Genetic traceability of wild and captive-reared totoaba stocks:
A tool for population recovery assessment and law enforcement

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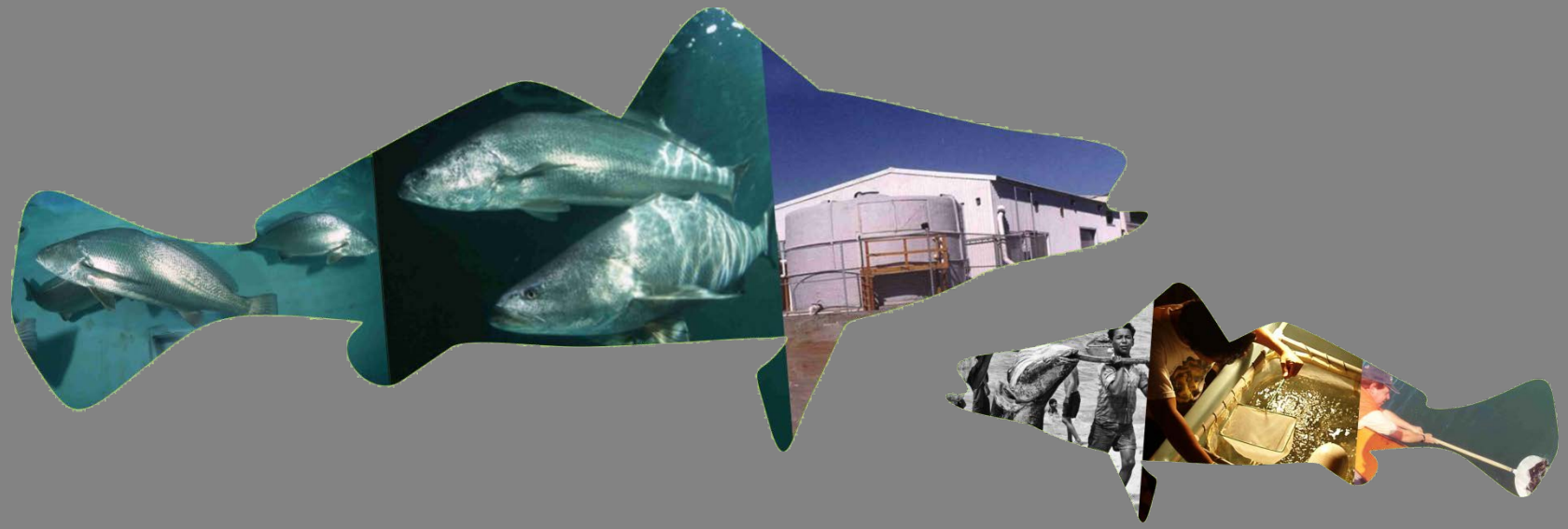
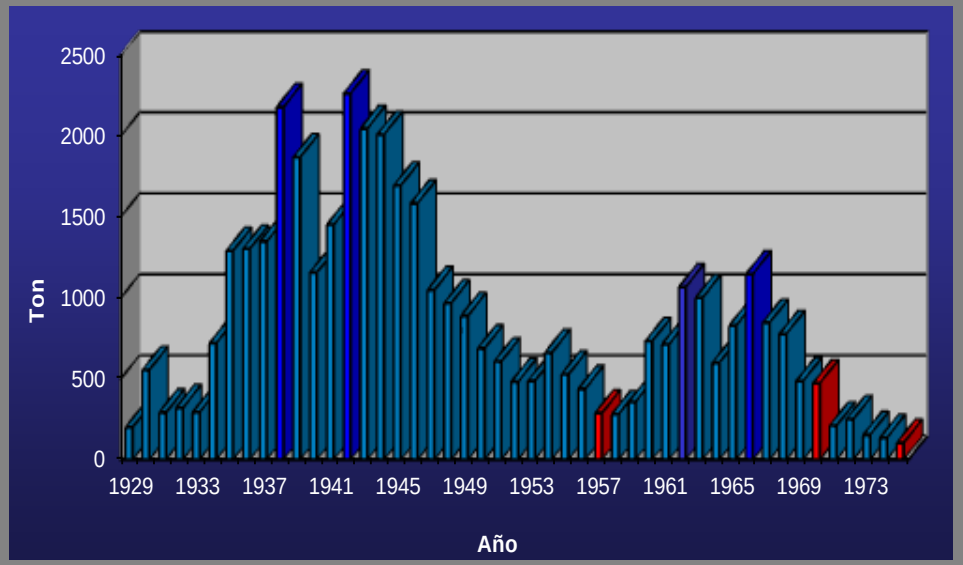


Background

Totoaba macdonaldi

Data:
- Max Age (L∞) 50 years.
- Max Rep. Length 2.30 m.
- Size at First Year 50 cm.
- Weight at First Year 1-3 kg.
- Max Weight 150 kg

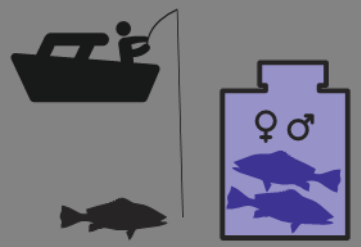


Habitat:
- Endemic to the Gulf of California
- Distribution / Migration Pattern

Importance: Historic/Ecologic/Economic

- Commercial and Sport Fishery
- Historical Value due to Swim Bladder
- NOM-059-ECOL-2001
- CITES Appendix I

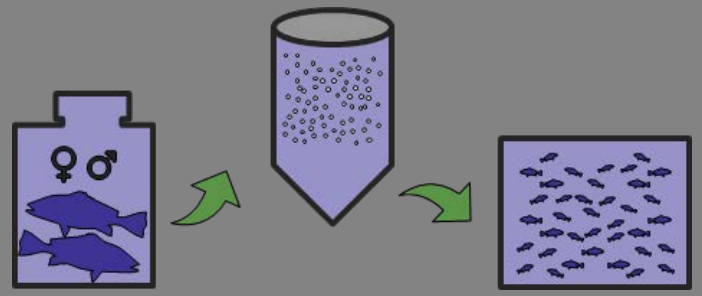
- Meat firm white low fat content.



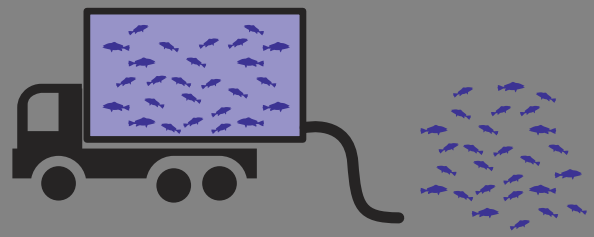
Brood Stock acquisition and maintenance

- Four collections over the past 20 yrs. (60 wild BS)
- Improvements in decompression procedure during capture, transport and acclimation results in 50-65% survival.
- Maturation in captivity with an optimal 10 years period of egg production once they are 8 y.o.
- Observed fecundity ~ 1 million eggs for a 10 kg female and an additional million for every kg of weight gain.
- Fertile eggs are produced through manipulation of environment and assisted by hormonal treatment.

Captive Breeding Biotechnology

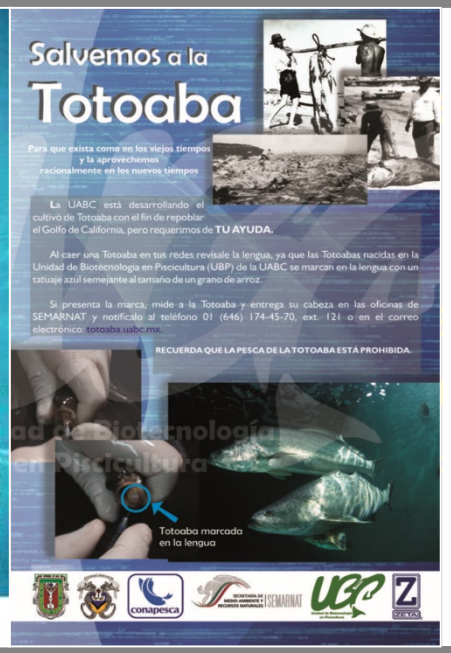


- **Selective breeding procedures: Pedigree**
- **Improved protocols for larvae to juvenile rearing:**
  - ✓ **T°C / Light / Stocking densities / Feeding with species specific nutritional requirements, etc.**
  - ✓ **Scaled up to produce 50,000 juveniles from each million eggs (5% survival).**
  - ✓ **Cannibalism / Size disparity / Deformities**
- **Base Unit (Lab) with overall costs estimates based on real production from BS capture to fingerlings.**
- **Current installed capacity**
  - ✓ **16 Wild-BS (1♀:1♂) , 38 F1-BS (14♀:10♂:14?)**
  - ✓ **Fertile egg production up to 35 millions**
  - ✓ **Larval rearing up to 1.5 million / run (max. 3 runs)**
  - ✓ **Limited space for weaning and grow out.**

