Gila River Basin Native Fishes Conservation Program - Technical Committee Meeting
December 11-13, 2018
US Forest Service Silver City Ranger Station
3005 Camino del Bosque, Silver City, NM 88061

Meeting Notes

Meeting Objectives

- Provide updates on 2018 accomplishments and 2019 work planned
- Discuss and prioritize 2020 proposed projects and Tier 2 projects
- Provide updates on other projects within the GRBNFCP

Next Meeting

- December 10-12, 2019 in the Verde Valley/Cottonwood, AZ

Participants – see last page

Throughout the two-day workshop there were many presentations given via powerpoint, these will be uploaded for viewing to the Gila River Basin Native Fishes Conservation Program website (https://www.usbr.gov/lc/phoenix/biology/azfish/).

2018 Annual Reporting - *(see website)*

Agency representatives presented work completed in fiscal year 2018, in-person and via webex; there was time after each presentation for question and answer and discussion.

- Arizona Game and Fish Department – *Tony Robinson and Brian Hickerson, Arizona Game and Fish Department (AGFD)*
    - Gila topminnow

        - AZGFD presented a table showing 13 sites (e.g. Tortilla Creek, Charlebois Spring, Bonita Creek and Spring Creek) that are doing well and numbers are increasing. They presented three sites that don't have a great prospects going forward (see presentation) were highly affected by drought, especially noted in Arnett Creek
- Desert Pupfish
    - Activities this year included monitoring nine sites. One of the sites (Bonito Creek) lacks habitat because a flood blew out the beaver dams and no fish were captured there this year, or the previous 3 years.
- Spikedace – 3 locations as of last week (12/4/2018);
    - This year AZGFD had three stockings (Fossil Creek, Spring Creek and Blue River (middle)) and four monitoring sites (Blue (lower), Spring Creek, Blue (middle), and Hot Springs Canyon).
    - Lower Blue River – Spikedace are doing well and are considered established. It took a few years for the conditions to be right for spawning and successful recruitment. A wet winter in 2016 likely contributed to the spike in numbers captured in 2017. More research on that needs to be done.
    - Spring Creek – chub may be a predator here (speculation) and may affect recruitment. Not yet considered established.
    - Middle Blue River – This is one of the newer spikedace projects (first stocked in 2017) fish held in cages for eDNA study before stocking. The habitat here looks promising for Spikedace. Source of fish is the Lower Blue, so stocking middle Blue is dependent on abundance in the lower Blue..
    - Hot Springs – the stream can be small during the hot months and habitat is limited during that time. Spikedace numbers have decreased since last stocked, and only 1 or 2 captured in the last two years.
- Loach minnow – 3 locations
    - This year AZGFD had no stockings, three monitoring sites (Blue (lower), Hot Springs Canyon, and Bonita), and two collections for ARCC.
    - Lower Blue River project is looking successful (augmentation); Loach Minnow abundance has consistently increased in the last few years
    - Hot Springs population appears relatively small but persistent over time
    - Bonito Creek hasn't resulted in captures by AZGFD since 2015, although BLM does have eDNA results showing positive for loach minnow in 2017. This area only had one stocking in 2014, unlike other locations. Habitat is affected by beavers, so this site may need more consideration for continuation.
- Roundtail Chub – all sites appear to be doing well.
    - This year AZGFD conducted one stocking (Harden Cienega), five monitoring sites (see table in presentation), and one fish health assessment.
    - In the lower Blue River, it was promising that the year-classes are maintaining numbers through the last couple years, and more chub were caught this year than in the last – resilient to drought; fish likely responded to months in 2016 that were all above base flow.
- Non-native species removal – 4 sites (Blue River, Redfield Canyon, Red Tank Draw, and La Barge Draw)
    - Continue to monitor (snorkel surveys) in Blue River to verify that there are NO sunfish before stocking native fish, it seems that green sunfish, channel catfish have been extirpated from this site and AZGFD did not observe any red shiners and fathead minnows in 2018 .
    - Redfield canyon – This site had been divided into 3 reaches for removal purposes. Need is to maintain removal of sunfishes particularly in the upper reach to protect existing native fish until the barrier is installed.
    - Red Tank Draw – needs more coordination with private land owners to address sunfish in tanks. Rarick Draw has a great barrier but there are still non-native bullhead and Fathead Minnow upstream. This is a "maintain the chub population" project (they are still

relatively abundant). Access to much of the drainage is difficult, but (if allowed) treating the upstream tinajas like ponds would be relatively easy. Seasonal flow connects this area to Wet Beaver Creek.

- Translocation sites – 14 sites assessed
  - Some sites had non-native populations and 4 sites did not have sufficient habitat for native fish species.
  - 2 sites, Romero Canyon and Upper Sabino Canyon appeared to have habitat for Gila topminnow and Roundtail chub; Romero Canyon would need some partner agency coordination to extend the range of the Roundatil Chub upstream.
- Discussion
  - Unable to collect Aravaipa spikedace and loach minnow for the Aquatic Research Conservation Center (ARCC) due to coordination issues, there is still opportunity to collect in 2018 for research at the ARCC in 2019
  - Loach minnow – collected 223 from Blue River and transported to ARCC. This number was determined as a percentage of last years catch. Anticipate higher numbers going forward as Loach Minnow rebound from the Wallow Fire

- Aquatic Research and Conservation Center (ARCC) – *Josh Walters and Kristopher Starr, ARCC*
  - 2nd year of spawning in the cage 1 raceways following renovation.
  - All research with Aravaipa fish (spikedace and loach minnow), pumps can simulate high flow events. There cannot be more replicates unless there are more fish for more lineages (3 for spikedace and 4 for loach minnow all held separately).
  - Try to compare the distinct nesting locations, vs. letting them disperse themselves – all larvae must be removed so they are not eaten by their "parents"
  - ARCC staff conducted spawning density trials this year to determine the relationship between broodstock density and larval production. Spikedace and loach minnow both appear to produce more fish when broodstock is less dense. Spikedace had a linear relationship while loach minnow exhibited an exponential decay.
  - Same study will be conducted again next year – lower densities are important, especially in the Aravaipa lineages.
  - Loach minnow (anecdotal findings) – almost all pertain to loach minnow (except algae)
    - Loach minnow in the wild don't live past 3 years, but at the ARCC there are fish that are 6 years old – those are still counted within the determination of brood stock
  - Tagging will help determine what year they come in to the ARCC
  - Post-stocking survival – could be done by ARCC staff that wouldn't impact the workplan of others
    - Concern of causing mortality by over sampling (electroshocking)
  - When there are too many fish at ARCC there is a risk of die off due to disease, if they stay another year, they lose a year of reproduction which is difficult for a short-lived species
  - Discussion
    - Have attempted a 50-50 male to female ratio
    - Used best guess for distance between nest sites, there is literature that suggests if there are landmarks the reduction in distance can be achieved – something to consider
    - Age of loach minnow
      - Is there data to show which age is reproducing?
      - There is anecdotal evidence that as they age they decrease in number of fish produced.

- New Mexico Department of Game and Fish (NMDGF) – *Bryan Ferguson, NMDGF*
  - West Fork Gila non-native removal
    - Propst et al. paper showing the removal of non-natives has been helping the populations of native fish.
    - Overall abundance of all fish increased from 2017 to 2018 (see table in presentation).
    - Yellow bullhead is coming back with large numbers of mostly young of year fish.

- Little Creek (repatriation) – stocked 12/10/2018 with 1187 more loach minnow
- Mule Creek (repatriation) – Roundtail chub seem to be persisting, encouraging, as they have not been stocked since 2014
- Burro Cienaga (repatriation) – with turbid water the efficiency of capturing topminnow decreases
- Gila Farm Pond – all fish found after shocking were non-native and no native fish detected
- Discussion
  - Mule Creek is encouraging for how many chub we are seeing, AGFD to give 2 more stockings, not a lot of them, but they are throughout
    - Dries at the mouth and starts to dry at the bottom, this may keep the non-natives out.
    - There are deep pools that can't be touched with a shocker, but eDNA for the small mouth may be helpful
- Middle Fork inventory was completed in the upper section in June.

- Bureau of Land Management, Safford – *Heidi Blasius, BLM*
  - Background
    - BOR and BLM Washington Office has helped fund the Bonita Creek project over the years
    - Barrier established in 2008
    - Lower Bonita Creek was treated with piscicide in 2008
    - In 2009, nonnatives, including Mosquitofish and Green Sunfish were found in the treated reach. Mechanical removal initiated at time of discovery of Green Sunfish.
    - Bonita Creek is closed to fishing, but still receives some fishing pressure from the surrounding communities.
  - Monitoring and Managing non-native fish species
    - 15 low water road crossings that provide administrative and recreational access throughout lower Bonita Creek
    - During mechanical removal, the creek was divided up by the road crossings to create manageable sections.
    - Beaver pools were notched during removal effort to reduce volume of water. Reducing depth of beaver pools was necessary for traps to be set with an air pocket to prevent drowning of non-targeted wildlife. Removal of dead and dying cattails (habitat for Green Sunfish) helped too.
    - Beaver dams reduced and in some cases prevented non-native fishes from moving upstream (act as a natural barrier).
  - Results
    - Over 47,000 net sets resulted in ~23,000 Green Sunfish removed between 2009 and 2018.
    - The 2016 effort resulted in crashing the population of Green Sunfish – a dedicated year-round crew through sufficient funding.
    - No Green Sunfish were captured in 2018
    - Continuing to set nets to determine whether Green Sunfish are completely eradicated – starting to see more Gila Chub and Gila Topminnow, Sonora Suckers are doing well.
    - Full collaborative effort
  - Discussion
    - Net setting in the upper sections was limited as to not impact native fishes
    - The barrier has created pooling on the apron (from beaver dams) and the Green Sunfish are starting to try to leap the barrier.

- eDNA Project Review - *Yvette Paroz, US Forest Service*
  - 5-year agreement with BOR
    - Expand sampling for Loach minnow and Spikedace
    - Develop a southwest eDNA database
    - Develop markers for 10 additional species
  - Locations for collection (prioritized areas)

        - San Francisco River – because of the fire and the effects it could have on loach minnow
        - Verde River
        - Eagle River
        - East Fork Gila River
        - Mainstem Gila River
        - East Fork Black River
    - Loach minnow sampling
        - In the San Francisco, species was found up to the Box Canyon
    - Spikedace
        - In the San Francisco River there is good habitat for putting more fish
        - Need verification on the "presence point" farthest west
    - Next markers for development – group's input
        - Redeye Bass
        - Smallmouth bass (not a cross with redeye bass) – for the Verde, and for Fossil Creek
        - If they can analyze 2 markers per year, this is dependent on fin clips (2-3) from a range of locations
        - Immediate Needs: more sunfish/centrarchids sunfish clips/bodies sent directly to the lab.
        - When sampling/monitoring – if possible, bring back fin clips of different species that are sampled
        - Re: gartersnakes – they are working on both species, but not with great luck
            - Army Corps of Engineers may want to contribute to something that is already happening
            - The problem is that the snakes don't spend enough time in the water to have the eDNA to be picked up, but the markers are developed
        - **Next 4 priority species**
            - Flathead catfish (new, potential invasions)
            - Red shiner (finish, more samples, fin clips)
            - Gila topminnow
            - Mosquitofish
            - Yellow/black/any bullhead
        - Yvette to send what material (and how much is needed) and where to send samples

- NAU habitat assessment project – *Tony Robinson presenting for Jack Torresdal from NAU*
    - Work done through a Heritage Grant – locations where spikedace and loach minnow have been stocked and why they succeeded or didn't

- Rangewide assessment of spikedace and loach minnow – *Keith Gido/Crosby Hedden (via webex) – Kansas State University*
    - This study was initiated in Sept 2018. Three main objectives
        - Identify habitat available for species across range
        - Quantify species needs for successful repatriation.
        - Identify location with high likelihood for successful repatriation.
    - Crew was able to get out this fall to survey.
    - Mesohabitat – riffle, ripple, pool, etc.
    - Loach minnow have no real trend regarding velocity and depth (broad scale/site/reach)
    - Spikedace are avoiding shallow areas and low velocity waters (broad scale/site/reach)
    - Mesohabitat
        - Loach minnow there is a trend
        - Spikedace there is no trend
    - Velocity – Loach minnow likes high velocity and spikedace is all over
    - Depth – Loach minnow and spikedace don't have a strong preference
    - Discussion
        - Results are consistent with past research (a decade ago)

        - Looking at quantifying the size of the river – sm/med or med/lg stream or river
            - Available in the national hydrography
        - Keith: Looking to get input on how this research can help with the goals of this group and its needs on what types of information would be helpful
        - Flows were elevated in October (discharge was measured at every site), will be interesting to conduct the sampling in base flows
        - Spatial distribution of habitat along a stream/river and how that effects the species found there.
        - More indices can be incorporated into this effort and research
        - In general, the invertebrate production exceeded the consumptive demand, but this was conducted before some of the high densities that have been shown recently (see Bryan's presentation)

- Barrier work – *Bill Stewart*
    - 12 barriers by 2023 – 8 are complete, 2 are in progress/paperwork and 2 more are planned (two on the Verde River)
    - Bonita Creek
        - Fix the pooling, could put a beaver dam analog further down, but those already exist naturally
    - Blue River
        - Problem with sedimentation, maybe get a crew in to clean up the excess sediment
        - Continue monitoring in this area for sunfish, especially with the modeling of 2yr and 10yr floods.
        - Trail cameras from June to October (generally)
    - Hot Springs
        - Erosion issue, working with Coconino National Forest to help fix it
    - Verde
        - First assessment of geology, geography, etc. happening soon
        - Lowest barrier right above Sycamore and upper site located above Hell's Canyon
        - Engagement with the Verde Front to have further discussion regarding recreation and barriers.

DAY 2 – December 12, 2018

Genetics Presentations and Discussion *(see website)*

Presentations from Wade Wilson from US Fish and Wildlife Services, and Tom Turner, from the University of New Mexico, were given on the most recent updates on genetics projects, management plans and habitat assessments for Gila topminnow, spikedace and loach minnow species.

- Gila topminnow Genetics Project and USFWS Genetic Management Plans – *Wade Wilson, USFWS*
    - Wade was funded in FY17 to conduct genetics on Gila topminnow and to assist with the development of a genetics management plan.
    - Alleles lost are those that occur with less frequency, in the example of Uvalde and Phantom Lake springs there were alleles lost a different locations, indicating some genetic drift.
        - Is it better to put rare-allele individuals into the wild, or propagate in the hatcheries?
            - If it is not marked it not possible to track specific individuals and their genotypes. With short-lived species, marking specific individuals is difficult.
    - Discriminant Analysis of Principal Components (DAPC) can show the degree of genetic divergence occurring among populations graphically – management implication/action – managers can change actions to alter gene flow among populations
    - Genetic Management Plans – used across the FWS with support from the Washington Office

        - Facilities – important to know if they can only hold fish as a refuge population, or if they have at the ability to propagate; previous facilities –can they be brought back in if necessary
        - Genetic and propagation management actions need to be tied back to recovery plans and/or SSAs
        - Need to define what you are going to propagate and at what size they will be released into the wild
        - Genetic risks – there are various risks (mixing of lineages, equipment failure), the plan needs to identify the risks and how each facility will mitigate them.
        - Augmentation plan – Additional plans such as an augmentation plan can be appendices.
        - Good genetic management requires constant communication/coordination between the propagation facilities, researchers, data collectors, and implementation
    - Discussion
        - ASU lineages replicated enough elsewhere – wait until the data is clear before taking topminnow out of that facility

- Range wide Genetic Assessment of Spikedace and Loach minnow presentation – *Tom Turner, University of New Mexico*
    - Tom was funded in FY18 to conduct rangewide genetics on spikedace and loach minnow using SNPs and to assist with the development of a genetics management plan.
    - Look across the genome (among and within) to help indicate how natural selection is happening across the landscape
    - Wanted to get up to 30 fin clips at each location
    - At the lab – DNA sequencing for all the samples, and look see where they differ in the sequencing at the nucleotide level
    - Tom provided an example from some previous work he has conducted on the Upper Gila
        - He looked at what happens with a large fire event to spikedace?
            - Fewer fish in fewer places
        - How different are populations on the landscape (F^{st})? Genetic diversity decreases with a large event and we would see genetic drift unless the more individuals come in from refuge (downstream repopulations) large populations with high diversity downstream can help repopulate an upstream small population with smaller genetic diversity Spikedace populations in the upper Gila should not be maintained separately in a hatchery; they are connected and should be kept together in propagation.
        - Loach minnow respond differently to large events and may need to be maintained separately.
    - Discussion
        - Slow repopulation generally, but after an event that movement upstream after seems to happen faster. Response to disturbance was interesting and disturbance may play a role in more robust repopulation
        - With new technology, there may be an opportunity to look at the sequencing of museum specimens and then look at long-term changes/trends
        - Should the upstream/West Fork and riverside/mainstem populations be kept separately at the hatchery?
            - They should be kept as one, unless there is an adaptive difference – they may need to be refreshed with wild stock. Currently, there are more being caught in the forks area and that is why they are being shipped to the hatchery. Try to get them from as many places as you can if they are genetically linked
                - 10 marker satellites originally, now Wade is using close to 20 markers.
                - Keep them separate for now, and wait for further data and analysis is complete
                - Option: keep the old mainstem population (hasn't been added to for the last 9 years) at the hatchery and then refresh with wild caught, this will

help especially with the potential Gila diversion (in between the 2 populations) in the next couple years.
        - Consider watching the alleles over time to see how much efficacy there is in those individuals
    - Concerns about overwhelming the small population with the diversity of the riverside population
    - Loach minnow – isolation by distance, there is no need to keep the West Fork and mainstem separate in the hatchery – this needs to be reevaluated regularly.
- Disturbance event response (same or different across species) – most species (native and non) respond similarly to disturbance in that they find refuge and then repopulate
- How can we recognize divergence in a bad way and evolution/adaptation in a good way?
    - If you take one individual and move it and monitor its changes in genes over time to see adaptation
    - In genes that do something (thermal response), that is when it is important to see how they change over time (adaptive differences)
        - Verde has been isolated the longest – and in considering the barriers, determining what the best management strategy.
    - Ensure that a bottleneck situation is not being replicated in the hatchery (example: Gila Trout)
    - Consider the wild populations, if they are not good habitat and alleles are being lost, then those are not good places to use for propagation – we don't want the hatchery population to look like (Phantom Lake Springs/Wade's presentation)
- How often do we need to check in with the hatchery populations and adjust management action?
    - By species, the genetic management plan will indicate how often a check in is necessary
- Thoughts on technology advancement and balancing the field determination of 2 distinct population.
    - The more markers you use the more differences you can detect
    - Colorado cutthroat – selection acts on the phenotype, not genotype – life history variations need to be maintained and their place/job in the ecosystem, using ecology and biology with the genetics (entire sequence) to determine a big picture and give context – then there are the societal values of the species

Monitoring Updates *(see website)*

Updates were given during this session on the existing monitoring contract (held by Marsh and Associates), the results from the 2018 season, as well as progress towards a 2019 Monitoring Workshop.

- Monitoring Contract – *Aaron Burgad, Marsh and Associates*
    - Monitoring was initiated in 1994 and focus was shifted further upstream to wild populations of listed and candidate species in 2012.
    - Objectives are to detect presence of focus species and evaluate community composition.
    - Stations are set up based on number of focus species present.
    - Sampling gear varied according to stream habitat, accessibility, etc.
    - Results from 2018 included 21 streams and 64 stations.
    - Morgan City Wash – green sunfish have invaded due to a breech in the barrier
    - Backpack electrofishing allowed for the catching of 0 and age one individuals
    - Collapsible Promar hoops were generally used in areas where access was only by backpacking
    - Suggestions
        - Use detected/not detected, not presence/absence
        - Combine chub species in the report (if appropriate)

        - Design the sampling based on the species you are interested in collecting and where (e.g. seine for spikedace versus shocker) – also consider keeping the sampling the same and detecting them where they are based on the method, not changing it for the species
    - Paul Marsh, who was not present at the meeting, reported that the refuge populations of Gila topminnow are doing well.
    - Web map
        - BOR has created a webmap to present results from monitoring.
        - 2012 to 2018 monitoring data; list shows each creek, the site sampled, what species were found and has a link to an abstract from the report.
        - Layers can be filtered by species and combine with non-native fish data collected.
    - Discussion
        - Based on the database or summary of database – most on summary reports, issues with the UTMs form the database
        - Will it be noted that the protocols will be changed? Yes
        - Standard sampling book through AFS, data has been eliminated because standardized methods were not used (in court cases), there is a need to have broad agreement on sampling protocols
        -

- 2019 Monitoring Workshop – *Kent Mosher, Bureau of Reclamation*

    - Monitoring Workshop
        - Discussion: Consideration of utilizing American Fisheries Society protocols. That may be decided later, also, there is an importance in determining frequency of sampling as well. Also for incorporation: needs for recovery, barriers and other factors.
        - Understanding that detection or non-detection by maintaining consistent gear type
        - More discussion needed on the sampling re-design and how/if it would go beyond the Marsh and Assoc. contract.
            - It needs to meet the needs of all agencies.
            - Suggested to involve Scott Bonar along the way, as AGFD is adopting AFS protocols, there needs to be feedback between this group and Those developing new protocols to adjust if needed
        - Maintain continuity of the monitoring time series while changing protocols, how have you thought about addressing that?
            - There is value in keeping a long-term dataset and identify gaps that new protocols could cover – the workshop is for broad discussion and to provide better direction for Marsh and Assoc. and future monitoring
        - Re: protocols
            - Regular specimen collection might be a good part of this and fin clipping and archiving
            - On a regular interval, this can help sew together a continuity issue, understand how the long-term data connects to a new/redesigned methodology
        - A planning team has been created. Team members include:
            - Jill Wick and Bryan Ferguson – NMDGF
            - Tony Robinson and Julie Carter – AZGFD
            - Doug Duncan and Kirk Young – USFWS
            - Kent Mosher and Bill Stewart – BOR

WaterSMART

- Grant Presentation - *Jessica Asbill-Case (See website)*
    - Drought Resiliency - State government may apply, 50-50 match
        - Annual cycle, opens in December
    - Cooperative Watershed Management Grant
    - 60-120 applicants

Ongoing Project Updates

- Spikedace and Loach minnow Recovery Plan - *Mary Richardson, USFWS*
    - Recovery Planning Implementation (RPI) Process overview *(See website)*
    - Challenges – nationwide numbers: ~1600 species and ~450 without recovery plans
        - Developing recovery plans – can be sued because species are listed faster than the recovery plans are developed
        - Budgets and workforce for this area is static or shrinking
        - Changes need public input and so changing a plan takes longer than developing a new one
    - Now three parts/documents
        - Species Status Assessment (SSA) – not a decision document, increases efficiencies because it is used in a variety of ways
        - Recovery Plan – includes criteria, actions and time/cost estimates
        - Recovery implementation strategy (RIS) – on the ground activities that complete the recovery actions
            - Criteria cannot be changed/revised in this document, has to be in the recovery plan
    - Gila topminnow – 5-year review will be complete in June of 2019
    - Spikedace – draft plan, no review, behind in development, approach is being worked out with partners, likely the first revision of the Recovery Plan will be at the end of 2019
    - Loach minnow – 6 years into the process, first revision currently, team member revisions are being incorporated, still working on recovery implementation strategy, will be following the new RPI process.
    - Biological report is a hybrid between an SSA and the old introduction. The SSA is more comprehensive with the work/input of partners.

- Gila topminnow – *Doug Duncan, USFWS*
    - Most topminnow work is funded by this program, most work is in AZ due to historical range and conducted by AGFD
    - Safe harbor agreement – Pinal county will use topminnow in their vector control program. The media attention coming with that should be beneficial
    - SSA commitment and Recovery Plan revision
        - SSA lead designation TBD (will come from the regional office)
        - Petition from AGFD to downlist to threatened
        - Implementation Plan will be within the recovery plan (because it is near done and will not be switching to the RPI process), the 5-yr review will be a 2-page document.

- Razorback sucker – *Kirk Young and Jess Gwinn, USFWS*
    - Big river fish are high priority in Region 6
        - SSA, then decisions (listings, recommendations) – received over 4,000 comments from the good outreach from partners
        - Razorback sucker was the 2nd fish out of that effort
        - 5-yr review with recommendation to downlist (another opportunity for public to comment, typically 45-day comment period), and is now in the queue after the Humpback Chub
            - Chub – late winter/early spring 2019 and razorback sucker after that
        - Interesting approaches to conservation, resiliency and viability – contact Jess with questions
            - 4d rule – alleviate regulatory burden in relation to conservation actions, looking for further input here.

- yy Male – *Chad Teal and Scott Bonar, USGS AZ Cooperative Fish and Wildlife Research Unit (see website)*
    - Specifically targets nuisance species – in the past with mechanical removal but extirpation is very difficult

- Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) gene editing in mosquitoes to eliminate malaria
- Daughterless carp – takes longer for extirpation
- Trojan y chromosome strategy
    - With a flood of yy males and the xx females will be extirpated
    - Modeled to be effective in different species and faster than daughterless carp
        - Still take decades for complete extirpation, but if combined with mechanical suppression the rate of extirpation increases.
    - BOR funding this work beginning in FY17
        - Red shiner and green sunfish – compare methods based on the 2 types of fish
        - Collection and then to U of A propagation lab
        - Sex chromosomes don't work
        - Sequence the entire genome and find the one area where they differ
        - RAD-Seq can sequence very quickly
        - Breeding to create the Trojans
    - Results
        - Conducting RAD sequencing to build RAD libraries for genetic sex determination.
        - Chad has been spawning red shiners and has initiated red shiner sex reversal trials in Oct 2018
            - 30-500 eggs every 3-5 days
            - 60 trial, and they are 4.5 month spawners
        - Green sunfish
            - Trials starting next month, same treatment as bluegill

- Discussion
    - Bluegill and green sunfish offspring is skewed towards mostly male
    - Need chromosomal determination?
        - Could have YYYY across chromosomes
    - Environmental sex determination?
        - That could be an issue, if they are recessive for sex determination in chromosome it could change the determination by heat
    - Testing the yy males
        - Lab trials first, genetic screening for all yy male progeny
        - Ponds and then stretches of streams for tests of fitness and effectiveness
    - Mosquitofish
        - Modeled the effectiveness, but takes a long time. Conclusion is the combine yy males and mechanical suppression to be most effective.
    - This could work on channel catfish
    - Field applications of this trial/technique?
        - Just brook trout at this point where there are actual releases
            - Currently being released in Bonito Creek in NM

Tier 1 Workplan

All final workplans are compiled and added to the website for viewing, they can be found at https://www.usbr.gov/lc/phoenix/biology/azfish/budget.html.

- Review of timeline of GRBNFCP process
    - (Page 7; https://www.usbr.gov/lc/phoenix/biology/azfish/pdf/2018-2022GRBStrategicPlan.pdf)
- Project/proposal evaluation form update – *Bill Stewart*
- Work plan template – "blurb guidance"
- AGFD Adjustments to FY19 – *Tony handed out AZGFD FY2019 work plan*

- Fresno Canyon – no work has been done in a while, Doug and Ross met with the landowner, pull out chub (less than 100 fish there) from there and send them to ARCC for breeding. Private landowner is on board now.
- Bonita – Loach minnow monitoring for only 2 more years. AGFD has not detected any loach minnow in last three years. This project could be taken off the list, depending on how many more years we do not capture any Loach Minnow. There is some decent Loach Minnow habitat downstream from Midnight Canyon to Red Knolls. BLM is continuing to monitor. FWS/Mary does not support more stockings at this point. Recommended to eliminate this project
- Gila topminnow stockings – BOR/FWS to make adjustments in the work plan
- Project 12 (Miscellaneous Stock Tank Surveys) can be merged into individual restoration projects – for example, Verde River drainage tank surveys, GIS study to prioritize sites/tanks. Recommended to add more detail to the Verde River plan.
    - Focus on tanks closest to the river, survey the wet ones or those that have fish on record, detect non-native fish
- Project 13 (Assess Potential Repatriation Waters) – Plan to assess Blue River tributaries before putting loach minnow in. Will evaluate streams in Pima County in the coming weeks.
    - Assessments: protocol developed by Mike Anderson, habitat survey in multiple 100-meter sections. Might be able to combine assessment data with Jack T. and Crosby's work.
- Project 14 (Expand Roundtail Chub Populaion in Harden Cienega Creek) – concern is that Green Sunfish have been detected above the barrier in the last two years (1 last yeart and 2 this year). Can likely move this project to long-term monitoring, or, keep this project but shift focus. Potential to work with NM, because source of Green Sunfish may be tanks on private land tanks (some in AZ and NM) – Could survey tanks beginning in 2020
- Project 15 – Eagle Creek - pre barrier survey planned for 2019. AGFD developed a draft monitoring plan. AGFD - There are fixed sites, monitored by M&A, there are Freeport McMoRan sites – one fixed site below the barrier, a number of sites above: each reach has 1 fixed and a few random sites
    - San Carlos Tribe relationship and potential for conservation on their land. USFWS recently talked with tribe about Eagle Creek (spikedace and loach minnow). With the implementation of the barrier there will be a need for surveys on tribal lands. Tribe isreceptive to doing some surveys in the drainage (*see Kirk Young's one page description*). The tribe would have the report to release as they want, but the information would be known for follow-up.
        - Tony & Doug are supportive of this project. Maintain consistency in projects/protocols and with eDNA (like it's done on the Forest), budget may be an issue that needs to be considered
        - Get methodology and sites that have been surveyed to Kirk as a starting point.
    - AGFD - There are fixed sites, monitored by M&A, there are Freeport McMoRan sites – one fixed site below the barrier, a number of sites above: each reach has 1 fixed and a few random sites
- Project 16 (Red Tank Draw Native Fish Restoration) – treat the tinajas in Red Tank Draw (Rarick Canyon) like tanks – Determine what is feasible, and if decide on chemicals, develop a treatment plan.
- Project 17 (Sharp Spring native fish restoration) – This project has been delayed because of lack of communication back from AZSP. AGFD hasn't completed any work there in years, because they have not gained approval. Evaluation form considers that all pieces are in place to start work immediately. More conversation needed on this.
- Project 18 (Boyce Thompson Ayer Lake native fish restoration) – another AZSP collaboration project that has been on hold for years now.
- Project 20 (Aquatic Research and Conseervation Center O&M) – Could bring chub from Sheehy Spring into ARCC. ARCC staff are receptive

- AZ BLM – will continue to spot check in Bonita for green sunfish, and then starting Aravaipa

- NMDGF – no changes to the FY19 workplan
    - eDNA on the East Fork, coordinate with Yvette/FS
- NM BLM – no changes – funding needed for Gila River mainstem and tributaries monitoring
- Report back to the Policy Committee with the FY19 changes proposed – send them to Bill

Proposed Projects for 2020

- AZ BLM – Bonita Creek and Aravaipa Creek continued
- NM BLM – Commit to Gila River mainstem and tributaries monitoring
    - Put in template and send to Bill
- NMDGF – same as FY19 with the addition of Long-term Fall Monitoring (October) with BLM, FS, NMDGF
    - Put in template and send to Bill
- AGFD
    - See attached 2020 AGFD Work Plan
    - **Addition**
        - Verde River restoration project: assess stock tanks tributaries, and prioritize high risk/threat stock tanks, assisting Matt with the planning process
- ARCC
    - Loach minnow nest spacing research
    - Post stocking mortality research
    - PVA modeling factor assessment – work with Tony AZGFD for proposal
- Q&A
    - What about the Black River?
        - There is a significant brook trout problem upstream of barrier and on tribal lands and until that is taken care of we can't repatriate loach minnow in the treatment area
    - Bear Wallow. Barrier repair work in progress. May be able to chemically treat within a few years.

Tier 2 Project revisit and brainstorm

- Discretionary to the tech committee and they can run the projects through the evaluation and if there is money available some of them can move forward.
- Review 2017 list and rollover, if any, from 2019 proposed projects
- Brainstorm to build upon Tier 2 list
- See updated table (Appendix A)

Information & Education Updates *(Appendix E)*

- Subcommittee
    - Federal agencies – met twice to discuss projects
    - It was suggested to open the committee up to state agencies, universities
- GRBNFCP website – *Kent Mosher, BOR*
    - Updated from the 2011 version
    - New logo from Josh Grant in NM – send comments to Stuart regarding logo changes
- Projects – *Kent Mosher, Bureau of Reclamation*
    - Emily Freed student with S. Bonar, and she comes with money to do projects in the schools, how they are collecting data, improvements, for school ponds
    - Send ideas for projects to Kent, if folks are interested in being on the sub-committee, let Kent know.
- Native Fish in the Classroom – *Stuart Wilkins, USFWS*
    - Stuart to send out the video
    - Curriculum does cover the release of fish in their range and why non-natives should not be released.

Plus/Delta

- Nice to have in a small town, not PHX/ABQ
- PPTs are nicer to digest the information, especially when there is a lot of info from one entity

- More refreshments

Next Meeting

- Cottonwood/Verde Valley
- Dates: December 10-12, 2019

Participants

Brian Hickerson	Arizona Game and Fish Department
Joshua Walters	Arizona Game and Fish Department
Julie Carter	Arizona Game and Fish Department
Kristopher Starr	Arizona Game and Fish Department
Tony Robinson (Committee Member)	Arizona Game and Fish Department
Heidi Blasius (Ex-officio Member)	Bureau of Land Management
Timothy Frey (Ex-officio Member)	Bureau of Land Management
Keith Gido (Phone)	Kansas State University
Crosby Hedden (Phone)	Kansas State University
Aaron Burgad	Marsh & Associates
Bryan Ferguson	New Mexico Department of Game and Fish
Jill Wick (Committee Member)	New Mexico Department of Game and Fish
Martha Cooper	The Nature Conservancy
Doug Duncan (Committee Member)	US Fish and Wildlife Service
Kirk Young	US Fish and Wildlife Service
Mary Richardson	US Fish and Wildlife Service
Jess Gwinn	US Fish and Wildlife Service
Shaula Hedwall (phone)	US Fish and Wildlife Service
Stuart Wilkins	US Fish and Wildlife Service
Wade Wilson	US Fish and Wildlife Service
David Probst	UNM
Dustin Myers	US Forest Service
Jerry Monzingo	US Forest Service
Yvette Paroz (Ex-officio Member)	US Forest Service
Matt O'Neill (Phone)	US Forest Service
Scott Bonar	USGS/University of Arizona
Chad Teal	University of Arizona
Tom Turner	University of New Mexico
Gregor Hamilton	University of New Mexico
Alex Cameron	University of New Mexico
Kent Mosher	US Bureau of Reclamation
Bill Stewart (Committee Member)	US Bureau of Reclamation
Andi Rogers (Facilitator)	Southwest Decision Resources
Carrie Eberly (Facilitator)	Southwest Decision Resources

Appendix A
Prioritized Tier 2 Projects from 2018 Technical Committee Meeting

** **$**=10k-50K, **$$**=50k-100, **$$$**= 100K and up. The number in (x) is the number of dots each project received.**

2018 Tier 2 Project	Est. Cost	Comments

1. eDNA – Developing the tool for further refinement for spikedace and loachminnow (7)	$$	• Yvette – there is funding for field crew support – this is the last year with extra funding for field support.
		• Expanding the research to go further downstream to find out persistence rates and limits
		• eDNA for ponds above Harden Cienaga for green sunfish to find out where they are coming from.
		• Crew that could expand out from the Gila River
		  o The labs have been getting false positives, so keep that in mind
		  o $75 for one species and $100 for 2 species sample
		• This is ripe for citizen science
		• Emery-Riddle AGFD work, red eye bass marker may be specific to AZ. Clips from those fish are needed (this could be covered under funding that exists)

2. Research lethal grid electroshocking for non-native removal (3)	$$$	Grad student to test high powered generators and/or electric seines
3. Factors (all species) that determine success/failure of re-established populations (3)	$$$	Spikedace and Loach Minnow – happening Interest in other species
4. On call mechanical removal crew (2)	$.5	• Addressing non-native detections immediately – all agencies having some participation in a short period of time • Beef up an effort for short term extra effort
5. Ammonia testing in a flowing system (1)	$$	• Support for alternative methods of killing fish, chemically • Human health and environment study fees/costs

Appendix A
Prioritized Tier 2 Projects from 2018 Technical Committee Meeting
** **$**=10k-50K, **$$**=50k-100, **$$$**= 100K and up. The number in (x) is the number of dots each project received.**

6. Reclamation equipment (1)	$.5	
7. Pupfish/top minnow pond habitat maintenance work (1); thinning of riparian vegetation for habitat improvement for Gila Topminnow	$	• Some sites identified on the Tonto • Schools in Tucson and PHX can be involved in the creation of these
8. Weir at Morgan City wash- replacement (0)	$$	
9. Hatchery research on year-round spawning (0)	$	Couple years out, small systems and cheap *Add outreach with a mobile fish tank for schools
10. Physical manipulation of substrate to promote spawning of loachminnow in the wild (0)	$$	
11. Contractor for compliance work (0)	$$	
12. T4 Springs improvement (0)	$$$	Doug to follow up with Laura re: drying up of spring
13. Bog hole improvement – topminnow, pupfish Gila chub (0)	$$$	
14. Understand how hatchery operations can influence/contribute to successful repatriation (0); research on flow conditioned pupfish	$$	Flow condition fish first, before stocking
15. Radio study to follow fish after flood events (0)	$$$	** being considered** natives and non-natives
16. Razorback movement/survival study in the Verde River (0)	$$$	Work with SRP, surveying with radio tags
17. Removal of non-native trout in Campbell Blue Creek (0)	$.5	
Items highlighted in blue – already happening		
Evaluate if hatchery/captive re-established populations are genetically representative of remnant populations (8)	$$$	This is happening for spikedace, loachminnow and topminnow. Keep it up!
Chub DNA project (6)	$$$	Happening, could drop in priority. Check back in with Tom to see if how it is progressing
Effective population size evaluation N_b (5)	$$	Tom Turner is going to be doing this work
Range wide Spikedace and Loach Minnow assessment-Looking at variables to determine where/why (4)	$$$	Combined with above
Non-native removal of yellow bullhead and green sunfish in Aravaipa (3)	$.5	Ongoing, add red shiner before green sunfish, not part of the work plan, but is currently funded

Appendix A

Prioritized Tier 2 Projects from 2018 Technical Committee Meeting

** **$**=10k-50K, **$$**=50k-100, **$$$**= 100K and up. The number in (x) is the number of dots each project received.**

Items highlighted in orange – eliminate from list		
Survey Upper Verde stock tanks (1)	$$	
eDNA (range wide) confirming non-native eradication-use of the tool (0)	$	
NEW additions (2018 meeting)		
1. Assess dispersal through canyons, is it related to flood event, demographic connectivity between populations (radio tag)		
2. Using Lidar and hydro modeling (riverside site) to see how habitat availability changes with changes in flow/discharge		
3. Mark and recapture for monitoring – validation of sampling techniques		

AGFD Work Accomplished 2018

Brian Hickerson and Tony Robinson

Overview

- Gila Topminnow

- Desert Pupfish

- Loach Minnow

- Roundtail Chub

- Spikedace

Major Projects:

- Blue River Native Fish Restoration
- Muleshoe CMA Native Fish restoration
- Bonita Creek
- Spring Creek

Gila Topminnow

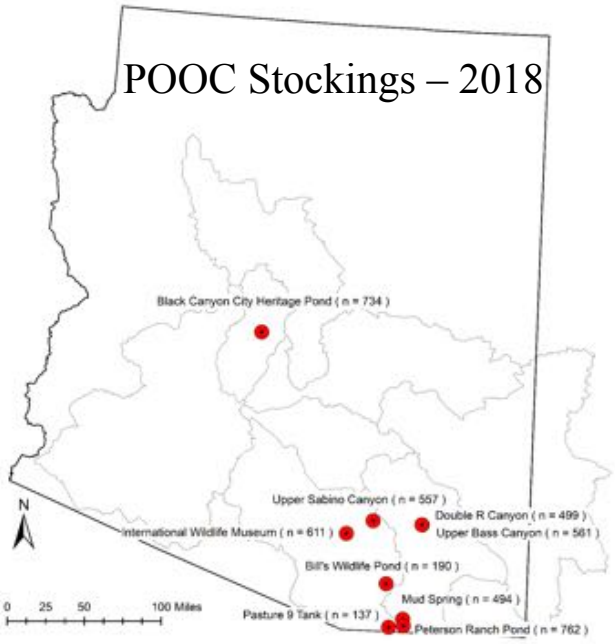
- 9 Stockings
- 24 Monitoring sites

Gila Topminnow Monitoring

WaterName	2017 Catch	2018 Catch	Year Last Stocked
Tortilla Creek	829	2,020	2017
Swimming Pool Tank - Robbins Butte	391	1,480	2015[2]
Black Canyon City Heritage Pond	--	1,429	2018
Spring Water Wetland - Las Cienegas	--	1,161	2013[2]
Stop Sign Tank - Robbins Butte	652	1,138	2015[2]
Egret Pond - Las Cienegas	1,204	993	2013[2]
Charlebois Spring	18	914	2017
Crescent Pond - Las Cienegas	618	652	2013[2]
Bonita Creek	388	646	2015[2]
Spring Creek	207	497	2016
Gaucho Tank - Las Cienegas	2,727	449	2014[2]
Hidden Water Spring	425	312	2016
Sabino Canyon (Lower)	103	276	2016
Murray Spring	10	54	2017
Pasture 9 Tank[1]	--	52	2018
Clyne Pond	82	12	2016
West Fork Pinto Creek	397	9	2017
Bill's Wildlife Tank - Las Cienegas	22	5	2018
Bass Canyon[1]	28	3	2018
Sheepshead Canyon	83	1	2016
Double R Canyon[1]	--	0	2018
Arnett Creek	90	6	2017
Rock Spring	0	0	2014[2]
Nogales Spring - Las Cienegas	3	0	2015[2]

[1]Population augmented after monitoring; [2]Three years of post-stocking monitoring completed.

