# United States Department of the Interior

NATIONAL PARK SERVICE
Mojave National Preserve
2701 Barstow Road
Barstow, California 92311


IN REPLY REFER TO:
N22 (MOJA)

January 22, 2009

Chris Kofron
Ventura Fish and Wildlife Office
2493 Portola Road, Suite B
Ventura, California 93003

Re: 2nd Annual Report, Permit TE-161225-0,

Dear Mr. Chris Kofron:

The following is our second annual report for the hybridization experiment with Mohave tui chub and arroyo chub under Endangered Species permit number TE-161225-0.

Background

The Mohave tui chub (*Siphateles bicolor mohavensis*), (Pisces: Cyprinidae), was listed as endangered October 13, 1970. The Recovery Plan was published Sept. 17, 1984. At the time of its listing the extirpation of Mohave tui chub (MTC) from the Mojave River was thought to have resulted from hybridization with arroyo chub (AC) (*Gila orcutti*). The Recovery Plan states, "[t]he exotic species of Gila invaded the Mohave River and subsequently hybridized with the Mohave tui chub. By 1970 genetically pure Mohave tui chubs had been eliminated from the river by hybridization and subsequent introgression." The work that this statement was based on (Hubbs and Miller 1943, Miller 1969) assessed hybridization through anatomy, such as scale radii. More recent work (Chen et al. 2006) found genetically pure AC in the Mojave River and genetically pure MTC in the three extant populations (Zzyzx, Naval Air Weapons Station at China Lake, and Camp Cady). Thus the viability of Mohave tui chub – arroyo chub hybrids is a research question that needs to be addressed prior to reestablishment of MTC in the Mojave River basin, or establishment of recovery populations in offstream waters that potentially could be invaded by arroyo chubs.

Abiotic factors may have contributed to extirpation of MTC from the Mojave River. Castleberry and Cech (1986) argue that physiological responses to high temperatures, rapidly fluctuating temperatures, and high flow velocities give arroyo chubs a competitive advantage over Mohave tui chubs in predator avoidance, feeding, and escaping stressful environmental conditions. Mohave tui chubs, on the other hand, may have a competitive advantage in low temperature and/or hypoxic conditions that typically occur in stratified lake environments, particularly during a winter freeze. Arroyo chubs are better swimmers than Mohave tui chubs and thus less likely to be swept downstream to the Soda Lake playa. In the flood-prone Mojave River the Mohave tui chub were frequently washed downstream to expire on the exposed terminal basin in great numbers. A combination of low fertility of hybrids and greater tolerance of high temperatures and higher flow velocities may explain the presence of pure arroyo chubs in the Mojave River drainage. This project seeks to address the issue of hybridization and competition between Mohave tui chub and Arroyo chub in an aquarium and an artificial pond environment. A better understanding of the viability of hybrids may be useful in selecting suitable habitats in the upper Mojave River drainage for recovery of the species.

The original objectives of this study proposal were to address the following two research hypotheses.

1) Are *Siphateles bicolor mohavensis* X *Gila orcutti* hybrids viable?

2) Will *Siphateles bicolor mohavensis* out-compete *Gila orcutti* in a still pond subject to seasonal conditions of low temperature hypoxia?

Now that we have discovered hitch (*Lavinia exilicauda*) established in the Mojave River we are also interested in potential competitive and hybridization relationships between this species and Mohave tui chub.

Narrative

*Hybridization experiment* – As reported in our first annual report dated January 31, 2008, we started this experiment with 10 laboratory spawned MTC, which had been released into the Fire Pond at Camp Cady on March 30, 2007 (Recovery Permit TE-086593-0) and which we obtained November 18, 2007 when California Department of Fish and Game (CDFG) was in the process of transferring MTC from the Fire Pond to Bud's Pond, and with 10 presumptive AC which we collected from the Mojave River at Afton Canyon between December 4 and 11, 2007.