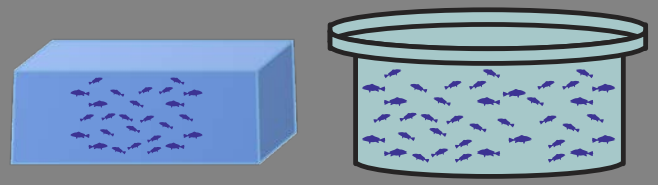


Experimental Releases

- Eight experimental releases into wild stock starting in 1997: Nearly 23,000 individuals.
- Physical / Genetic tagging:
  - Evaluation of stock enhancement feasibility
  - First genetically tagged recaptures in 2014
- Awareness: Community participation



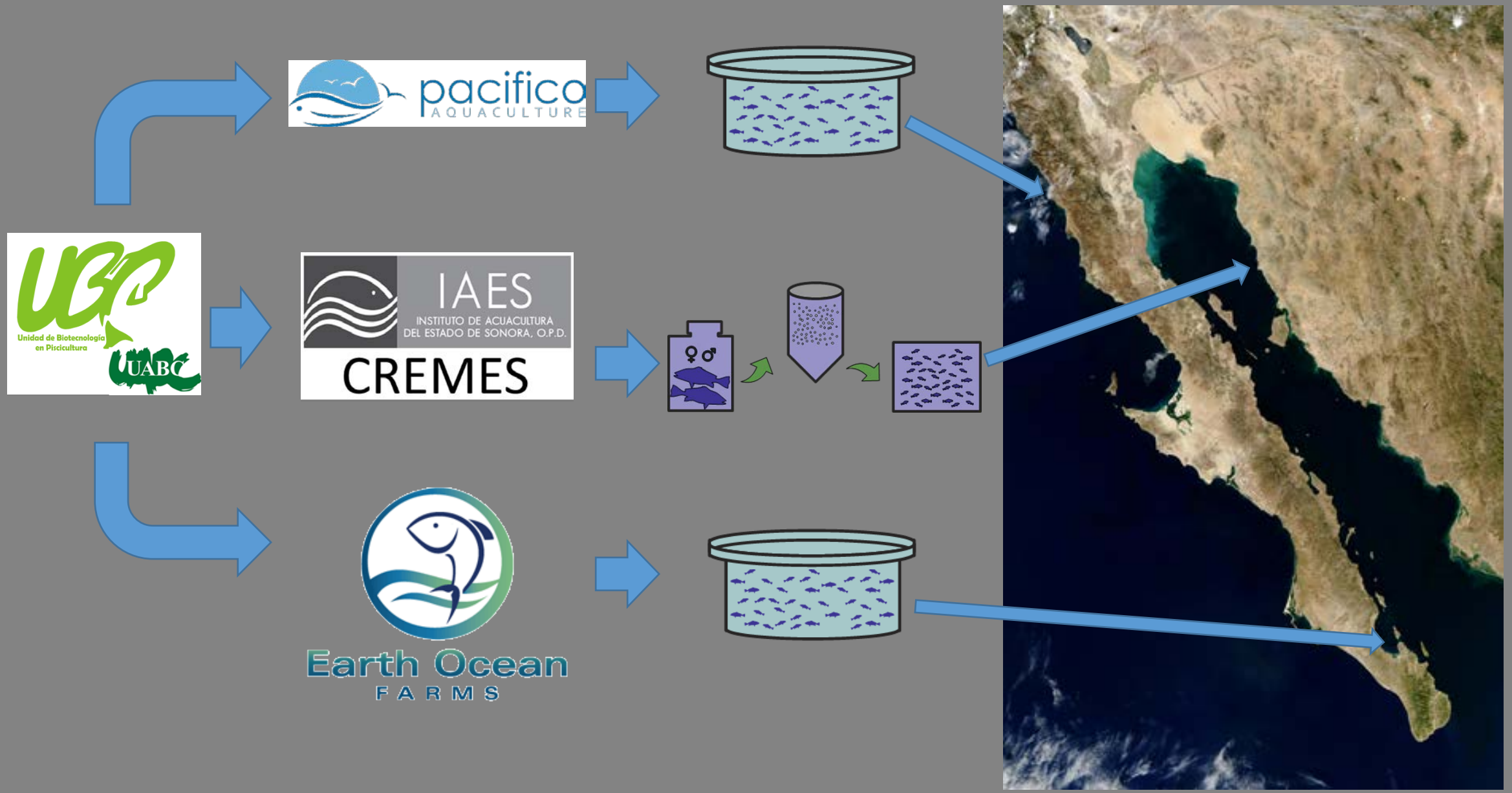
Mariculture Development



- **Land-based grow out trial ***
  - ✓ **PEZCO / PROVIPSA**
  - ✓ **2010 First authorization to sell captive-bred totoaba.**
  - ✓ **Selection of F1 generation animals for aquaculture-oriented brood stock.**
  - ✓ **UABC: First captive breeding unit registered as an Environmental Management Unit (UMA).**
- **Marine farms development ***
  - **Delly / Pacifico / EOF: Lots from UABC´s UMA**
  - **Surface and submerged cages**
  - **Current development of cost-effective diet for grow out**

* **All productive and commercial activities under NOM-059 regulation (SEMARNAT-DGVS).**

Current Totoaba UMAs



Genetic Markers: Objectives

Selective captive breeding program:

- ✓ Constrain inbreeding and pedigree selection
- ✓ Evaluate performance of wild and captive reared broodstock

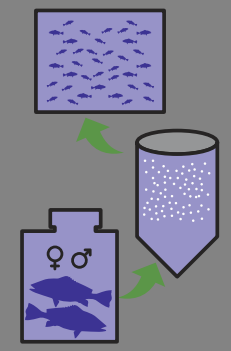
Genetic forensics:

- ✓ Species ID for detection of illegal products
- ✓ Individual traceability to regulate commercialization
- ✓ Ensure transparency in law enforcement

Population dynamics:

- ✓ Estimate current and historical population effective size
- ✓ Demographic trend inference

Captive Breeding Program

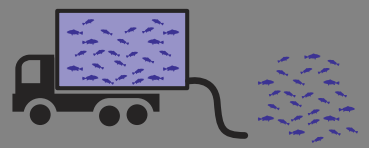


Genetic diversity and relatedness:

- ✓ High nuclear and mitochondrial diversity (*Ho* = 0.71; *H* = 0.91)
- ✓ Very low relatedness within broodstock (*R* = 0.02)

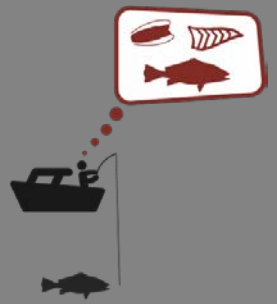
Parental assignment:

- ✓ 100% assignment using only 12 out of 24 tested STRs
- ✓ Low probability of identity among full sibs (1 : 1,500 millions)



Experimental releases to wild stock:

- ✓ Nearly 23,000 color-tagged fingerlings (90% genetic tag)
- ✓ Two seized bladders tracked back to captive breeding