POOC Stockings – 2018



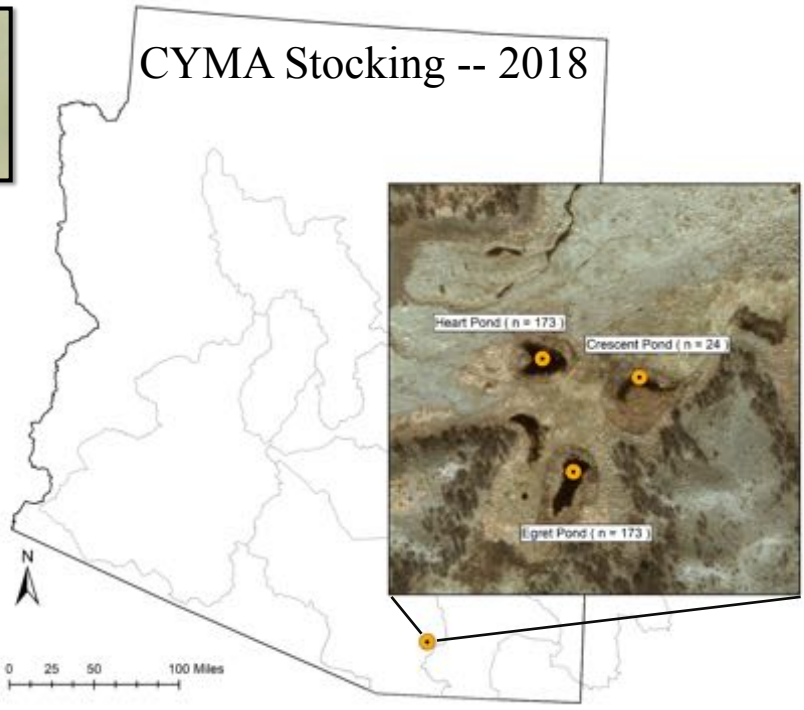
Desert Pupfish

- 3 Stockings
- 9 Monitoring Sites

Desert Pupfish Monitoring

Water Name	2017 Catch	2018 Catch	Year Last Stocked
Black Canyon City Heritage Pond	23	504	2017
Twin Tank (Robbins Butte)	507	286	2010[2]
Cottonwood Tank (Robbins Butte)	38	172	2010[2]
Heart Pond (LCNCA)[1]	87	83	2018
Crescent Pond (LCNCA)[1]	12	63	2018
Mint Spring	56	55	2016
Cottonwood Tank (LCNCA)	34	47	2017
Egret Pond (LCNCA)[1]	25	40	2018
Bonita Creek	0	0	2015[2]

[1]Population augmented after monitoring; [2]Three years of post-stocking monitoring completed.



Spikedace

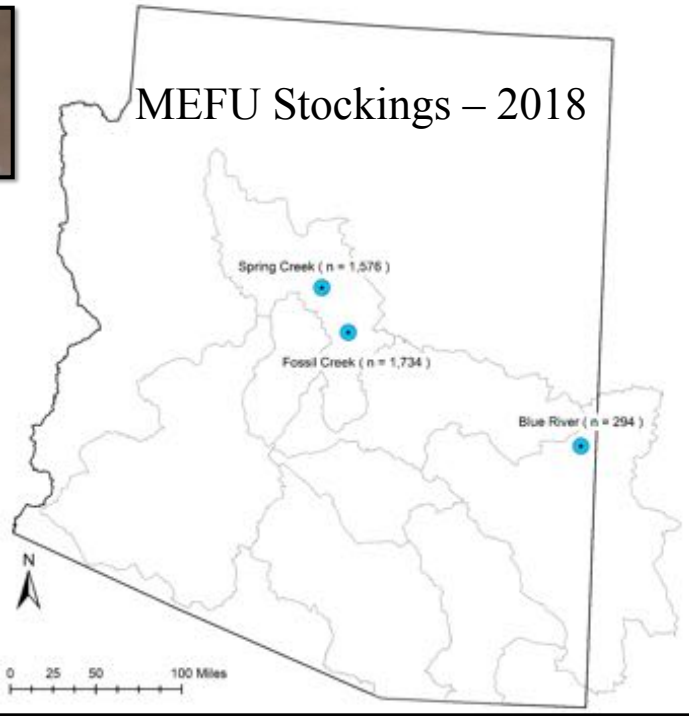
- 2 Stockings
- 4 Monitoring Sites
- No Collections in 2018

Spikedace Monitoring

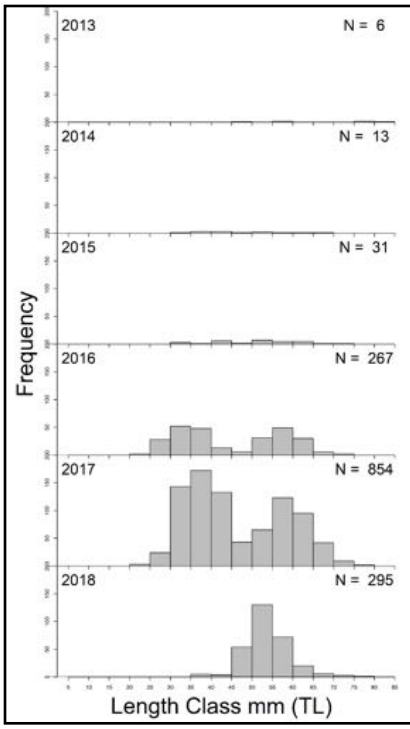
Water Name	2017 Catch	2018 Catch	Year Last Stocked
Blue River (Lower)	858	292	2015[2]
Spring Creek[1]	11	20	2018
Blue River (Middle)[1]	--	6	2018
Hot Springs Canyon	4	1	2011[2]

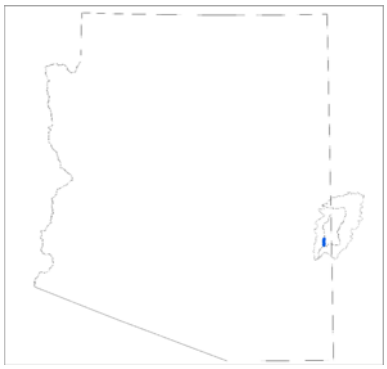
[1]Population augmented after monitoring; [2]Three years of post-stocking monitoring completed.

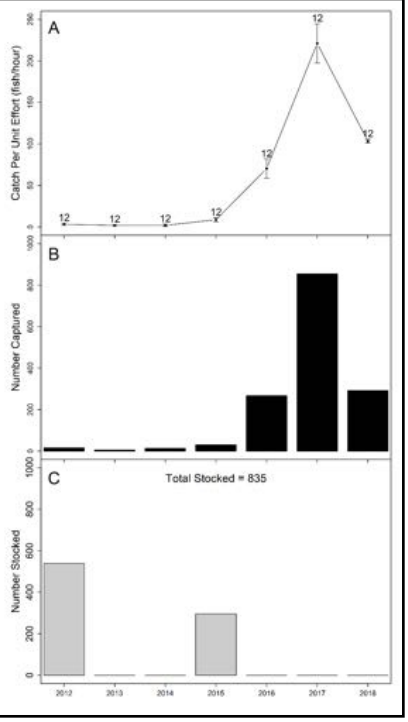
MEFU Stockings – 2018



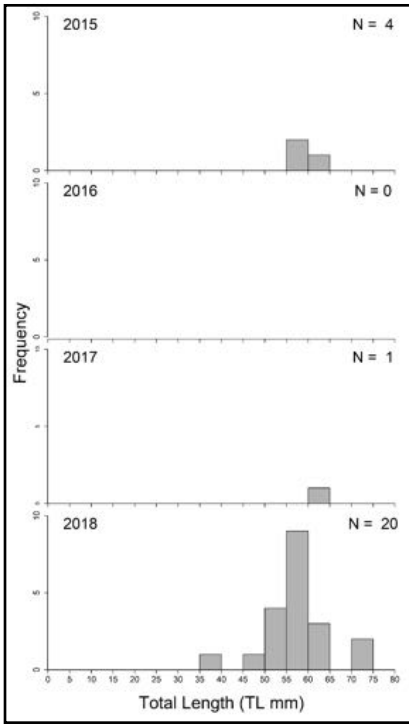
Lower Blue River MEFU

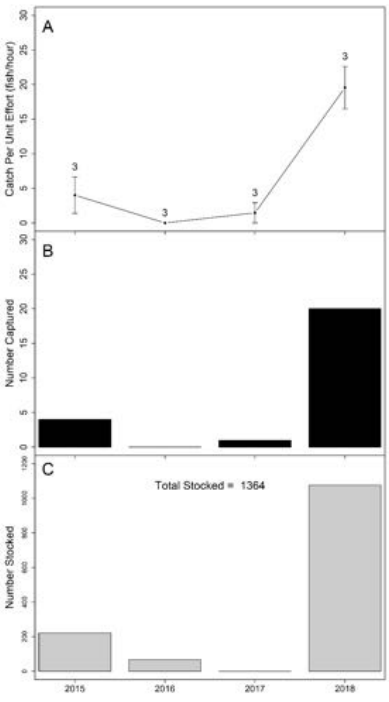




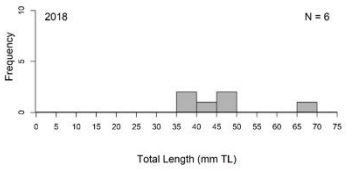


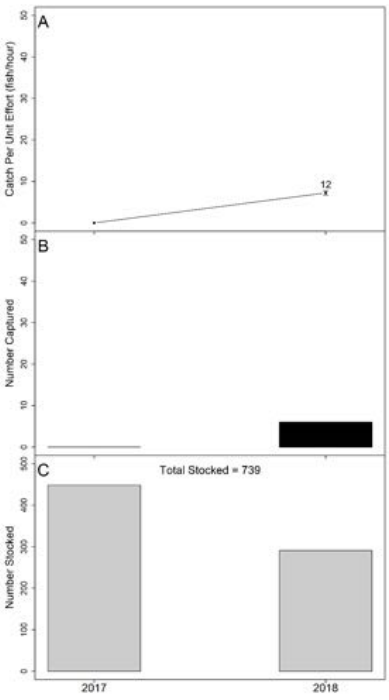
Spring Creek MEFU



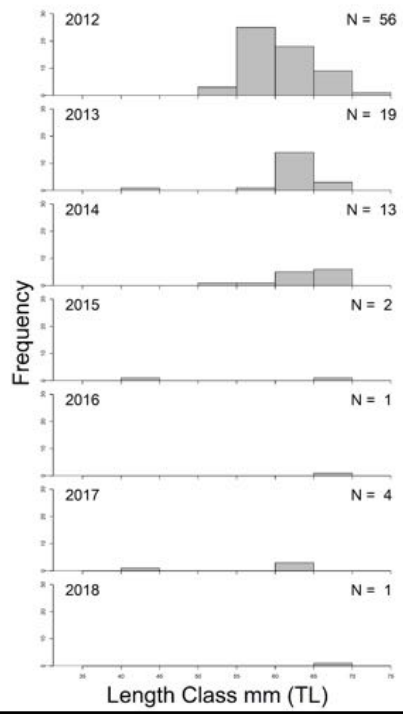


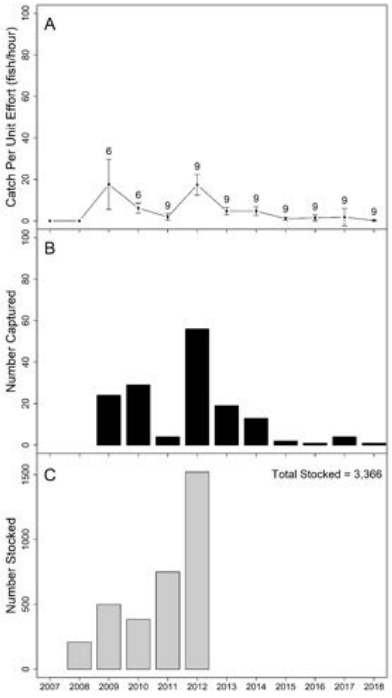
Middle Blue River MEFU





Hot Springs MEFU





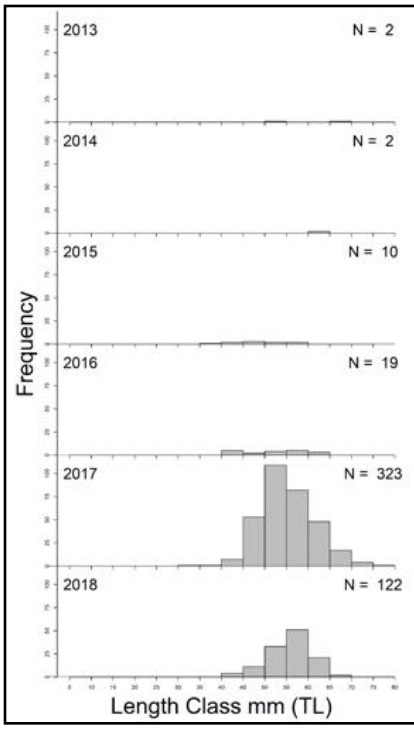
Loach Minnow

- No Stocking in 2018
- 3 Monitoring Sites
- 2 Collections

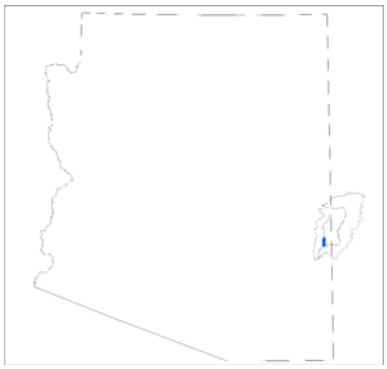
Loach Minnow Monitoring

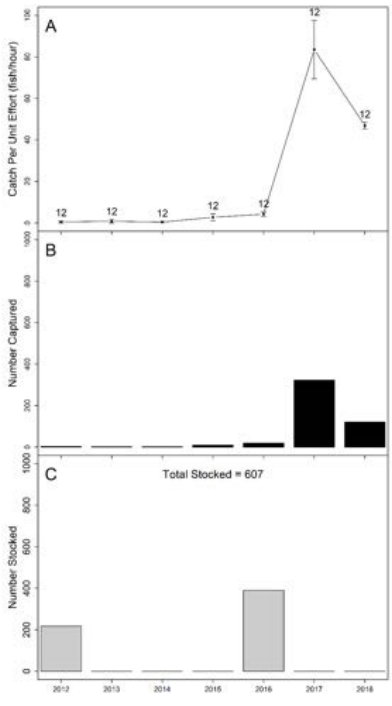
Water Name	2017 Catch	2018 Catch	Year Last Stocked
Blue River (Lower)	326	121	2016
Hot Springs Canyon	21	25	2011[2]
Bonita Creek	0	0	2014[2]

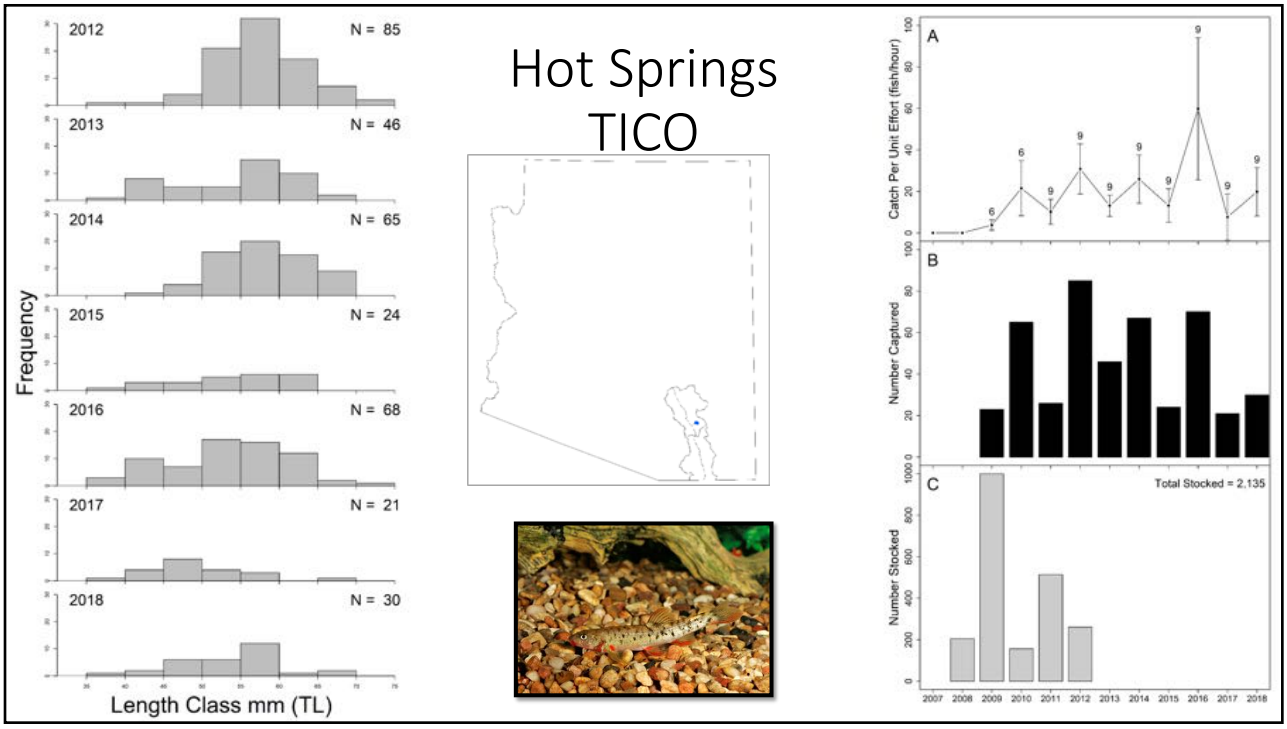
[1]Population augmented after monitoring; [2]Three years of post-stocking monitoring completed.

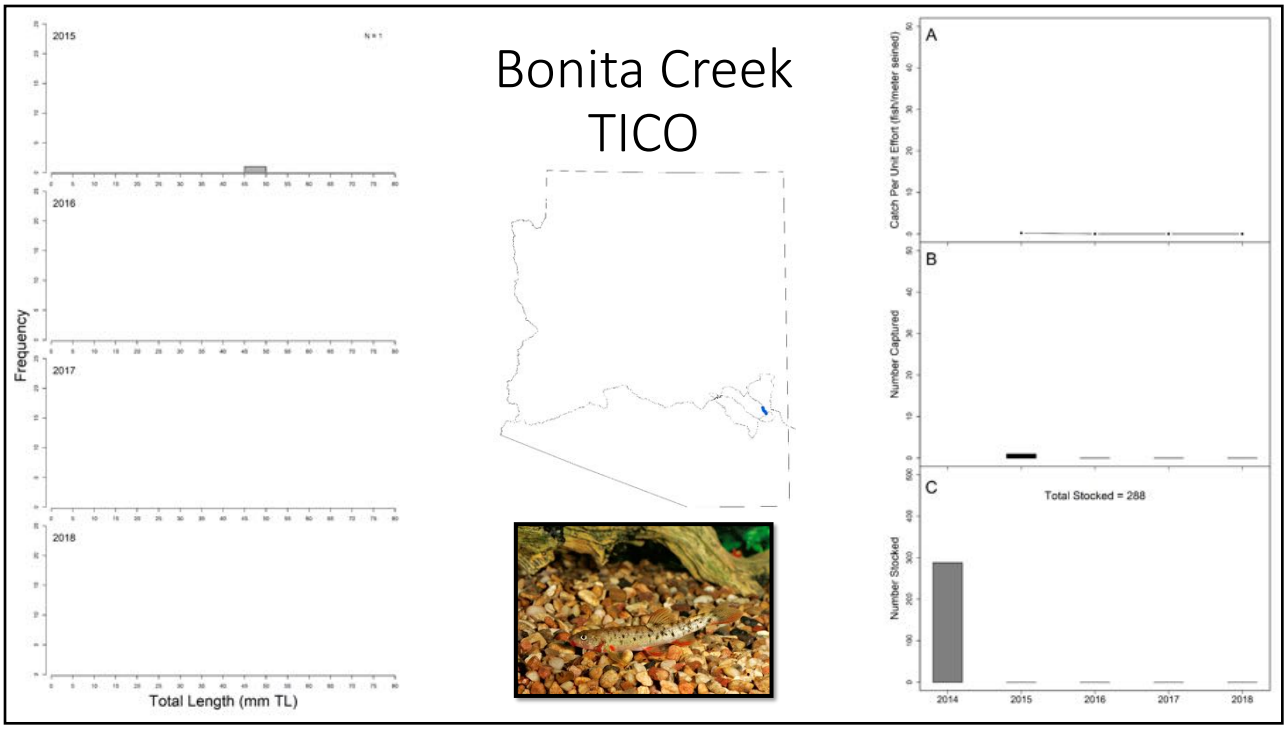


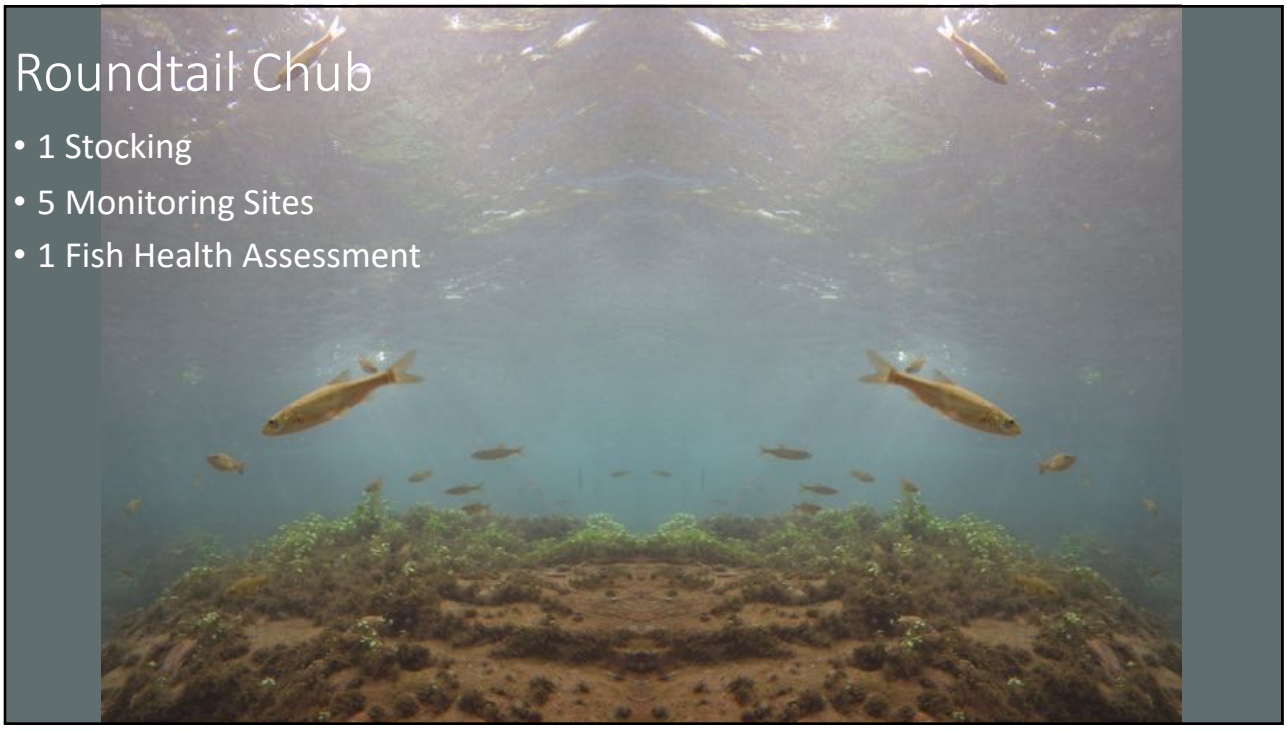
Lower Blue River TICO






Hot Springs
TICO
2012 N = 85
2013 N = 46
2014 N = 65
2015 N = 24
2016 N = 68
2017 N = 21
2018 N = 30
Frequency
Length Class mm (TL)
A
Catch Per Unit Effort (fish/hour)
B
Number Captured
C
Total Stocked = 2,135
Number Stocked


Bonita Creek
TICO
2015
2016
2017
2018
Frequency
Total Length (mm TL)
A
Catch Per Unit Effort (fish/meter seined)
B
Number Captured
C
Total Stocked = 288
Number Stocked



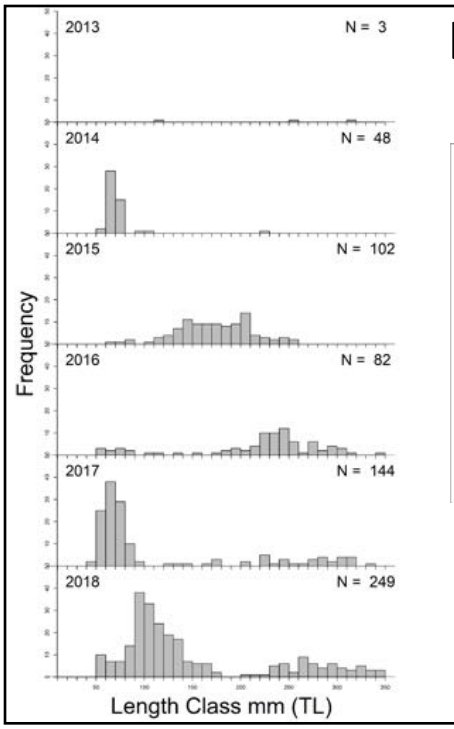
Roundtail Chub Monitoring

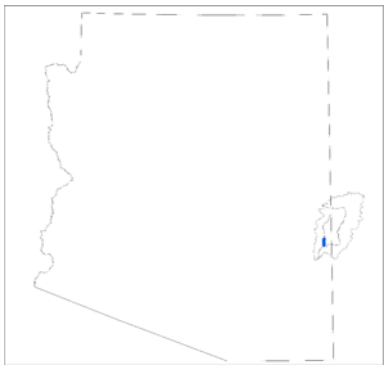
Water Name	2017 Catch	2018 Catch	Year Last Stocked
Blue River (Lower)	151	334	2011[2]
Harden Cienega[1]	391	304	2015[2]
Quinsler Pond (Blue River)	274	145	2015[2]
Spring Water Wetland	--	75	2017
Blue River (Middle)	57	38	2016

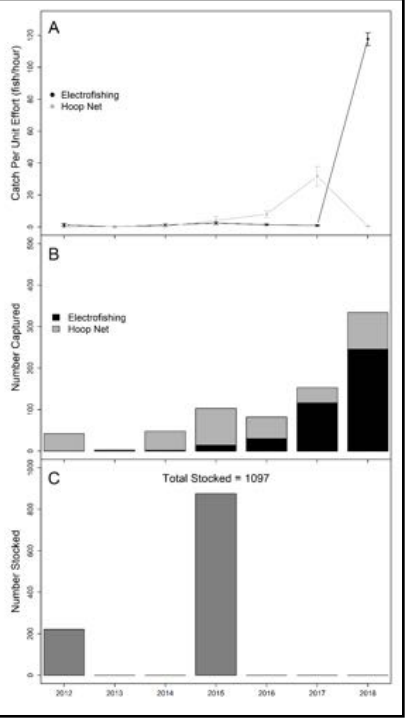
[1]Population augmented after monitoring; [2]Three years of post-stocking monitoring completed.

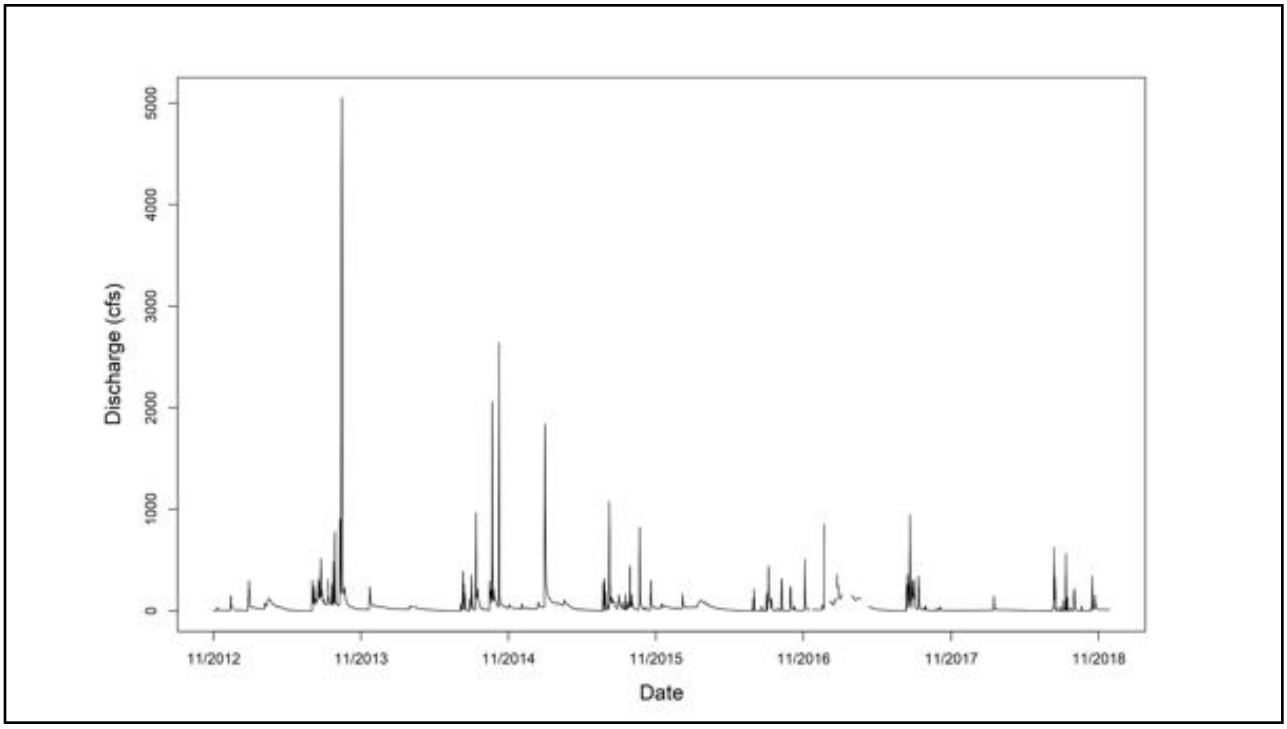
GIRO Stocking -- 2018

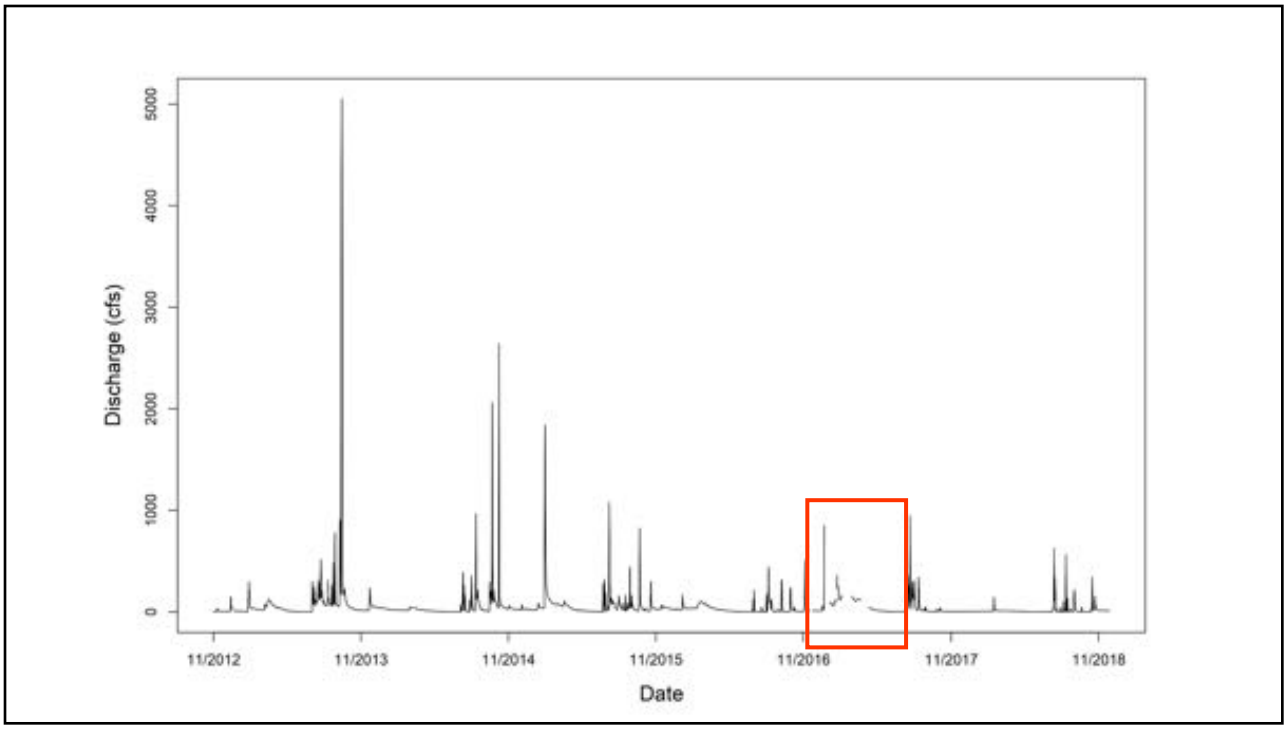
Lower Blue River GIRO

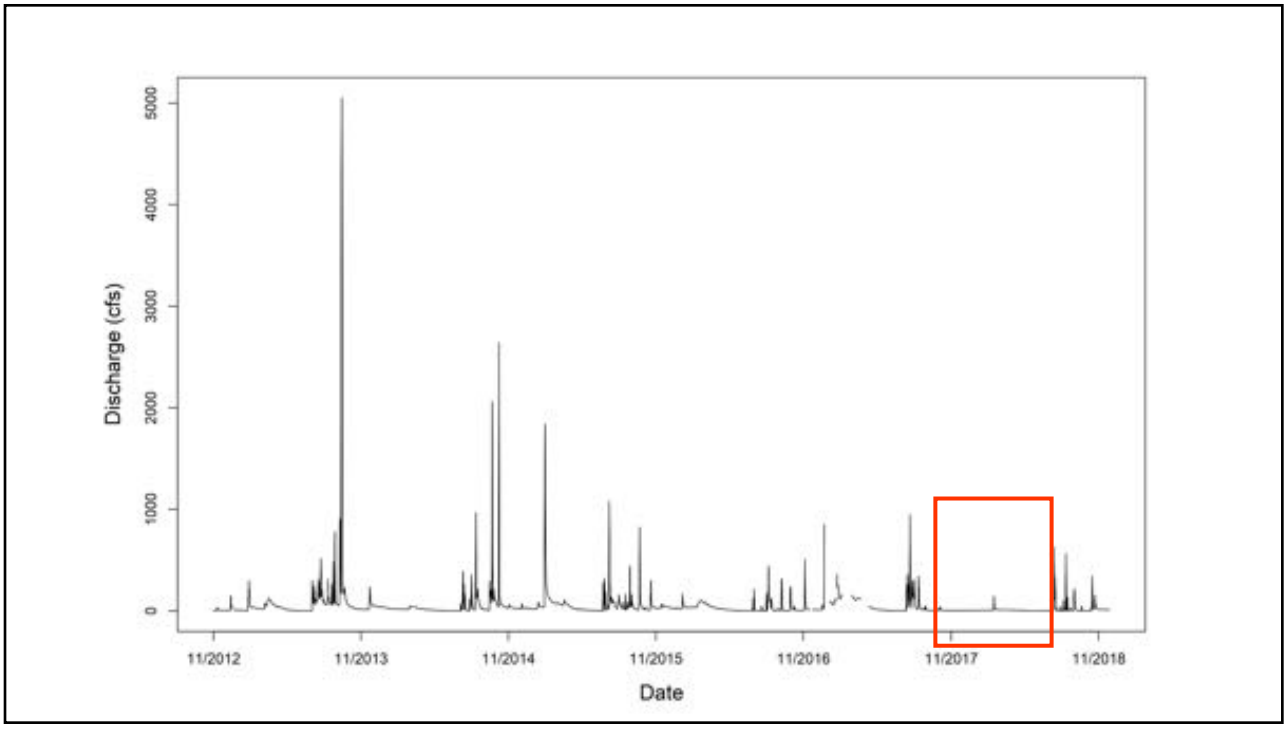




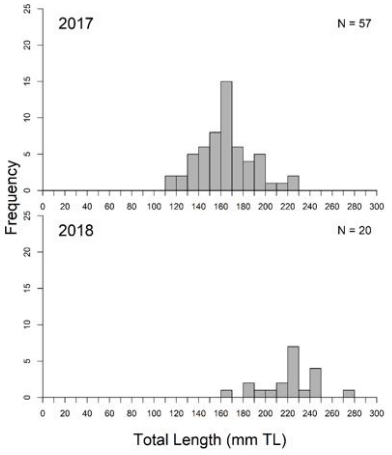


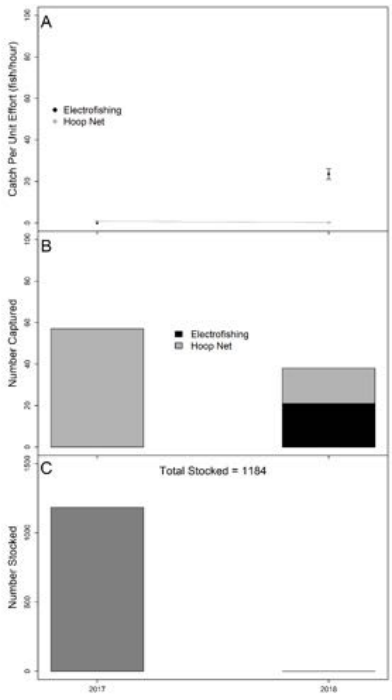

Discharge (cfs)
0
1000
2000
3000
4000
5000
11/2012
11/2013
11/2014
11/2015
11/2016
11/2017
11/2018
Date