On December 27, 2007 we started a tank chiller on the second 50 gallon tank containing arroyo chub at the Desert Discovery Center (DDC) and cooled it from about 13°C to 9°C in 3 days. On January 4, 2008 we changed the photoperiod from 14 hours of light to 10 hours of light. This was only on the tank lights. The two 50 gallon tanks were in a storeroom where people enter and leave during the day, turning the room lights on and off. We set the on cycle of the lights to correspond to daytime activities at the Center. Sujan M. Henkanaththegedara, doctoral student with Dr. Craig Stockwell from North Dakota State University and his assistant, Nathan A. Stroh, came to the DDC on January 28 to inspect the fish. Stockwell and Henkanaththegedara are funded by the National Park Service to conduct research on the Lake Ecology of Lake Tuendae at Zzyzx on Mojave National Preserve (Recovery Permit TE126141-0) Henkanaththegedara and Stroh observed spawning behavior (and possibly tubercle formation) in the Mohave tui chub in the 50 gallon tank where we were not controlling temperature. Water temperature in this tank had remained in the range of 15 to 20°C but all of the Mojave tui chubs appeared to have developed spawning characteristics including rust colored fins and tubercles on the heads of the males. Sujan and Nate returned March 10 to separate the Mohave tui chub by sex on the basis of tubercles on the male's heads. We had 6 male and 4 female Mohave tui chub. We marked males with a left pectoral fin clip and females with a right pectoral fin clip then put 2 males and 2 females in the 50 gallon tank and the rest in the 125 gallon tank. Fin clips were air dried and saved for later genetic work.

As of mid March the warming cycle in the 50 gallon tank containing the presumed arroyo chubs, which we started February 4, had produced no signs of spawning behavior. We began feeding California black worms on March 21 and heating the tank between 27°C and 30°C on a three day cycle March 28 (Tres 1992). It was also apparent that the fish collected from Afton Canyon were infected with anchorworm (*Lernaea cyprinacea*). We treated two fish with 3% salt solution for 24 hours in a separate container February 26 and another March 24.

Henkanaththegedara and Stroh identified 8 of the fish collected from Afton Canyon as hitch (*Lavinia exilicauda*) and two as arroyo chub. At this point we appeared to have 3 species instead of two, had separated the MTC by sexes for spawning in one of the 50 gallon tanks, and had only 3 tanks. We therefore decided to move the two AC to the 125 gallon tank with the six MTC. Our reasoning was that we were eventually going to combine MTC and AC in one tank for hybridization. On April 7, a single anchorworm was noted on the small arroyo chub just moved to the 125 gallon tank. This fish was quarantined for treatment in a 3% salt bath for two days then returned to the 125 gallon tank April 12. On April 29 anchorworm again appeared in the 125 gallon tank containing 6 Mohave tui chub and 2 arroyo chub. We removed 4 anchorworms from 3 Mohave tui chub with tweezers on April 30. All fish were observed every day and fed vigorously up to May 11. No unusual behavior or physical changes were noted. On the morning of May 12 all fish in the 125 gallon tank were in bad shape, with swollen whitish skin discoloration around the dorsal fin and down the sides with some isolated spots. The first MTC mortality was noted that day along with one of the two AC.

All remaining fish in the 125 gallon tank were dipped in a Methylene Blue bath solution of 50 ppm the afternoon of May 12. Due to the uncertainty of the problem and unclear instructions for medication, the fish were returned to the 125 gallon tank. We treated the 125 gallon tank with CLOUT, fish medication for parasitic and protozoan infections, for 24 hours starting on May 13. All the fish except the one remaining arroyo chub had anchorworms hanging from bottom, top, sides, and lower jaw. Their skin was swollen and had a whitish color. We did a 30% water change on May 14 because of high nitrate, and to reduce the possibility of toxic levels of CLOUT in preparation of a second 24 hour treatment. On May 14, nitroband was added to reduce nitrate, and a second CLOUT treatment started. We disinfected the chiller with bleach solution and cooled the

tank from 27°C to 20°C over the weekend. The second MTC mortality was found May 16, and the third May 20. We tried oxytetracycline treatment on the 3 remaining Mohave tui chubs in the 125 gallon tank. Terramycin was weighed out to make a 100ppm solution in 10 gallons of water in a plastic cooler to which we attached a bubbler. We left the fish in this bath for about 6 hours. They seemed to do well in the bath but, when removed exhibited obvious signs of stress. Two of the fish didn't have much left for dorsal fins and had large black masses of what looked to be bacterial infection around the dorsal insertion. One had what looked like a whitish fungal infection trailing off what remained of its dorsal fin. We repeated the treatment for about 8 hours. The three remaining MTC appeared to have hundreds of anchorworms on the dorsal fin, along the sides, hanging off the chin, protruding from their nares, and even from the edge of the eye. In contrast, the one remaining arroyo chub in the tank appeared completely free of worms. We also tried treating weekly with Trifon, another organo-phosphoric acid. The fourth MTC mortality was found May 26 and the fifth May 31. Following a conference call June 2 our authority to remove MTC from the wild was revoked, although it was acknowledged that we had not actually removed any MTC from the wild. All MTC in our possession had been spawned in captivity. The sixth and last MTC in the 125 gallon tank died June 10. We euthanized the remaining AC and sent all specimens to the California Nevada Fish Health Center c/o Lisa Ratcliff June 19, 2008.