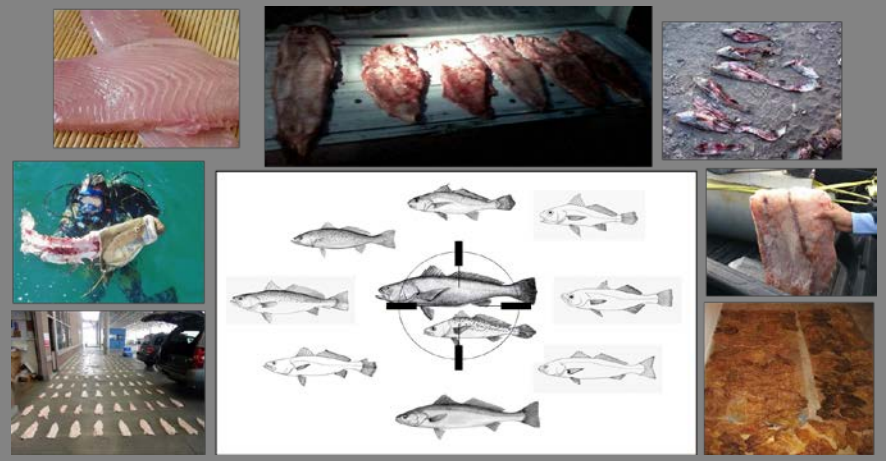
Genetic Forensics

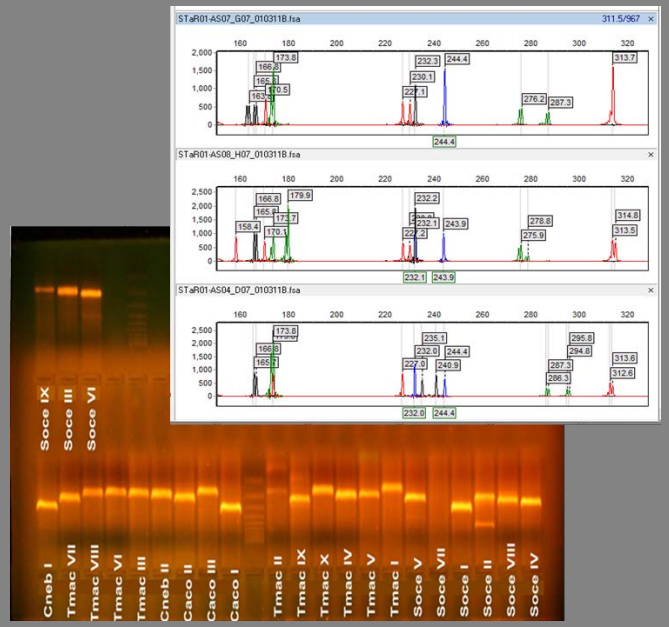
Mitochondrial DNA sequencing:

- ✓ Data base for Gulf of California and Baja California sciaenids
- ✓ PCR quick and specific totoaba ID protocol (2-4 hours)

STRs (24 microsatellite loci):

- ✓ High resolution: Guaranteed individual-traceability



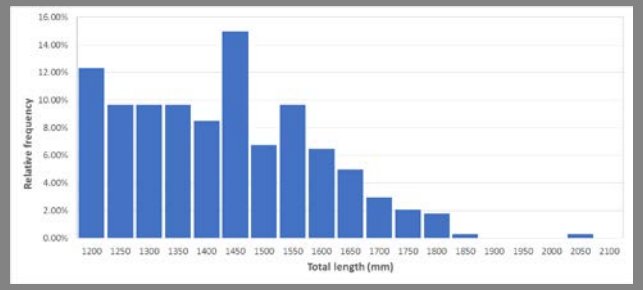


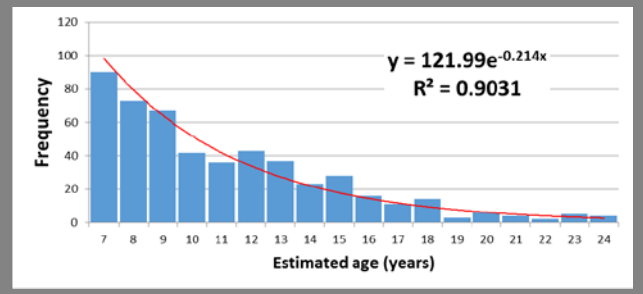
Wild stock dynamics

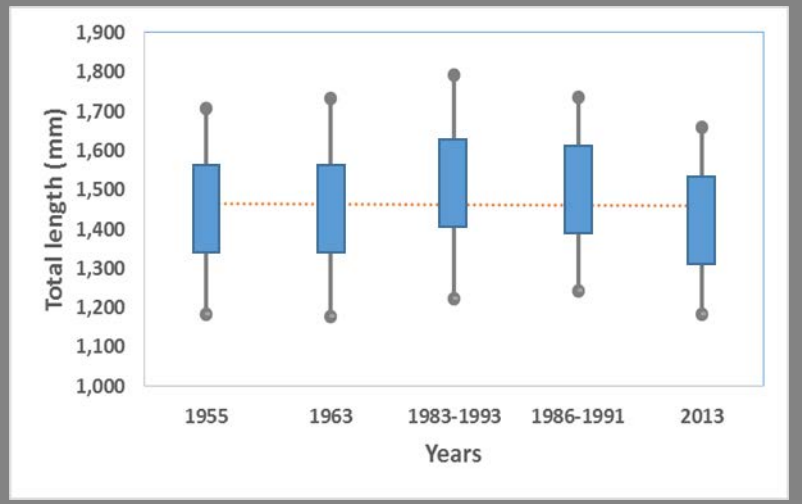
Morphometric data from seizures: No sign of stock depletion

- ✓ No significant shifts in size and age structure in the last 60 years
- ✓ Age of first reproduction, individual growth rate and adult mortality are also the same than in early 50's. All above suggest a stable stock.

USFWS / PROFEPA / PGR



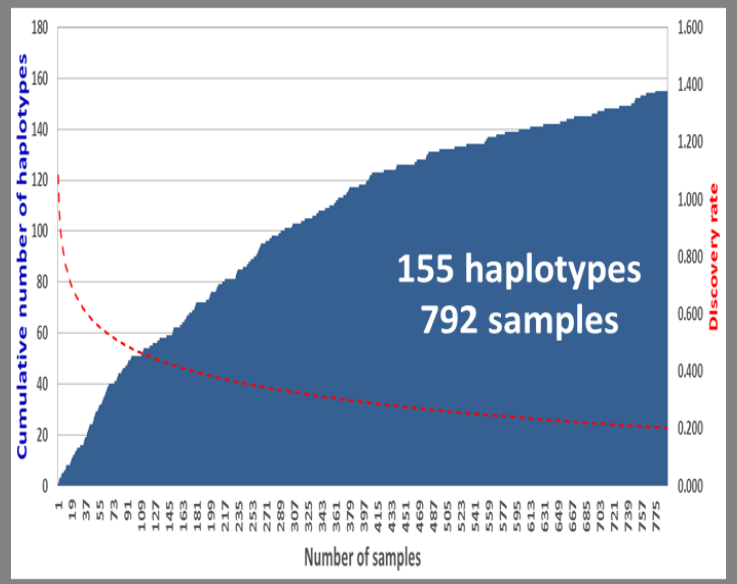


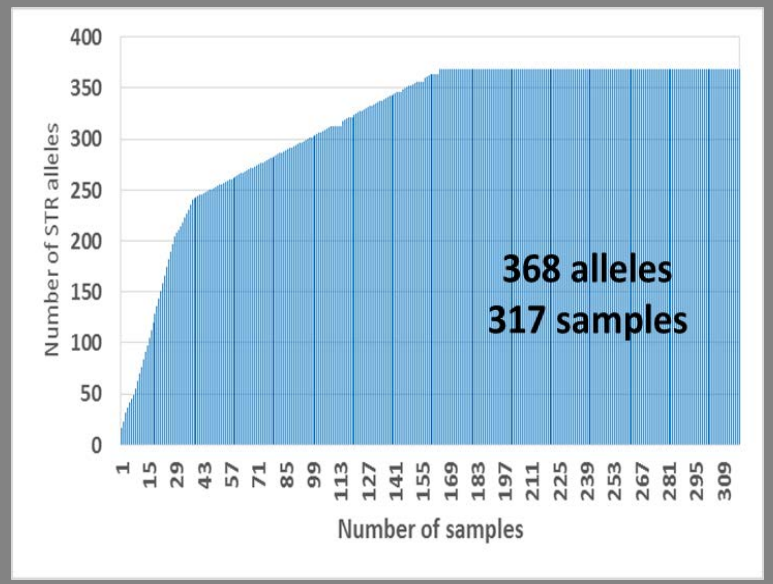


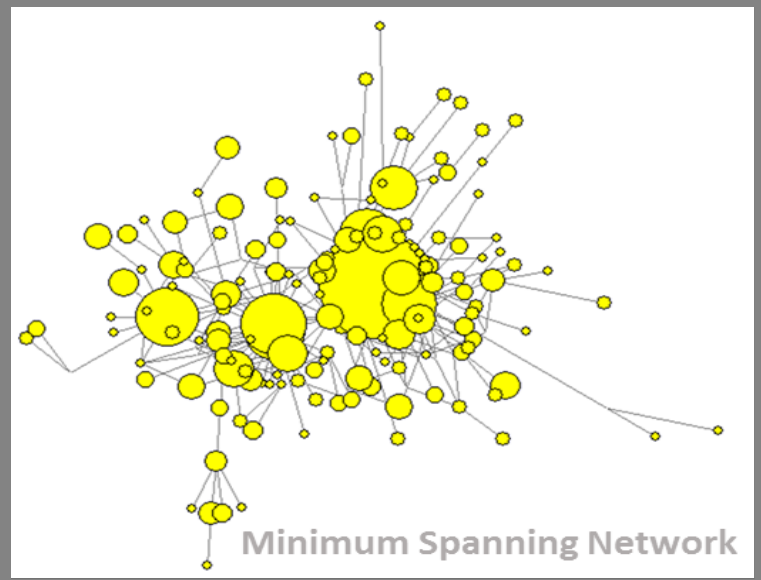
Wild stock dynamics

Genetic diversity and demographic history:

- ✓ High genetic diversity is not congruent with assumed fishery collapse.
- ✓ Demographic tests do not support a recent severe bottleneck.



