell | 23 |
| 5. Green River | 29 |
| 6. Upper Colorado River | 29 |
| Samples collected in the |  |
| mid to late 80 's, early 90 's |  |



## Levels of variation: microsatellites

| Allelic Richness |  |  |  |  |  |  | - Highest diversity downstream, in |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Locus Mohave |  | Mead | Powell | Green- <br> Yampa | Upper Colorado | Total |  |
| Xte1 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |  |
| Xte2 | 2.40 | 2.94 | 2.00 | 1.60 | 2.90 | 2.73 | Kes Mohave and |
| Xte4 | 6.04 | 3.87 | 5.31 | 5.24 | 1.00 | 5.13 |  |
| Xte7 | 8.62 | 6.87 | 4.79 | 3.80 | 2.59 | 7.16 | Mead |
| Xte8 | 12.18 | 10.69 | 8.78 | 8.08 | 7.45 | 11.20 |  |
| Xte10 | 8.42 | 7.99 | 7.93 | 7.11 | 4.00 | 8.07 | consete |
| Xte11 | 10.16 | 8.87 | 8.74 | 9.29 | 3.90 | 9.96 | - Coisistein win |
| Xte12 | 7.96 | 7.93 | 6.81 | 7.26 | 4.00 | 7.80 | mtDNA |
| Xte14 | 6.23 | 4.81 | 5.31 | 5.11 | 1.00 | 5.13 |  |
| Xte16 | 15.44 | 13.00 | 11.28 | 7.50 | 5.56 | 12.54 |  |
| Xte17 | 10.22 | 9.00 | 5.67 | 7.93 | 3.00 | 8.72 |  |
| Xte18 | 6.74 | 4.94 | 4.00 | 4.52 | 3.68 | 5.84 |  |
| Xte19 | 10.34 | 8.75 | 7.62 | 4.63 | 2.90 | 8.80 |  |
| Xte20 | 17.95 | 18.42 | 11.38 | 8.68 | 2.68 | 15.00 |  |
| Xte22 | 17.33 | 14.55 | 10.64 | 10.62 | 4.97 | 14.57 |  |
|  | 9.47 | 8.31 | 6.82 | 6.22 | 3.44 | 8.31 |  |

## Population structure: microsatellites

$F_{\text {ST }}=0.06$ Jackknife values mean $=0.06 \pm 0.01$

From mtDNA
$F_{\text {ST }}=0.21$

## STRUCTURE - Example

- Color = group identifier
- Columns = individuals
- Height = probability of assignment to specific groups
k=4 (50 replicates)



## Geographic structure

- Consistency across replicates
- $K=2$ or 3 ?
- Upper Colorado River
- Above and below Grand Canyon?
- Admixture
- Mohave and Mead not different from each other


## Levels of focus/interest

- Geographic structure among extant populations
- Lake Mohave
- Lake Mead


## How do we preserve the population in Lake Mohave?

- Need to increase population sizes while maintaining genetic diversity
-How?
- Larvae have to be representative of wild adults
- Repatriates have
 to reproduce


## Lake Mohave

## Conservation management plan

- Initiated in mid-1990's
- Capture naturally produced larvae across regions throughout the spawning season to represent genetic diversity in the lake


Hoover (Boulder) Dam


- Reared in captivity
- Hatcheries
- Backwaters

- Release into the wild
- PIT tag
- Monitoring

- Native Fishes Work Group
- Genetics



## mtDNA

- Previously published studies of mtDNA have shown that genetic variation is being maintained by this sampling strategy
- For example, variation among larval samples within areas, not among areas

| Year | \# samples | gene diversity | allelic richness |
| :--- | :---: | :---: | :---: |
| 1997 | 13 | 0.703 | 4.79 |
| 1998 | 17 | 0.634 | 4.69 |
| 1999 | 11 | 0.639 | 4.37 |
| 2000 | 15 | 0.691 | 4.69 |
| 2001 | 9 | 0.688 | 4.54 |
| 2002 | 14 | 0.657 | 4.37 |
| 2003 | 14 | 0.693 | 4.70 |
| 2004 | 21 | 0.643 | 4.61 |
| 2005 | 17 | 0.695 | 5.11 |
| 2006 | 23 | 0.684 | 4.95 |
| 2007 | 12 | 0.721 | 5.81 |
|  |  |  |  |
| ANOVA |  |  |  |
| F |  | 0.83 | 1.19 |
| P |  | 0.60 | 0.30 |