Discharge (cfs)
0
1000
2000
3000
4000
5000
11/2012
11/2013
11/2014
11/2015
11/2016
11/2017
11/2018
Date



Middle Blue River GIRO





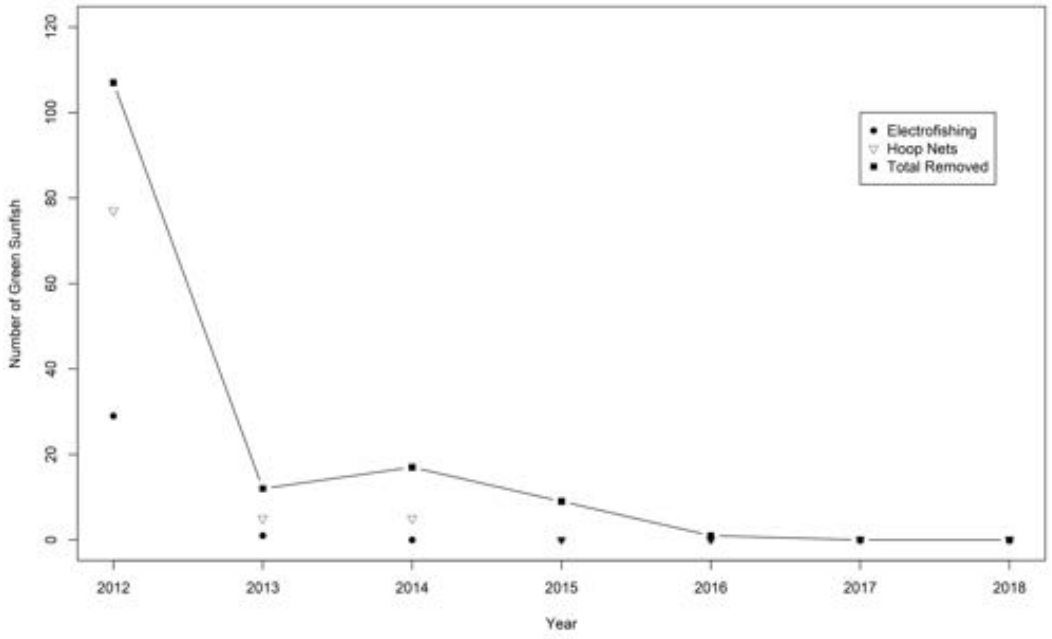
Nonnative Species Removal

- Blue River
- Redfield Canyon
- Red Tank Draw
- La Barge Spring

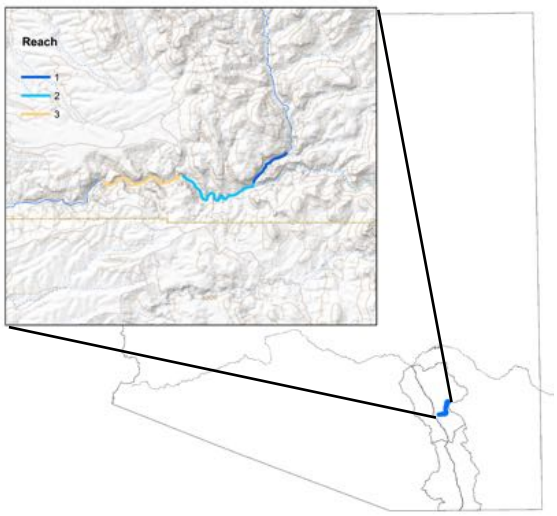
Blue River

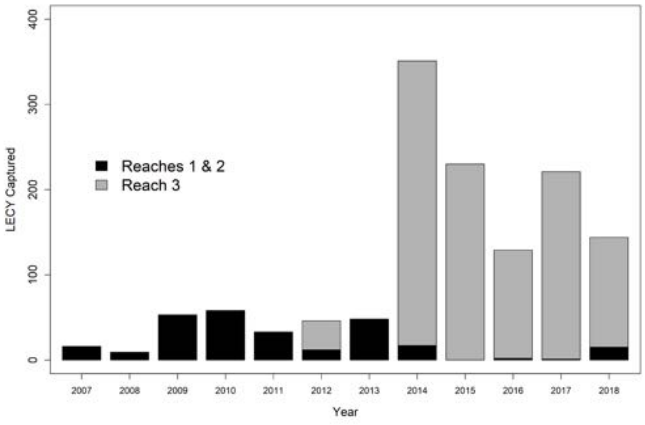
- Green Sunfish not detected since June 2016
- Channel Catfish not detected since 2013
- Red Shiner and Fathead Minnow not detected in 2018

Blue River LECY Removal

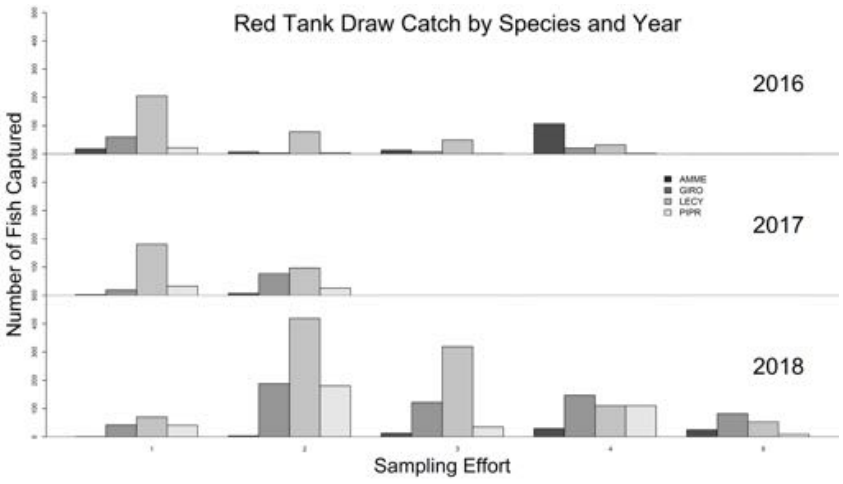


Redfield Canyon

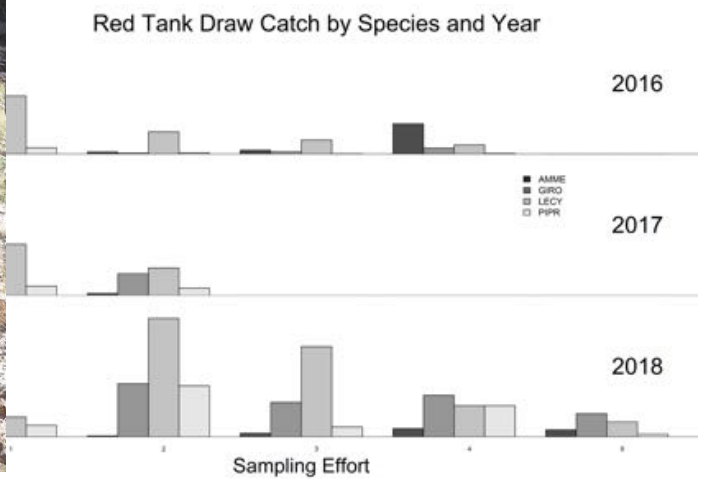




Red Tank Draw



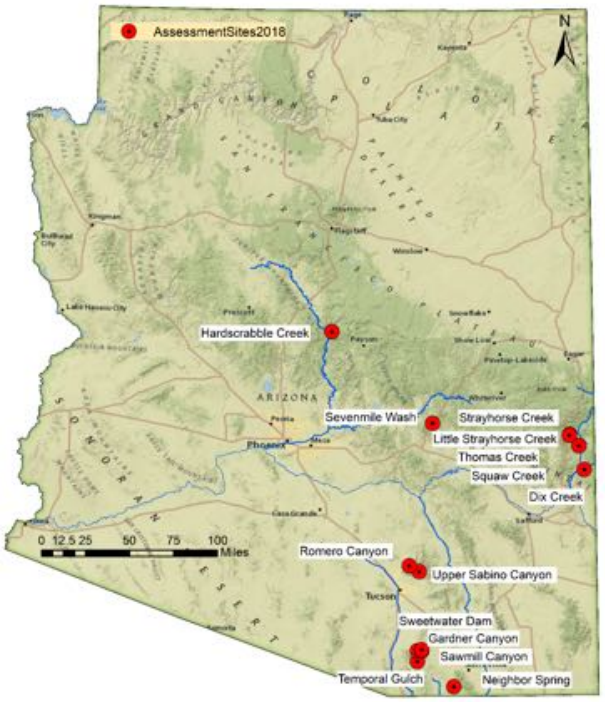
Red Tank Draw



Newly discovered barrier in Rarick

2018 Assessment Sites

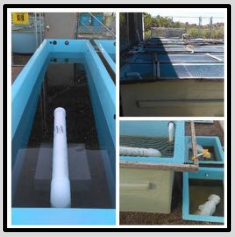
- 14 Sites total
- Nonnatives present
  - Hardscrabble Creek (MIDO, LECY)
  - Neighbor Spring (LECY)
  - Temporal Gulch (LECY)
  - Sweetwater Dam (CAAU, GAAF)
  - Sawmill Canyon (GAAF)
- Not suitable for any species
  - Strayhorse Creek
  - Little Strayhorse Creek
  - Squaw Creek
  - Sevenmile Creek



The Aquatic Research and Conservation Center

2018 Spikedace and Loach Minnow captive propagation review

Infrastructure used for 2018 spawn season



Cage 1 raceways
Number of raceways: 20
Length: 15'
Width: 3'
Depth: 2'
Gallons: 673
Pump (Gpm): 75 -250

Cage 2 circular tanks
Number of tanks: 24
Diameter: 6'
Depth': 2
Gallons: 423

Cage 2 raceways
Number of tanks: 5
Length: 15.5'
Width: 1.8'
Depth: 5"
Gallons: 88.5
Pump (Gpm): 12

Other Facility specs
Water source: Artesian well
Gallons per minute: ~70

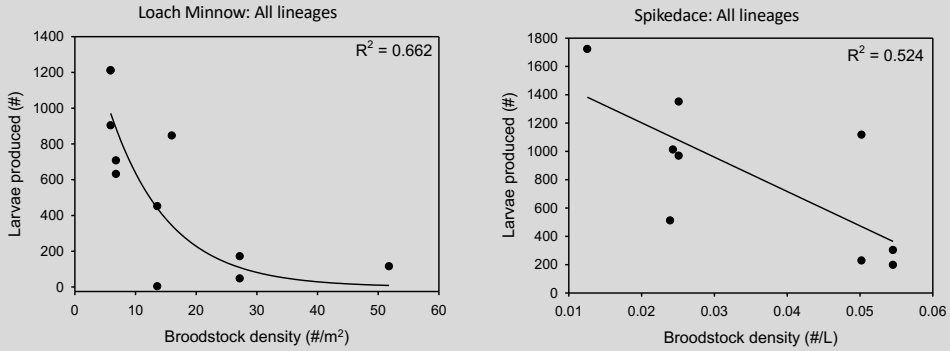
2018 Propagation research overview

Objective: Determine the relationship between broodstock density and larval production per raceway.

Methods: Using a total of 12 large raceways in cage 1, test 3 different brood stock densities (low, medium, high) for both target species with two replicates of each density. For Spikedace, density is determined as fish/L and fish/m^2 for Loach Minnow. All other parameters were matched between each raceway including the ones not used in this study. Larval fish were removed and counted weekly. Flows were set at a constant 75 gpm for all cage 1 raceways with a single high flow event once per day for 4 minutes.

Loach Minnow: Spawning substrate consisted of small chip gravel 2 inches thick. Nest were constructed using medium sized cobble stones laid out in 6 inch circles spaced 15 inches from edge of nest to edge of nest. Nest were placed in alternating patterns for the larger raceways in cage 1 and down the center for the raceways in cage 2. Three cinder blocks were placed at the up stream end for cage 1 raceways to distribute the recirculation flow and keep spawning substrate in place.

2018 density study results



Results: For both species, larval fish production was negatively related to bloodstock density. Spikedace exhibited a linear relationship. Loach Minnow conversely exhibited a negative exponential decay relationship. 2018 resulted in 2 times the total Spikedace production from previous years and 8 time the total Loach Minnow production. This includes all lineages.

Anecdotal findings

1. Loach Minnow stocking densities are likely more related to the number of fish per nest than actual density
2. The optimal Loach Minnow density seems to be one spawning pair per nest with a few extra adults in the raceway.
3. Nest spacing can likely be reduced to increase the number of nests per raceway and the number of adult individuals.
4. Larval fish removal is significantly more difficult for Loach Minnow and could result in egg disturbance if not done carefully.
5. Algae levels in all raceways were lower than normal for an unknown reason possibly contributing to a more successful spawn for both species.
6. Higher densities may be achieved when adding cobble to the entire raceway on top of the chip gravel. This allows fish to space themselves as there are no distinct nests available. While this has the potential to increase the number of adults in a raceway, it further complicates larval fish removal and will still likely show the same relationship in terms of fish per nest site and larval production due to the territoriality of male Loach Minnow.

Factors that likely contribute to fluctuations in spawning success

1. Brood density
2. **Effective brood stock size**
3. **Age structure of broodstock**
4. **Male:Female Ratio**
5. Substrate types and spacing
6. Flow rate
7. Temperature
8. Algae growth
9. Disease outbreaks
10. Predation and stress

Broodstock Management

Key
- Optimistic collections will still occur
- No fish were brought in
- Fish were brought in but kept counts below desired 500
- Fish were brought in brining counts near 500

Wild caught fish brought to ARCC

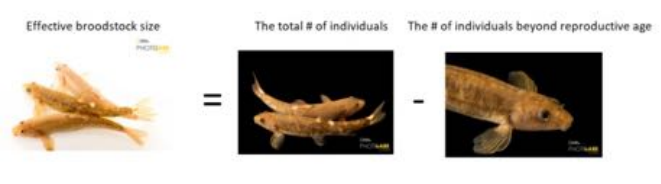
Taxa	Basin	Extant Lineage/Stream	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Meda fulgida	Gila	upper Gila River, NM	840		148									
Meda fulgida		Gila River Forks (East, West, Middle)			17	250	148			XXX				
Meda fulgida	San Pedro	Aravaipa Creek (& Turkey Creek)	258		200		XXX			28	150	80	163	
Tiaroga cobitus		Gila River Forks (East, West, Middle)			46	100	434			61			110	
Tiaroga cobitus	San Francisco	San Francisco River, NM (& Tularosa River, Negrito Creek)						XXX	41					
Tiaroga cobitus		Blue River (& Campbell Blue Creek, Dry Blue Creek, Pace Creek, Dutch Blue Creek)		30	91		27	XXX	XXX			12		222
Tiaroga cobitus	San Pedro	Aravaipa Creek (& Deer and Turkey Creeks)	254		110		XXX	XXX		48	50	200	100	

ARCC brood stock counts

Taxa	Basin	Extant Lineage/Stream	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	
Meda fulgida	Gila	upper Gila River, NM								[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	No wild genetics in 9 years
Meda fulgida		Gila River Forks (East, West, Middle)								250	[illegible]	[illegible]	[illegible]	83	No wild genetics in 4 years
Meda fulgida	San Pedro	Aravaipa Creek (& Turkey Creek)								480	412	263	382	331	
Tiaroga cobitus		Gila River Forks (East, West, Middle)								57	[illegible]	[illegible]	128	97	
Tiaroga cobitus	San Francisco	San Francisco River, NM (& Tularosa River, Negrito Creek)								[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	No wild genetics in 5 years
Tiaroga cobitus		Blue River (& Campbell Blue Creek, Dry Blue Creek, Pace Creek, Dutch Blue Creek)								[illegible]	[illegible]	214	[illegible]	117	
Tiaroga cobitus	San Pedro	Aravaipa Creek (& Deer and Turkey Creeks)								340	316	297	490	438	

Effective broodstock size

Who is contributing to spawn?
At what rates are they contributing?
How can we better track the production of individuals in the hatchery?



Are the individuals that are not contributing negatively impacting propagation?

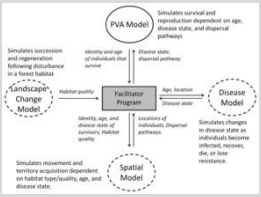
Future needs:
1. Better tracking of wild and hatchery stock throughout the facility
2. Ability to track the year fish were brought on station
3. Frequent genetic testing of offspring, broodstock and wild fish

2019 Research plans

- Ongoing density study
  - Same experimental plan as 2018
- VIE Feasibility and Tag Retention
  - Both species
  - Laboratory-based
  - 6 months post-tagging
- Optimal Feed Rates
  - Both species
  - Laboratory-based
- Paired aquaria spawning
  - Loach Minnow
  - Single-nest pairing
- Hormone-induced spawning
  - Spikedace
  - Carp Pituitary Extract

Long term research

- Loach Minnow nest spacing
  - Using optimal density
  - Determine optimal nest spacing
- Post-stocking survival and movement
  - Spring Creek
  - VIE-tagged Spikedace
  - Two sites each below and above stocking site
  - Sampling up to 3 months post-stocking
  - 1000 fish
- Population Viability Analysis Model
  - Life history parameters needed to refine model
  - Can all be done in laboratory
  - Fecundity, temp. tolerances, etc.



Planning for success

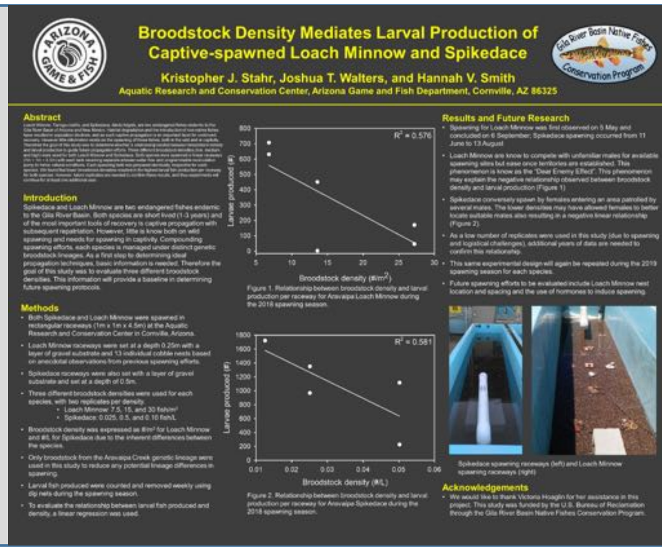
Uh oh, we have too many fish!

Things to consider:

1. **If no stocking sites available what are the alternatives?**
2. **Discuss options for stocking in advance**
3. **Short lived fish require quick action**
4. **Tank space at the ARCC is limited**

- **Stocking in streams with invasive species?**
- **Stocking more individuals into available systems?**
- **After genetic testing, stock offspring on source populations?**

Until we conduct research to determine why these species do or do not establish in the systems that have been renovated and optimized for them all options should be on the table.


Broodstock Density Mediates Larval Production of Captive-spawned Loach Minnow and Spikedace
Kristopher J. Stahr, Joshua T. Walters, and Hannah V. Smith
Aquatic Research and Conservation Center, Arizona Game and Fish Department, Cornville, AZ 86325
Abstract
Introduction
Spikedace and Loach Minnow are two endangered fishes endemic to the Gila River Basin. Both species are short lived (1-3 years) and of the most important tools of recovery is captive propagation with subsequent repatriation. However, little is know both on wild spawning and needs for spawning in captivity. Compounding spawning efforts, each species is managed under distinct genetic broodstock lineages. As a first step to determining ideal propagation techniques, basic information is needed. Therefore the goal of this study was to evaluate three different broodstock densities. This information will provide a baseline in determining future spawning protocols.
Methods
Both Spikedace and Loach Minnow were spawned in rectangular raceways (1m x 1m x 4.5m) at the Aquatic Research and Conservation Center in Cornville, Arizona.
Loach Minnow raceways were set at a depth 0.25m with a layer of gravel substrate and 13 individual cobble nests based on anecdotal observations from previous spawning efforts.
Spikedace raceways were also set with a layer of gravel substrate and set at a depth of 0.5m.
Three different broodstock densities were used for each species, with two replicates per density.
Loach Minnow: 7.5, 15, and 30 fish/m²
Spikedace: 0.025, 0.5, and 0.10 fish/L
Broodstock density was expressed as #/m² for Loach Minnow and #/L for Spikedace due to the inherent differences between the species.
Only broodstock from the Aravaipa Creek genetic lineage were used in this study to reduce any potential lineage differences in spawning.
Larval fish produced were counted and removed weekly using dip nets during the spawning season.
To evaluate the relationship between larval fish produced and density, a linear regression was used.
R² = 0.576
Larvae produced (#)
Broodstock density (#/m²)
Figure 1. Relationship between broodstock density and larval production per raceway for Aravaipa Loach Minnow during the 2018 spawning season.
R² = 0.581
Larvae produced (#)
Broodstock density (#/L)
Figure 2. Relationship between broodstock density and larval production per raceway for Aravaipa Spikedace during the 2018 spawning season.
Results and Future Research
Spawning for Loach Minnow was first observed on 5 May and concluded on 6 September; Spikedace spawning occurred from 11 June to 13 August.
Loach Minnow are know to compete with unfamiliar males for available spawning sites but ease once territories are established. This phenomenon is know as the "Dear Enemy Effect". This phenomenon may explain the negative relationship observed between broodstock density and larval production (Figure 1).
Spikedace conversely spawn by females entering an area patrolled by several males. The lower densities may have allowed females to better locate suitable mates also resulting in a negative linear relationship (Figure 2).
As a low number of replicates were used in this study (due to spawning and logistical challenges), additional years of data are needed to confirm this relationship.
This same experimental design will again be repeated during the 2019 spawning season for each species.
Future spawning efforts to be evaluated include Loach Minnow nest location and spacing and the use of hormones to induce spawning.
Spikedace spawning raceways (left) and Loach Minnow spawning raceways (right)
Acknowledgements
We would like to thank Victoria Hoaglin for her assistance in this project. This study was funded by the U.S. Bureau of Reclamation through the Gila River Basin Native Fishes Conservation Program.

2018 NM Projects Update

GRBNFCP Technical Committee Meeting
December 11, 2018

Project 1
Removal of nonnative fishes from West Fork Gila River

- Project began in 2006
- 4 km reach on DGF Heartbar Property
- Single Pass removal, 2 shockers
- Propst et al. 2014

Project 1
Removal of nonnative fishes from West Fork Gila River

WF Gila NNR Results

June 2017

Species	Number Caught	Density (fish/m^2)
Native		
Desert Sucker	1393	0.05
Gila Trout	12	0
Loach Minnow	1802	0.064
Longfin Dace	871	0.031
Roundtail Chub	1	0
Sonora Sucker	807	0.029
Speckled Dace	692	0.025
Spikedace	495	0.018
Nonnative		
Common Carp	3	0
Rainbow Trout Hybrid	1	0
Red Shiner	1	0
Yellow Bullhead	17	0

June 2018

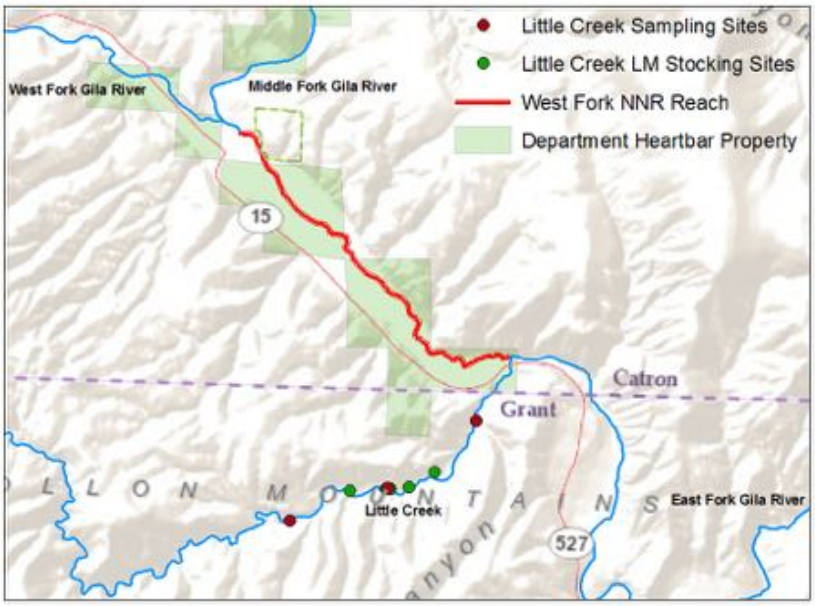
Species	Number Caught	Density (fish/m^2)
Native		
Desert Sucker	2810	0.110
Gila Trout	1	0
Loach Minnow	2522	0.099
Longfin Dace	2472	0.097
Roundtail Chub	4	0
Sonora Sucker	2316	0.091
Speckled Dace	1326	0.052
Spikedace	1512	0.059
Nonnative		
Green Sunfish	1	0
Rainbow Trout Hybrid	6	0
Smallmouth Bass	6	0
Western Mosquitofish	110	0.004
Yellow Bullhead	384	0.015

Project 2
New Mexico T&E Repatriations and Monitoring

Little Creek
Loach Minnow Repatriation

Date	Number Stocked	Stocking Source
November 18th, 2014	267	ARCC (Gila Forks)
September 3rd, 2015	62	WF Gila River
November 15th, 2016	125	WF Gila River
November 30th, 2017	159	ARCC (Gila Forks)
November 30th, 2017	103	WF Gila River

Sampled July 19th, 2018 (3 Loach Minnow Captured at one site)
Sampled May, 2017 (45 were captured and were present in all 3 sites)



Project 2
New Mexico T&E Repatriations and Monitoring

Mule Creek
Roundtail (Gila) Chub Repatriation

Date	Number Stocked	Stocking Source
June 27, 2012	120	Harden Cienega via ARCC
November 21, 2013	119	Harden Cienega via ARCC
November 13, 2014	60	Harden Cienega via ARCC

Nonnative fish found in 2016 (3 Smallmouth Bass, 3 Green Sunfish, 1 Black Bullhead)

Sampled April 11th, 2018
Chub Minimum Total Lengths: 107, 107, 110 (Previous smallest 117 in 2014)

Species	Number Captured	CPUE (fish/hr)
Black Bullhead	1	1.34
Desert Sucker	191	255.61
Longfin Dace	19	25.43
Roundtail Chub	16	21.41
Sonora Sucker	230	307.81
Speckled Dace	13	17.40

Project 2
New Mexico T&E Repatriations and Monitoring

Other 2018 projects:

Turkey Creek: Sampled April 10th 2018 for Chub tissue samples, 45 Chub captured in 3 pools at a rate of 252.73 fish/hr.

Burro Cienega (Gila Topminnow stocked 2008, last surveyed 2012): Sampled July 18th 2018 (4 Gila Topminnow Captured)

Project 2
New Mexico T&E Repatriations and Monitoring

Other 2018 projects:

Gila Farm Pond (Roundtail Chub stocked 2008, last surveyed 2012): 1 Largemouth Bass, 2 Yellow Bullhead, 25 Common Carp, 121 Green Sunfish

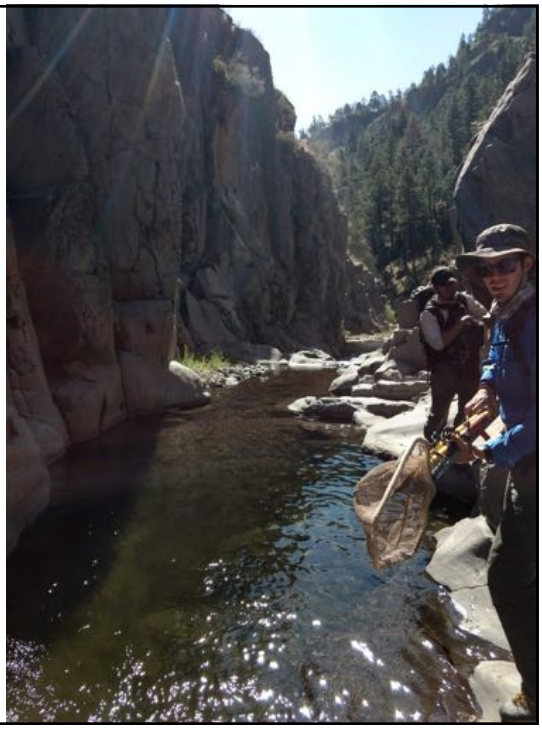
Other Spikedace/Loach Minnow Stockings:
San Francisco River (LM and SD): This winter TBD

Saliz Canyon: Loach Minnow stocked 2016 and 2017
Impacted by Owl Fire, Not suitable Loach Minnow habitat right now

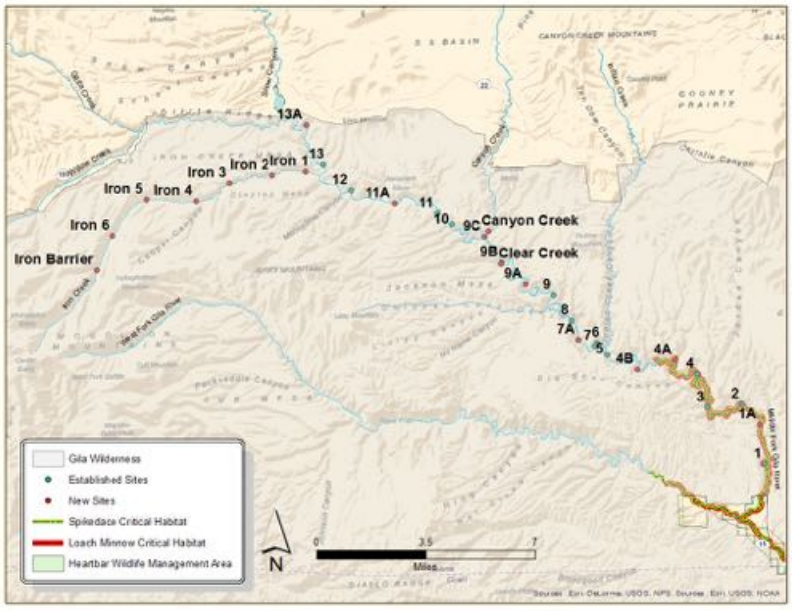
Project 3
Remote Site Inventory

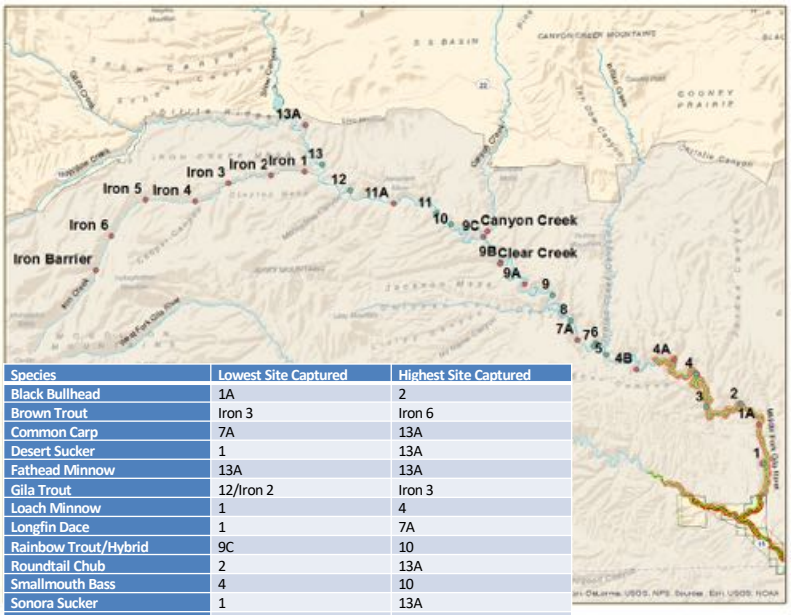
Middle Fork Gila River

- Inventory completed in 2008
- Updated surveys in lower section in June 2017
- Updated surveys in upper section in June 2018
- Two crews for one week of sampling



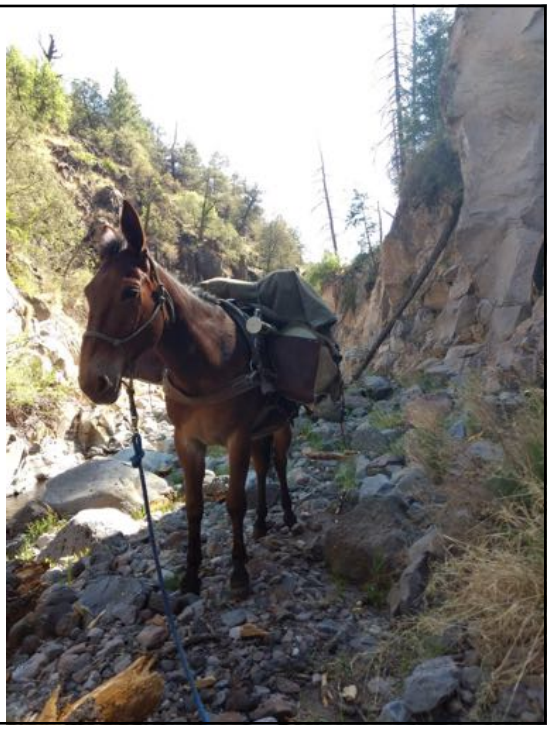
Project 3
Remote Site Inventory





Species	Lowest Site Captured	Highest Site Captured
Black Bullhead	1A	2
Brown Trout	Iron 3	Iron 6
Common Carp	7A	13A
Desert Sucker	1	13A
Fathead Minnow	13A	13A
Gila Trout	12/Iron 2	Iron 3
Loach Minnow	1	4
Longfin Dace	1	7A
Rainbow Trout/Hybrid	9C	10
Roundtail Chub	2	13A
Smallmouth Bass	4	10
Sonora Sucker	1	13A
Speckled Dace	1	13A
Spikedace	1	3
Western Mosquitofish	1	1
Yellow Bullhead	1A	7

Questions?



USDA U.S. FOREST SERVICE
Caring for the land and serving people

Rocky Mountain Research Station
National Genomic Center
Southwestern Region
Bureau of Reclamation

eDNA For Southwest Aquatic Species

3 Main Objectives for Agreement

Expanded sampling for Spikedace and Loachminnow

- 2 years of support for field sampling (Gila NF primarily)
- Funding for approximately 300 samples (2 species) – Rangewide/Multiagency
  - 79 Samples Collected 2018

Southwest eDNA Database Development

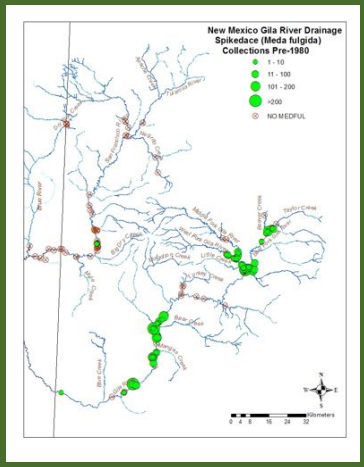
Development of 10 additional species markers.