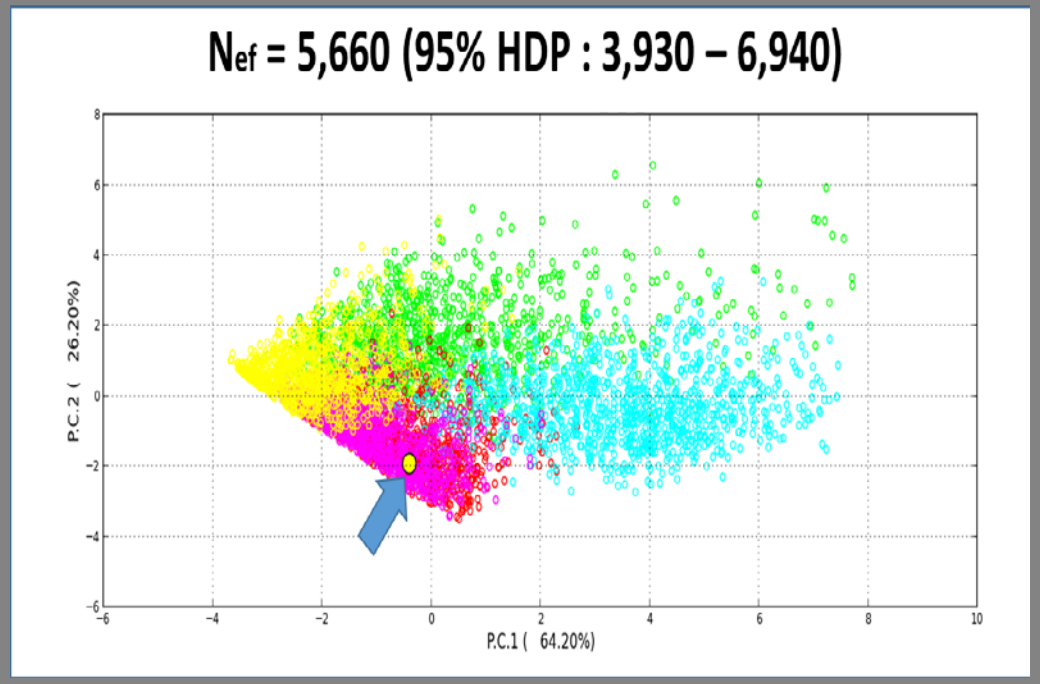


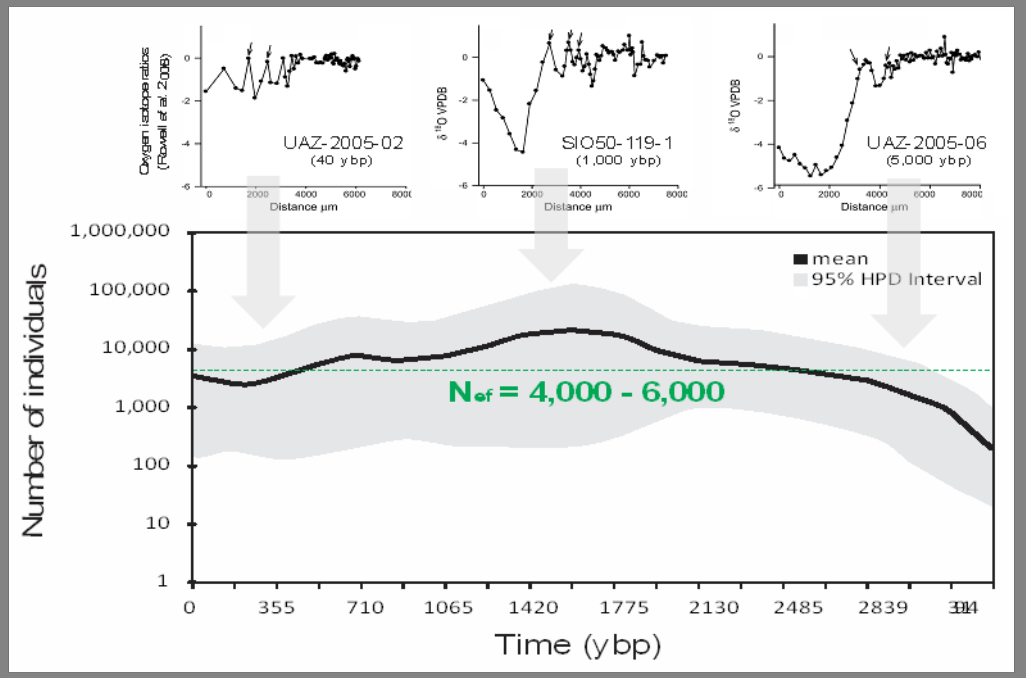


Wild stock dynamics

Effective Population Size (Ne)

- ✓ **Estimated Ne supports a stable demography and ensures long term conservation of genetic diversity** (Enríquez *et al.* 2008, Valenzuela-Quiñones *et al.* 2014, UABC 2015).
- ✓ **Major demographic events depicted from genetic data did not occurred within the timeframe of anthropogenic impact to totoaba population** (Valenzuela-Quiñones *et al.* 2014, UABC 2015).