# Distribution of variation among larval samples 

|  |  | among samples |  |  |  |  |  |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | among samples |  |  |  |  |  |  |  | within areas |  | among area |  |
| Year | \# samples | N | FST | P | FSC | P | FCT | P |  |  |  |  |  |
| 1997 | 13 | 339 | 0.083 | $<0.0001$ | 0.101 | $<0.0001$ | -0.021 | 0.840 |  |  |  |  |  |
| 1998 | 19 | 485 | 0.043 | $<0.0001$ | 0.046 | $<0.0001$ | -0.003 | 0.500 |  |  |  |  |  |
| 1999 | 13 | 294 | 0.041 | $<0.0001$ | 0.053 | $<0.0001$ | -0.013 | 0.715 |  |  |  |  |  |
| 2000 | 16 | 367 | 0.049 | $<0.0001$ | 0.058 | $<0.0001$ | -0.009 | 0.758 |  |  |  |  |  |
| 2001 | 10 | 230 | 0.100 | $<0.0001$ | 0.101 | $<0.0001$ | -0.001 | 0.522 |  |  |  |  |  |
| 2002 | 14 | 348 | 0.020 | $<0.0001$ | 0.024 | $<0.0001$ | -0.004 | 0.651 |  |  |  |  |  |
| 2003 | 14 | 370 | 0.06 | $<0.0001$ | 0.037 | $<0.0001$ | 0.023 | 0.069 |  |  |  |  |  |
| 2004 | 24 | 559 | 0.147 | $<0.0001$ | 0.138 | $<0.0001$ | 0.01 | 0.240 |  |  |  |  |  |
| 2005 | 17 | 437 | 0.059 | $<0.0001$ | 0.058 | $<0.0001$ | 0.001 | 0.380 |  |  |  |  |  |
| 2006 | 24 | 598 | 0.062 | $<0.0001$ | 0.063 | $<0.0001$ | -0.0004 | 0.430 |  |  |  |  |  |
| 2007 | 13 | 308 | 0.043 | $<0.0001$ | 0.054 | $<0.0001$ | -0.012 | 0.740 |  |  |  |  |  |
| average |  |  | 0.064 |  | 0.067 |  | -0.003 |  |  |  |  |  |  |

## Variation among samples within areas, not among areas

## Distribution of variation among larvae, adults, and repatriates

## SOURCE

Among groups
$F_{\mathrm{ST}}=0.003$
Among samples within groups $\quad F_{s c}=0.004$
Among larvae, repatriates, and adults $\quad F_{C T}=\mathbf{- 0 . 0 0 1}$
No differences among larvae, repatriates, and adults!

## What about nuclear genome?

- Genotyped 1560 Larvae (1997-2009) at 15 microsatellite loci
- 7000+ larvae samples
- Random sample of 120 from each year
- Allows us to examine nuclear DNA across years

- Larval allelic richness
- Varied from 16.22 (2004) to 17.32 (1997)
-Average = 17.10
- Allelic diversity has been maintained


## Larval Allelic Richness




Year Collected

## Variation among larval samples

- Smaller than estimates from mtDNA
-Consistent with mode of inheritance
- Very little difference among samples within regions ( $\mathrm{F}_{\mathrm{sc}}$ ) or among regions ( $\mathrm{Fcc}_{\mathrm{ct}}$ )
-Consistent across years

| Year | $F_{S C}$ | $F_{C T}$ |
| :--- | :---: | :---: |
| 1997 | 0.010 | 0.001 |
| 1998 | 0.009 | 0.002 |
| 1999 | 0.011 | 0.003 |
| 2000 | 0.013 | -0.001 |
| 2001 | 0.016 | 0.001 |
| 2002 | 0.008 | 0.000 |
| 2003 | 0.005 | 0.008 |
| 2004 | 0.014 | 0.001 |
| 2005 | 0.007 | 0.001 |
| 2006 | 0.013 | 0.004 |
| 2007 | 0.003 | -0.001 |
| 2008 | 0.012 | 0.002 |
| 2009 | 0.005 | 0.000 |
| average | 0.010 | 0.002 |

## Sampling - adults

- Genotyped 979 adult ( 300 wild and 679 repatriate) fin clips at 15 microsatellite loci
- Repatriates stocked in years 1992-2008
- Allows us to examine nuclear DNA across stocking year classes

- Adult allelic richness
- Varied from 7.07 (2008) to 8.04 (2003)
- Average of 7.57 (allele numbers corrected for sample size)
- Allelic diversity has been maintained across stocking years and relative to wild adults

Adult Allelic Richness


# Distribution of variation among stocking years 

- $\mathrm{F}_{\mathrm{ST}}$ (among year variation) $=0.004$
- Similar genetic composition among repatriates across stocking years


## Conclusions Lake Mohave

- All measures of genetic variation consistent among samples of larvae and repatriates
- What about transmission from larvae to adults?
- Requires direct comparison which has not been completed yet



## Transmission of variation from larvae to repatriates?

- Cannot compare allelic richness directly from these analyses because of different sample sizes
- Gene diversities are comparable
- Repatriate diversity of 0.75 (SD = 0.01)
- Larval diversity of 0.74 ( $\mathrm{SD}=0.01$ )
- Therefore, program is maintaining genetic variation!