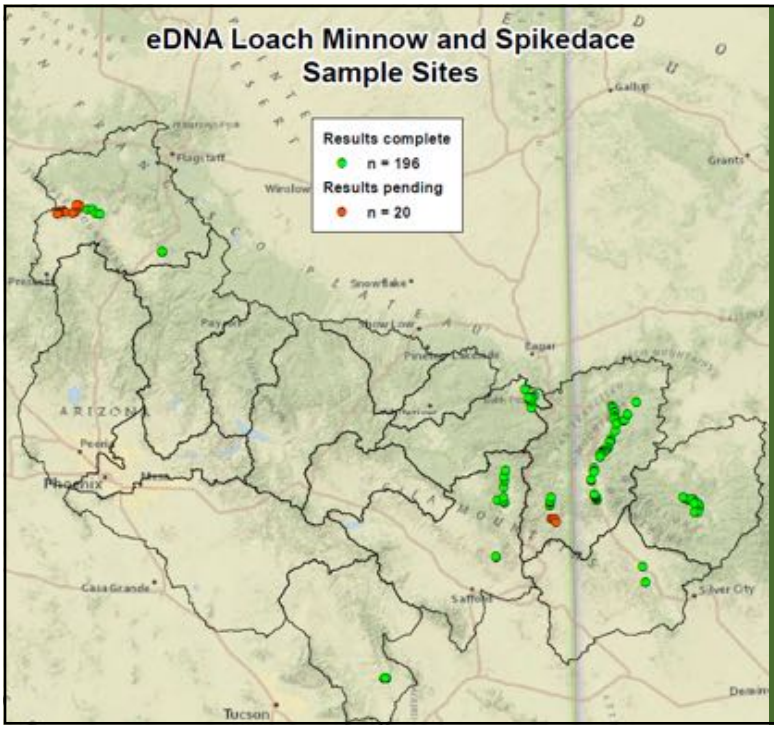
Prioritized Sampling

Prioritized populations that distribution of SD/LM is unknown or locations with high priority for management actions.

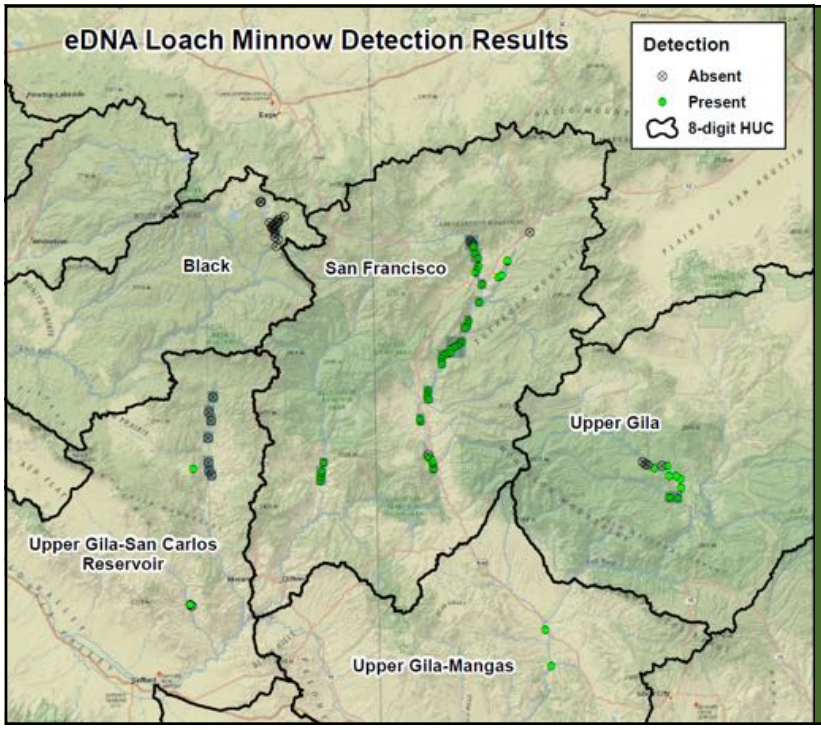
Some of the higher priority areas selected-

- San Francisco River
- Verde
- Eagle
- East Fork Gila River
- Mainstem Gila River
- East Fork Black River



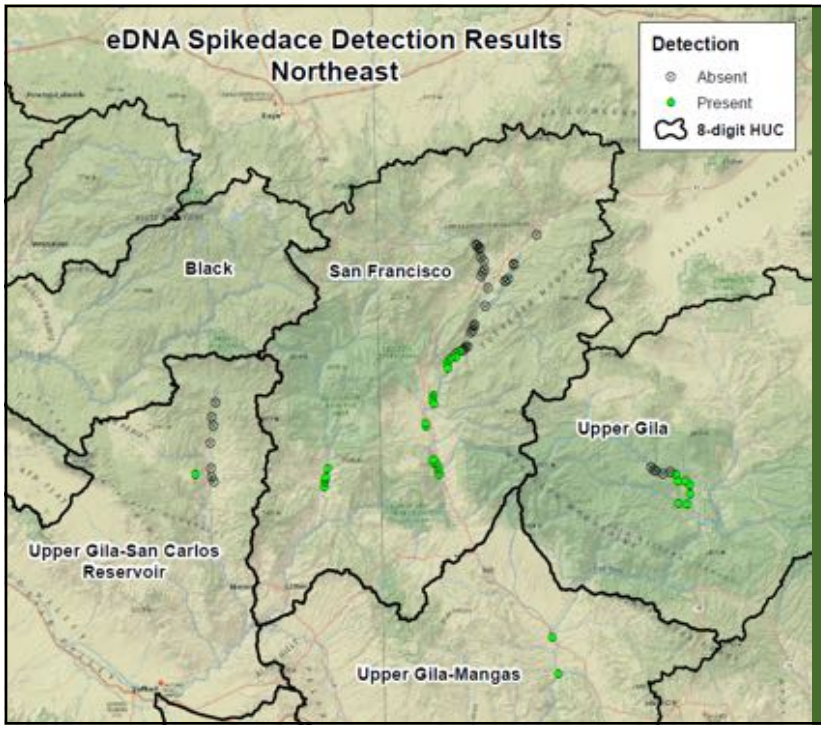


Current Sample Locations



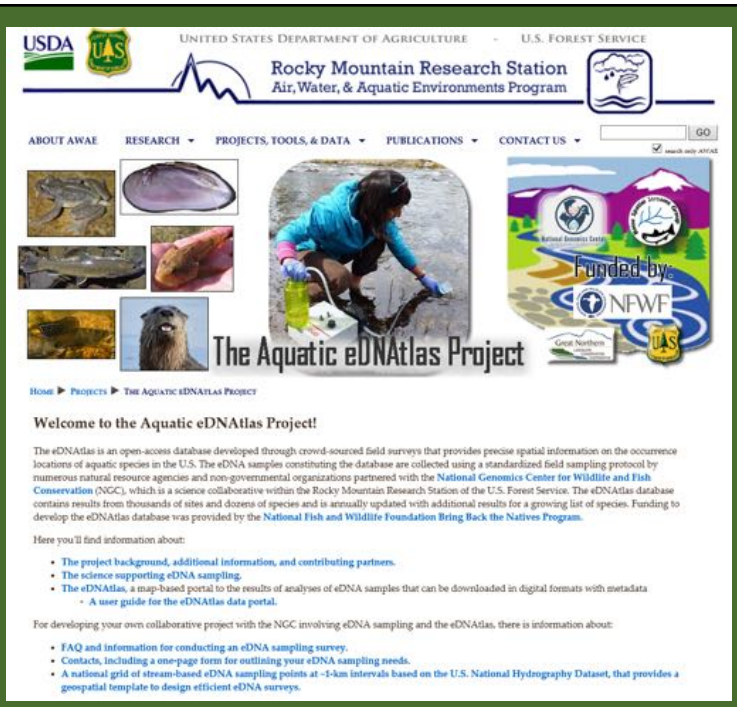
Loach Minnow

Systematic Sampling Examples



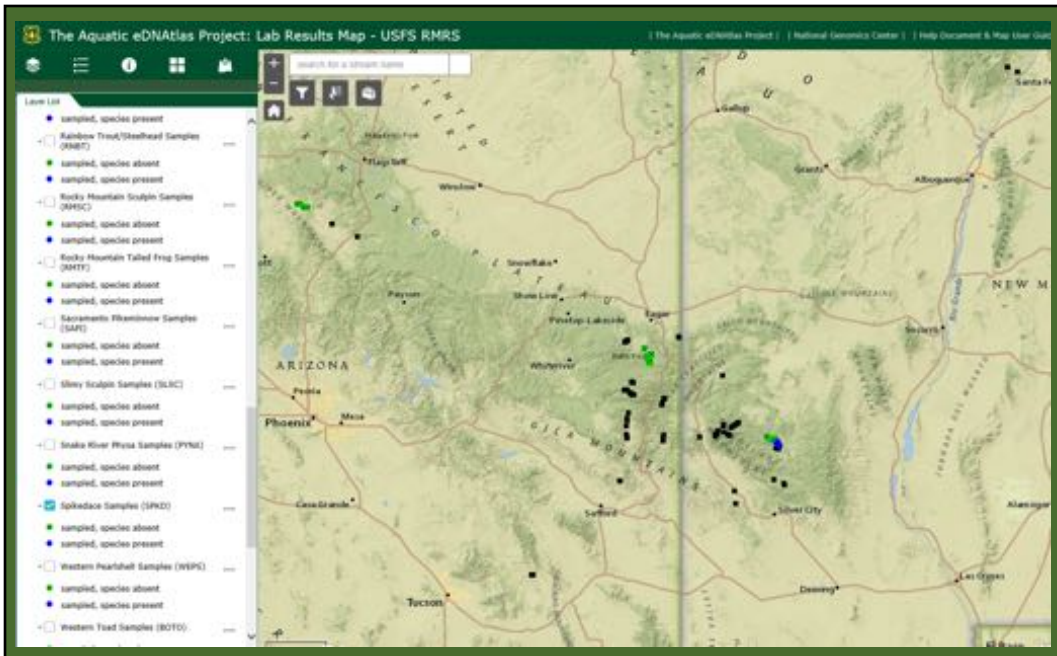
Spikedace

Systematic Sampling Examples

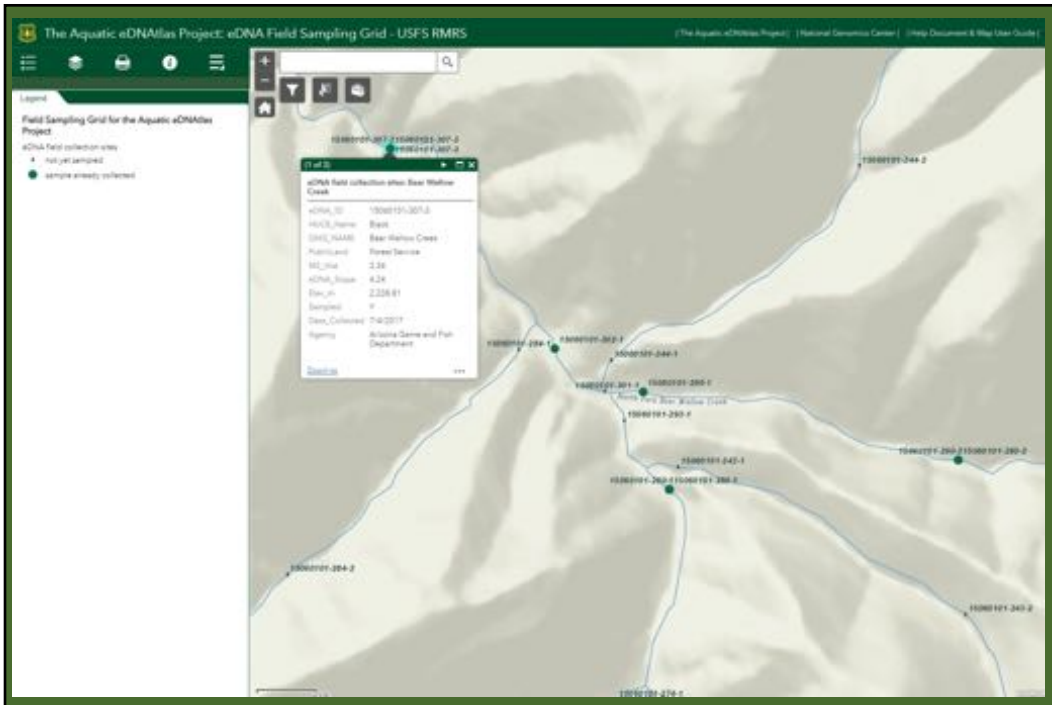


eDNA Atlas

- Some information in.
- Another download expected before end of year for species and sample sites.
- AZ/NM Streams have all been subdivided into 1 km sample sites each with some habitat information (slope and elevation).



Example of Output



Watersheds Mapped

Sampling Grid

National Genomic Center Markers	In Process Markers NGC/USFS
Any Salmonid	Green sunfish
Boreal Toad / Western Toad Complex	Desert sucker
Brook Trout	Bluehead sucker (CO)
Brown Trout	Sonora sucker
Loach Minnow	Razorback sucker
North American River Otter	Red shiner
Northern Pike	Roundtail chub
Rainbow Trout/Steelhead	Mosquitofish
Rio Grande Chub	Gila topminnow
Rio Grande sucker	Desert pupfish
Smallmouth Bass	White Sands pupfish
Spikedace	Channel catfish
Yaqui Catfish	Sonoran tiger salamander
Partner Verified Markers	
California floater	Other partners working on Gartersnakes, Jumping Mouse
Dreissenid mussels (zebra/quagga)	Unsure of progress – will have to be verified by NGC.
Chitrid (BD)	

Need to prioritize future marker development.

- Green Sunfish and Roundtail Chub in process.
- DOE developing some on list.
- What are our next priorities?

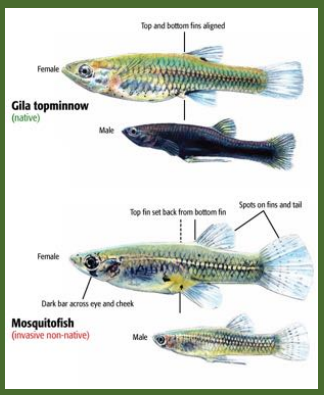
Things to consider….

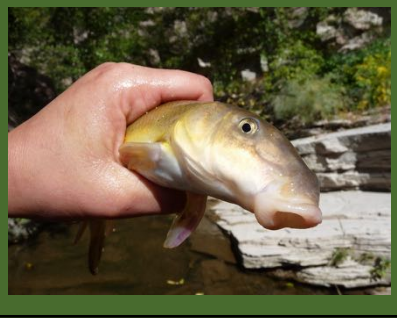
Species that are difficult to detect.

Management needs for Gila Basin Projects.

Recovery information needs.

Group Discussion of Priorities





Habitat assessments for Spikedace and Loach minnow

Ben Koch and Jack Torresdal

Center for Ecosystem Science & Society
Northern Arizona University

Funding by Arizona Game and Fish Department Natural Heritage Program

Purpose: To improve the success of repatriations and translocations of endangered fish in Arizona.

- Spikedace (*Meda fulgida*) and loach minnow (*Rhinichthys cobitis*) are among the rarest of native fish species remaining in southwestern streams.
- Repatriations / translocations are integral to their recovery, yet efforts to date have had mixed success.
- The objective of this project is to identify a suite of easily-measurable quantitative metrics to identify stream reaches most likely to support populations of our two focal species.
- These data will help prioritize new stream reaches for successful spikedace and loach minnow repatriations / translocations.

Reaches surveyed for this project (2016-2018) include unoccupied, previously occupied, and currently occupied sites for each species:

- Spring Creek (Oak)
- Spring Creek (Tonto)
- Upper Tonto (Kohl's)
- Campbell Blue
- Upper Blue River
- West Fork Black River
- North Fork East Fork Black
- West Clear Creek
- Fossil Creek (upper)
- Fossil Creek (Mazatzal)
- Redfield Canyon
- Upper Verde River
- Bonita Creek (above barrier)
- Bonita Creek (Midnight Canyon)
- Blue River (Juan Miller crossing)
- Eagle Creek (Honeymoon)
- Aravaipa
- Hot Springs Canyon

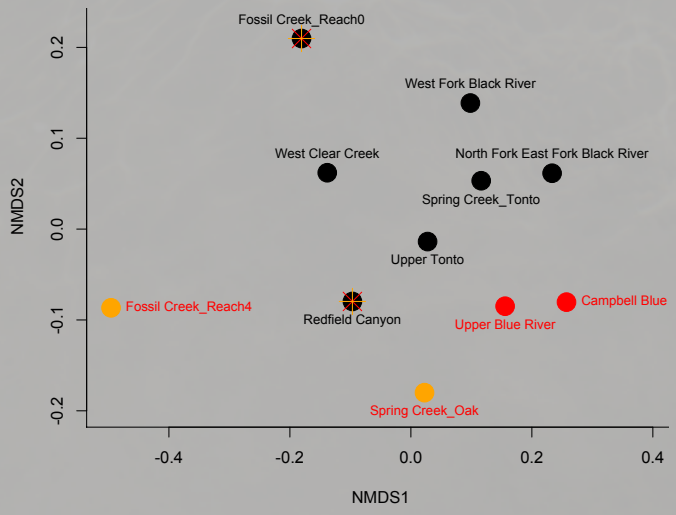
What we measure at each site:

(reaches: 250-500m total length)

- 10 transects per reach:
  - width, depths, substrate type, substrate embeddedness, canopy cover, large woody debris, overhanging vegetation, dominant riparian vegetation
  - pebble counts: measure *B*-axis of 100 particles per transect to generate a quantitative measure of substrate size (& variability) for the reach
  - % habitat type: run / riffle / eddy / pool / glide backwater
- Suspended organic matter concentrations (morning, mid-day, late afternoon)
- Discharge
- Fish abundance / composition / size structure (snorkel surveys)
- Macroinvertebrate abundance & composition (family-level)
- Primary productivity (O_2 & incident light) for 1-4 weeks
- Sub-hourly stream temperature for 1 year

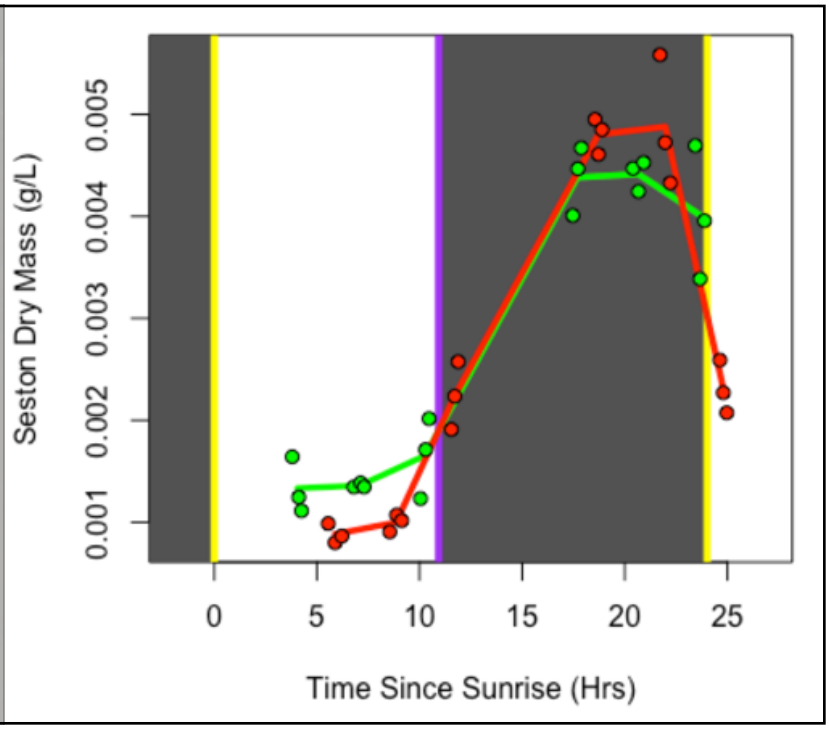
2017 data (preliminary)

- unoccupied
- spikedace occupied
- loach minnow occupied
- failed repatriation



Future field experiment planned for 2019 (funding just secured from AWPF):

Hypothesis:
Native fish (e.g., suckers) may facilitate spikedace by supplying food particles via bioturbation.

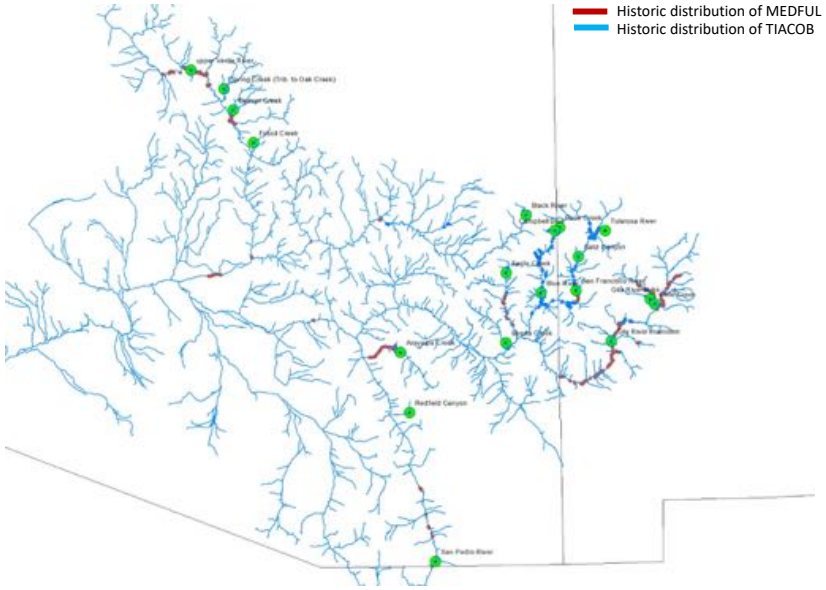


Questions:

Jack Torresdal (jtorresdal@gmail.com)

Objectives

- Identify habitat available for species across range
  - Multiple spatial scales
- Quantify species needs for successful repatriation
- Identify locations with high likelihood for successful repatriation



Methods

- Reach - 1 Kilometer
- Site – 3 100 m sections
  - Upper, Middle, and Lower sections of the reach
- Mesohabitat - 8-10 sampled per section
  - ~1 – 10 m^2
  - Depth, Velocity, substrate measured at 9 points within each mesohabitat

Methods

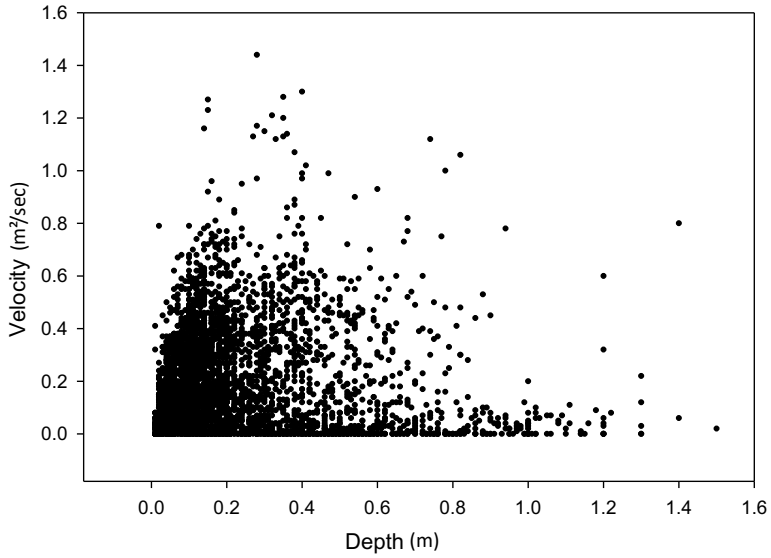
- Habitat availability
  - Transects
  - ~200 points per reach

Roth et al. 1996

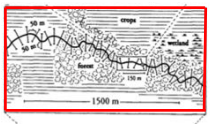
Fish Communities

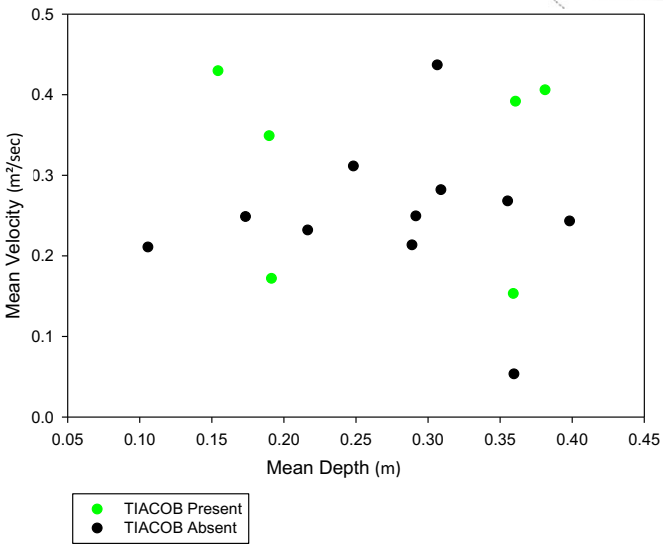
Site	# Native Species (Total)	# Non-Natives Species (Total)	# TIACOB	# MEDFUL
Blue	7 (444)	1 (58)	11	180
Aravaipa	7 (879)	0	96	127
West Fork	6 (317)	1 (2)	30	89
Riverside	5 (417)	6 (75)	83	78
Spring	5 (217)	1 (14)	0	24
Fossil	6 (66)	1 (1)	0	14
Cambell	5 (393)	2 (5)	11	0
Little Creek	5 (246)	2 (15)	1	0
Beaver	1 (6)	3 (33)	0	0
Black	2 (92)	3 (62)	0	0
Bonita	2 (36)	4 (139)	0	0
Eagle	4 (605)	1 (67)	0	0
Saliz	2 (38)	0	0	0
San Fransisco	4 (10)	5 (34)	0	0
San Pedro	2 (152)	3 (256)	0	0
Tularosa	2 (9)	0	0	0
Upper Verde	4 (13)	6 (65)	0	0

Habitat Availability - Depth/Velocity

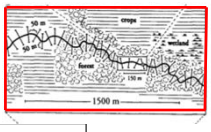


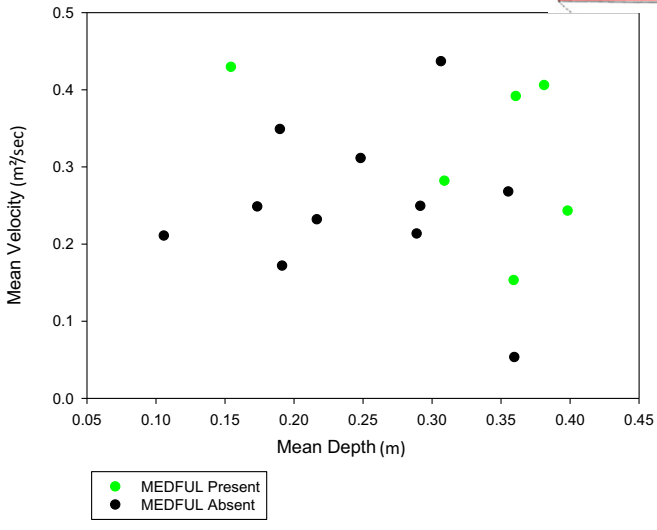
TIACOB Reach Level



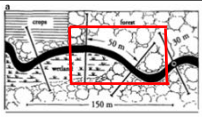


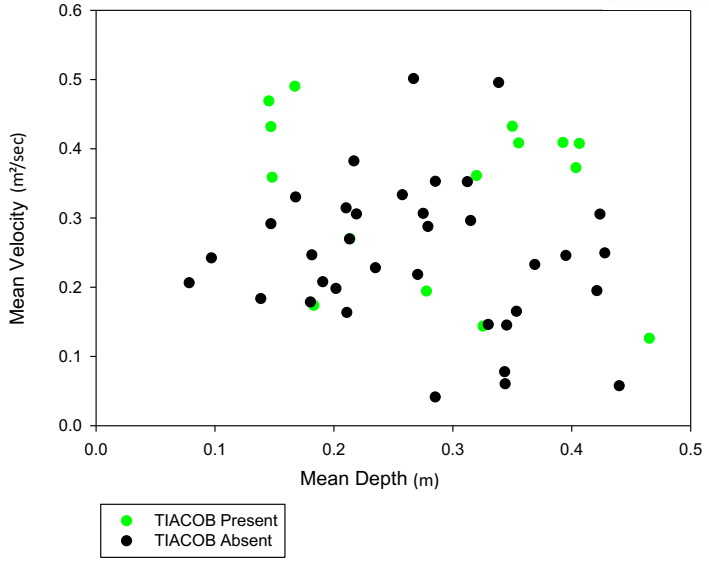
MEDFUL Reach Level



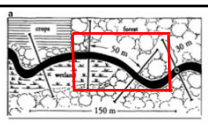


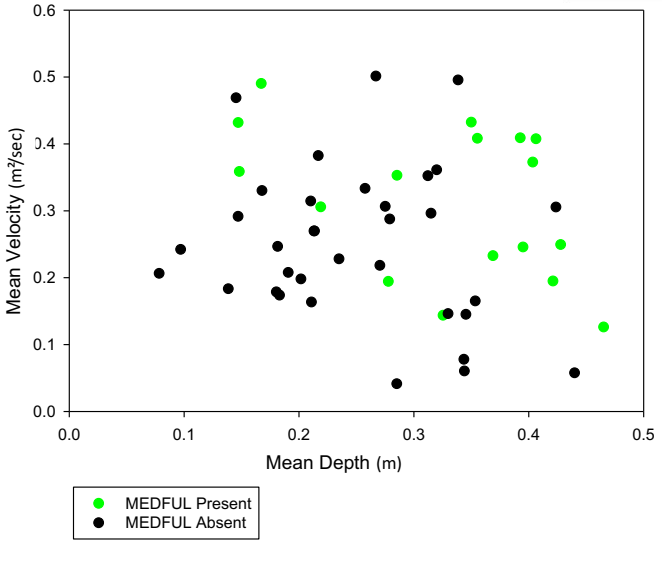
TIACOB Site Level



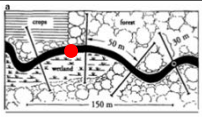


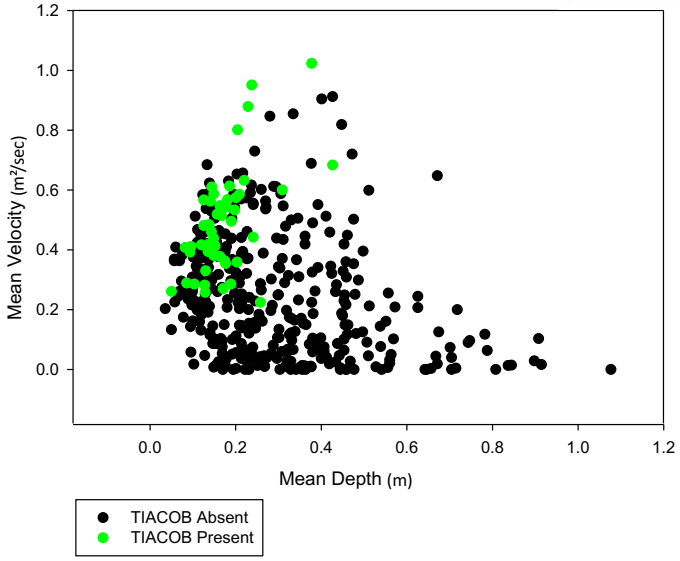
MEDFUL Site Level



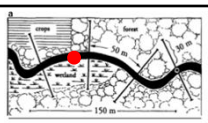


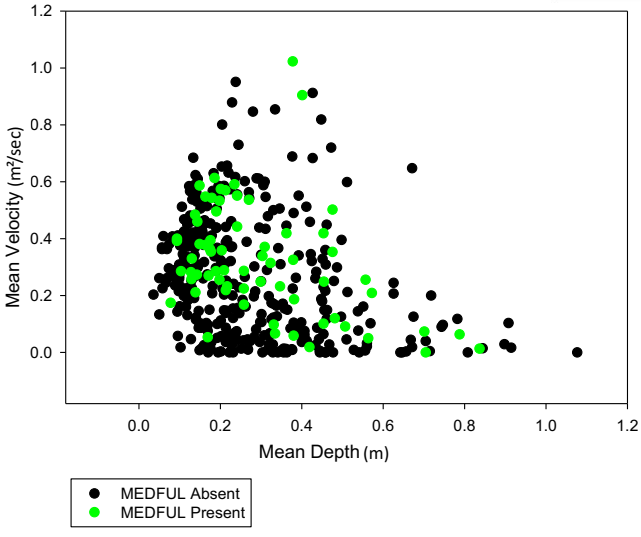
TIACOB Meso-Habitat Level



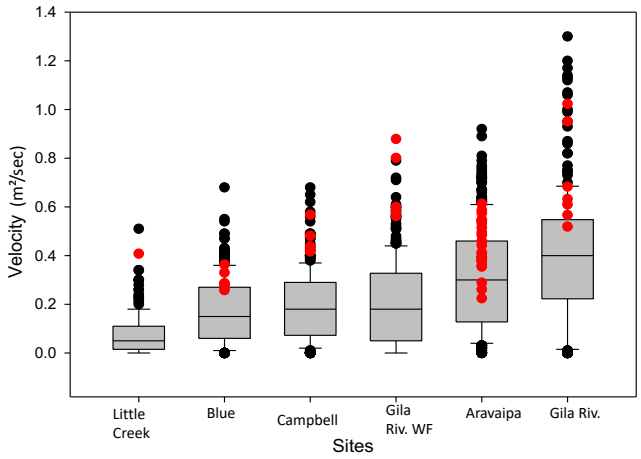


MEDFUL Meso-Habitat Level

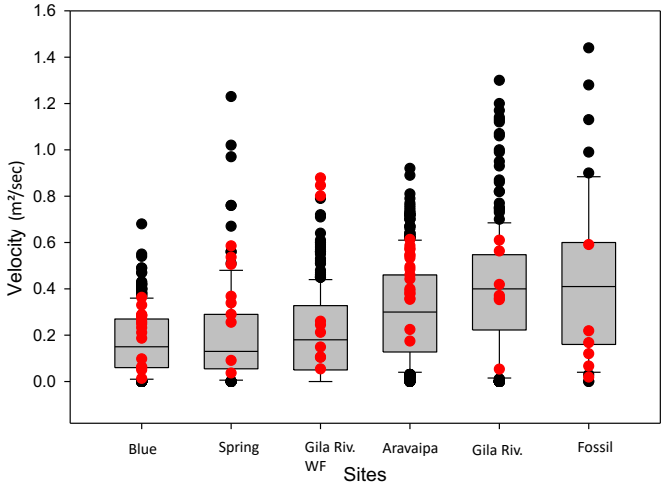




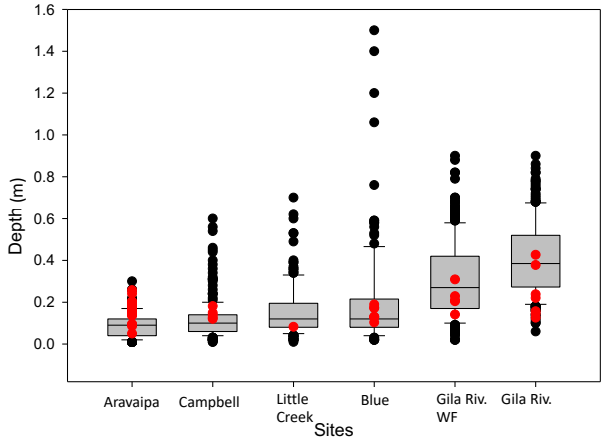
TIACOB Occupancy - Velocity



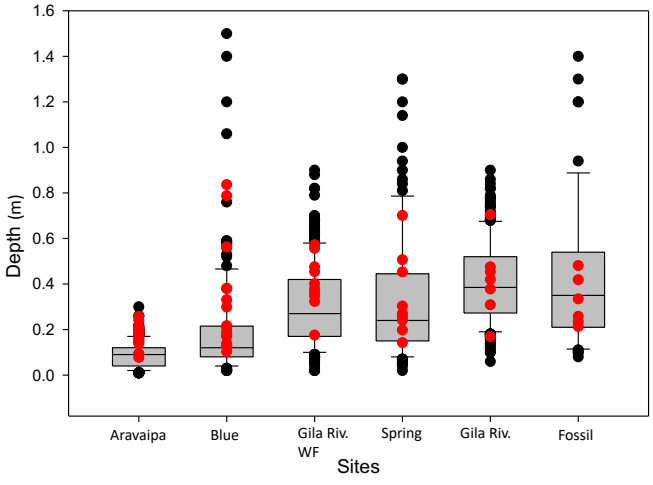
MEDFUL Occupancy - Velocity



TIACOB Occupancy - Depth



MEDFUL Occupancy - Depth



Conclusions

- More data to collect
- 3 More Sampling Trips
  - June 2019
  - October 2019
  - June 2020

Acknowledgments

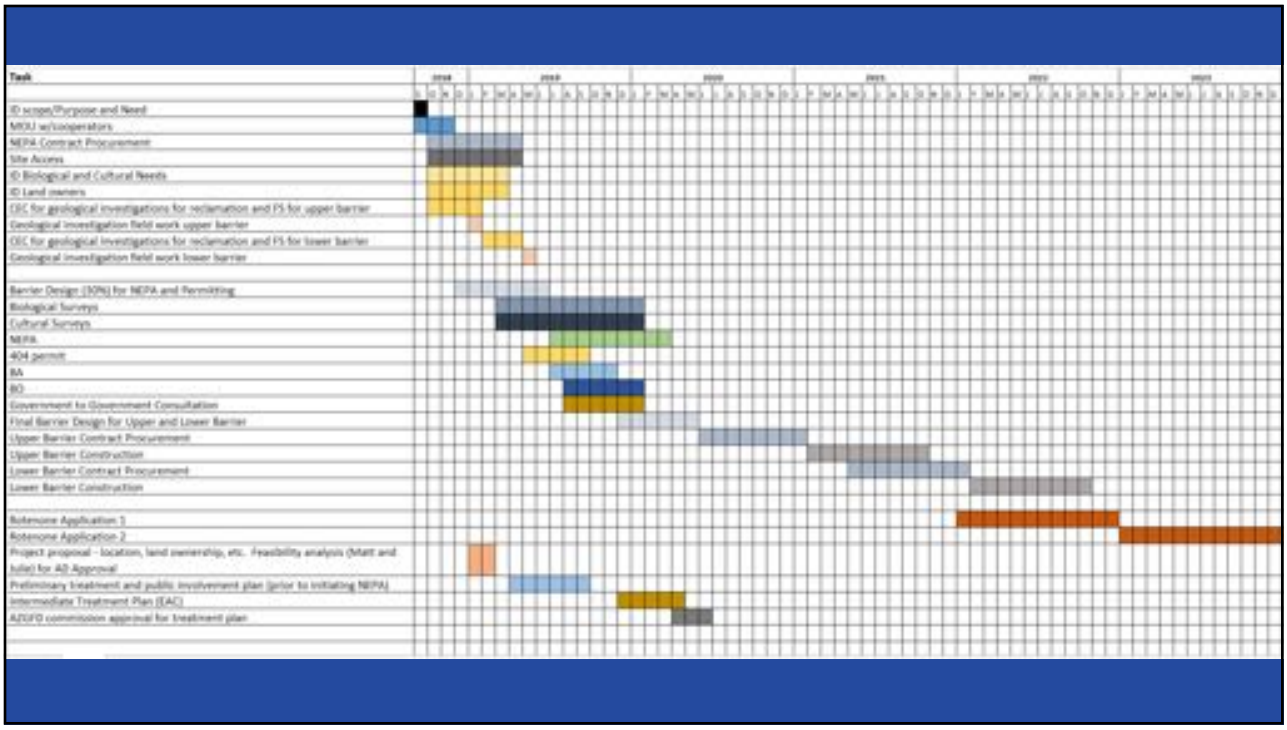
- Scott Clark
- Gregor Hamilton
- Steve Davenport
- Heidi Blasius
- Tony Robinson
- Martha Cooper
- Peter Reinthal
- Mark Haberstich