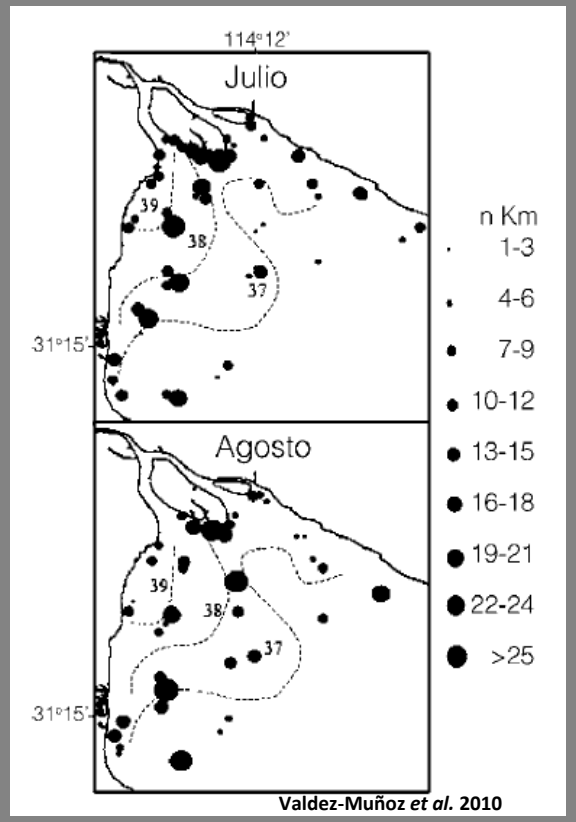




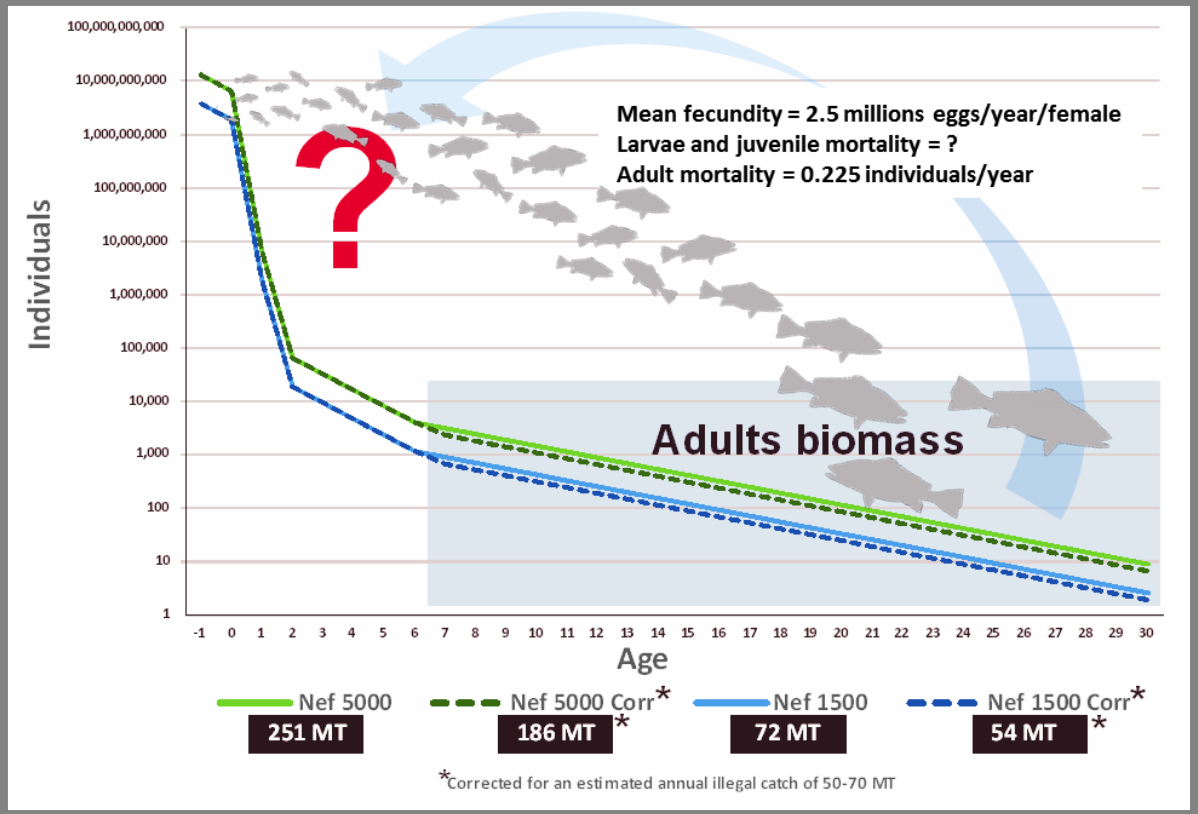
Wild stock dynamics

Demographic trend simulations:

- ✓ **Simulations based on effective population size estimates and current knowledge on totoaba life history suggest juvenile mortality as the main issue in stock recovery.**



Valdez-Muñoz *et al.* 2010

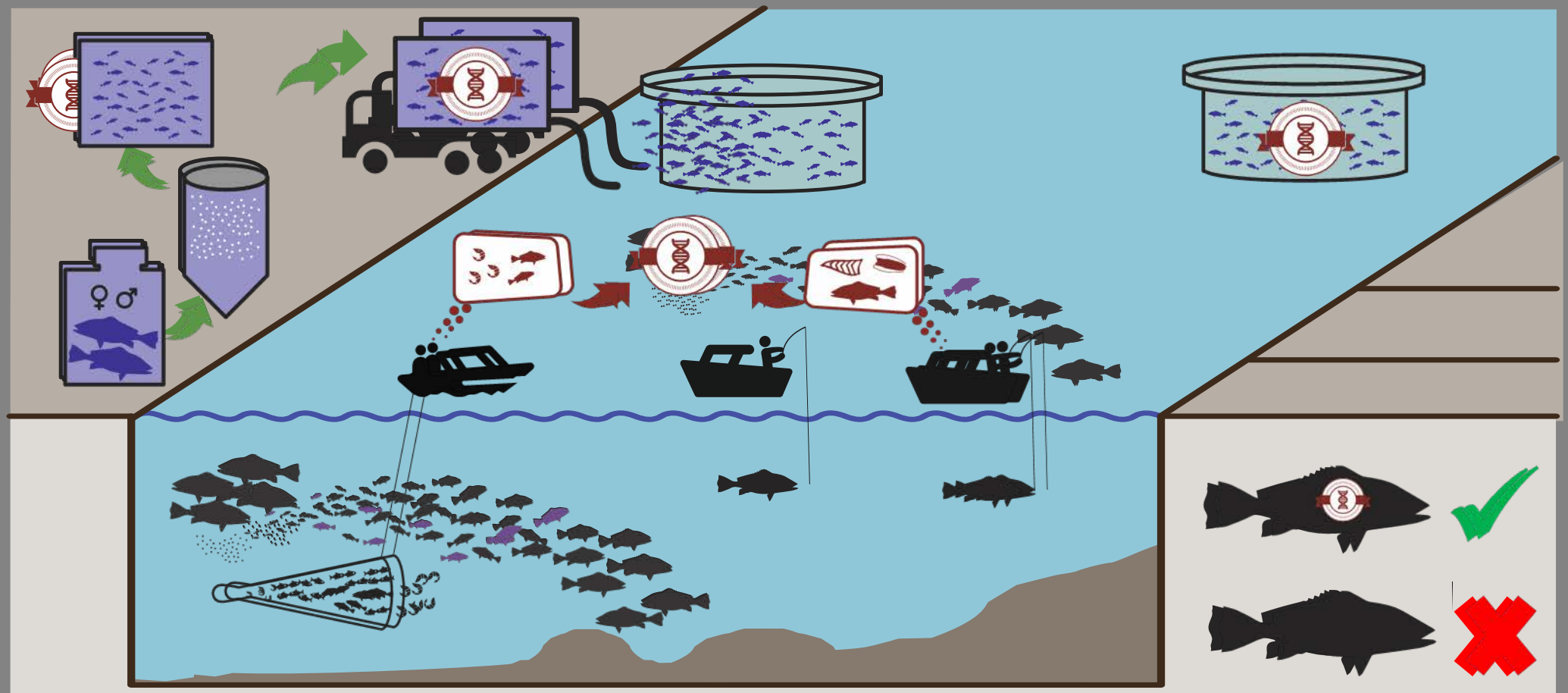


Summary

- ✓ **Captive breeding technology is fully developed and has spread.**
- ✓ **Traceability can be guaranteed, down to individual level.**
- ✓ **Current available data suggest totoaba wild-stock is not critically endangered.**
- ✓ **First two recaptures of captive-reared totoaba: Survival to adult stage.**

Stock Enhancement Feasibility

Genetically Monitored Stock Enhancement



Long-term wild stock genetic monitoring

Requested Specific Outcomes

- **Collaborate to complete the standardization, optimization and validation of a high-resolution and low-cost genetic markers set for genetic traceability of totoaba captive-reared and wild stocks.**

- **Improve and scale-up, with support from the corresponding Governmental Agencies, current breeding and rearing capabilities at Environmental Management Units (UMAs) in order to properly start a stock enhancement pilot program.**

- **Set an agreement with the Mexican Federal Governmental Agencies to establish a mandatory genetic traceability program. Building up a traceability data base from all available sources (captive breeders, fingerlings by-catch, seizures, scientific collections and recreational catch, if any) is critical for law enforcement and long-term genetic monitoring wild stock.**

Species	IUCN	N	n_H	π_n	H	Annual Catch	Reference
Atractoscion aequidens	NE	104	32	0.005	0.85		Henriques et al. 2014
Atractoscion aequidens	NA	40	19	0.008	0.90		Henriques et al. 2014
Atractoscion nobilis	LC	59	32	0.010	0.96	1,500 MT	Ríos (2010), SAGARPA (2013)
Collichthys lucidus *	NA	151	55	0.034	0.89	320,000 MT	SNFRI (2001), Song et al. (2013)
Cynoscion acoupa	LC	297	83	0.003	0.89	15,000 MT	Fundación PROZEE (2006), Rodrigues et al. (2008)
Cynoscion nebulosus *	NA	280	60	0.024	0.85	4,500 MT	NMFS (2000), Anderson y Karel (2010)
Larymichthys polyactis *	NA	127	125	0.013	1.00	388,000 MT	FAO (2010), Wu et al. (2012)
Nibea albiflora	NA	65	37	0.008	0.97		Han et al. 2008a
Pennahia argentata *	NA	132	113	0.026	1.00		Han et al. 2008b
Sciaenops ocellatus *	NA	209	134	0.030	0.98	700 MT	Van Voorhees et al. (1992), Seyoum et al. (2000)
Cynoscion reticulatus	LC	42	31	0.016	0.99		Ríos 2012
Cynoscion othonopterus	V	92	23	0.004	0.69	2,600 MT	Rodriguez-Quiroz et al. (2010), Ríos (2012)
Totoaba macdonaldi	CE	74	42	0.013	0.98		Quezada 2009
Totoaba macdonaldi	CE	792	155	0.013	0.96	60 MT	UABC 2014