## Levels of focus/interest

- Geographic structure among extant populations
- Lake Mohave
- Lake Mead


## Samples

- Far fewer samples
- Larvae
- Echo Bay (EBL) 1997 - N = 25
- Echo Bay (EBL) 2002 - N = 30
- Las Vegas Bay (LVL) 2002 - N =27
- Adults
- Mixed locations from late 80 's - N = 13
- Echo Bay (EB) 2002 - N = 11
- Las Vegas Bay (LV) 2002 - N =18

|  | N |  | gene diversity allelic richness |  |
| :--- | :---: | :---: | :---: | :---: |
| larvae |  |  |  |  |
| Echo Bay - 97 | 25 | 0.610 | 2.84 |  |
| Echo Bay - 02 | 30 | 0.706 | 3.83 |  |
| Las Vegas Bay -02 | 27 | 0.641 | 3.22 |  |
| adults |  |  |  |  |
| mixed - late 80s | 16 | 0.125 | 1.69 |  |
| Echo Bay - 02 | 11 | 0.710 | 4.00 |  |
| Las Vegas Bay -02 | 18 | 0.660 | 4.07 |  |

- Generally lower diversity in larvae (excluding 80’s adult sample)
- Increased diversity in newer samples, especially for the adults
- Lower gene diversity than Lake Mohave (ave. = 0.74)


## Distribution of variation

## among Lake Mead samples

- $F_{\text {ST }}$ (total among group variation) $=0.216$
- $\mathrm{F}_{\mathrm{SC}}$ (among samples within adults or larvae) $=0.221$
- $\mathrm{F}_{\mathrm{CT}}($ adult vs. larvae) $=0.007$
- Lots of variation, with differences due to individual samples (similar to Lake Mohave)
- Much larger than Lake Mohave
- Largest estimate among larval samples within years was $\mathrm{F}_{\mathrm{ST}}=$ 0.147 (ave = 0.064)
- Among adults and larvae $F_{S T}=0.003$
- Comparable to estimate for entire Colorado drainage $\mathrm{F}_{\mathrm{ST}}=0.21$


## What about nuclear genome?

|  | larvae |  |  | adults |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | EBL - 97 | EBL - 02 | LVL - 02 | mixed 80s | EB - 02 | LV - 02 | TOTAL |
| Xte1 | 1.99 | 2.00 | 1.94 | 2.00 | 2.00 | 2.00 | 1.99 |
| Xte2 | 1.00 | 1.00 | 1.00 | 2.69 | 2.00 | 2.56 | 1.54 |
| Xte7 | 4.14 | 5.36 | 7.05 | 7.48 | 6.00 | 6.54 | 6.88 |
| Xte8 | 6.29 | 7.98 | 8.11 | 10.48 | 9.00 | 10.49 | 9.14 |
| Xte11 | 8.06 | 8.90 | 6.96 | 9.36 | 10.00 | 8.91 | 9.21 |
| Xte12 | 7.40 | 7.88 | 6.14 | 7.65 | 6.00 | 8.32 | 8.04 |
| Xte16 | 9.14 | 9.20 | 8.59 | 11.00 | 9.00 | 10.81 | 11.44 |
| Xte17 | 8.60 | 8.78 | 8.33 | 11.00 | 9.00 | 8.01 | 9.92 |
| Xte18 | 5.33 | 5.72 | 5.31 | 5.85 | 4.00 | 5.74 | 5.59 |
| Xte19 | 7.92 | 7.09 | 5.70 | 7.79 | 8.00 | 6.79 | 7.94 |
| Xte20 | 9.99 | 9.86 | 9.76 | 13.83 | 13.00 | 12.35 | 12.54 |
| Xte22 | 8.13 | 9.16 | 9.46 | 12.03 | 11.00 | 13.19 | 11.69 |
| Xte24 | 9.07 | 9.47 | 10.45 | 9.36 | 8.00 | 14.12 | 11.62 |
| Xte25 | 2.00 | 2.37 | 2.41 | 2.85 | 2.00 | 2.95 | 2.43 |
| average | 6.36 | 6.77 | 6.52 | 8.10 | 7.07 | 8.06 | 7.85 |

- Allelic richness
- Less diversity in larvae than adults


## Distribution of variation among samples

- $F_{S T}$ (total among group variation) $=0.031$
- $\mathrm{F}_{\text {SC }}$ (among samples within adults or larvae) $=0.033$
- $F_{C T}($ adult vs. larvae) $=-0.005$
- Slightly higher than Lake Mohave
- Average $F_{s c}=0.010$
- Average $F_{C T}=0.002$
- Lower than estimate from entire drainage
- $F_{S T}=0.06$


## Conclusions Lake Mead

- Less diversity in larvae than adults
- More variation among samples
- Sampling effect?
- Increased mtDNA diversity over time
- Sampling effect?
- Additional input source?
- Require additional sampling to understand dynamics of this system