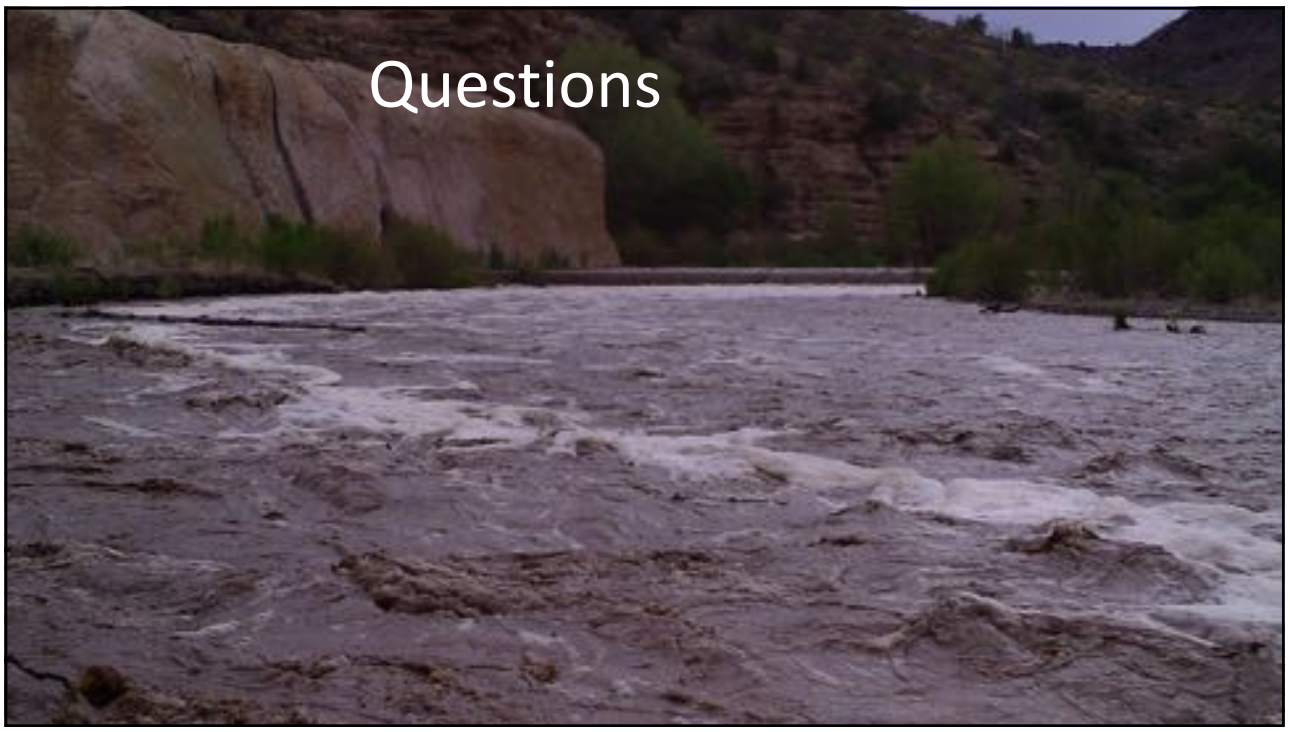
Fish Barriers

1. Aravaipa Creek (2001)
2. Fossil Creek (2004)
3. Cottonwood Creek (2004)
4. Bonita Creek (2008)
5. Hot Springs Canyon (2010)
6. Blue River (2012)
7. Spring Creek (2015)
8. W. Fork Black River (2016)
9. Eagle Creek (2019)
10. Redfield Canyon (2019)
11. Verde River #1 (2021)
12. Verde River #2 (2022)

Hot Springs


Verde River
Paulden
Perkinsville
Del Rio
Chino Valley
Granite
Lonesome Valley
Granite Dells
Prescott Valley
Clarkdale
Cottonwood
Page Springs
Beaverhead Flat
White Hills
Middle Verde
Sedona


Task
ID scope/Purpose and Need
MOU w/cooperators
NEPA Contract Procurement
Site Access
ID Biological and Cultural Needs
ID Land owners
CEC for geological investigations for reclamation and FS for upper barrier
Geological investigation field work upper barrier
CEC for geological investigations for reclamation and FS for lower barrier
Geological investigation field work lower barrier
Barrier Design (30%) for NEPA and Permitting
Biological Surveys
Cultural Surveys
NEPA
404 permit
BA
BO
Government to Government Consultation
Final Barrier Design for Upper and Lower Barrier
Upper Barrier Contract Procurement
Upper Barrier Construction
Lower Barrier Contract Procurement
Lower Barrier Construction
Rotenone Application 1
Rotenone Application 2
Project proposal - location, land ownership, etc. Feasibility analysis (Matt and Julie) for AZI Approval
Preliminary treatment and public involvement plan (prior to initiating NEPA)
Intermediate Treatment Plan (EAC)
AZGFD commission approval for treatment plan


Questions

Presentation Overview

- Gila Topminnow Genetics Project
- Overview of Genetic Management Plans (U.S. Fish and Wildlife Service template)

Genetic Characterization of Refuge, Reestablished, and Natural Populations of the Gila Topminnow in Arizona

Gila Topminnow Genetics: Overview

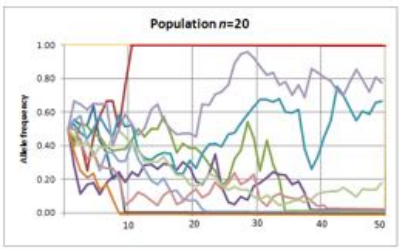
- **Funded by BOR, IA: R17PG00086**
- **Period of Performance FY2018-2022 (five years)**
  - Year 1-2: collect samples and develop markers
  - Year 3-5: genotype, analyses, draft documents
- **Objectives:**
  - Quantify the current genetic diversity within and among extant populations of Gila Topminnow.
  - Compare current diversity estimates to those previously reported in an effort to examine genetic drift and overall loss of genetic diversity.
  - Develop a genetic management protocol for management of captive stock and augmentation efforts.

Gila Topminnow Genetics: Methods

- **Collect approximately 2,050 samples from 41 Priority populations (50 individuals each).**
  - **Priority 1 populations** = 10 extant populations: Cienega Creek, Fresno Canyon, Coal Mine Canyon, Bylas Spring, Middle Springs, Salt Creek, Monkey Spring, Cottonwood Spring, Lower Santa Cruz
  - **Priority 2** = 6 ASU Animal Facility refuge populations
  - **Priority 3a** = 10 Long-lived refuge sites that have not been augmented
  - **Priority 3b** = 3 Long-lived sites that have been augmented
  - **Priority 4** = 12 Reestablished sites with over 500 overwintering adults
- **Develop new microsatellite markers and use in conjunction with the microsatellite markers used in previous studies for historical comparisons (genetic drift).**

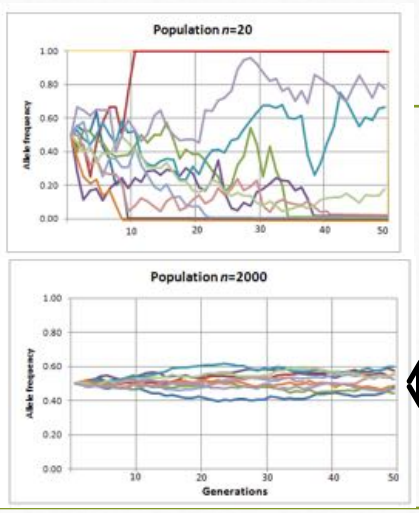
Genetic Drift – Overview and Example

Genetic Drift

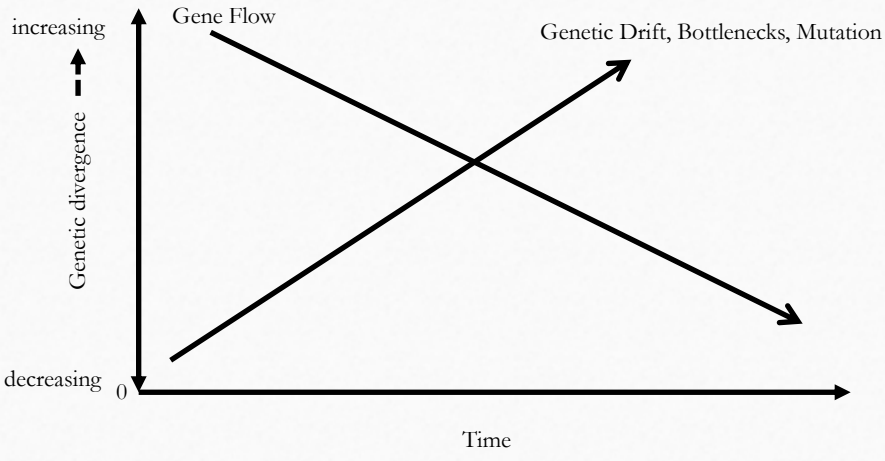


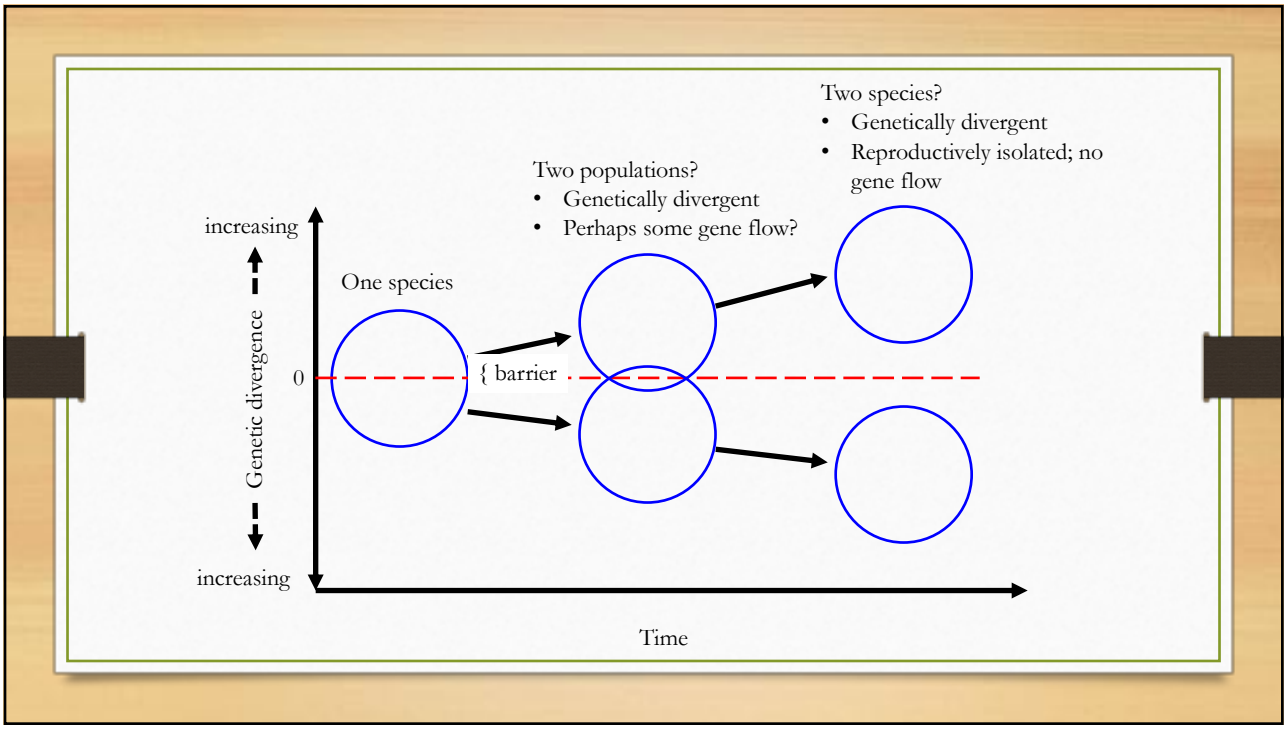
Large variance in allele frequency. Some lost some fixed.

Genetic Drift



Reduced variance in allele frequency

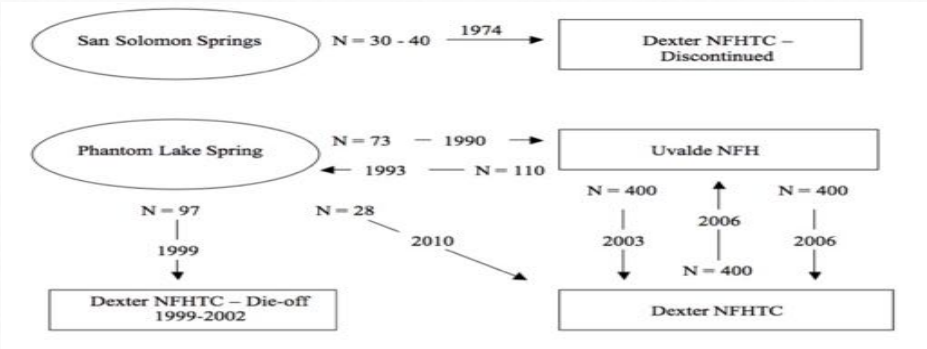




Drift and Gene Flow Example

- Comanche Springs Pupfish – TX
  - Fish species lives in spring fed system
  - Water pumping decreased flow of spring and decreased amount of habitat
  - Male have territories (0.5m) and defend them and build nests
  - Females choose male and spawn on nest
  - Transfer of wild fish to hatchery in 1970s

Drift and Gene Flow



Locus	Allele	Population/Allele Frequency: Uvalde 2002	Uvalde 2011	Southwestern ARRC	Phantom Lake Springs
Cdw16					
	333	0	4.4	7.2	1.8
	337	15.2	11.8	11.5	10.7
	341	3.0	1.5	1.4	0
	345	6.1	1.5	9.6	44.6
	349	3.0	0	0	0
	361	13.6	13.2	9.1	21.4
	365	3.0	7.4	4.8	0
	369	3.0	0	2.4	0
	377	33.3	44.1	38.5	17.9
	381	1.5	0	1.9	0
	385	0	0	0.5	0
	389	1.5	1.5	0.5	0
	397	15.2	14.7	12.0	3.6
	401	0	0	0.5	0
	405	1.5	0	0	0
Gata02					
	326	0	0	0.5	0
	330	0	1.5	2.4	22.2
	334	0	0	3.4	1.9
	338	30.0	9.1	40.4	16.7
	342	16.0	22.7	17.3	14.8
	346	38.0	39.4	26.9	11.1
	354	0	1.5	0	0
	358	16.0	24.2	9.1	33.3
	362	0	1.5	0	0
Gata09					
	172	1.5	2.9	0	0
	188	21.2	11.8	17.2	28.6
	262	0	13.2	6.9	8.9
	278	0	0	3.4	0
	282	0	1.5	1.0	0
	286	13.6	17.6	11.8	14.3
	290	4.5	2.9	1.5	0
	294	12.1	2.9	6.4	0
	298	0	0	1.5	0
	302	0	2.9	0	0
	308	10.6	7.4	12.7	25.0
	312	6.1	4.4	8.8	5.4
	316	12.1	10.3	4.9	7.1

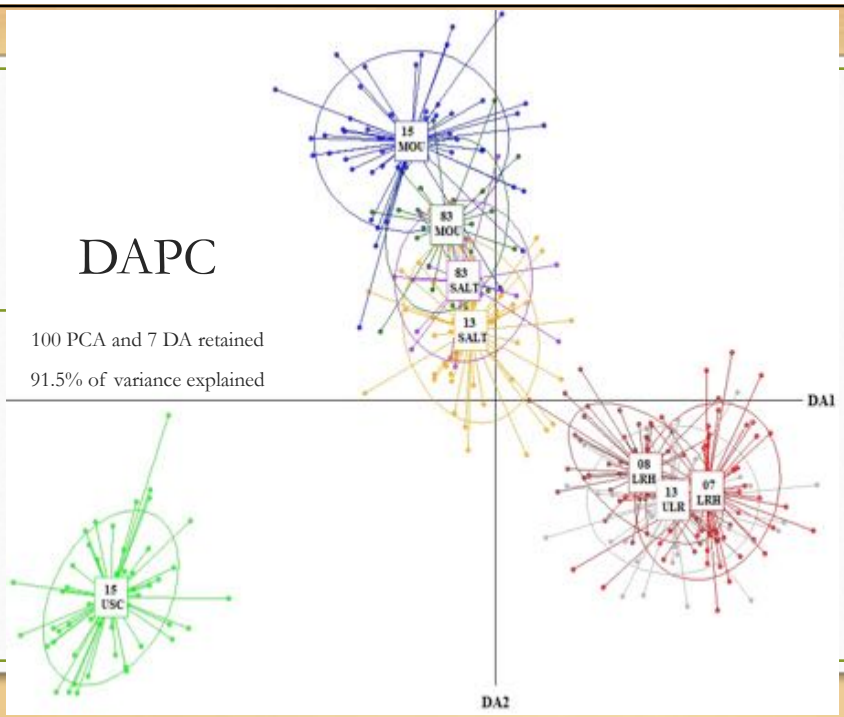
Discriminant Analysis of Principal Components

- Can use DAPC plots to examine population relationships and temporal patterns.
- Another pupfish example – translocation program to maintain additional populations and diversity.

DAPC

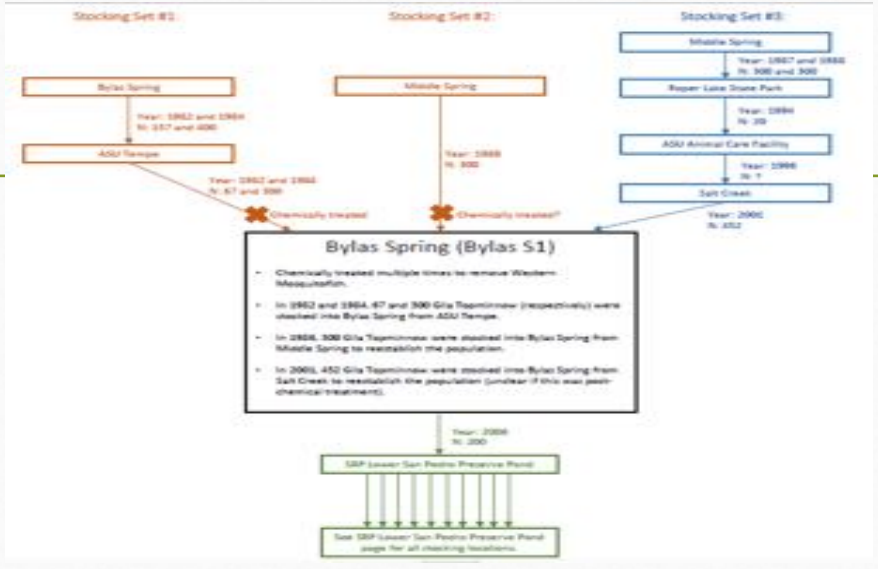
100 PCA and 7 DA retained

91.5% of variance explained



Gila Topminnow

- What are patterns in source and other populations?
  - Previous studies indicate diversity is being maintained, but good genetic monitoring programs periodically evaluate status.



Gila Topminnow Genetics: 2018 Update

- **Samples collected from 20 populations**
  - **Priority 1 populations** = extant populations (4 of 10)
  - **Priority 2** = 6 ASU Animal Facility refuge populations (6 of 6)
  - **Priority 3a** = 10 long lived refuge sites that have not been augmented (6 of 10)
  - **Priority 3b** = 3 Long-lived sites that have been augmented (0 of 3)
  - **Priority 4** = 12 Reestablished sites with over 500 overwintering adults (4 of 12)
- **Extractions started**
- **Microsatellite marker development data should be received next week**

Genetic Management Plans

U.S. Fish and Wildlife Service template

Genetic Management Plans

- **U.S. Fish and Wildlife Service - Fish and Aquatic Conservation Program (FAC)**
  - 2017 – developed a template to use across FAC Program for use with all aquatic species
  - Developed from Rio Grande Silvery Minnow (New Mexico) Plan

Template Sections

- **Introduction -** biology, life history, status, and population(s) description for the species of interest relevant to the propagation or captive process.
- **Propagation and Management Facilities**
  - **Current facilities –** capabilities, capacity, constraints
  - **Previous facilities –** background, history
- **Relationship to Recovery Plans and Recovery Programs –** specific goals or actions
- **Captive Propagation Goals –** life stage, target size, numbers, methods, timeline

Template Sections - continued

- **Genetic Management Goals -** relate back to Management Plans with specific tasks that need completed
- **Genetics and Captive Population Management**
  - **Genetic Risks to Captive Populations – Evaluate each:** Broodstock collection, loss due to human or equipment failure, inbreeding, population/lineage homogenization, genetic drift, disease transmission
  - **Genetic status –** genetic profiles of wild and captive populations, year founded, # individuals
- **Health Monitoring –** how, who, tests, # individuals

Template Sections - continued

- **Future Research Needs**
- **Literature Cited**
- **Appendices (e.g. Augmentation Plan)**

Thank You!

- **Bureau of Reclamation**
- **Arizona Game and Fish Department**
- **Marsh and Associates**

Questions?

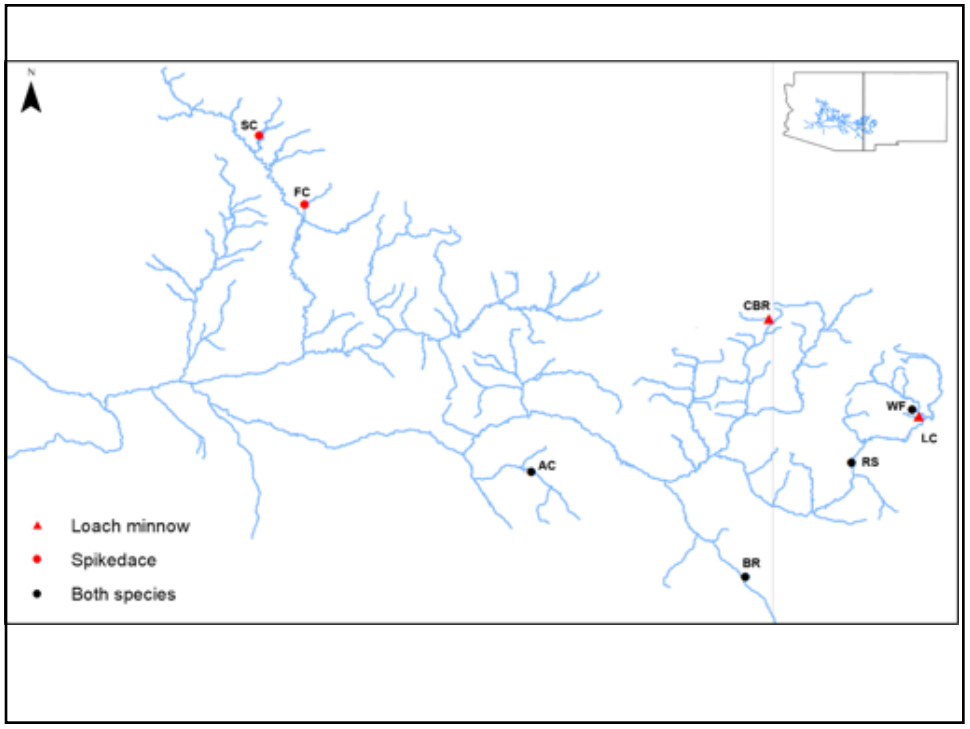
Range-wide genetic assessment of Loach Minnow and Spikedace

Loachminnow, *Tiaroga cobitis*
W.H. Brandenburg

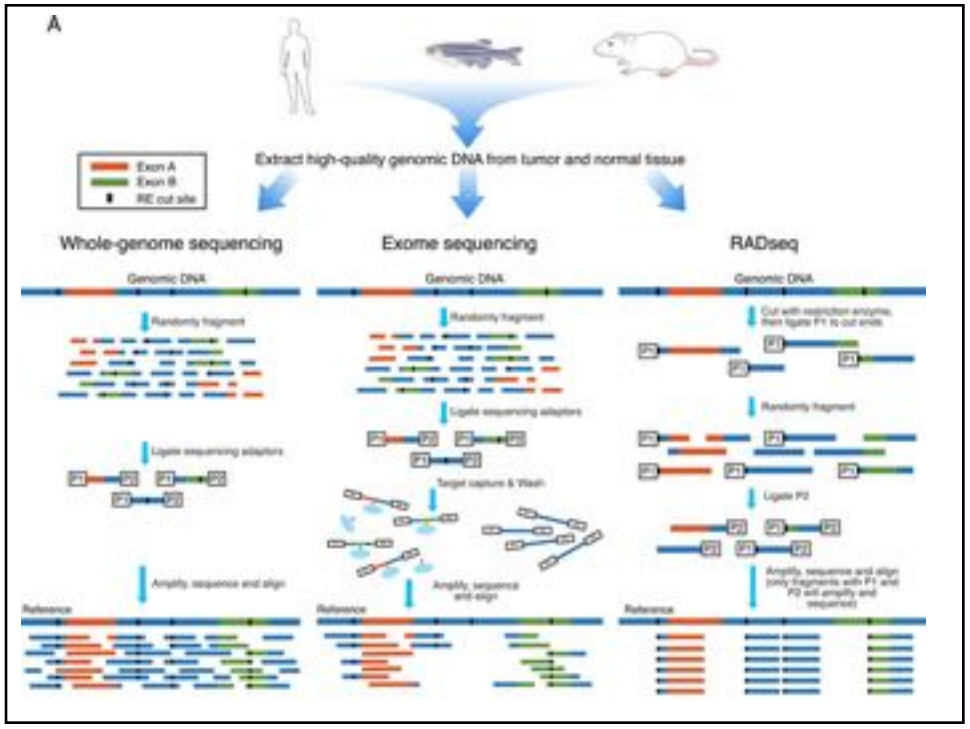
Thomas Turner, Megan Osborne, Alex Cameron
Museum of Southwestern Biology
University of New Mexico

Study Objectives

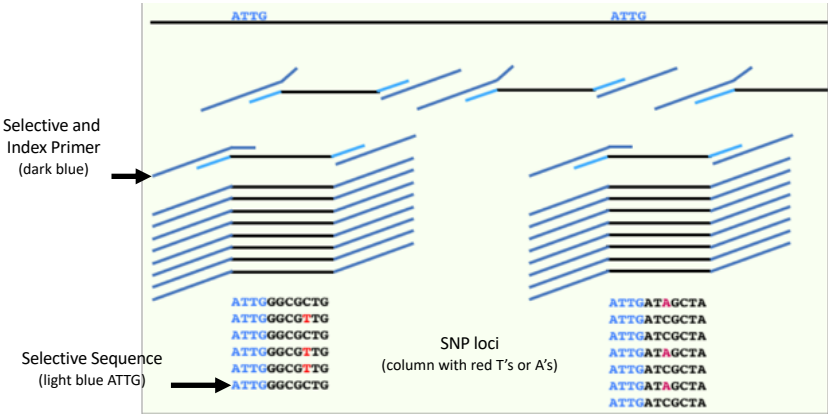
1. Characterize genetic variability using SNPs
   a) Evaluate differences among wild populations
   b) Evaluate differences between wild populations, replicated populations and captive stocks.
2. Estimate genetic effective population size (N_e) and other metrics
3. Assist with updates of brood stock management plans for both species



Site	Site Code	Species	Sample Size
Spring Creek	SC	Spikedace	31
Fossil Creek	FC	Spikedace	14
Aravaipa Creek	AC	Spikedace	28
		Loach minnow	28
Blue River	BR	Spikedace	30
		Loach minnow	12
Riverside Gila	RS	Spikedace	30
		Loach minnow	31
West Fork Gila	WF	Spikedace	33
		Loach minnow	30
Campbell Blue	CBR	Loach minnow	11
Little Creek Gila	LC	Loach minnow	1
San Francisco	SFR	Loach minnow	10



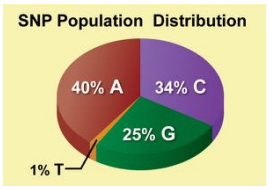
SNPsaurus nextRAD Sequencing

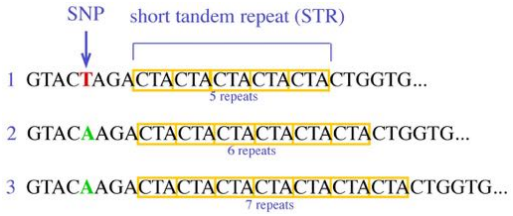


- Selective primers to amplify only the loci in the genome with selective sequence (instead of restriction enzyme)
- Reduces loss during library creation (reduction in number of steps)
- Subsequent sequencing of library allows SNP genotyping

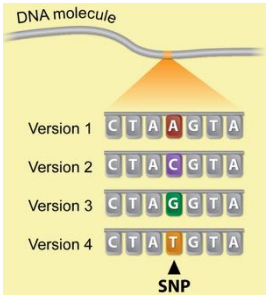
What are SNPs?

- Single Nucleotide Polymorphisms
  - Type of genetic variant
  - Substitutions of a single nucleotide (A, T, C, or G)
  - ≥ 2 versions present in ≥1% of population
  - Widespread throughout genome; primarily non-coding
  - Most commonly bi-allelic (e.g. A versus T)





http://www.le.ac.uk/ge/maj4/SNP_STR.jpg



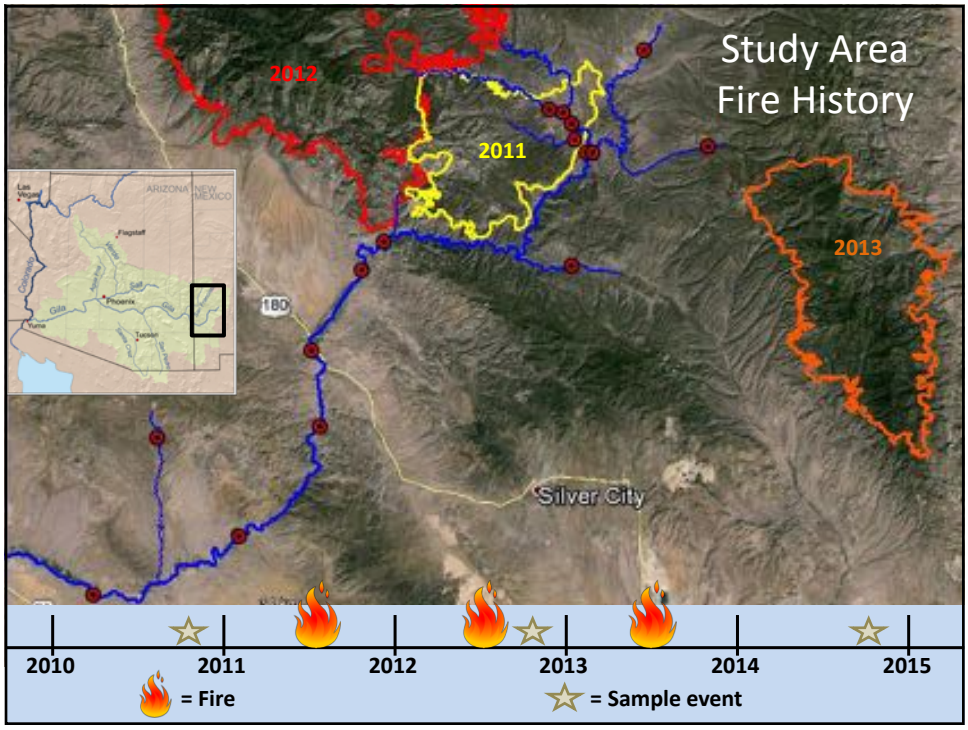
Expected Outcomes

- Examination of population differences over thousands of genetic loci
- Development of targeted SNP loci for both species
- Evaluation of hatchery stocks

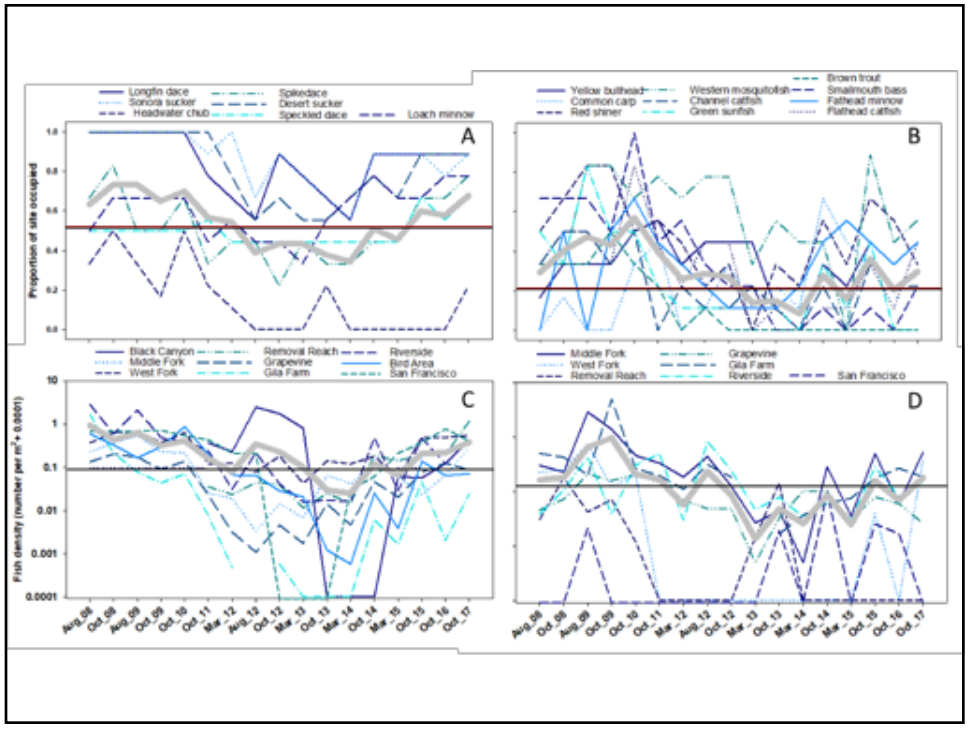


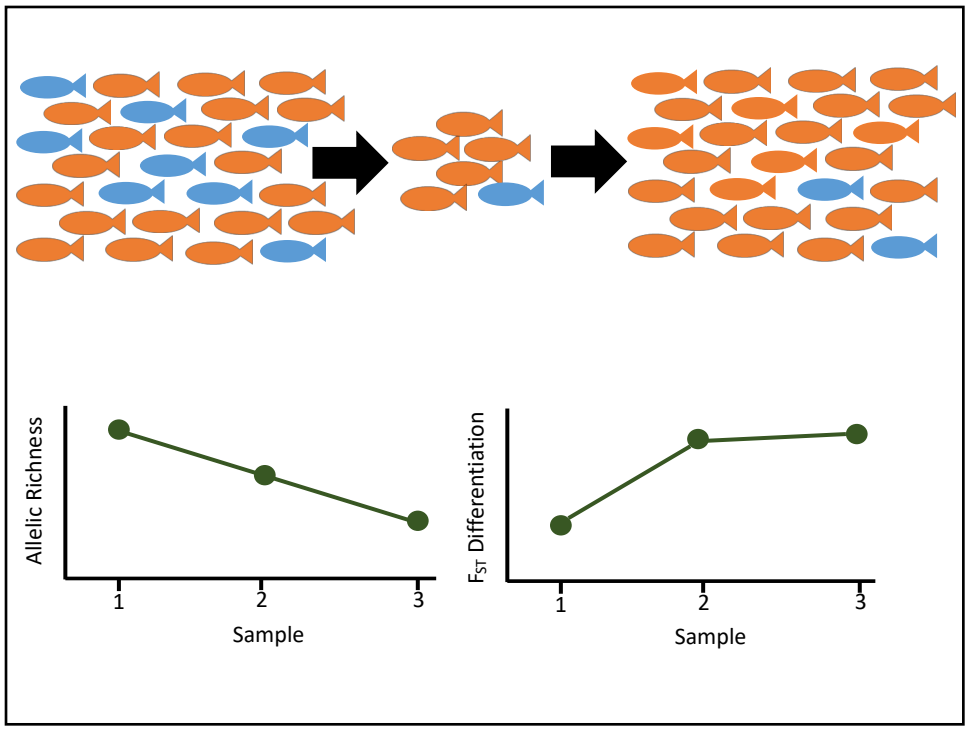

Demographic Impacts

- Pre-disturbance
- Disturbance
- Post-disturbance
- Recovery


Study Area
Fire History
2012
2011
2013
Silver City
180
2010
2011
2012
2013
2014
2015
= Fire
= Sample event


Smallmouth bass
imgkid.com
Sonora sucker
Desert sucker
Yellow Bullhead
www.tnfish.org
Gila River Fish Community
Speckled dace
Longfin dace
Spikedace
Loach minnow
Headwater chub


Longfin dace
Spikedace
Sonora sucker
Desert sucker
Headwater chub
Speckled dace
Loach minnow
A
Yellow bullhead
Western mosquitofish
Brown trout
Common carp
Channel catfish
Smallmouth bass
Red shiner
Green sunfish
Fathead minnow
Flathead catfish
B
Proportion of site occupied
Black Canyon
Middle Fork
West Fork
Removal Reach
Grapevine
Gila Farm
Riverside
Bird Area
San Francisco
C
D
Fish density (number per m² + 0.0001)