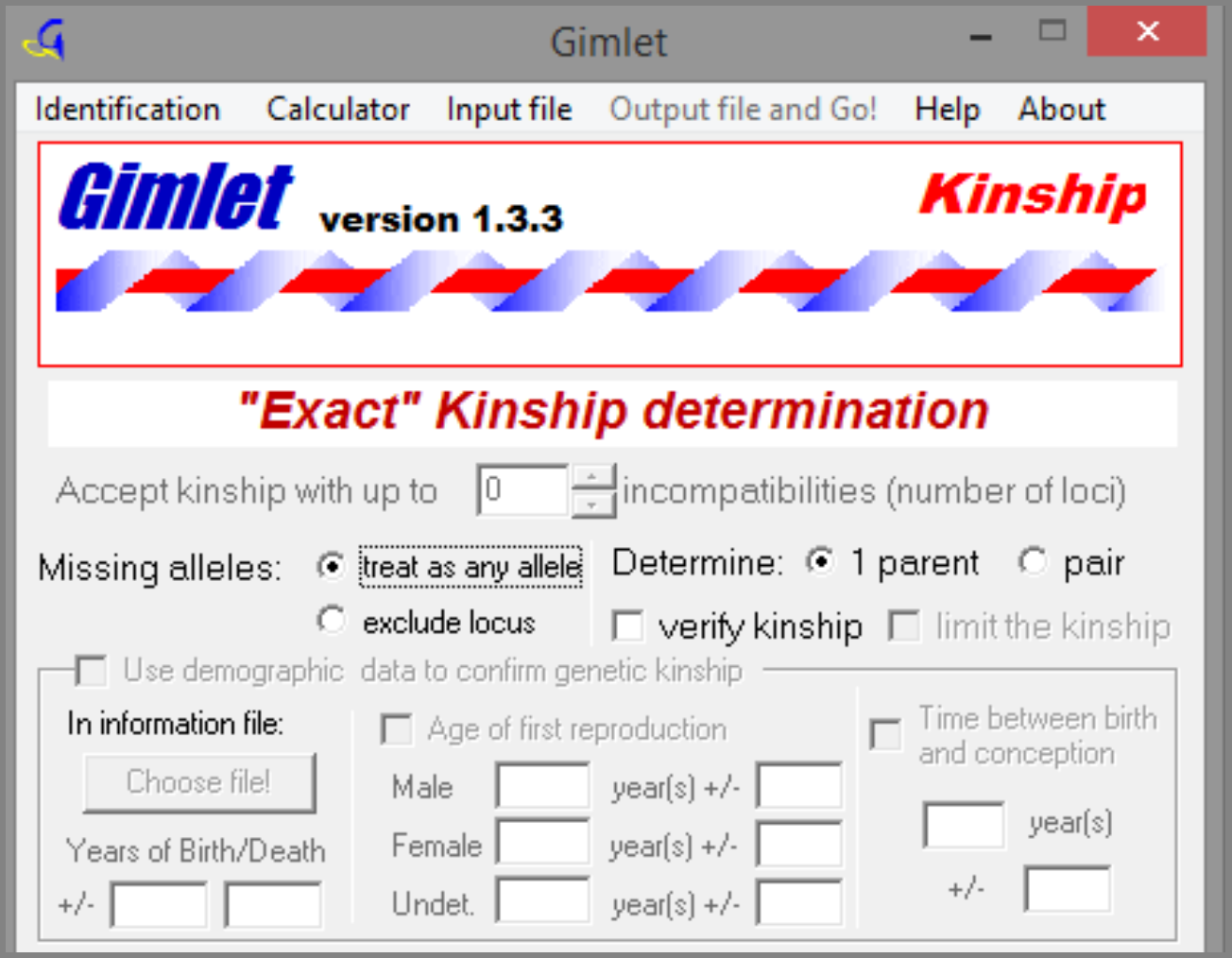
Year/Color	N	Current age
1997	200	17 y.o.
1999	600	15 y.o.
2000	1200	14 y.o.
* 2001	2100	13 y.o.
2002	1600	12 y.o.
* 2007	2000	7 y.o.
2012	2070	2 y.o.
2013	12000	1 y.o.

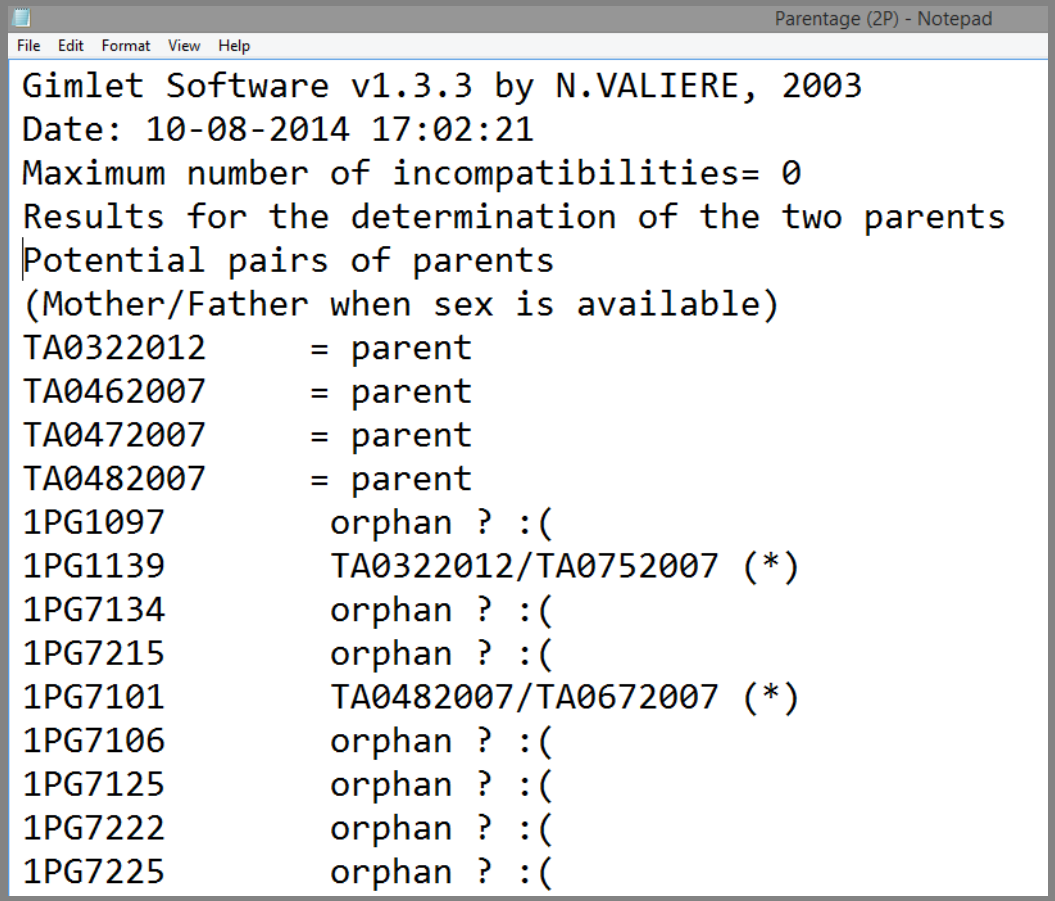
Unidad de Biotecnología en Piscicultura

22,970

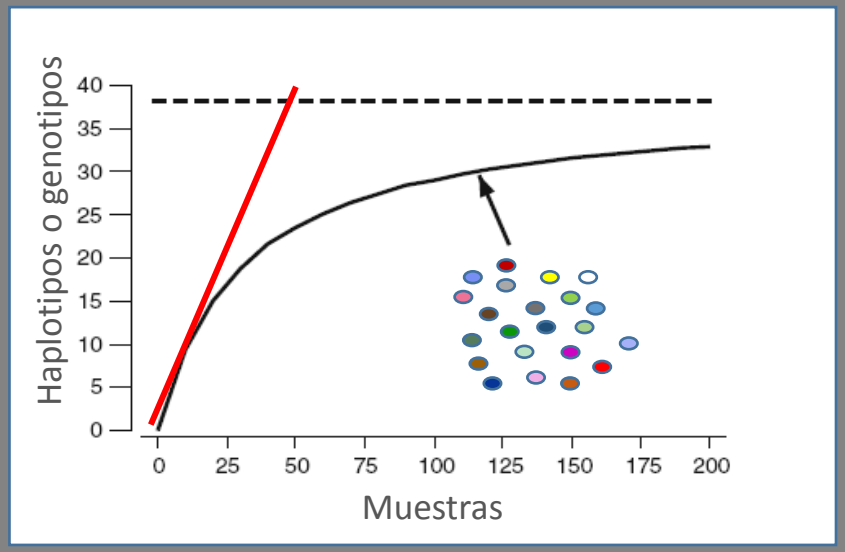
individuals tagged and released to the wild

Cohort	# Released	Current age	
1997	200	17	
1999	600	15	
2000	1,200	14	
2001	2,100	13	1 Recapture? UBP (18 STRs)
2002	1,600	12	
2007	2,000	7	1 Recapture UBP (24 STRs)
2012	2,070	2	
2013	12,000	1	