*Additional observations on arroyo chub* – We collected 12 AC from the Upper Mojave Narrows May 12 (described below in *field work*) and transported them to the DDC for continuation of the initial hybridization experiment. These fish were maintained in a cooler fitted with an aerator and treated for external parasites and tapeworm. Due to a lack of aquariums to house the newly captured AC and the anchorworm outbreak on the MTC, the AC were maintained in the cooler until July 14. To improve conditions for the AC the lid to the cooler was propped open approximately ½ inch to allow natural light and air circulation. Four AC managed to jump out of the cooler through this crack, even though the water level was less than a third of the depth of the cooler, and perished on the floor over the weekend of June 28. After the last MTC in the large tank died, the tank was disassembled, cleaned, disinfected and reassembled on July 9. After an adjustment period, the remaining AC kept in the cooler were moved to this large tank on July 14.

The AC responded well in the large tank until August 23 when large quantities of ants were found floating on the water surface in the tank. Hundreds of ants were massed in balls clinging to plastic asparagus plant leaf tips projecting above the water. It took several days to remove the ants and treat the floor surrounding the setup so as to prevent more ants from invading the tank. AC began to show adverse signs and began to die soon afterward. The first mortality was noted August 26, two more died by August 28. A water change was initiated that replaced one third of the volume each day over three days. The remaining three fish persisted but their health deteriorated. Behavior consisted of loss of balance, laying on their sides or back, swimming in darting and spiraling motions across the tank. One mortality occurred September 9 and the last two AC were euthanized on September 16 after exhibiting the abnormal behavior for over a week and death appeared imminent.

Other problems at the DDC in addition to the outbreak of ants led us to reconsider the use of this facility for the experiment. Part of the problem of dealing with the anchorworm was the inadequately controlled high air temperature in the building. Our one chiller was only sufficient to cool one of our 50 gallon tanks. Control of the light cycle is difficult in a building where many different people enter the storeroom where the two smaller tanks are kept. We have moved the 50 gallon tank with the four remaining MTC and the 125 gallon tank to the climate-controlled Mojave National Preserve Headquarters building at 2701 Barstow Road, Barstow, California. Spawning will be attempted in a dark, locked room where we have complete control over light and temperature. We will keep hitch in the remaining 50 gallon tank at the DDC until such time as we have spawned MTC. We will then replace the hitch in the display tank with spawned MTC.

*Field work* – The discovery of presumed hitch in our tank containing fish from Afton Canyon led us to organize an interagency collecting expedition to update our understanding of fish community composition in the Mojave River. Agencies and institutions involved included the National Park Service, California Department of Fish and Game, the Natural History Museum of Los Angeles County, the Bureau of Land Management, the U.S. Forest Service, North Dakota State University, and the Lewis Center for Educational Research. We sampled at the Upper Mojave Narrows on May 12, Afton Canyon May 13, and Deep Creek September 22 and 23. Voucher specimens are permanently archived at the Natural History Museum of Los Angeles County.

Following the September 22-23 collecting expedition Mojave National Preserve staff returned to Devil's Hole in Deep Creek in the San Bernardino National Forest (see *Results* for sample locations) on October 29 and collected 22 arroyo chubs. These fish were treated for parasites and are currently housed in a three level 325 gallon breeding tank purchased by Mojave National Preserve for this project. One AC will be randomly selected, euthanized, and sent to the California Nevada Fish Health Center along with another previous mortality to be tested for parasites.

Prior to our December 4-11, 2007 discovery of hitch, all fish collected from Afton Canyon had been reported as either *Siphateles bicolor mohavensis* or *Gila orcutti* or their hybrids except for 7 black bullhead (*Ameiurus melas*) reported by Timothy Brown in 1978. Twenty seven fish collected on May 22, 2002 (LACM 55990-1) were all identified as *Gila orcutti* with the possible exception of one (Jeffrey A. Seigel, Natural History Museum of Los Angeles County, personal communication). Another 72 fishes were collected from Afton Canyon on April 6-8, 2005. Seventy one were identified as *Gila orcutti* (LACM 56881-1) and one was identified as a hitch (LACM 56881-2) (*Lavinia exilicauda*). Henkanaththegedara et al. (2004) noted that brown bullhead were the first reported non-native fish species in the Mojave River drainage. By 2002, 23 non-native fishes were reported in the drainage (Marchetti et al. 2004, Moyle 2002). Non native species richness appears to be highest at the Silverwood reservoir on the West Fork which ties in to the State Water Project.