Allelic Richness
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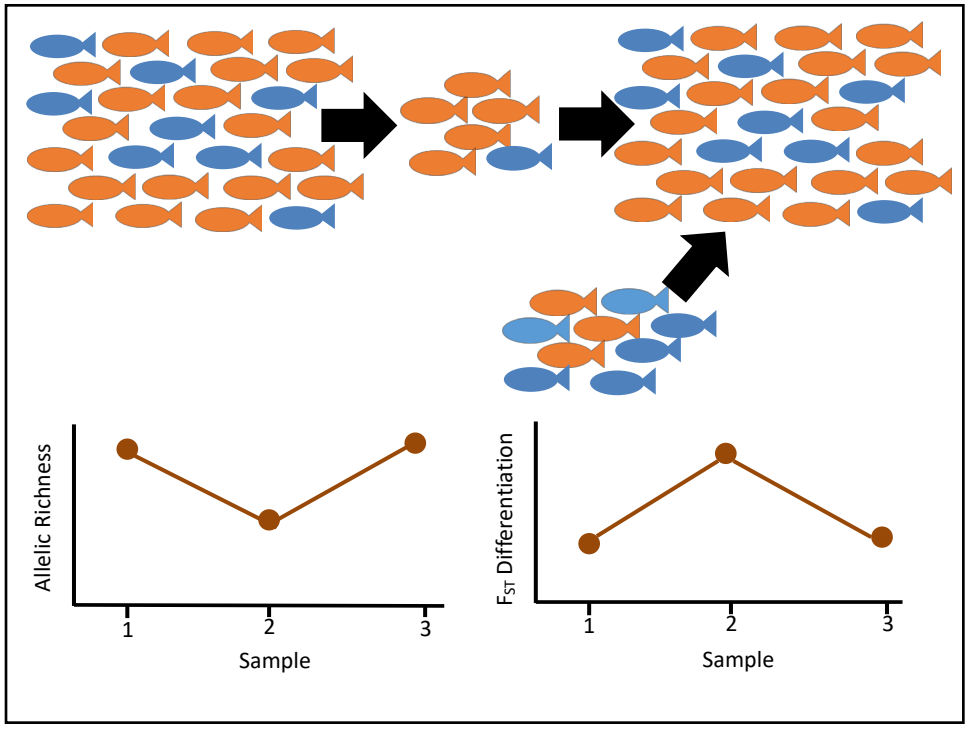
2
3
Sample
F_ST Differentiation
1
2
3
Sample


Allelic Richness
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F_{ST} Differentiation
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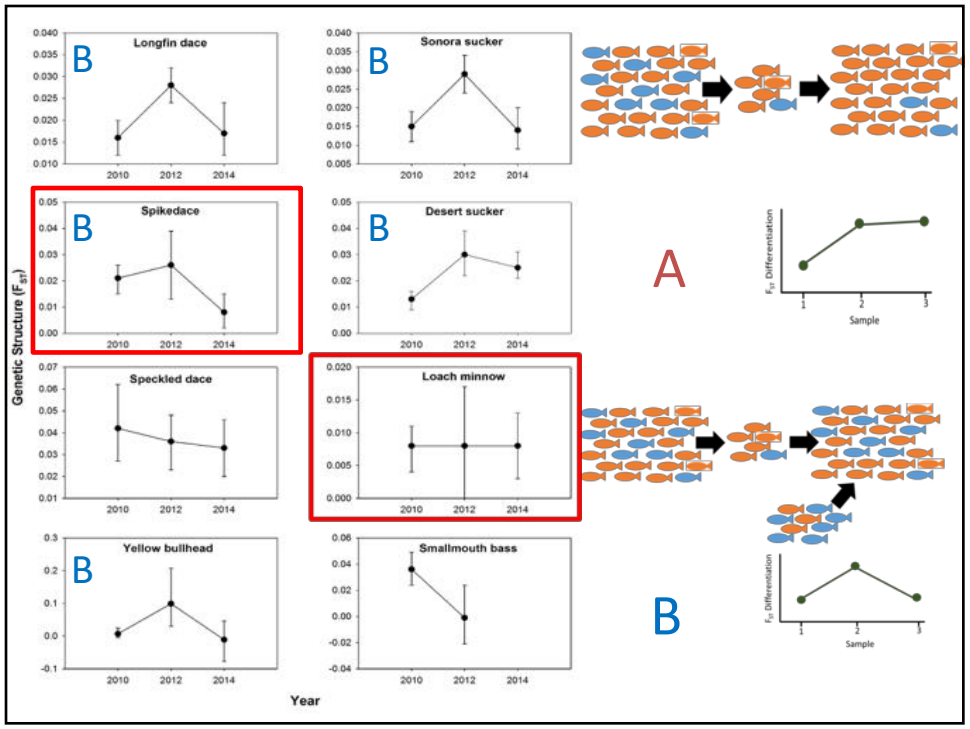
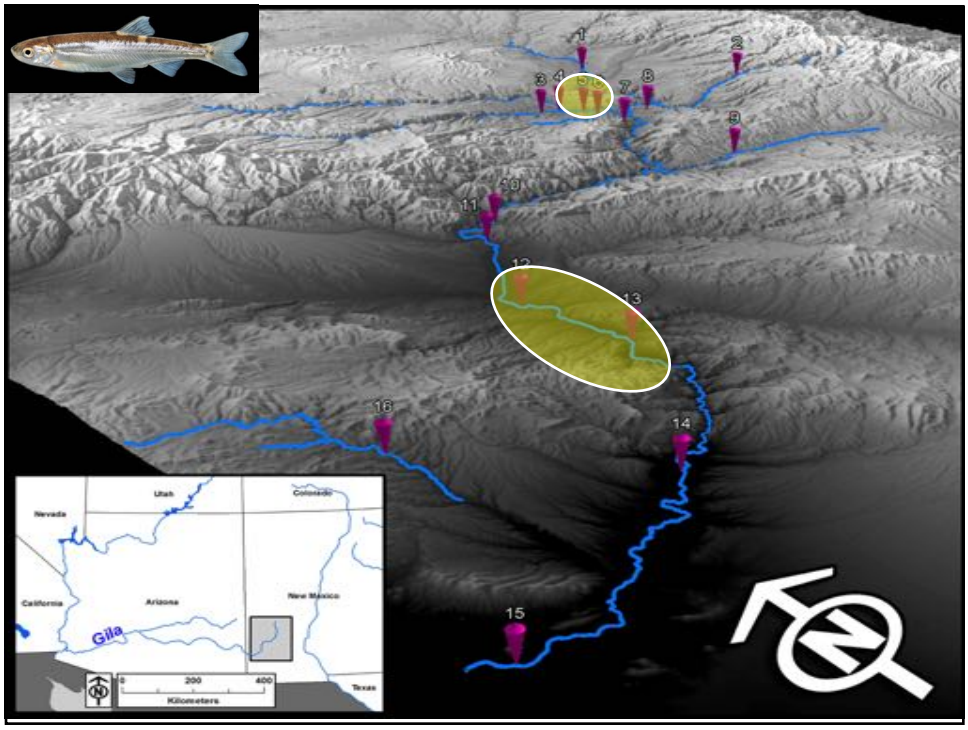

Longfin dace
B
Sonora sucker
B
Spikedace
B
Desert sucker
B
A
Speckled dace
Loach minnow
Yellow bullhead
B
Smallmouth bass
B
Genetic Structure (F_{st})
Year
2010
2012
2014
Sample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Texas
Gila
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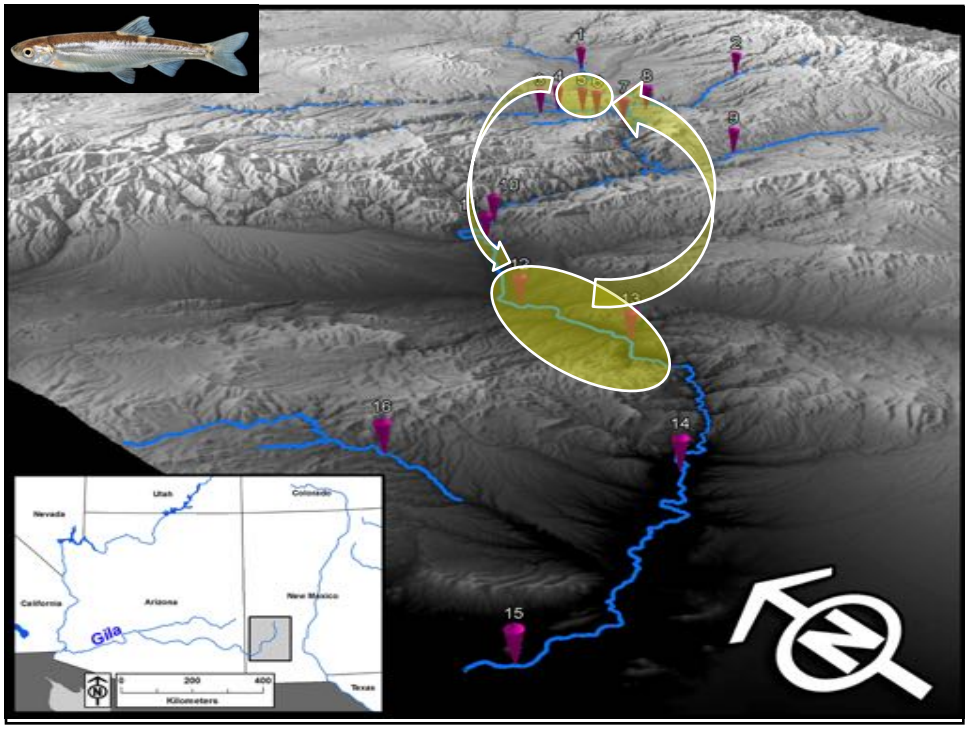
400
Kilometers
N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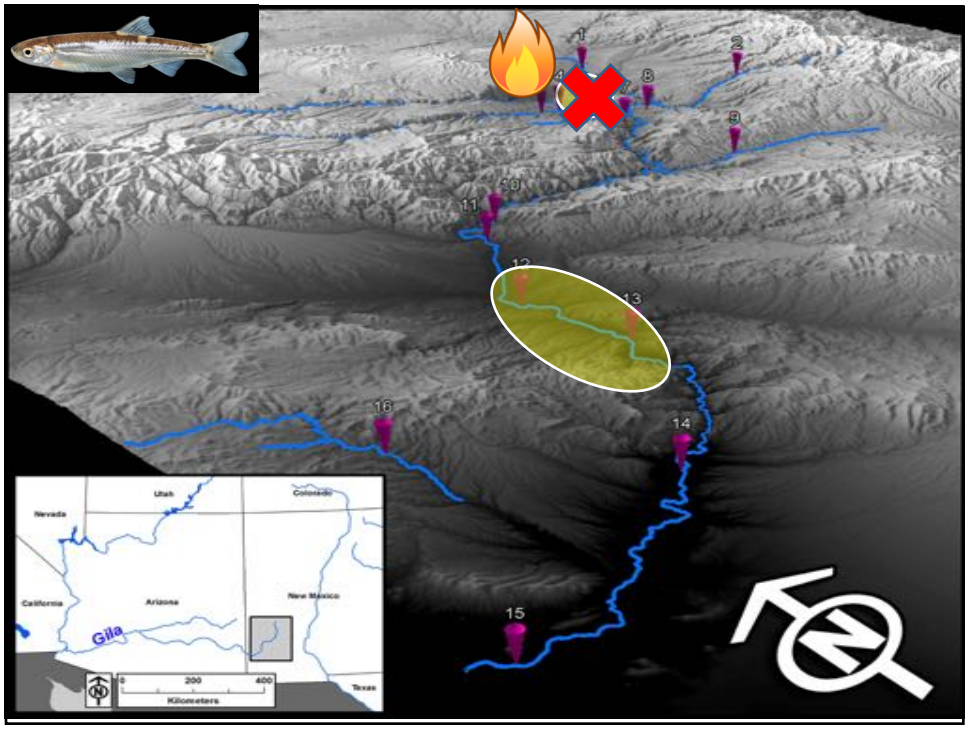
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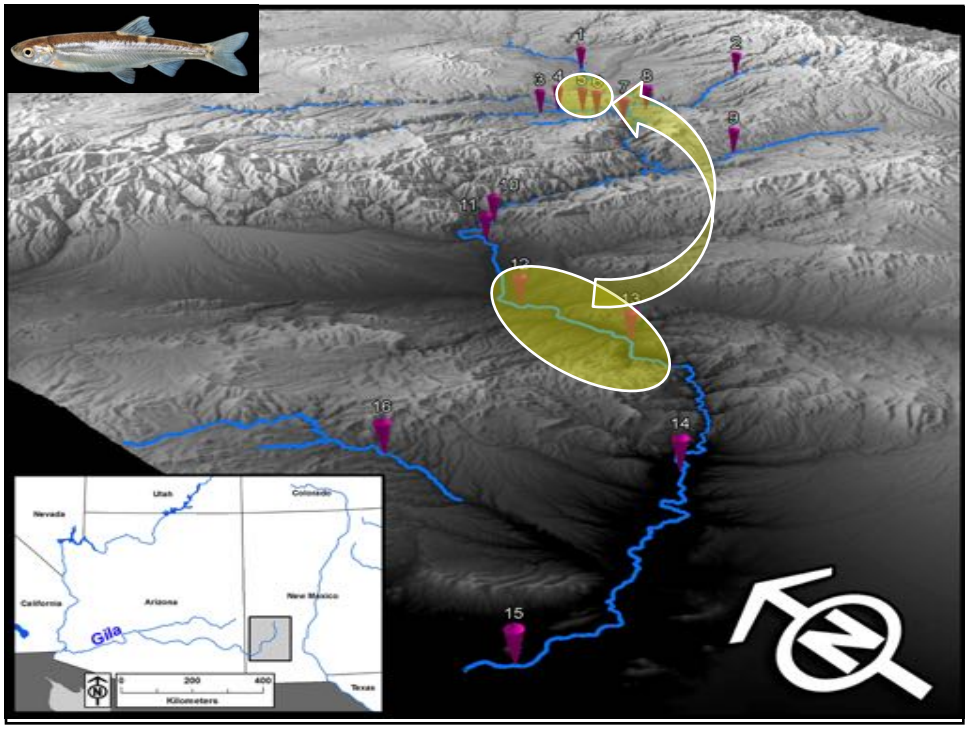
Kilometers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Nevada
California
Arizona
New Mexico
Texas
Gila
200
400
Kilometers

Gila River Basin Native Fish Monitoring - 2018

AARON A. BURGAD, BRIAN R. KESNER, AND PAUL C. MARSH

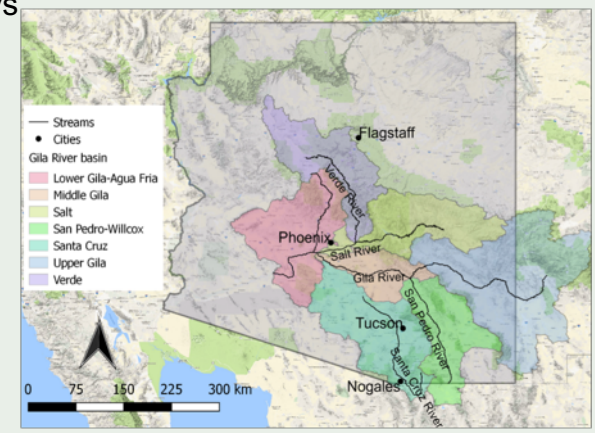
MARSH & ASSOCIATES, LLC

Long-term monitoring program

- Initiated in 1994 by Bureau of Reclamation
  - Central Arizona Project
    - Provide baseline data on the distribution and abundance of non-native fishes
- 2012
  - Focus shifted further upstream on wild populations of federal-listed/candidate fishes
- Objectives
  1. Detect the presence of each focus species and determine the upper and lower extent
  2. Evaluate fish community composition

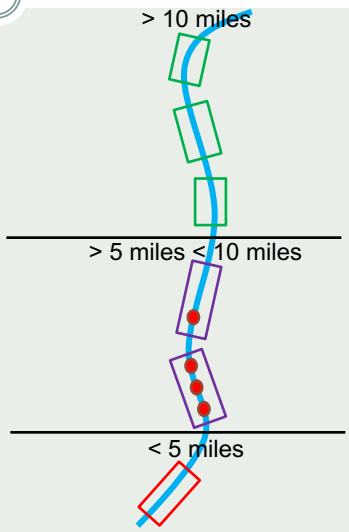
Methods

- 2018 stream surveys
  - Pre-selected streams
    - 24
- Focus species
  - Gila topminnow
  - Loach minnow
  - Spikedace
  - Gila chub
  - Roundtail chub



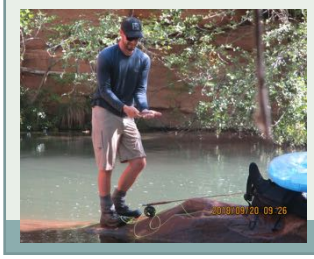
Sampling protocol

- Monitoring protocol
  - Clarkson et al. (2011)
- Terminology
  - Stream reach
    - Determined based on stream length
  - Station
    - Determined based on presence and abundance of focus species
- Abundance
  - >25 – 1 station (100 m)
  - <25 – next station (500 m)

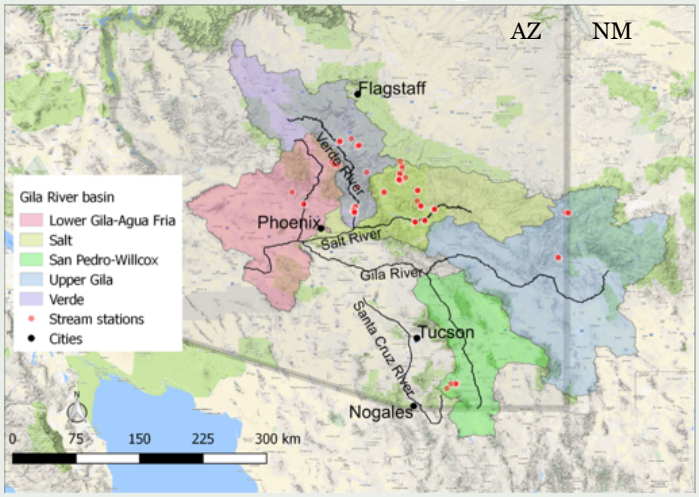


Sampling methods

- Sampling gears
  - Backpack electroshocking
  - Promar collapsible hoop nets
  - Collapsible minnow traps
  - Large hoop nets
  - Seines
  - Dip net
  - Angling (fly rod)

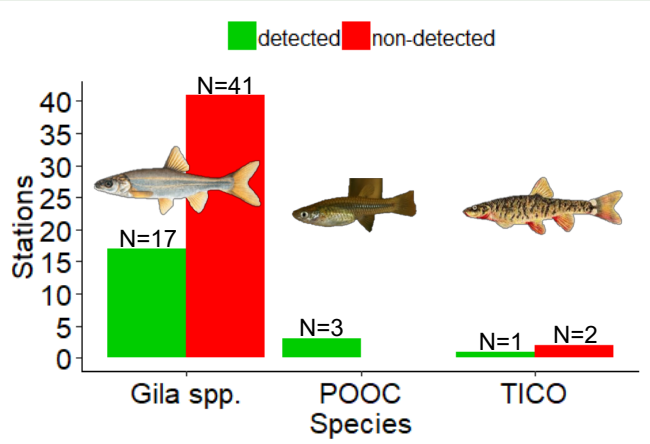


Results – basins, streams, and stations sampled

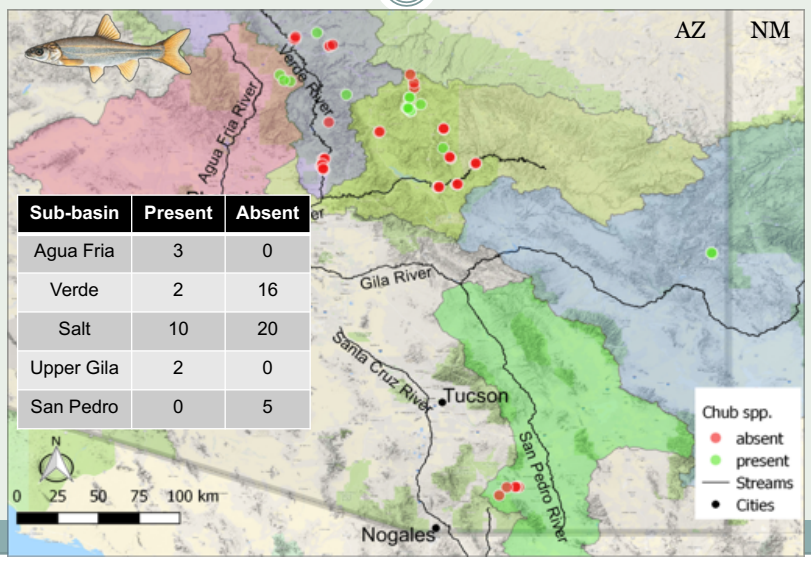


- Sub-basins
  - 5
- Streams
  - 21
- Stations
  - 64

Focus species detection

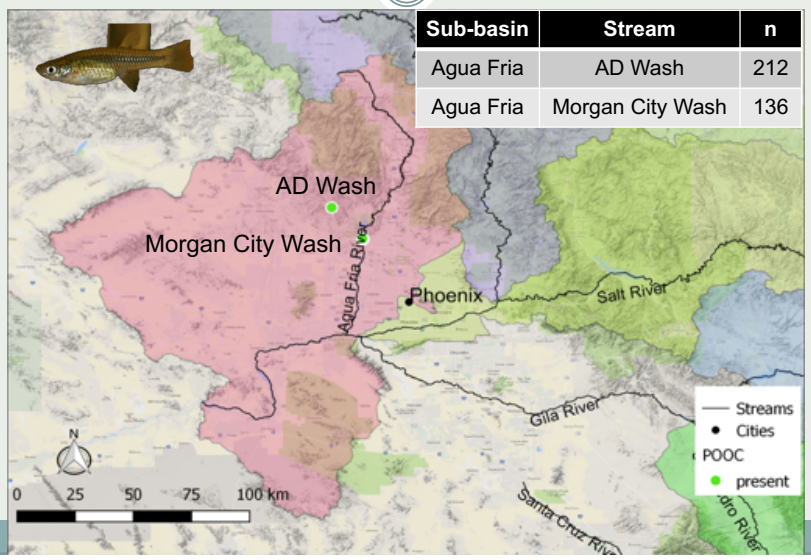


Gila spp. distribution



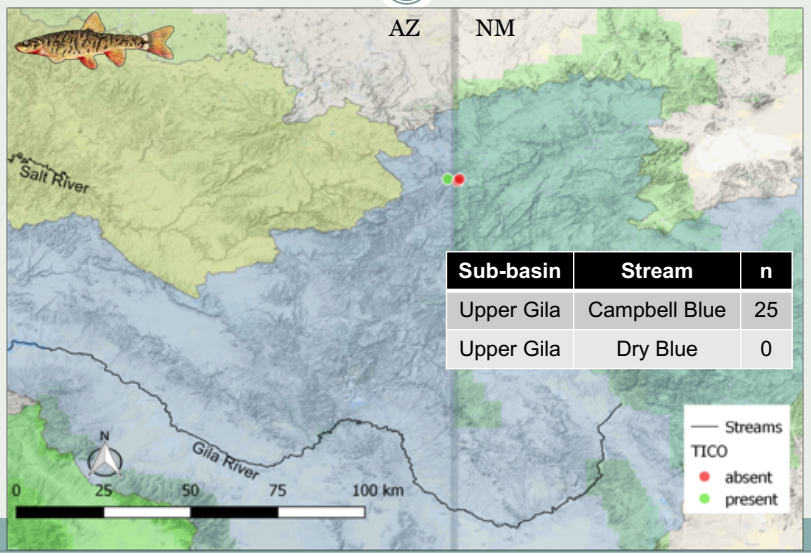
Sub-basin	Present	Absent
Agua Fria	3	0
Verde	2	16
Salt	10	20
Upper Gila	2	0
San Pedro	0	5

POOC distribution



Sub-basin	Stream	n
Agua Fria	AD Wash	212
Agua Fria	Morgan City Wash	136

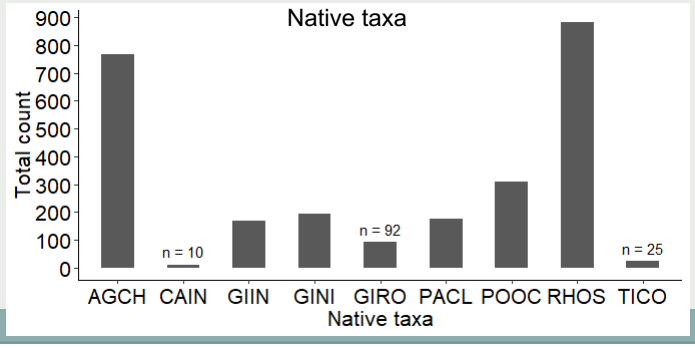
TICO distribution



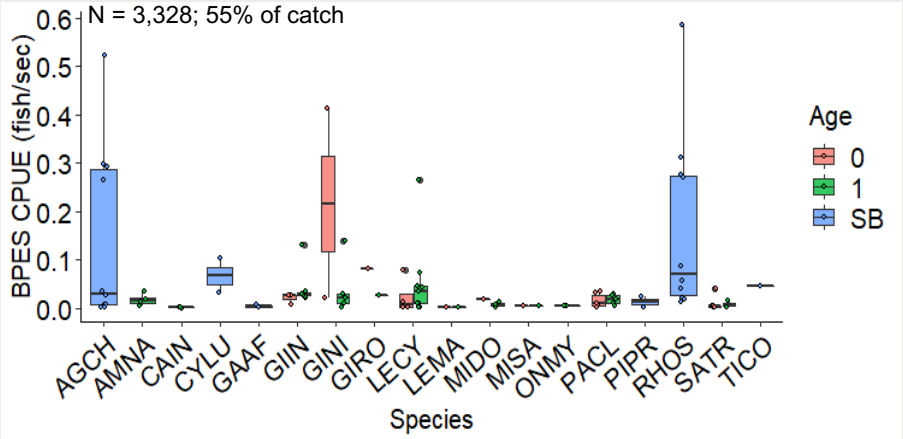
Sub-basin	Stream	n
Upper Gila	Campbell Blue	25
Upper Gila	Dry Blue	0

Community composition

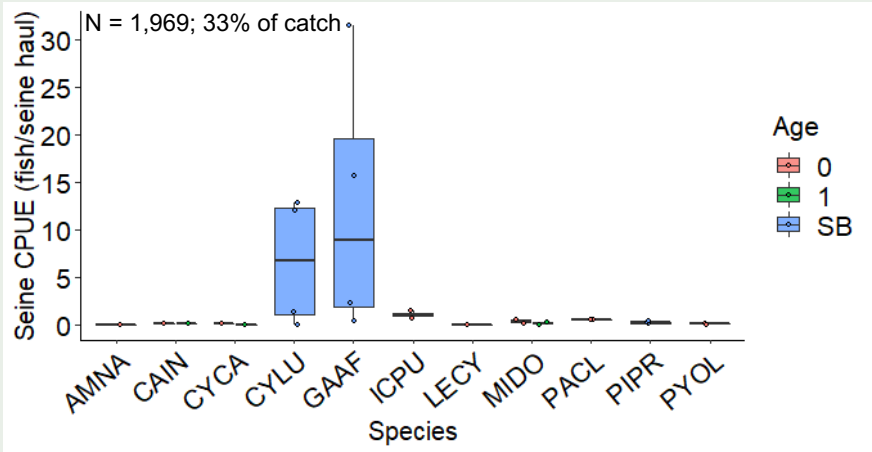
- Total catch
  - 23 species
    - 6,017 individuals
      - 44% native, 56% nonnative



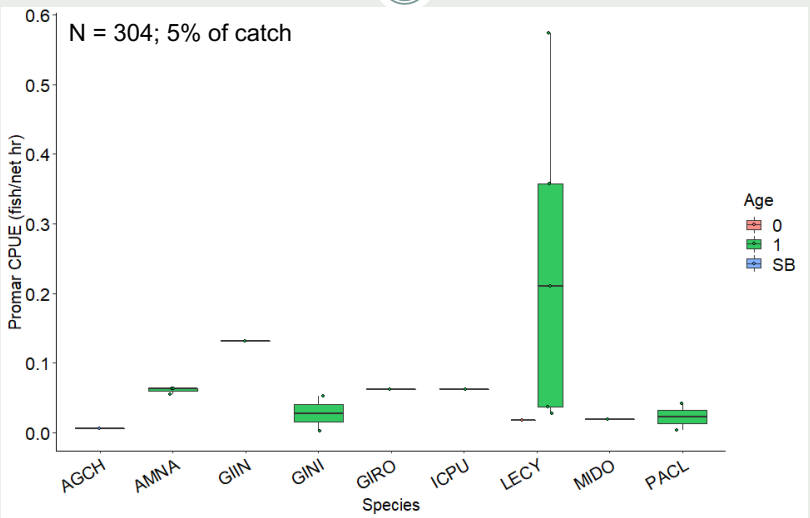
Backpack electroshocking catch



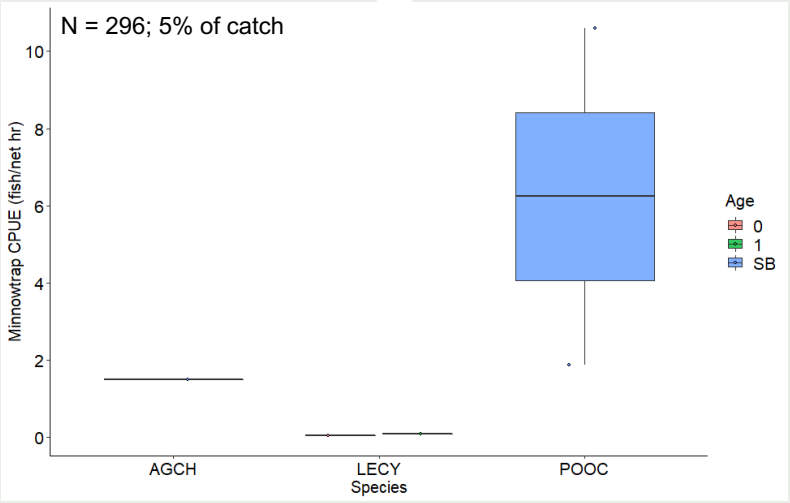
Seine catch



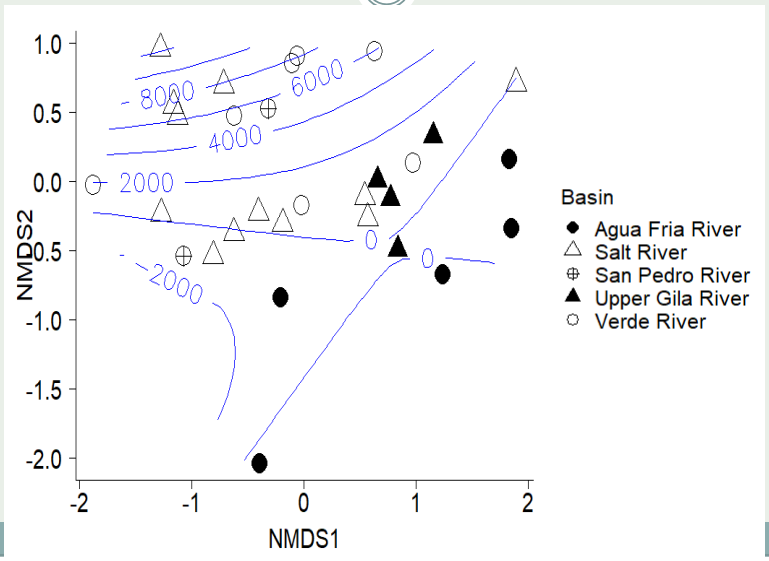
Collapsible promar hoop net catch



Minnowtrap catch



Non-metric multidimensional scaling



Proposed 2019 streams

Proposed Streams

Sub-basin	Stream(s)	Focus species
Agua Fria	3	POOC
Salt River	Black River	TICO
San Pedro	2	GIIN, POOC
Santa Cruz	5	GIIN, POOC
Upper Gila	3	GIIN, GIRO, TICO

Carryover Streams

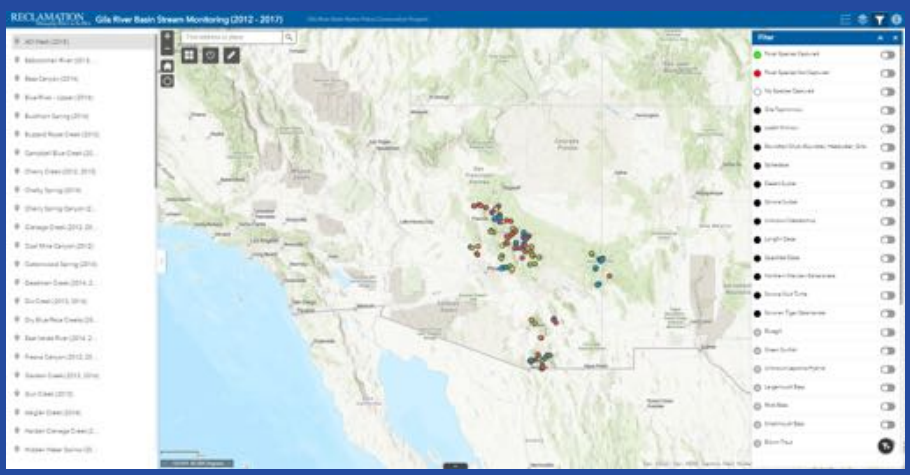
Sub-basin	Stream(s)	Focus species
Salt	Salome Creek	GIRO
San Pedro	2	GIIN, POOC
Upper Gila	Whitewater Creek	TICO
Verde	Wet Beaver Creek	GIRO

Gila topminnow refuge populations

- Stocks being maintained are doing well and thriving
  - Bylas
  - Cienega Creek
  - Parker Canyon (only until genetics testing is completed)
  - Red Rock
  - Sharp Spring
  - Rio Yaqui (Yaqui topminnow)


Acknowledgements
BOR
Bill Stewart
Kent Mosher
KA Ranch
Edie Johnson
Chris Stratton
SRP
Marc Wicke
Horner Mountain Ranch
Bryce Beeler
NMGFD
Michael Ruhl
Bryan Ferguson
Marsh & Associates
Lara McCall
Jake Rennert
Ben Miller
Taylor Haas
U.S. Forest Service
Anthony Bush
Albert Sillas
Janie Agyagos
Tim Hendricks
AZGFD
Curtis Gill
Tony Robinson
Julie Carter
Brett Montgomery
John Dickson
Sharon Lashway
Scott Rogers
Matt Rinker
Ross Timmons
Josh Walters

Gila River Basin Native Fish Monitoring Services Web App



Gila River Basin Native Fish Monitoring Workshops

Objective:

- Develop Gila River Basin Native Fish Sampling Plan

POC:

- Jason Kline (SWCA)
- Kent Mosher (USBR)

Gila River Basin Native Fish Monitoring Workshops

Main Components:

- Questionnaire
- Workshop #1
  - Present, evaluate, and discuss Questionnaire results.
  - Define goals/purpose of surveys by water body.
  - Discuss sampling design.
- Recruit Expert Panel for Workshop #2
- Workshop #2
  - Review outcome of first workshop.
  - Host panel discussion with sample design experts.
  - Prioritize and develop sampling standards for each body of water.
- Gila River Basin Native Fish Sampling Plan

Gila River Basin Native Fish Monitoring Workshops

Timeline:

- **Questionnaire (January 2019)**
- **Workshop #1 (April 2019)**
- **Workshop #2 (October/November 2019)**
- **Gila River Basin Native Fish Sampling Plan (April 2020)**

Questions?