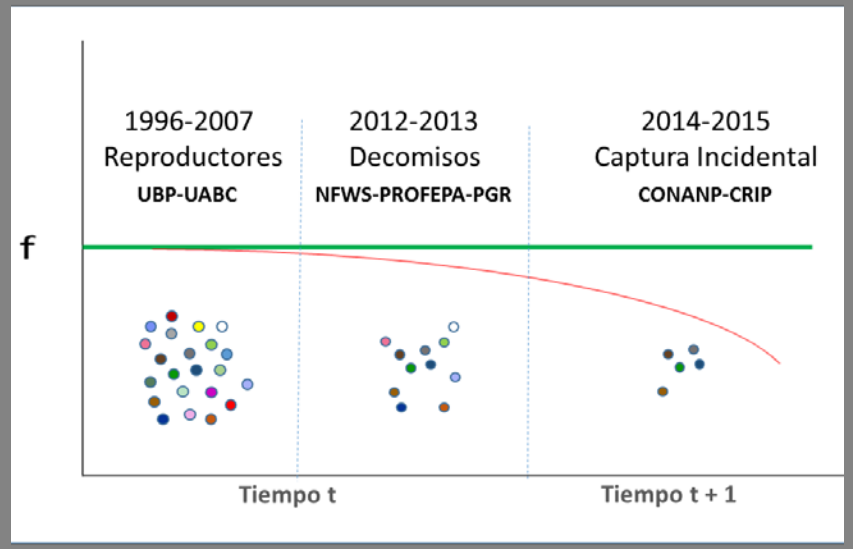

Gimlet
Identification
Calculator
Input file
Output file and Go!
Help
About
Gimlet
version 1.3.3
Kinship
"Exact" Kinship determination
Accept kinship with up to
0
incompatibilities (number of loci)
Missing alleles:
treat as any allele
exclude locus
Determine:
1 parent
pair
verify kinship
limit the kinship
Use demographic data to confirm genetic kinship
In information file:
Choose file!
Years of Birth/Death
+/-
Age of first reproduction
Male
year(s) +/-
Female
year(s) +/-
Undet.
year(s) +/-
Time between birth and conception
year(s)
+/-


Parentage (2P) - Notepad
File Edit Format View Help
Gimlet Software v1.3.3 by N.VALIERE, 2003
Date: 10-08-2014 17:02:21
Maximum number of incompatibilities= 0
Results for the determination of the two parents
Potential pairs of parents
(Mother/Father when sex is available)
TA0322012 = parent
TA0462007 = parent
TA0472007 = parent
TA0482007 = parent
1PG1097 orphan ? :(
1PG1139 TA0322012/TA0752007 (*)
1PG7134 orphan ? :(
1PG7215 orphan ? :(
1PG7101 TA0482007/TA0672007 (*)
1PG7106 orphan ? :(
1PG7125 orphan ? :(
1PG7222 orphan ? :(
1PG7225 orphan ? :(

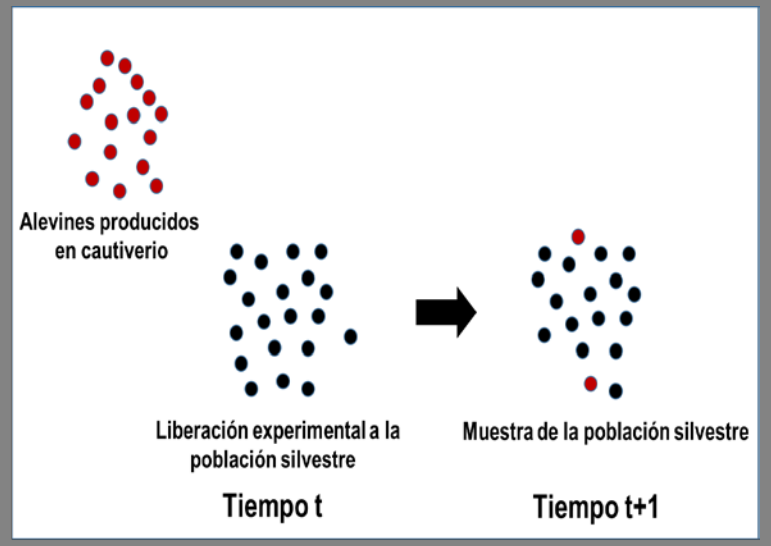
Rarefaction (Discovery rate)