Results

The six MTC that died following the outbreak of anchorworm in the 125 gallon tank and the one AC that was euthanized were sent to the California Nevada Fish Health Center. None of the laboratory spawned MTC had tapeworm but although all fish had been treated with praziquantel (6 mg/l) for 24 hours the AC tested positive for Asian tapeworm (*Bothripcephalus acheilognathi*).

Representative vouchers of all fishes collected from the Mojave River are deposited in the Natural History Museum of Los Angeles County. Parasitology work was done by Lisa Ratcliff at the California - Nevada Fish Health Center, 24411 Coleman Fish Hatchery Road, Anderson, CA 96007.

*Upper Narrows* – We collected seven fish species from the Mojave River above the Upper Narrows near the Lewis Center for Educational Research on May 12, 2008. They are: mosquitofish (*Gambusia affinis*), threespine stickleback (*Gasterosteus aculeatus*), brown bullhead, and green sunfish (*Lepomis cyanellus*). Fifty four cyprinids were collected, vouchered and identified on the basis of fin–ray, gill raker and pharyngeal tooth counts; arroyo chub, hitch, and hitch/arroyo chub hybrids (Jeffrey A. Seigel, personal communication). Twenty seven arroyo chubs (LACM 56833-3, 10 specimens; LACM 56834-3, 11 specimens; LACM 56838-2, 6 specimens), 21 hitch (LACM 56833-5, 4 specimens; LACM 56834-6, 11 specimens; LACM 56838-5, 6 specimens) and 6 hitch/chub hybrids (LACM 56833-4, 1 specimen; LACM 56834-5, 3 specimens; LACM 56838-4, 2 specimens) were collected from the Lewis Center on May 12 plus one larva (8.9 mm SL) that is probably an arroyo chub collected the next morning. These numbers may be meaningful in that they show the relative abundance of each minnow at that locality. This at least provides us with a baseline and it might be worthwhile to collect there in the future to see how these numbers change. Certainly hitch is now a dominant part of the River's fish fauna; a new factor influencing any strategy to reintroduce MTC.

Of the 20 mosquitofish, 20 threespine stickleback, and 20 hitch sent to the California Nevada Fish Health Center, Asian tapeworm was found in 30% of hitch (N=6) and *Schistocephalus spp.* was found in 10% of threespine stickleback (N=2).

*Afton Canyon* – The minnow population in Afton Canyon has seemingly undergone a rapid change in the last 6 years. In May 2002 we collected 27 *Gila orcutti* (LACM 55990-1). In April 2005, 72 fishes were collected; 71 arroyo chubs (LACM 56881-1) and one hitch (LACM 56881-2). Of 107 fish collected and vouchered from Afton Canyon on May 13, 2008 there were 69 hitch (LACM 56880-3), 18 arroyo chubs (LACM 56880-1), and 20 hitch/chub crosses (LACM 56880-2). Additionally, a small slough south of the Campground and west/southwest of the first Afton Canyon crossing was also sampled on May 13. A riparian area was noticed while driving east on Afton Canyon road, south of the fork to the Campground. Nearly 0.5 km south of the fork was a small, shallow swamp/slough off of the main flow of the river. Schools of very small fish were noticed in the shallows and collected with dipnets. No larger juveniles or adults were observed. Fifty four fish were

collected, identified and vouchered at LACM. They are all identified as arroyo chub (LACM 56893-1, 54 specimens, 7.6-18.3 mm SL).

*Deep Creek* – Interagency crews qualitatively electrofished and trapped three locations in Deep Creek, September 22-23, 2008 to evaluate species composition. The locations sampled (in upstream to downstream order) were Devil's Hole, below Warm Springs, and above Mojave Forks Dam between the USGS Gage and the flood control dam. Identification in the field suggested the following species. At Devil's Hole (34°18'03"N 117°07'37"): rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), arroyo chub, black bullhead, and threespine stickleback were found. In addition, the Eurasian snail *Radix auriculata* was collected. This mollusk is not a new or unusual find in California. At Warm Springs (34°20'36"N 117°10'30"W) arroyo chub and black bullhead were collected. Above the Forks Dam (34°20'39"N 117°13'47"W) black bullhead, hitch (LACM 56864-3, 23 specimens; LACM 56865-3, 14 specimens), green sunfish (*Lepomis cyanellus*), black bass (*Micropterus salmoides*), arroyo chub (LACM 56864-2, 1 specimen), and prickly sculpin (*Cottus asper*) were found. There were 384 specimen collected and kept from Devil's Hole (LACM 56868-1) and all are identified as arroyo chub. There also were 279 arroyo chub collected and kept from Warm Springs (LACM 56867-2). The apparent absence of hitch and/or hybrids from the upper two sites suggests that hydraulic features between Warm Springs and the Forks may provide an effective natural barrier to upstream fish migration. One possible barrier is a large cataract about 1.7 miles upstream from Mojave Forks dam (Matthew Huffine, Lewis Center for Educational Research, personal communication). The failure to collect trout and stickleback at the lower two sites may result from unsuitable water temperatures in mid-summer. The numbers seem to indicate a shift in the predominate minnow in Deep Creek; that is, the arroyo chub-dominant upper Devil's Hole/Warm Springs area (663 arroyo chub, no hitch) and the hitch-dominant lower Fork's Dam area (1 arroyo chub, 37 hitch). It seems unlikely that these numbers are an artifact of seasonality or collecting bias.