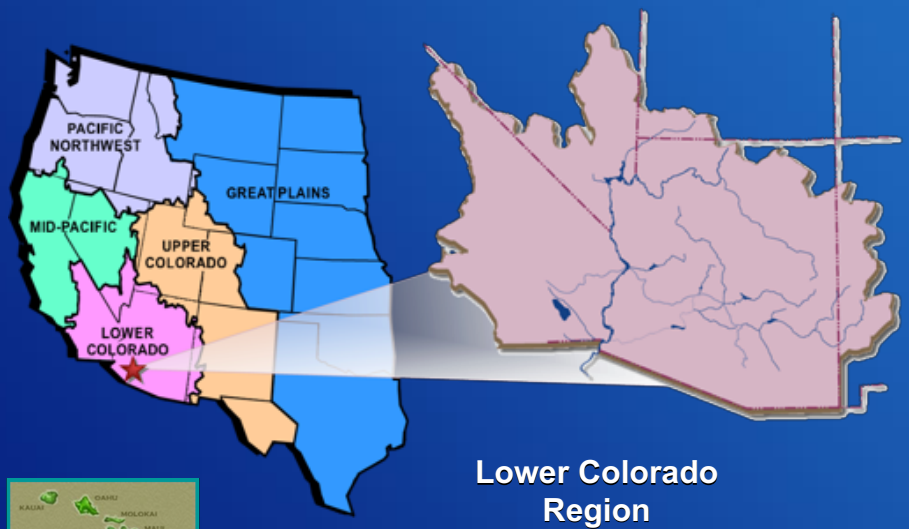
RECLAMATION

Managing Water in the West

WaterSMART Grants

U.S. Department of the Interior
Bureau of Reclamation

Reclamation Regions



Lower Colorado Region

RECLAMATION

Phoenix Area Office Service Area

Reclamation Mission

To manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

RECLAMATION

Water Related Facilities and Uses

Hoover Dam

CAP

Environmental Uses

Agricultural Use

Municipal Use

RECLAMATION

Overview

- **Reclamation Grant Programs**
- **NEPA**
- **How do I get a grant?**
- **What's a FOA?**
- **Suggestions to improve your proposals**

RECLAMATION

WaterSMART Program

Sustain and Manage America's Resources for Tomorrow

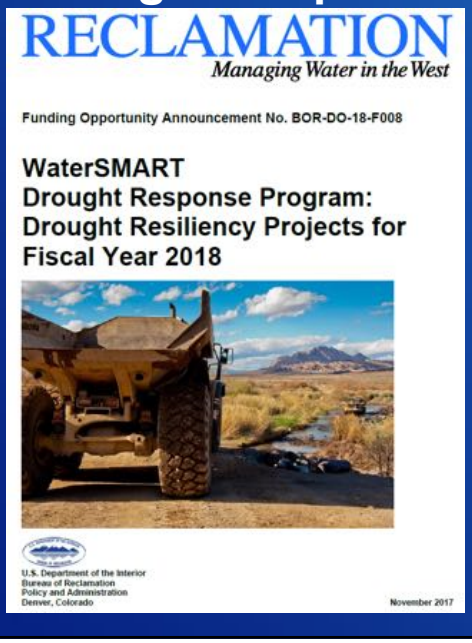
- Working to achieve a sustainable water strategy to meet the Nation's water needs
- Managed out of Reclamation's Denver Office
- Reclamation Wide programs

RECLAMATION

WaterSMART Program

RECLAMATION

Drought Response Program



RECLAMATION

Drought Response Program

- 2015 City of Phoenix, AZ
  - Drought Resiliency Project - Deer Valley ASR Well Project
- 2016 City of Gallup, NM
  - Drought Contingency Plan

RECLAMATION

Drought Response Program

- Task C—Projects that Provide Protection for Fish, Wildlife, and the Environment
  - These projects seek to mitigate or minimize the potential drought-related impacts to ecosystems and to provide a sustainable environment for those species that are most vulnerable to periods of deficient water supplies

RECLAMATION

Cooperative Watershed Management Program

Sierra Streams Institute in north-central California

Land Trust of the Treasure Valley in Idaho

Flathead Basin Commission in western Montana

Clark Fork Coalition near Missoula, Montana

Blackfoot Challenge in western Montana

Hood River Soil and Water Conservation District in Oregon

Middle Colorado Watershed Council in Western Colorado

Western Slope Conservation Center in Colorado

Rio Grande Restoration, Inc. in northern New Mexico

Friends of the Teton River, Inc. in Idaho

San Juan Resource Conservation and Development in Colorado

RECLAMATION

Cooperative Watershed Management Grants

- Phase I
  - 2016
    - Tse Si Ani Chapter: Working Across Tribal Borders
  - 2018
    - Alter Valley Conservation Alliance
    - Coconino Plateau Watershed Partnership
    - Friends of the Rio de Flag
    - Industrial Development Authority of Gila County (Cobre Valley)
    - Watershed Management Group

RECLAMATION

Cooperative Watershed Management Grants

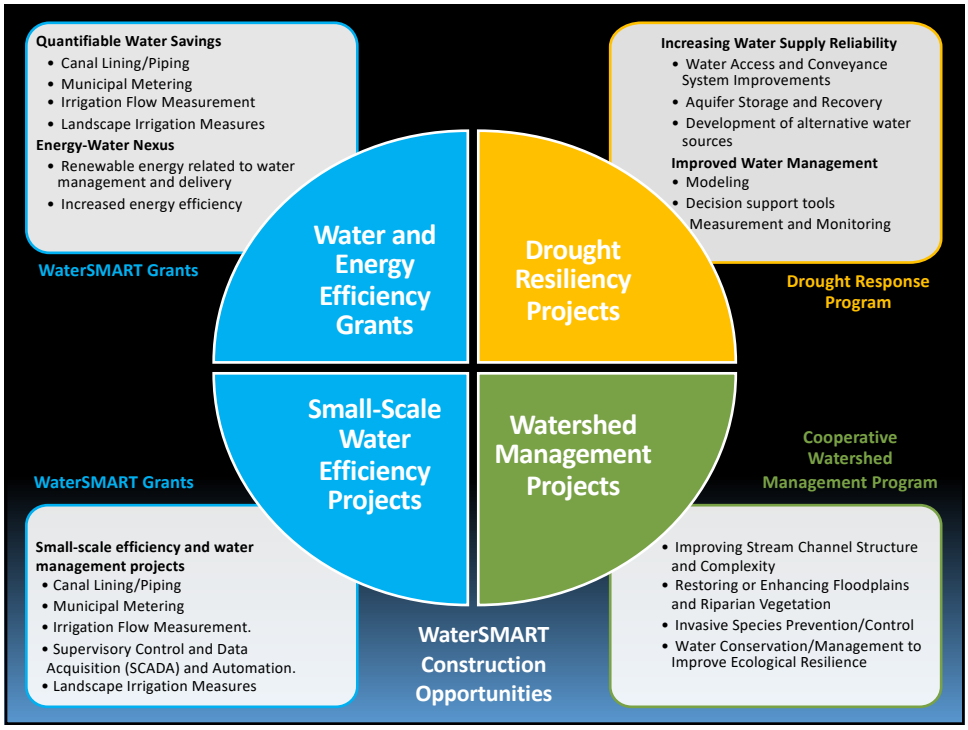
- **Phase II:**
  - **2017**
    - **Cienega Watershed Partnership received funding to complete streambank and riparian restoration along Cienega Creek and its tributaries in Pima County, Arizona**

RECLAMATION

Cooperative Watershed Management Grants

- Improving stream channel structure and complexity;
- Improving channel/floodplain connectivity;
- Protecting and stabilizing stream and river banks;
- Reducing erosion;
- Improving water delivery systems to increase efficiency or other projects to address water supply needs;
- Providing fish passage;
- Removing invasive species and restoring vegetation;
- Influencing water temperature or improving the timing or volume of available flows at particular locations to improve aquatic conditions;
- And other watershed management projects that will address water supply needs, water quality concerns, and restoration needs in the watershed.

RECLAMATION



NEPA Process

- NEPA- National Environmental Policy Act
- All projects funded require NEPA
- Expect NEPA to cost at least 5% of the budget and minimum 3 months to complete. GET ESTIMATES

RECLAMATION

How do I get a grant?

www.grants.gov

****KEEP APPLYING****

Request for Proposals (RFP)/Request for Application (RFA)/
Funding Opportunity Announcement (FOA)

Give yourself time to write the proposal!

RECLAMATION

IMPORTANT

READ THE FOA!!

Read everything in the Funding Opportunity Announcement and
ask questions if something doesn't make sense.

RECLAMATION

FOA Table of Contents

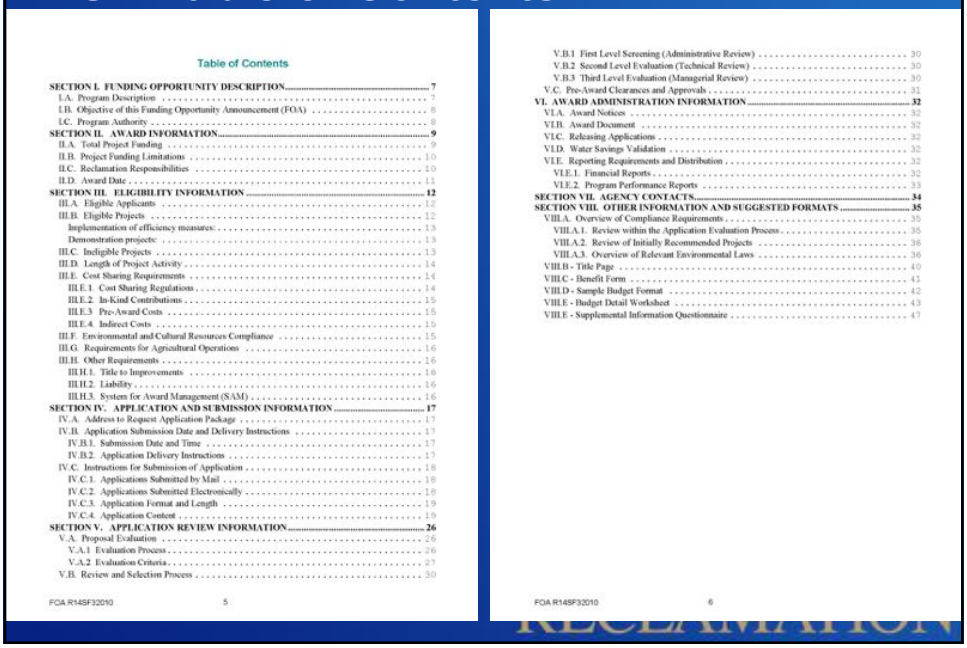


Table of Contents

FOA R14SF32010 5

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Tips

- **Apply for multiple grants, if it is the same project selected for multiple awards, we all talk with each other and the grant opportunity providing the most benefit will be awarded.**
- **If it is a large project, consider phasing your project and applying for funding in sequential years.**
- **Don't assume that the Grantor knows you. The committee is made up of people from all over the 17 western states.**
- **Focus on the Evaluation Criteria.**
- **Ask for help!**

RECLAMATION

Tips

- **Understand that the 50/50 cost share is non-federal funding, so you could apply for state funding for the same project. The state funding can count as your cost share portion.**
- **If your application does not get awarded, ask for a de-brief to help prepare a better proposal next year.**
- **Make sure SAM and ASAP registration is current at least two months before due date as it expires each year.**
- **If it is a multiple year project look at doing NEPA upfront**

RECLAMATION

RECLAMATION

Managing Water in the West

Questions?

http://www.usbr.gov/WaterSMART/

Presented by:

Jessica Asbill-Case
Water Resources Program Manager
Phoenix Area Office

Email: jasbillcase@usbr.gov
Phone: 623-773-6273

U.S. Department of the Interior
Bureau of Reclamation

Species Recovery - RPI and APG

December 12, 2018

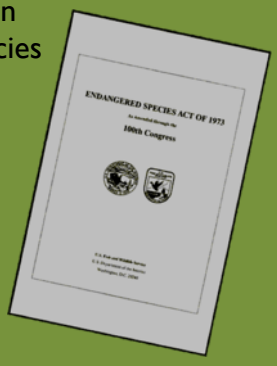
Objectives

Discuss:

- The Recovery Planning Implementation Process
- The Agency Priority Performance Goals Exercise
- Recovery Changes and GRBNFCP Priority Species
- Draft Spikedace and Loach Minnow Recovery Plans

Recovery

- A formal process under Section 4(f) of the Endangered Species Act;
- A means whereby the ecosystems upon which endangered and threatened species depend may be conserved.



Legally Required Components to Include in a Recovery Plan

The Secretary . . . shall, to the maximum extent practicable . . . incorporate in each plan –

(i) **site-specific management actions** …
(ii) **objective, measurable criteria** …
(iii) **estimates of the time required and the cost** to carry out those measures.

Challenges

Fish and Wildlife Service has:

~1600 listed species;

>100 species listed in the last five years;

~450 species without a recovery plan;

Static or Shrinking Budgets;

Reduced Workforce.

More Challenges

Developing recovery plans was taking too long:

- Species listed faster than we can complete recovery plans.

Existing plans were not readily updatable to incorporate new information

- Revisions took as long as developing new plans. So, many plans are becoming outdated.

Conclusion: We need a new approach to recovery.

New Process – Recovery Planning and Implementation (RPI)

RPI is a revised approach to recovery that:

1. Maximizes efficiency of completing recovery documents;
2. Maximizes efficiency of recovery implementation;
3. Maximizes integration of our efforts across the T & E species program.

Old Vs. New Approach to Recovery

Recovery Plan

- Introduction
- Criteria
- Actions
- Time and Cost Estimates

VS.

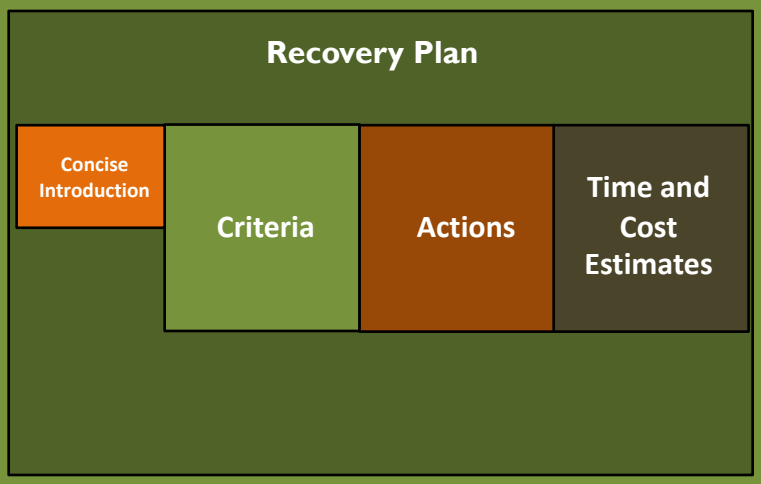
- I. SSA
- II. Recovery Plan
  - Brief Introduction
  - Criteria
  - Actions
  - Time and Cost Estimates
- III. Recovery Implementation Strategy

Part I - The Species Status Assessment (SSA)

Biological Information:	Current Conditions:	Factors Influencing Species Needs:	Species Viability Now and into the Future:
• Species Description • Life History • Taxonomy • Habitat	• Range & Distribution • Genetic Representation • Current Populations	• Threats description	• Resiliency • Redundancy • Representation

Part I - SSA

Part II - The Recovery Plan



Part III - Recovery Implementation Strategy (RIS)

A separate document which is:

- A nearer-term, revisable implementation document.
- A description of who does what, when, where, and how.

The RIS contains the on-the-ground activities or specific tasks necessary to complete recovery actions.

Part III - RIS

The RIS:

- Is separate from the Recovery Plan;
- Is worked on with partners and updated with partners;
- Does not require public review. This allows:
  - **Ability to adjust** in response to new information, completion of some activities, initiation of others, etc.;
  - **Opportunity for adaptive management**;
  - **Reduction** in the number of plan revisions;
  - **Savings of time and money** currently spent on recovery plan revisions;
  - **Avoiding** current situation of plans becoming outdated.

The Recovery Plan will still be published for public review.

Implementation of the RPI

September 2016 – Director's Memorandum: All new recovery planning efforts will implement RPI.

- Some Field Offices may not be able to develop SSAs for all currently outstanding RP development needs.
- Some leniency for recovery plans already well underway.
- Region 2 will be using the RPI to maximum extent practicable for recovery plans.

Agency Priority Performance Goals (APG)

Two Agency Priority Performance Goals from:

- DOI's Strategic Plan for FY 2018-2022
- Memoranda from Headquarters

APG #1 - State Participation

1) By 9/30/2018, 100% of proposed species listings based on best available information that includes state input and/or data provided through state participation in Species Status Assessments.
2) For SSAs developed in support of a listing decision, invite each state that has jurisdiction over the species to participate in the development of the SSA;
3) State participation determined on a case-by-case basis: may be document review; may be full technical team involvement.

APG #2: Quantitative Recovery Criteria

By 9/30/2019, 100% of all Fish and Wildlife Service recovery plans will have quantitative delisting criteria for what constitutes a recovered species. Criteria provide:

- Targets for identifying and implementing recovery actions to achieve recovery;
- A means of measuring progress towards recovery;
- Ability to recognize when recovery has been achieved.

APG #2: Quantitative Recovery Criteria

The APG States:
Recovery plans will have quantitative criteria for what constitutes a recovered species.
(Quantitative *downlisting* criteria are insufficient).

The APG Does Not State:
How many quantitative delisting criteria are necessary per plan.

Loach Minnow Example

Existing (1991) Loach Minnow Criterion on Reintroductions:

"Reintroduce populations to selected streams within historic range."

Revised as a quantitative criterion:

In addition to replications required for downlisting, replicate representative populations from each of the 5 RUs into new, protected streams as follows:
Verde/Lower Salt – 1 additional replicate;
Upper Salt River – 1 additional replicate;
San Pedro/Lower Gila River – 1 additional replicate;
Etc…

APG Batching

APG Batches:

	Target	New Target
Batch 1	09/30/18	1/2019 or 2/2019
Batch 2	12/14/18	1/2019
Batch 3	3/29/19	

APG #2: Quantitative Recovery Criteria

56 Species in Region 2 needing quantitative criteria, including:

- 12 species in Arizona
- 9 species in New Mexico

Coupled with species needing initial recovery plan, and species needing recovery plan revisions.

R2 APG Recovery Tasks

# Species	Recover Product
21	Five Year Review
14	Quantitative Delisting Criteria
3	Draft Recovery Plans
5	Draft Recovery Plan, 1st Rev.
2	Recovery Outlines
4	Final Recovery Plan
21	Final Delisting Criteria
1	Final Rule or Withdrawal
71	

Relevance to GRBNFCP

Razorback sucker, Gila topminnow, spikedace and loach minnow are:

- All GRBNFCP priority species;
- Affected by the RPI, APG, or both;
- Have significant portions of their recovery efforts carried out by GRBNFCP.

GRBNFCP Priority Species

Razorback Sucker

- Coordinating with Region 6 (FWS) and States in the Upper Basin
- 5-year review underway
- Revising Recovery Plan based on results of 5-Year Review

GRBNFCP Priority Species

Gila Topminnow

- 5 Year Review – June 1, 2019
- Draft Recovery Plan, 1st Revision – 2019

GRBNFCP Priority Species

Spikedace

- Quantitative Delisting Criteria – 7/2019
- Recovery Plan -1st Revision – 12/2019

GRBNFCP Priority Species

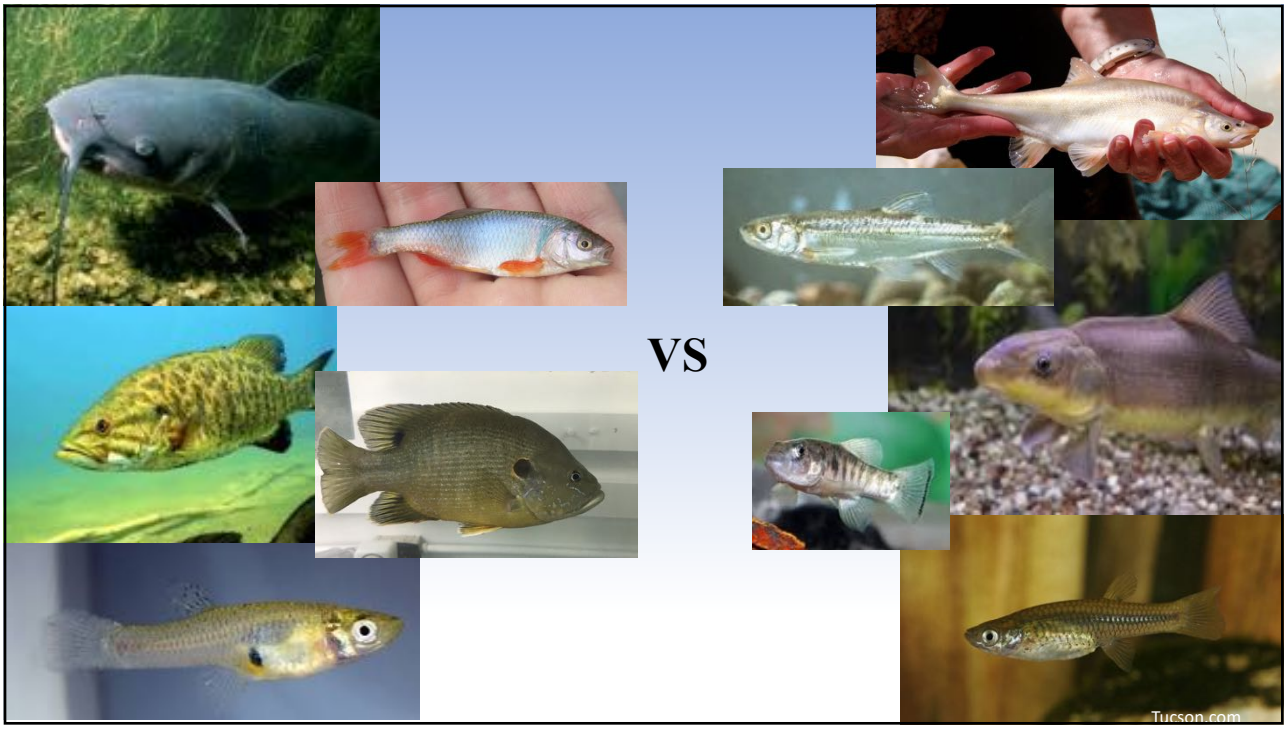
Loach Minnow

- Quantitative Delisting Criterion – Under Regional Office Review
- Recovery Plan -1st Revision – 6/2019

Developing Trojan Sex Chromosome Carriers (YY males) to Control Nuisance Fish Populations in the Southwest

Chad Teal
USGS AZ Cooperative Fish and Wildlife Research Unit-
chadteal@email.Arizona.edu

Dr. Scott Bonar- USGS AZ Cooperative Fish and Wildlife Research Unit
Dr. Dan Schill- Fisheries Management Solutions, Inc.
Dr. Melanie Culver- USGS AZ Cooperative Fish and Wildlife Research Unit



Mechanical Removal

Chemical Treatments

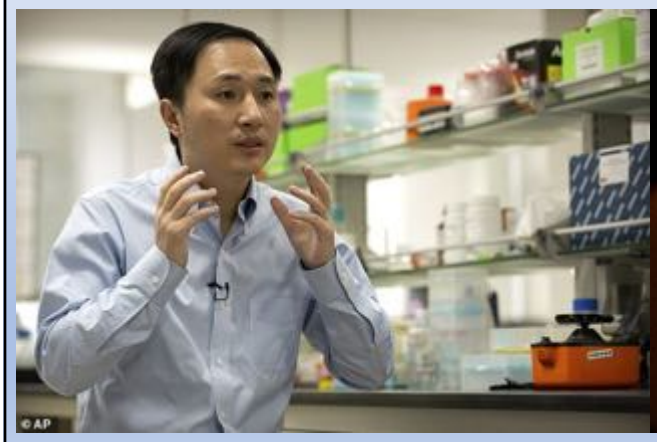
Genetic Approaches

CRISPR Cas 9/Gene Drive- effective but controversial

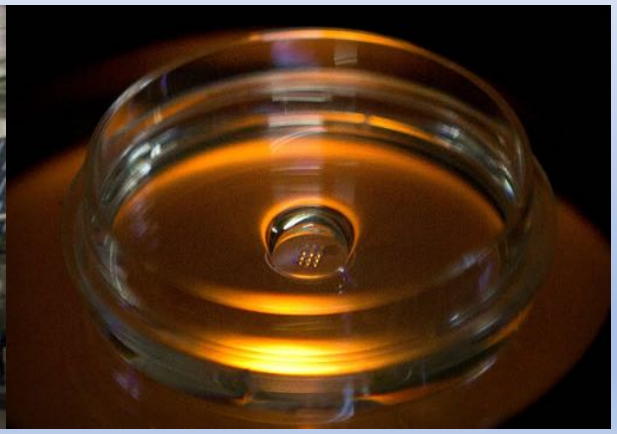
Daughterless carp- takes a long time

CRISPR- Clustered Regularly Interspaced Short Palindromic Repeats

Cas 9- an enzyme that uses CRISPR sequences as a guide to recognize and cut DNA strands that are complimentary to the CRISPR sequence… then you put in the sequence you want!

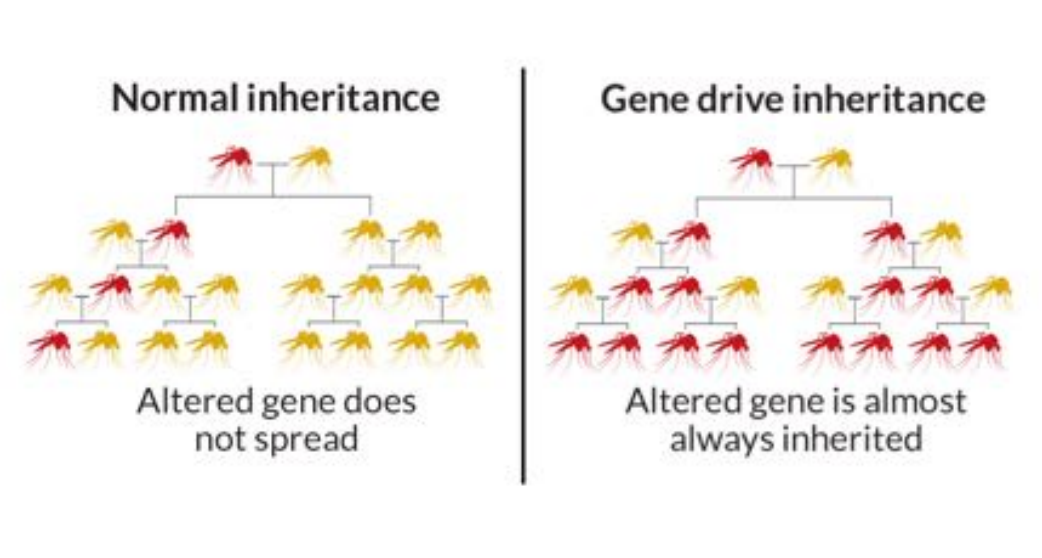


www.dailymail.co.uk.com



www.nytimes.com

CRISPR + Gene Drive



Could it spread into non targeted populations?

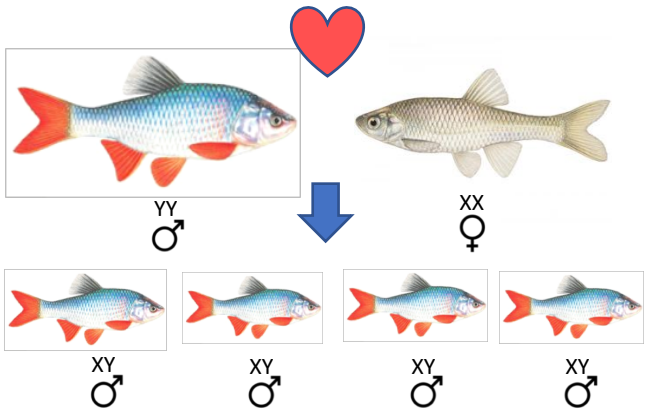
Daughterless Carp

Release individuals with copies of aromatase inhibitor gene resulting in all male progeny. This takes more time for extirpation than Trojan Y Chromosome Strategy.

Trojan Y Chromosome (TYC) Strategy

How the TYC strategy works

release of YY individuals into a population to skew sex ratio towards all male



Advantages of TYC over other genetic approaches:

- Faster than daughterless carp strategy

- Genetic control of nuisance populations without the use of transgenic individuals

- If YY individuals enter a non targeted population then the effect is easily reversed

ELSEVIER

SCIENCE DIRECT

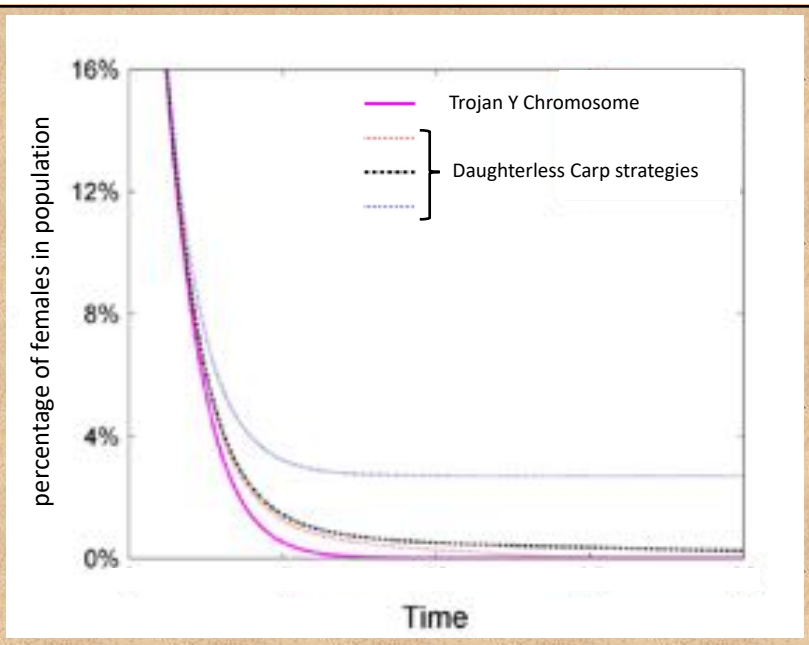
Journal of Theoretical Biology 241 (2006) 333–341

Journal of Theoretical Biology

www.elsevier.com/locate/yjtbi

A model describing the effect of sex-reversed YY fish in an established wild population: The use of a Trojan Y chromosome to cause extinction of an introduced exotic species

Juan B. Gutierrez[a,*], John L. Teem[b]



Teem et al. (2014)

Production of a YY Male Brook Trout Broodstock for Potential Eradication of Undesired Brook Trout Populations

Daniel J. Schill, Jeff A. Heindel, Matthew R. Campbell, Kevin A. Meyer & Elizabeth R. J. M. Mamer

Simulated Effects of YY-Male Stocking and Manual Suppression for Eradicating Nonnative Brook Trout Populations

Daniel J. Schill, Kevin A. Meyer & Michael J. Hansen

YY Males Brook Trout Introductions with Manual Suppression of Wild Population

Suppression rate (%)	Stocking rate (%)	Years
		Streams
0	10	>50
	25	>50
	50	12
25	10	>50
	25	13
	50	6
50	10	13
	25	6
	50	4
75	10	6
	25	4
	50	4

Schill et al. (2017)

THE UNIVERSITY OF ARIZONA

U.S. Bureau of Reclamation

Gila River

Species Selection

***Cyprinella lutrensis*- Red Shiner**

- 3 year life span
- Highly invasive
- Competes with native cyprinids

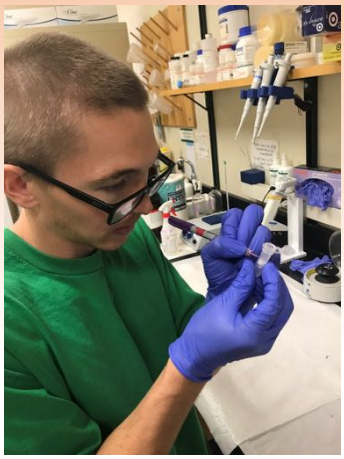
***Lepomis cyanellus*- Green Sunfish**

- 7-9 year life span
- Highly invasive
- Voracious predator

Project Goals

- Aquaculture of species feasible?
- What is their sex determination? XY/XX, ZW/ZZ, polygenic (Autosomal, Environmental?
- Can we find reliable Sex ID markers?
- Can we induce sex reversal with hormones?
- Are the Trojan sex chromosome carriers viable?

Male and female red shiner used for DNA samples

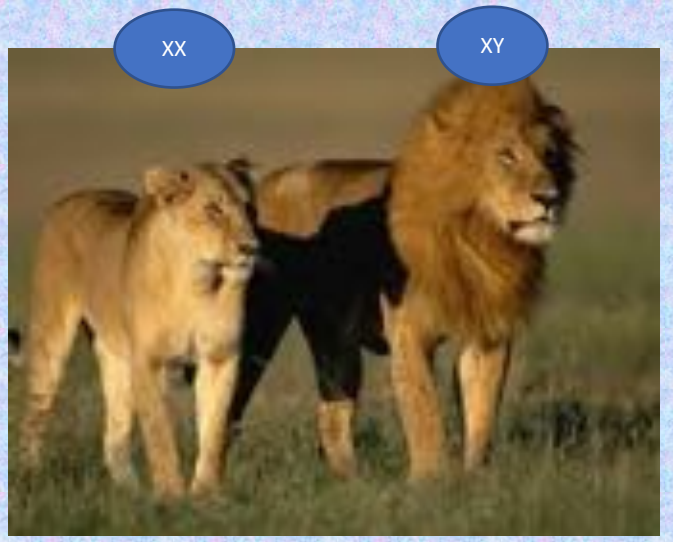


Technician, Colby Roberts, labeling DNA samples.


Methods


Collection
THE UNIVERSITY OF ARIZONA
Photo credit: Heidi Blasius

Sex Determination Systems



Fish Sex Determination- much more complicated

***G. affinis*- western mosquitofish**

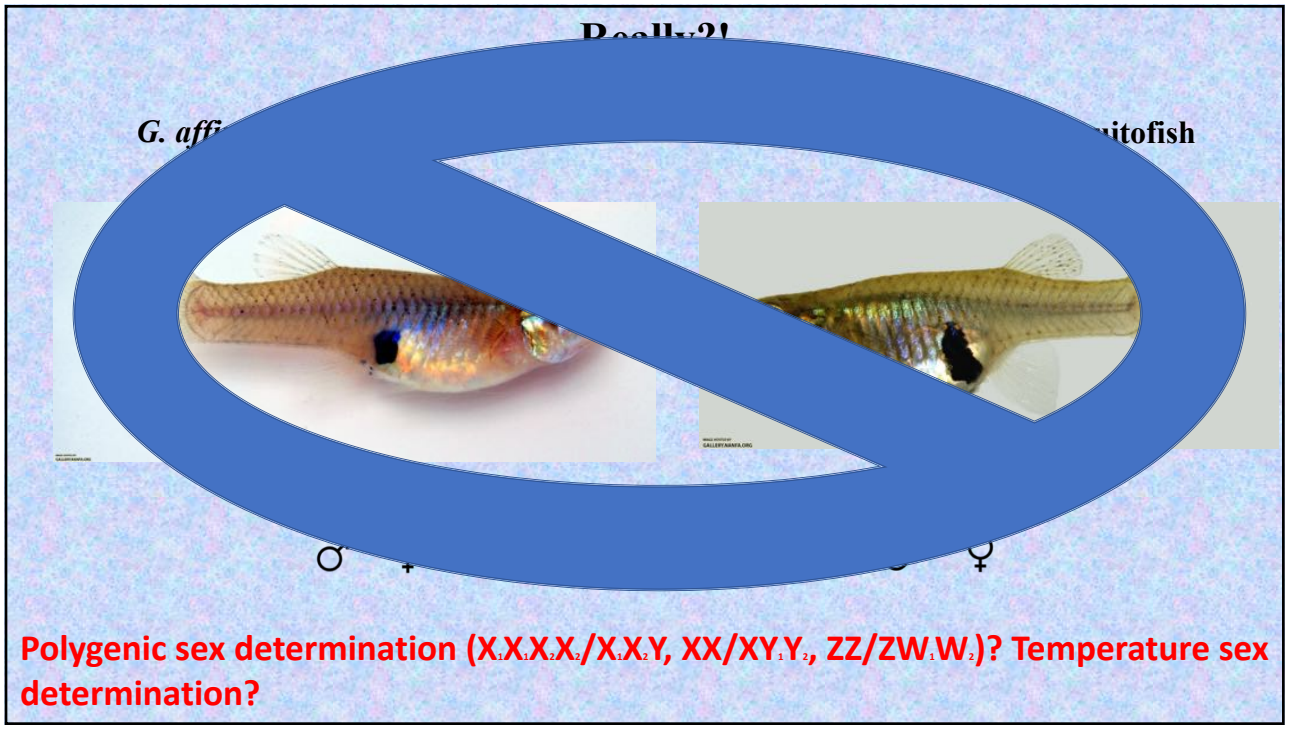
ZZ / ZW

♂ ♀

***G. holbrooki*- eastern mosquitofish**

XY / XX

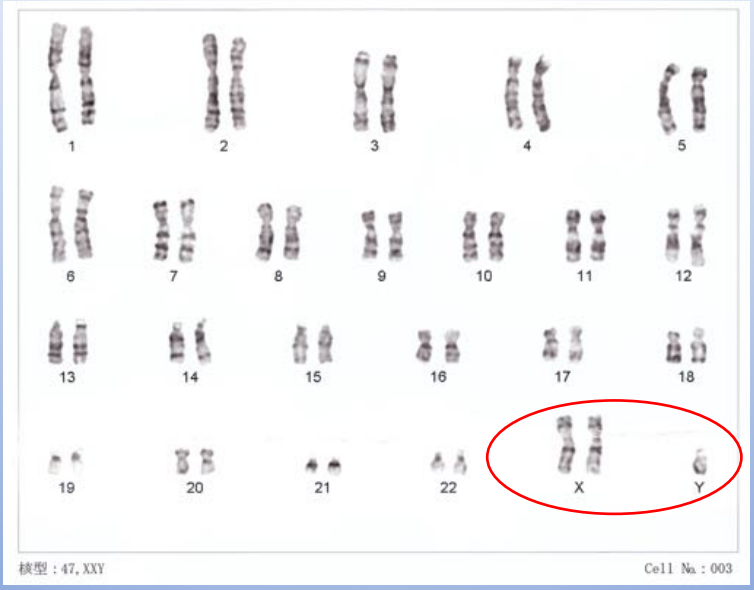
♂ ♀



Polygenic sex determination ($X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2, ZZ/ZW_1W_2)? Temperature sex determination?

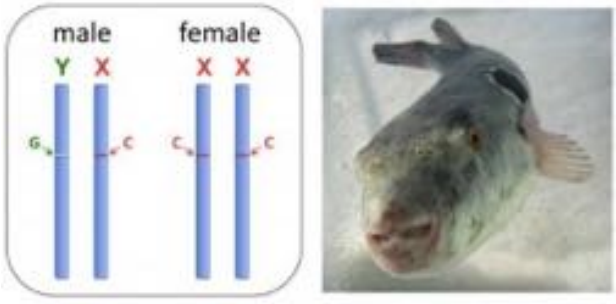
How do we find the sex determination system?

Human Chromosomes



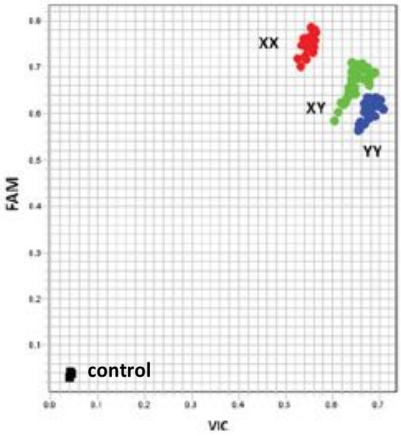
commons.wikimedia.org

Sequenced whole genome!