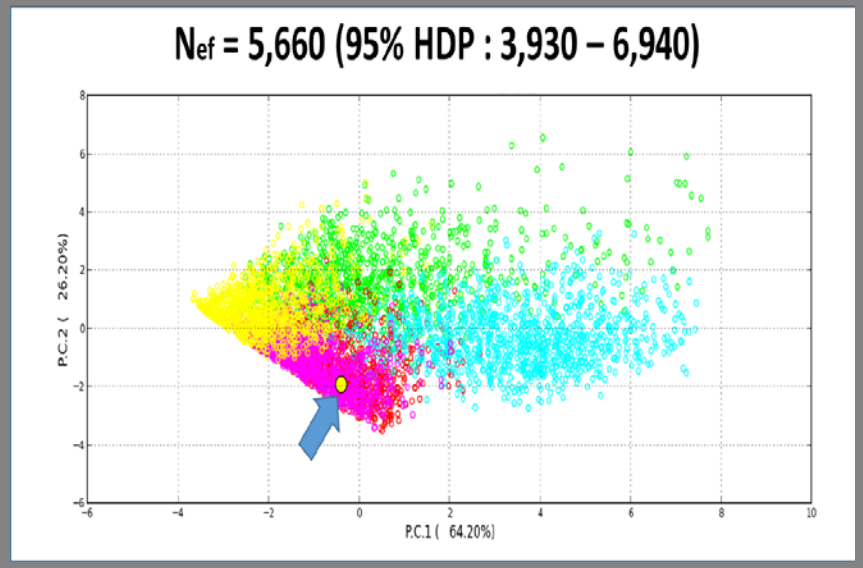
Temporal Shifts in Allele Frequency



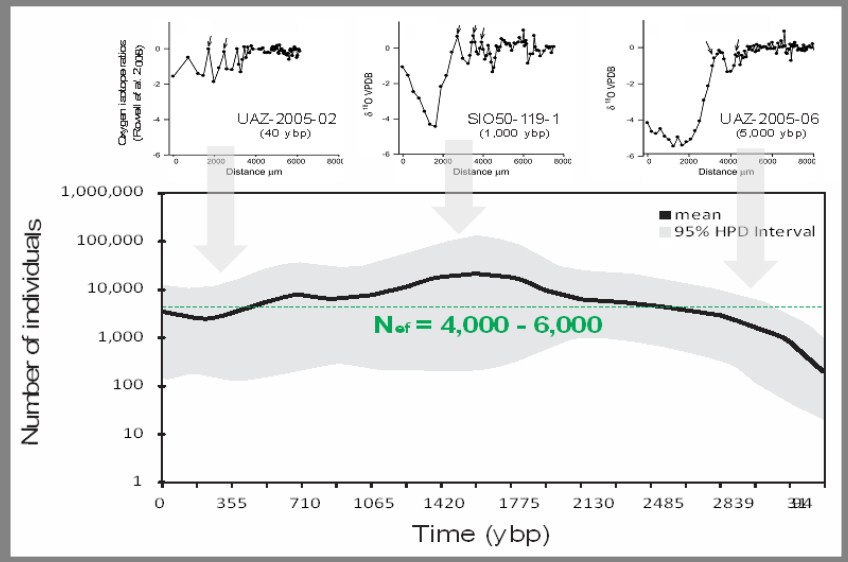
Capture - Recapture

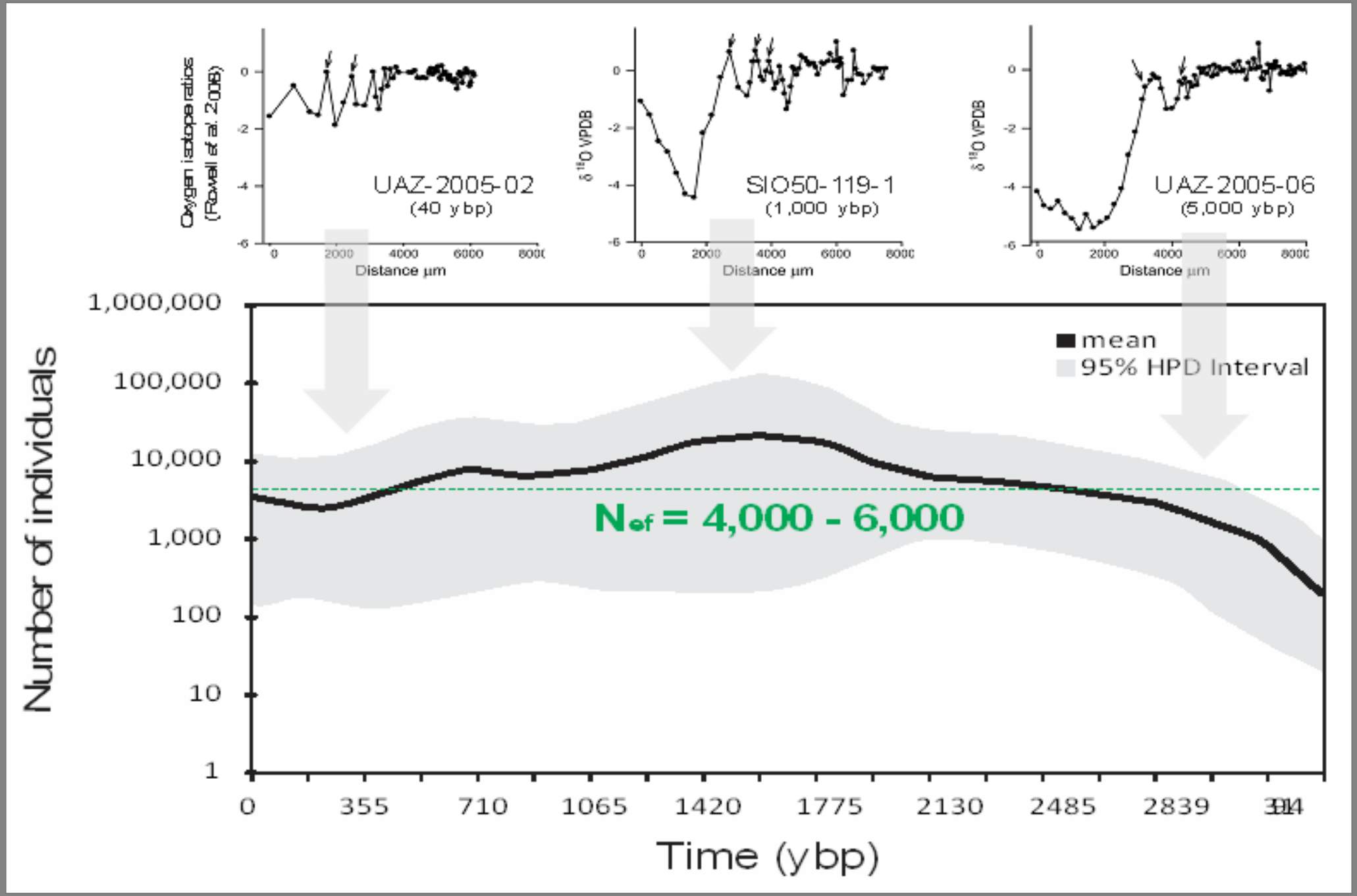


Aproximate Bayesian Computation Past Inference



Coalescent Bayesian Skyline Plot



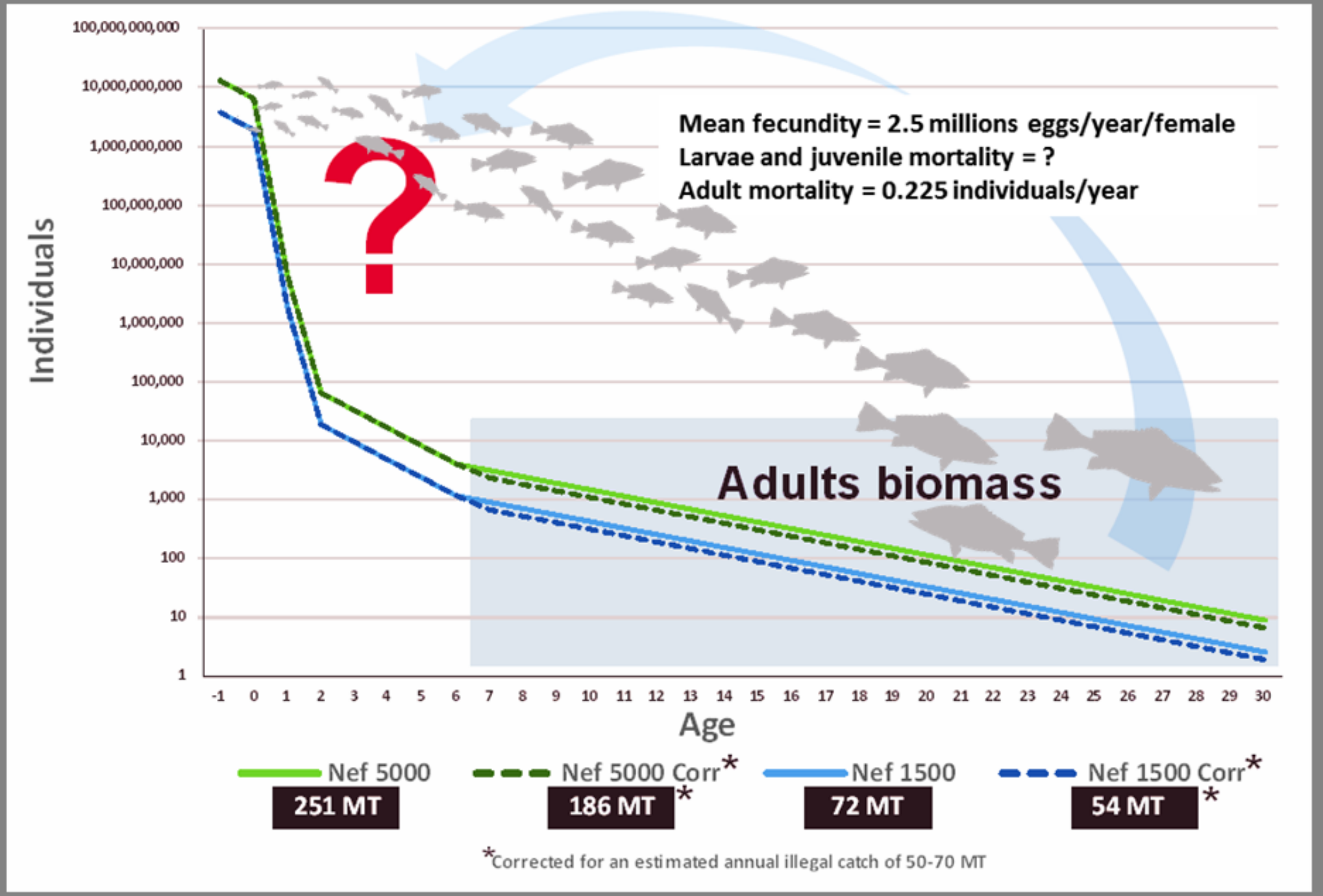

Oxygen isotope ratios (Rowell et al. 2008)
UAZ-2005-02
(40 ybp)
Distance µm
δ18O VPDB
SIO50-119-1
(1,000 ybp)
Distance µm
δ18O VPDB
UAZ-2005-06
(5,000 ybp)
Distance µm
mean
95% HPD Interval
Nef = 4,000 - 6,000
Number of individuals
1,000,000
100,000
10,000
1,000
100
10
1
0
355
710
1065
1420
1775
2130
2485
2839
Time (ybp)

Effective population size (breeding adults)

Markers	Method	n	N_{ef}	N_e	Reference
STRs (16)	MsVar	180	-	2,670	Valenzuela-Quiñones *et al.* 2014
STRs (16)	Nei	180	-	1,900	Valenzuela-Quiñones *et al.* 2014
STRs (16)	LD Ne Estimator	180	-	2,760	Valenzuela-Quiñones *et al.* 2014
STRs (16)	OneSamp	180	-	1,800	Valenzuela-Quiñones *et al.* 2014
STRs (16)	DIYABC	180	-	2,680	Valenzuela-Quiñones *et al.* 2014
STRs (24)	DIYABC	317	-	4,250	UABC 2015
mtDNA (1)	Temporal Method	573	5,370	10,740	UABC 2015
mtDNA (1)	DIYABC	792	5,560	11,120	UABC 2015
mtDNA (1)	BEAST-BSP	792	4,500	9,000	UABC 2015

Census population size for 1 y.o. Cohort

Markers	Method	n	N	Reference
Multilocus genotype (2)	Capture-Recapture	119	150,000	UABC 2015


Individuals
100,000,000,000
10,000,000,000
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Age
Mean fecundity = 2.5 millions eggs/year/female
Larvae and juvenile mortality = ?
Adult mortality = 0.225 individuals/year
Adults biomass
Nef 5000
251 MT
Nef 5000 Corr*
186 MT*
Nef 1500
72 MT
Nef 1500 Corr*
54 MT*
*Corrected for an estimated annual illegal catch of 50-70 MT