Conclusions and Recommendations

- Our first attempt at spawning MTC and AC in aquariums indicated that a simple application of temperature and light manipulation in our tanks may not be sufficient to induce reproductive readiness in arroyo chub. We followed the Tres (1992) protocol with the AC and did not observe any signs of spawning behavior. On the other hand, the MTC developed spawning colors and the males developed tubercles with no artificial manipulation, although we did not document gamete production.
- A single 24-hour treatment with praziquantel (6 mg/l) is not sufficient to remove Asian tapeworm from wild-caught AC. Multiple treatments with complete replacement of water may be required.
- The laboratory-spawned MTC had no resistance to anchorworm carried by AC collected from Afton Canyon. Parasite control will likely be a significant issue in further attempts to hybridize MTC with other cyprinids.
- Non-native fishes appear to be entering the Mojave River drainage via the California Aqueduct through Silverwood Reservoir.
- Non-native fish species richness appears to be highest in Deep Creek near the Forks Dam, and in the Mojave River at the Upper Narrows.
- Hitch and bullheads appear to be more common in areas directly above the Forks Dam whereas arroyo chub and trout appear to be more common upstream at Devil's Hole and the Warm Springs areas.
- Hitch appear to be expanding in the Mojave River and hybridizing with and replacing arroyo chub.
- In order to better frame the recovery potential of Mohave tui chub, species composition above and below potential migration barriers in Deep Creek, the upstream extent of arroyo chub in the perennial headwaters, and the species composition in all Mojave basin reservoirs should be determined.

Future Directions

- We have moved our 125 gallon tank and one of the 50 gallon tanks to our Headquarters Building in Barstow. The purpose of this move is to allow us to better control temperature and light during the spawning protocol. We have left one 50 gallon tank at the DDC with hitch for display. Once we are successful at spawning MTC we will replace the hitch display with spawned MTC.
- We plan to implement the pond experiment at the DDC. But given the problems we experienced with anchorworm the experiment must be carefully designed and managed in order to obtain useful information. We will submit a permit modification request in 2009 in collaboration with Michael

Glenn, USFWS Ventura Office, describing our intended experimental design. We will at that time request reauthorization to collect juvenile MTC from Lake Tuendae.

Acknowledgements

Numerous individuals, agencies, and institutions are contributing to the growing effort to improve the status of the fish native to the Mojave River. The primary authors of this report: Debra Hughson, Neal Darby, Steve Parmenter, and Jeff Seigel could not have done this work without the full support of the U.S. Fish and Wildlife Service, the U.S. Forest Service, the National Park Service, California Department of Fish and Game, the Bureau of Land Management, and the Natural History Museum of Los Angeles County. Individuals who assisted with the work and directly contributed information to this report also include Sujan Henkanaththegedara and Lisa Ratcliff. A great deal of the credit for the progress made towards recovery goes to Judy Hohman and Michael Glenn at the USFWS Ventura Office. Matthew Huffine and the Lewis Center for Educational Research have become central figures in the recovery effort. Through cooperation with USFWS and CDFG a new population of MTC was established at the Lewis Center near the Upper Narrows in October, 2008. Craig Stockwell and his students at North Dakota State University have undertaken much useful research with direct management utility towards the recovery effort. In addition, we would like to thank Jason Dungan, Nathan Stroh, Jane Lester, Kim Milliron, Gar Abbas, Susan Williams, Robert Bryson, Robert Hilborn, and Christopher Otahal.

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If you have any questions or comments on this annual progress report, please do not hesitate to contact at me 760-252-6105 or Neal Darby at 760-252-6146. Steve Parmenter can be reached at the California Department of Fish and Game Inland Deserts Region Field Office in Bishop at 760-872-1123.

Sincerely,

/s/ Debra Hughson

Debra Hughson, Ph.D.
Science Advisor

cc:

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