(Takashi et al 2012)

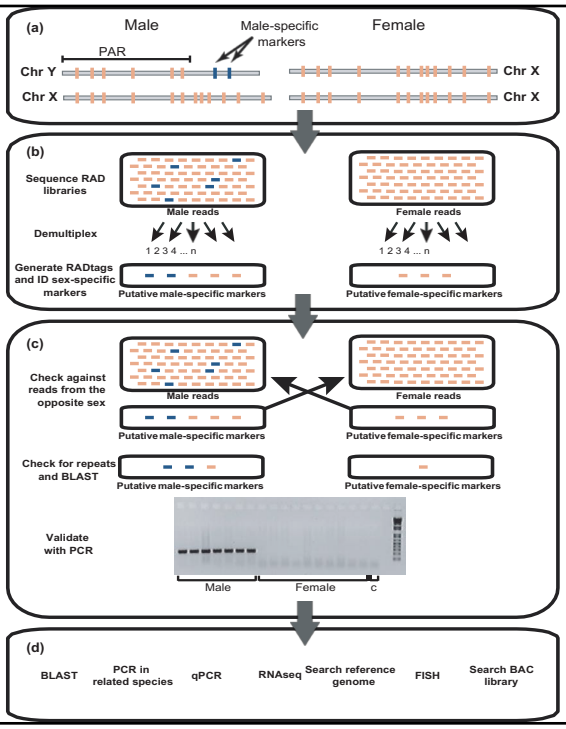
other methods can be used to find sex ID markers



(Schill et al 2016)

Restriction Site-Associated DNA Sequencing (RAD-Seq)

HiSeq 2500 Sequencing System- Next Generation Sequencer



- **Restriction Enzymes cut up the genome**
- **Compare the fragment sequences between male and female samples to find genetic sex differences**

Gamble and Zarkower 2014

Sex ID Marker Development

Using RAD-seq to recognize sex-specific markers and sex chromosome systems

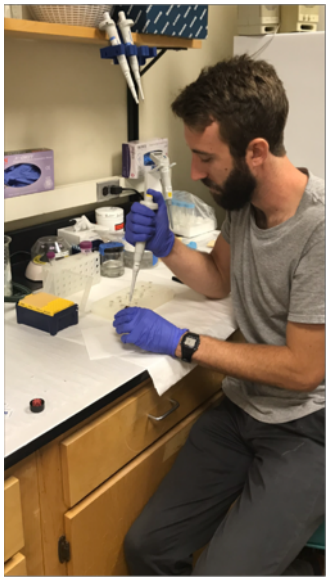
TONY GAMBLE
Department of Biological Sciences, Marquette University, Milwaukee, WI 53201, USA

Genomic characterization of sex-identification markers in *Sebastes carnatus* and *Sebastes chrysomelas* rockfishes

BENJAMIN L. S. FOWLER and VINCENT P. BUONACCORSI
Department of Biology, Juniata College, 1700 Moore Street, Huntingdon, PA 16652, USA

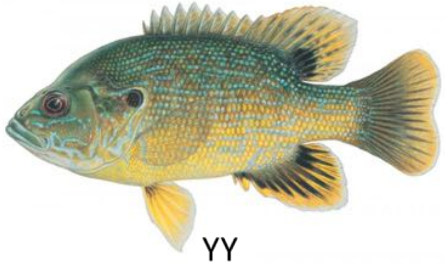
Mapping the sex determination locus in the hāpuku (*Polyprion oxygeneios*) using ddRAD sequencing

Jeremy K. Brown[1†], John B. Taggart[1†], Michaël Bekaert[1], Stefanie Wehner[1], Christos Palaiokostas[2], Alvin N. Setiawan[3], Jane E. Symonds[3,4] and David J. Penman[1*]



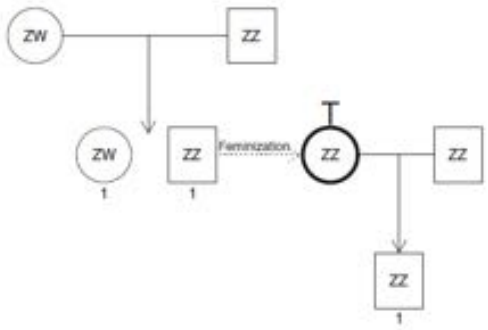
How to Create the Trojan Sex Chromosome Carriers

YY

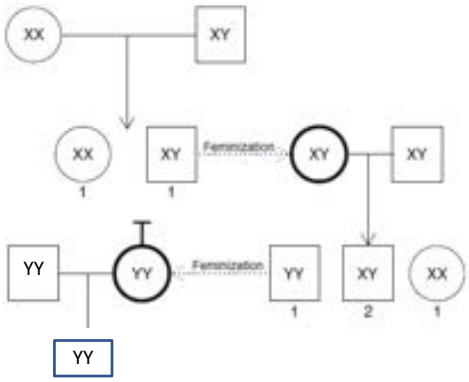


YY

G. affinis- western mosquitofish

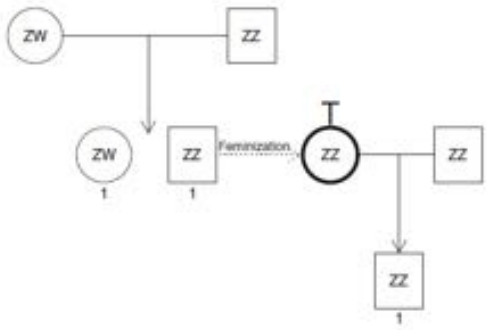


G. holbrooki- eastern mosquitofish



(Senior et al., 2013)

G. affinis- western mosquitofish



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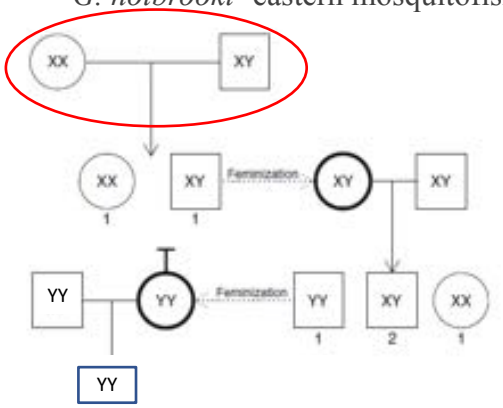

Senior et al. (2013)

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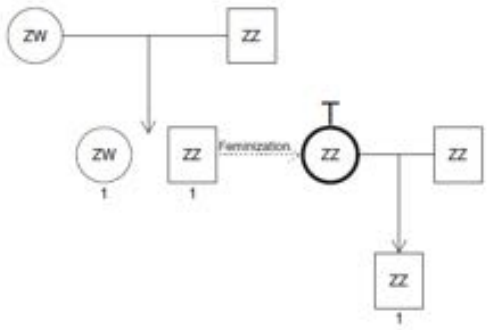

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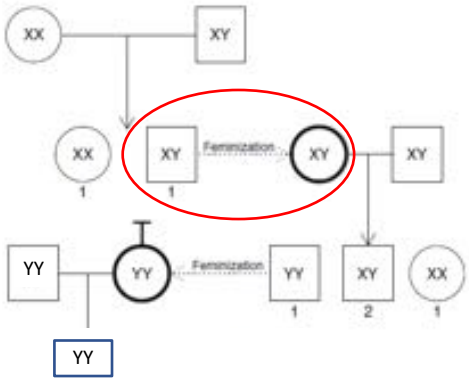

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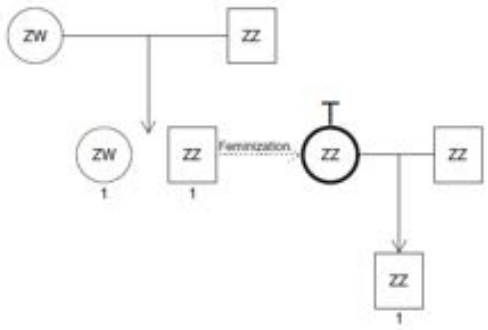

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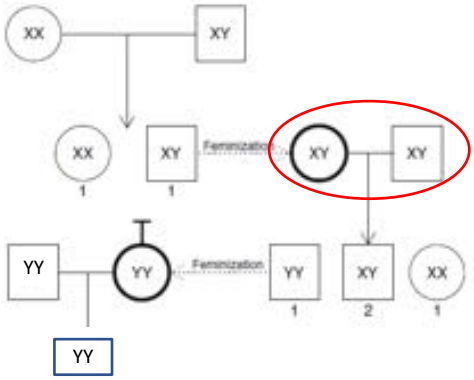

Senior et al. (2013)

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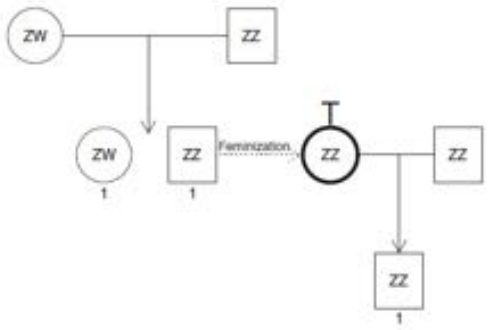

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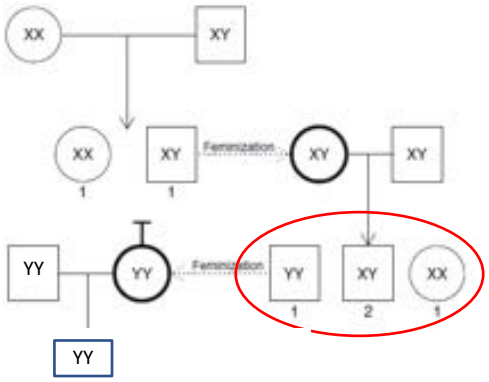

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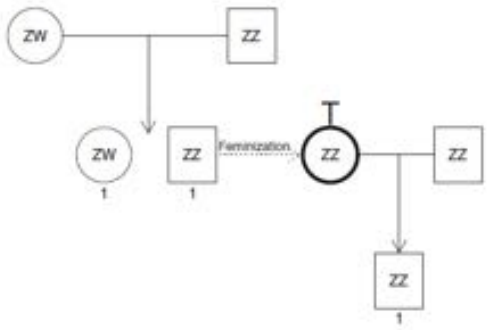

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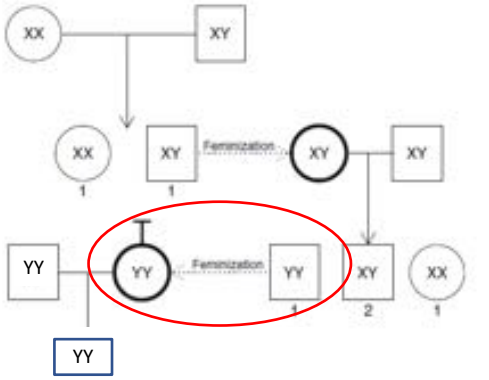

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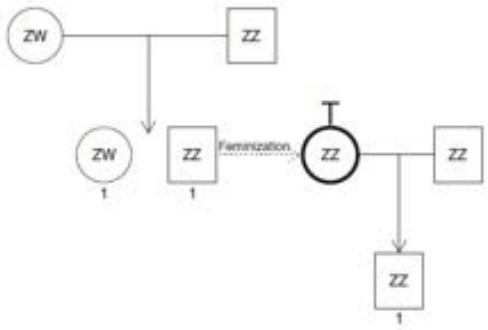

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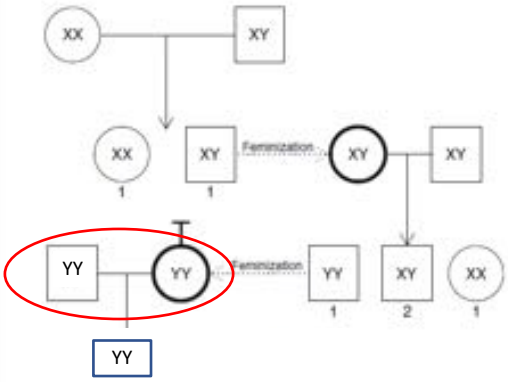

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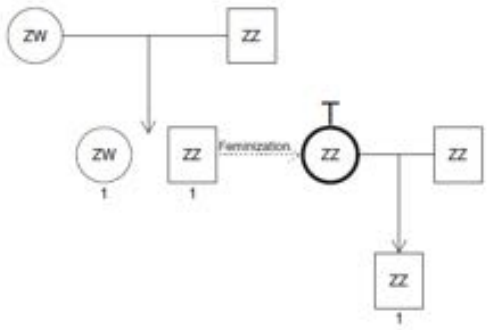

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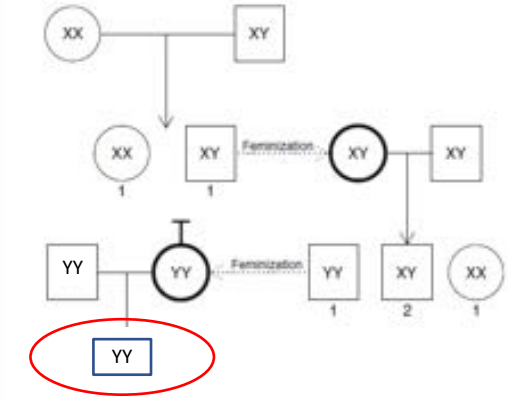

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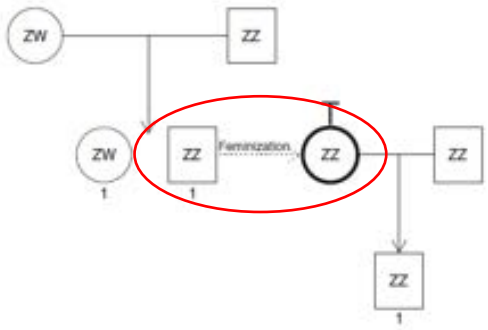

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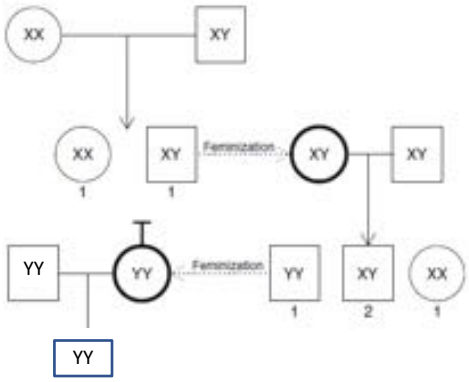
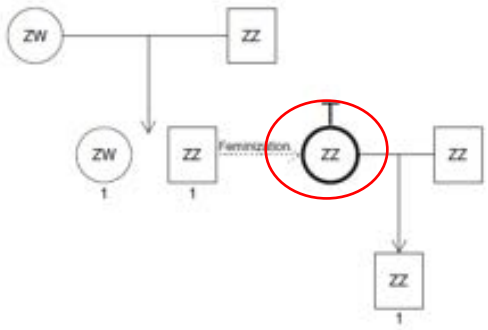
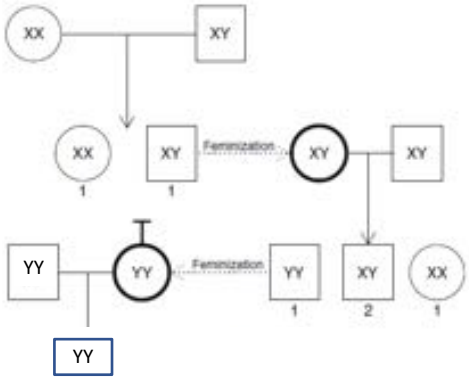


Senior et al. (2013)

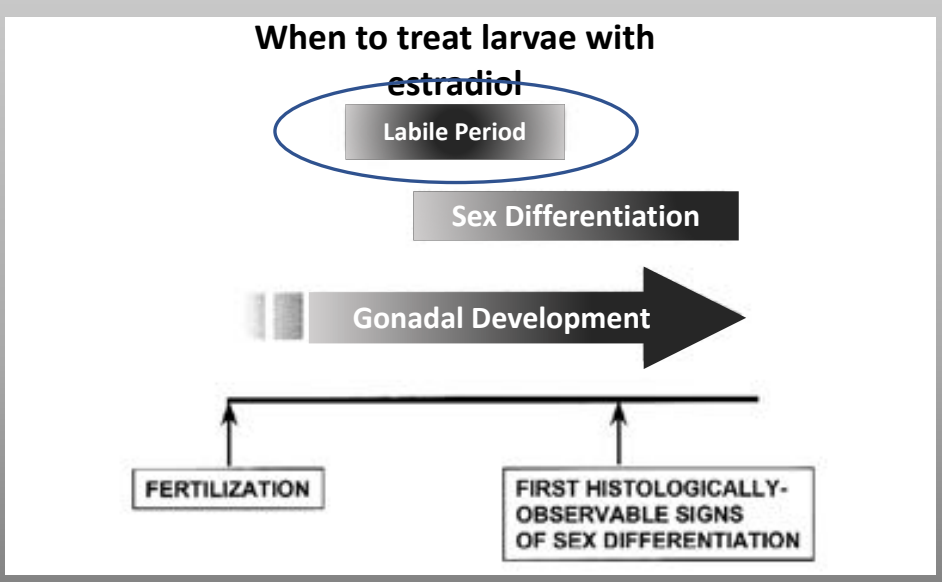
G. affinis- western mosquitofish



G. holbrooki- eastern mosquitofish



Senior et al. (2013)



Piferrer 2010

Genetics: Sex determination and Sex ID marker Development

- Extracted DNA samples from known males and females

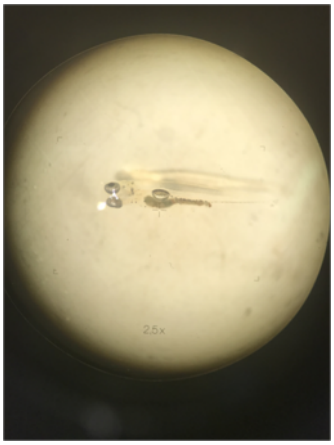
- Selected the restriction enzymes for ddRAD-Seq based on reference genomes - goldfish and zebrafish for red shiner and yellow croaker for green sunfish

- Acquired necessary materials to build RAD libraries

Breeding

Spawning tile with red shiner eggs

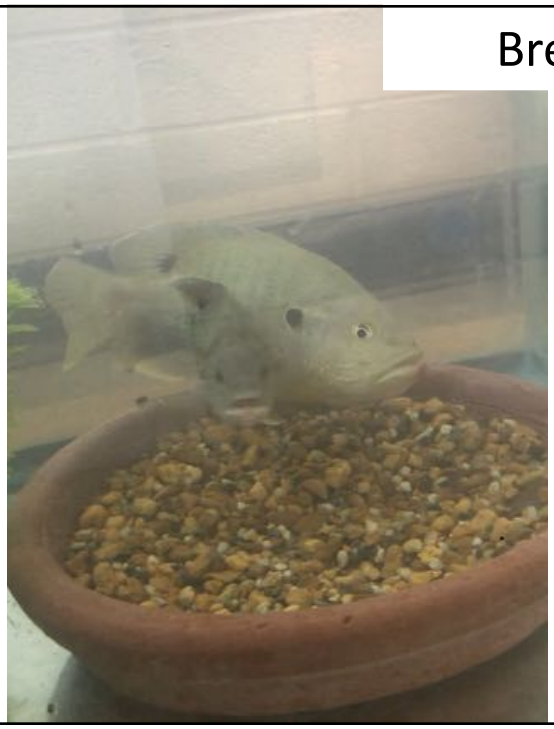
Larval Rearing



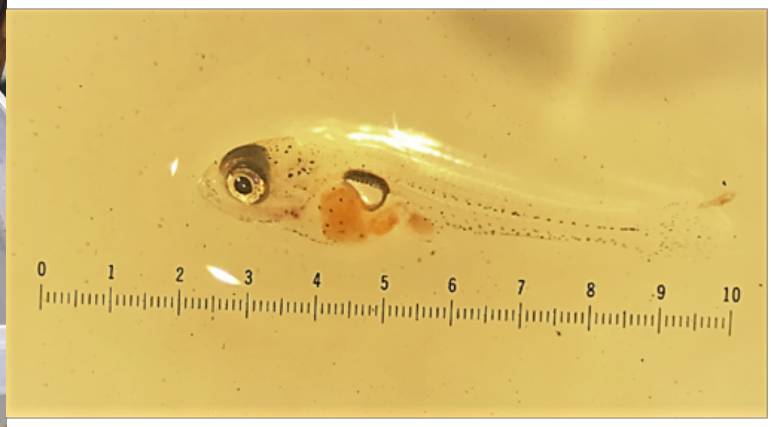
8 dph red shiner

Red shiner juveniles 30 dph

Breeding



Larval Rearing



Artificial spawning nest in incubation tank

19 dph green sunfish

Red Shiner Sex Reversal Treatments

- Started red shiner sex reversal trial October 2018
- High dose (100 mg 17β-Estradiol/kg) and low dose (50 mg 17β-Estradiol/kg)
- 60 day treatments

Green Sunfish Sex Reversal Treatments

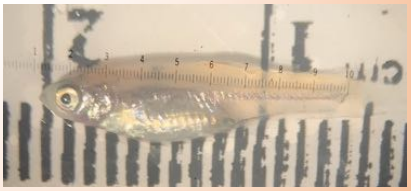
- Beginning green sunfish trials early 2019
- 150 mg 17β-Estradiol/kg of feed (Wang 2008)
- 30 day treatments

Breaking News!

Red shiner can reach sexual maturity in 4.5 months.

Next steps

- Did sex reversal work?
- Breed XY females with XY males
- Find sex ID markers
- Test sex ID markers on larger portion of the population



red shiner 24 dph

adult red shiner at UA propagation lab

Acknowledgements

- Scott Bonar- USGS AZ Cooperative Fish and Wildlife Research Unit
- Bill Stewart – US Buruea of Reclamation
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- Melanie Culver- USGS AZ Cooperative Fish and Wildlife Research Unit
- Alex Erwin- UA Genetics student, School of Natural Resources and Environment
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- Colby Roberts- Research Technician
- Nathan Burg- AZ Game and Fish Dept
- Heidi Blasius- US Bureau of Land Management

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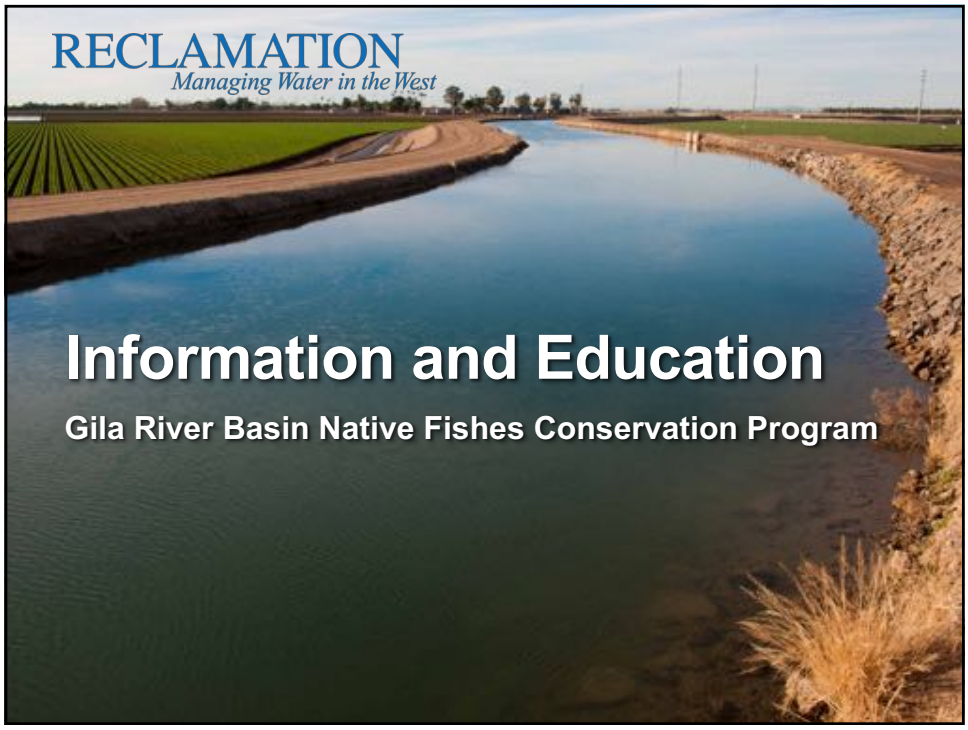


I & E Sub-Committee

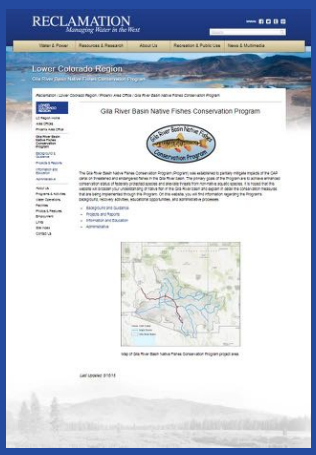
- Kent Mosher (USBR)
- William Stewart (USBR)
- Yvette Paroz (USFS)
- Doug Duncan (USFWS)
- Mary Richardson (USFWS)
- Stuart Wilkins (USFWS)

Current Projects

- Website
- Logo Redesign
- Native Fish in the Classroom
- Gila Topminnow & Desert Pupfish Citizen Science

Website

https://www.usbr.gov/lc/phoenix/biology/azfish



Logo Redesign

Logo Redesign

Native Fish in the Classroom

Funds to NMFWCO for:

- Development of GRB based Curriculum
- Classroom Tank Supplies
- Classroom Visits
  - Salary/travel for tank set-up, presentations, and activities
- Fish Collection/Release
  - Salary/travel
  - School Bus Rental

Gila Topminnow & Desert Pupfish Citizen Science

AGFD:

- Host workshops to train individuals in identifying and monitoring Gila Topminnow and Desert Pupfish.
- First workshop tentatively scheduled for March 2019.

Reclamation:

- Purchase monitoring kits that individuals can check out from regional offices to monitor populations.

Other Projects Under Consideration

- Trinkets (Stickers, fish stress ball, etc.)
- Snorkeling Program on Gila, Coronado, Coconino and/or Tonto NF
- Southwest Fish Art Contest
- Water quality curriculum at schools with native fish ponds

Photo Credit: Wildlife Forever

Questions?

Native Fish in the Classroom: Using Gila Trout to Engage Youth

Angela Palacios James

Fish Biologist

New Mexico Fish and Wildlife Conservation Office

WHAT IS NATIVE FISH IN THE CLASSROOM?

- Native fish in classes
- Activities related to native fish species of New Mexico

GOALS AND ASPIRATIONS

- Raise awareness of aquatic ecosystems
- Connect youth to nature in their community
- Develop and encourage sensitivity for the natural world
- Develop and encourage stewardship
- Expose students to careers in natural resources
- Provide a useful learning tool

AWARENESS AND SENSITIVITY

- Awareness
  - Expand awareness by providing a memorable experience to encourage environmental sensitivity
- Environmental sensitivity
  - A necessary building block in the development of environmentally literate citizens

GETTING IT INTO THE CLASS

- Includes multiple scholastic disciplines
  - Science, math, social studies, geography, art
- Work with teachers schedule
- Simplify process to minimize work load

NMFWCO RESPONSIBILITIES

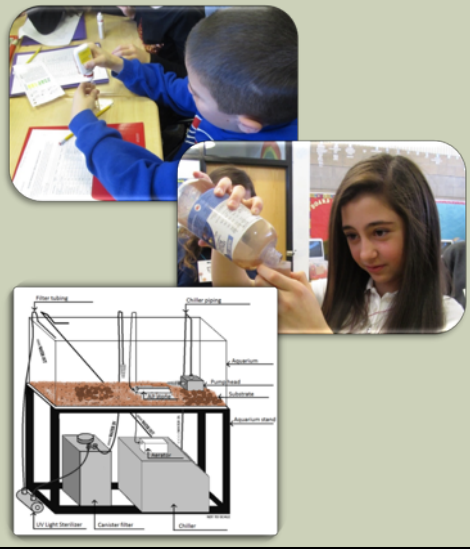
- Permits
- Fish
- Technical support
- In-class presentations
- Reference materials
- Field Release

TEACHER RESPONSIBILITIES

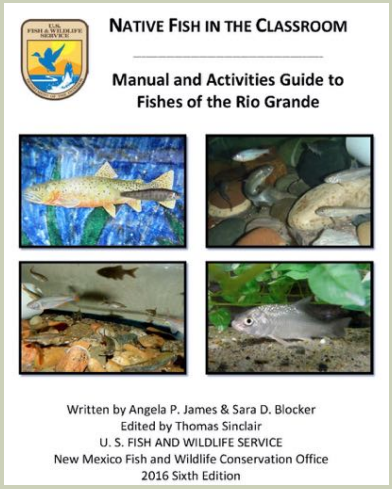
- Monitor student participation and care of aquariums
- Apply and modify activities to suit classroom needs
- Notify USFS or NMFWCO of any issues.

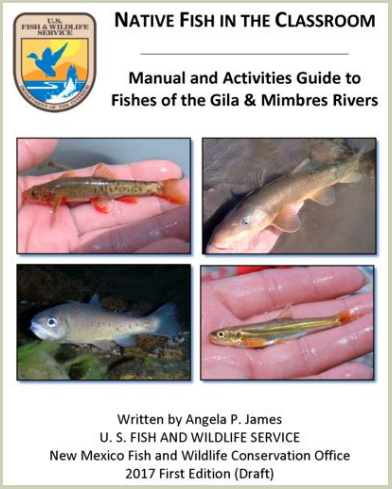
STUDENT RESPONSIBILITIES

- Feed fish
- Monitor fish health
- Monitor and maintain water quality
- Maintain a fish journal
- Complete in-classroom activities
- Participate in fish release



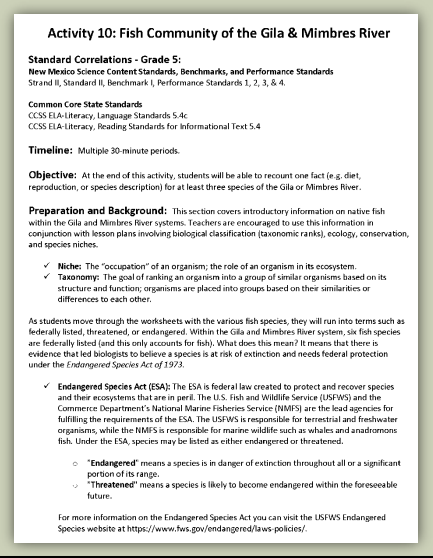
MANUAL AND ACTIVITY GUIDES





BREAKING DOWN AN ACTIVITY

- Standard Correlations
  - New Mexico Science Standards
  - Common Core
- Timeline
- SMART Objective
- Preparation and Background
- Activity
- Student reference & worksheets



Activity 10: Fish Community of the Gila & Mimbres River

Standard Correlations - Grade 5:
New Mexico Science Content Standards, Benchmarks, and Performance Standards
Strand II, Standard II, Benchmark I, Performance Standards 1, 2, 3, & 4.

Common Core State Standards
CCSS ELA-Literacy, Language Standards 5.4c
CCSS ELA-Literacy, Reading Standards for Informational Text 5.4

Timeline: Multiple 30-minute periods.

Objective: At the end of this activity, students will be able to recount one fact (e.g. diet, reproduction, or species description) for at least three species of the Gila or Mimbres River.

Preparation and Background: This section covers introductory information on native fish within the Gila and Mimbres River systems. Teachers are encouraged to use this information in conjunction with lesson plans involving biological classification (taxonomic ranks), ecology, conservation, and species niches.

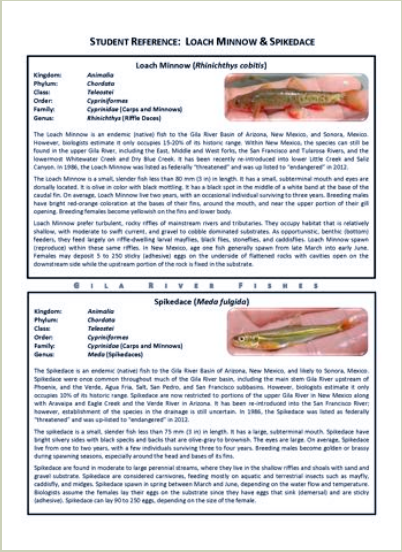
- ✓ **Niche:** The "occupation" of an organism; the role of an organism in its ecosystem.
- ✓ **Taxonomy:** The goal of ranking an organism into a group of similar organisms based on its structure and function; organisms are placed into groups based on their similarities or differences to each other.

As students move through the worksheets with the various fish species, they will run into terms such as federally listed, threatened, or endangered. Within the Gila and Mimbres River system, six fish species are federally listed (and this only accounts for fish). What does this mean? It means that there is evidence that led biologists to believe a species is at risk of extinction and needs federal protection under the *Endangered Species Act of 1973*.

- ✓ **Endangered Species Act (ESA):** The ESA is federal law created to protect and recover species and their ecosystems that are in peril. The U.S. Fish and Wildlife Service (USFWS) and the Commerce Department's National Marine Fisheries Service (NMFS) are the lead agencies for fulfilling the requirements of the ESA. The USFWS is responsible for terrestrial and freshwater organisms, while the NMFS is responsible for marine wildlife such as whales and anadromous fish. Under the ESA, species may be listed as either endangered or threatened.
  - "**Endangered**" means a species is in danger of extinction throughout all or a significant portion of its range.
  - "**Threatened**" means a species is likely to become endangered within the foreseeable future.

For more information on the Endangered Species Act you can visit the USFWS Endangered Species website at https://www.fws.gov/endangered/laws-policies/.

STUDENT MATERIALS



STUDENT REFERENCE: LOACH MINNOW & SPIKEDACE

Loach Minnow (*Rhinichthys cobitis*)

Kingdom:	*Animalia*
Phylum:	*Chordata*
Class:	*Teleostei*
Order:	*Cypriniformes*
Family:	Cyprinidae (Carps and Minnows)
Genus:	*Rhinichthys* (Riffle Daces)

The Loach Minnow is an endemic (native) fish to the Gila River Basin of Arizona, New Mexico, and Sonora, Mexico. However, biologists estimate it only occupies 15-20% of its historic range. Within New Mexico, the species can still be found in the upper Gila River, including the East, Middle and West forks, the San Francisco and Tularosa Rivers, and the lowermost Whitewater Creek and Dry Blue Creek. It has been recently re-introduced into lower Little Creek and Salt Canyon. In 1986, the Loach Minnow was listed as federally "threatened" and was up listed to "endangered" in 2012.

The Loach Minnow is a small, slender fish less than 80 mm (3 in) in length. It has a small, subterminal mouth and eyes are dorsally located. It is olive in color with black mottling. It has a black spot in the middle of a white band at the base of the caudal fin. On average, Loach Minnow live two years, with an occasional individual surviving to three years. Breeding males have bright red-orange coloration at the bases of their fins, around the mouth, and near the upper portion of their gill opening. Breeding females become yellowish on the fins and lower body.

Loach Minnow prefer turbulent, rocky riffles of mainstream rivers and tributaries. They occupy habitat that is relatively shallow, with moderate to swift current, and gravel to cobble dominated substrates. As opportunistic, benthic (bottom) feeders, they feed largely on riffle-dwelling larval mayflies, black flies, stoneflies, and caddisflies. Loach Minnow spawn (reproduce) within these same riffles. In New Mexico, age one fish generally spawn from late March into early June. Females may deposit 5 to 250 sticky (adhesive) eggs on the underside of flattened rocks with cavities open on the downstream side while the upstream portion of the rock is fixed in the substrate.

G I L A R I V E R F I S H E S

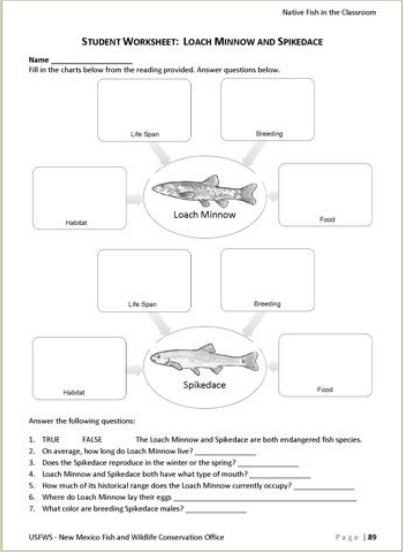
Spikedace (*Meda fulgida*)

Kingdom:	*Animalia*
Phylum:	*Chordata*
Class:	*Teleostei*
Order:	*Cypriniformes*
Family:	Cyprinidae (Carps and Minnows)
Genus:	*Meda* (Spikedaces)

The Spikedace is an endemic (native) fish to the Gila River Basin of Arizona, New Mexico, and likely to Sonora, Mexico. Spikedace were once common throughout much of the Gila River basin, including the main stem Gila River upstream of Phoenix, and the Verde, Agua Fria, Salt, San Pedro, and San Francisco subbasins. However, biologists estimate it only occupies 10% of its historic range. Spikedace are now restricted to portions of the upper Gila River in New Mexico along with Aravaipa and Eagle Creek and the Verde River in Arizona. It has been re-introduced into the San Francisco River; however, establishment of the species in the drainage is still uncertain. In 1986, the Spikedace was listed as federally "threatened" and was up-listed to "endangered" in 2012.

The spikedace is a small, slender fish less than 75 mm (3 in) in length. It has a large, subterminal mouth. Spikedace have bright silvery sides with black specks and backs that are olive-gray to brownish. The eyes are large. On average, Spikedace live from one to two years, with a few individuals surviving three to four years. Breeding males become golden or brassy during spawning seasons, especially around the head and bases of its fins.

Spikedace are found in moderate to large perennial streams, where they live in the shallow riffles and shoals with sand and gravel substrate. Spikedace are considered carnivores, feeding mostly on aquatic and terrestrial insects such as mayfly, caddisfly, and midges. Spikedace spawn in spring between March and June, depending on the water flow and temperature. Biologists assume the females lay their eggs on the substrate since they have eggs that sink (demersal) and are sticky (adhesive). Spikedace can lay 90 to 250 eggs, depending on the size of the female.



Native Fish in the Classroom

STUDENT WORKSHEET: LOACH MINNOW AND SPIKEDACE

Name _____

Fill in the charts below from the reading provided. Answer questions below.

Answer the following questions:

1. TRUE FALSE The Loach Minnow and Spikedace are both endangered fish species.
2. On average, how long do Loach Minnow live? _____
3. Does the Spikedace reproduce in the winter or the spring? _____
4. Loach Minnow and Spikedace both have what type of mouth? _____
5. How much of its historical range does the Loach Minnow currently occupy? _____
6. Where do Loach Minnow lay their eggs _____
7. What color are breeding Spikedace males? _____

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IN-CLASS PRESENTATIONS

- NFIC Introduction
- Fish Anatomy
- Estimating Pinto Fish Populations
- Case of the Missing Cutthroats

THE CULMINATION

Yesterday you were in our classrooms
Safe and sound and swimming.
Yesterday you were in our classrooms
Not worrying about Rainbow Trout too.

Today you are free in the river
Without a tank around you.
Today you are free in the river
Eating lots of mayflies as well.

Tomorrow we'll continue to protect you
We won't let you become extirpated.
Tomorrow we'll continue to protect you
You won't be extinct on our watch.

Yesterday we learned about you
Your anatomy, your predators, your prey.
Yesterday we learned about you.
Rounded tail? Maybe not. What surprise!

Today, we watch you go freely
As we wonder what the water levels are.
Today we watch you go freely
Missing you as you leave.

Tomorrow we will miss you even more
When we'll have no need for a siphon.
Tomorrow we'll miss you even more
As you swim forever in our hearts.

YESTERDAY,

TODAY,

TOMORROW

By Kaya Perce

Monte Vista Elementary School

2016-2017
School Year

PARTICIPATION SUMMARY

- Bosque School – 7 years
- Valle Vista Elementary – First five years
- Monte Vista and Emerson Elementary - 6 years
- 9 of 10 schools are set to return this year
- Entire student body of San Lorenzo Elementary

School Year	Schools	Classrooms	Students
2011-2012	4	6	130
2012-2013	5	9	200
2013-2014	6	14	319
2014-2015	6	14	339
2015-2016	7	19	429
2016-2017	10	18	516
2017-2018	11	21	481
		Total Students:	**2414**

PARTNERS

QUESTIONS & CONTACT INFORMATION

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3800 Commons Avenue NE,
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https://www.fws.gov/southwest/fisheries/nmfwco/education.html