

Conservation and Management of the Santa Ana River Watershed: Estimating Genetic Diversity of the Santa Ana Speckled Dace (*Rhinichthys osculus*)

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INTRODUCTION

One of the largest river basins in the Southern California region is the Santa Ana River watershed which covers 6,900 km² in parts of three California counties including San Bernardino, Riverside, and Los Angeles (SAWA 2009; SAWPA 2004) (Figure 2). Watersheds, as a collective drainage system for multitudes of smaller tributaries, provide a large part of the water resources to heavily populated areas. The U.S. Environmental Protection Agency lists 153 watershed systems within California. The study of these watersheds is integral to the health of the growing California population. The persistence of species that reside in the waters of these systems is indicative of the stability of the waterways. To this end, studying the evolution of species within these waterways is necessary. One such species is the small native minnow fish, *Rhinichthys osculus*, the speckled dace.

The Santa Ana Speckled Dace is a small, cyprinid fish approximately 80mm (Figure 1) in length and inhabits environments containing shallow cobble, gravel riffles and mixed sand habitats with overhanging riparian flora which provide a defensive measure to the fish from predators (Moyle & Marchetti 2006); although they can inhabit environments that are fairly open. The Santa Ana Speckled Dace once occupied the majority of the Santa Ana, San Gabriel and Los Angeles River systems but due to anthropogenic effects, their habitat has become highly fragmented. They were reported extirpated from the Los Angeles River system in the early 1990's which was most likely attributed to the urbanization of the watershed and creation of water divisions (SAWPA 2004). The populations of the Santa Ana Speckled Dace are highly effected by climatic events such as fire and floods. In 1995, the Santa Ana Speckled Dace was listed as a species of special concern by California Department of Fish and Game. Then in 1998 it was listed as a species of concern by the United States Forest Service. The Santa Ana Speckled Dace was not listed as a federally protected endangered species due to the lack of formal peer reviewed taxonomic description including a genetic description of the taxon and population level diversity (Moyle & Marchetti 2006).



Figure 1. *Rhinichthys osculus*, the Santa Ana Speckled Dace (University of California Agriculture and Natural Resources, 2012)

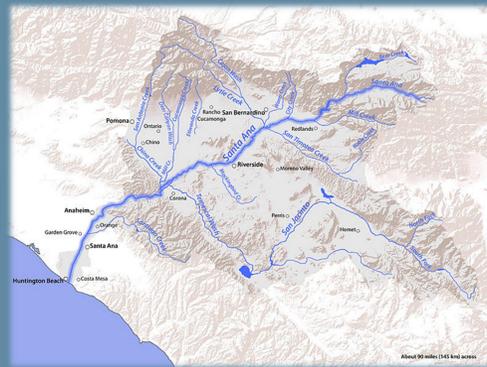


Figure 2: Santa Ana River Watershed with all drainages identified (taken from USGS).

METHODS

COLLECTION

In collaboration with the United States Forest Service and California Department of Fish and Wildlife, *R. osculus* samples were obtained from various sites within the Santa Ana watershed including Plunge Creek, City Creek, Twin/Strawberry Creek, Cajon Creek, Lytle Creek and Indian Creek wit. *R. osculus* samples were also acquired for the San Gabriel Watershed, Central Coast, Owens River and Colorado River populations using proper electroshocking technique under accordance with permits issued to the Metcalf Lab by the United States Forest Service. For each specimen that has been collected by the Metcalf Lab, GPS coordinates were taken from the sampling location on the designated tributary.

MOLECULAR METHODS

Each *R. osculus* sample underwent DNA extraction using published extraction protocols. DNA extractions were then visualized using agarose gel electrophoresis. Each sample underwent amplification using PCR and the corresponding LI-COR labeled primer for mtDNA's cytochrome-b and d-loop. PCR amplicons were then sequenced and analyzed on a LICOR model 4300 automated DNA analyzer.

EFFECTS OF FIRE AND FLOOD

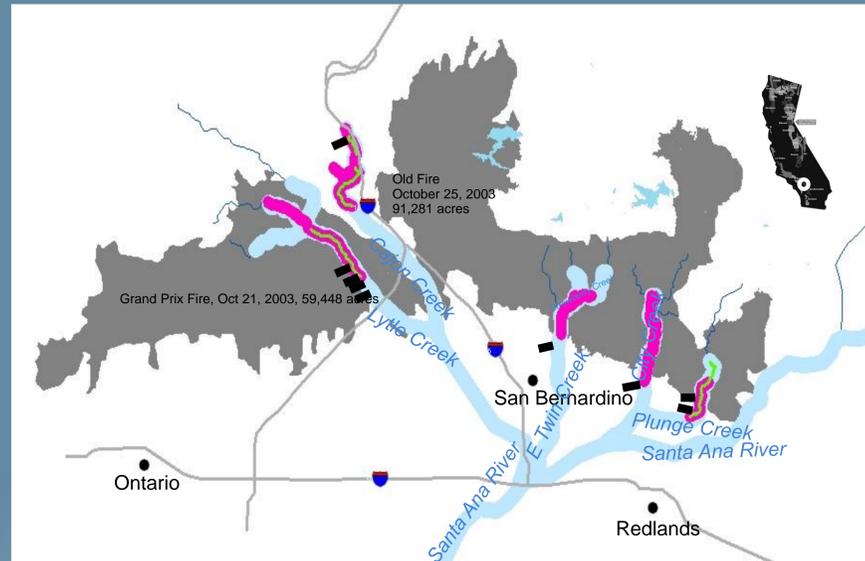


Figure 3: Santa Ana Speckled Dace in the Fire Affected Areas*:

- Current (post-fire and flood)
- Recent (pre-fire)
- Historic
- Fish Passage Barrier

In October 2003, two major fires affected the five northern most tributaries of the Santa Ana River Watershed system. Following these fires were times of heavy pluvial rains which resulted in flooding within these tributaries. Prior to the fires and flood of 2003 and 2004, the Santa Ana Speckled Dace had reported populations in Lytle Creek, Cajon Creek, Twin/Strawberry Creek, City Creek and Plunge Creek. Following the events of 2003-2004, the populations within Twin/Strawberry and City Creek were extirpated (Figure 3). Upon phylogenetic analysis of mtDNA's d-loop marker (Table 1, Figure 4), the greatest amount of genetic variation as well as unique haplotypes occurred within these extirpated populations.

RESULTS

Table 1: D-loop haplotypes within and among drainages of the Santa Ana Watershed. Fifteen haplotypes were observed from 46 individuals.

Drainage	Sample size sequenced for d-loop as of today	Number of haplotypes (A-O)	Number of haplotypes unique to the drainage	Pre-2002 Dace	2002-2003 Dace	Post Fire and Flood Dace Persistence 2003-2007	Loss of Unique Haplotypes
Lytle	N=10	C(9), N(1)	N(1)	Y	Y	Y	?
Cajon	N=12	A(1),C(7), F(3), O(1)	O(1)	Y	Y	Y	?
E Twin/ Strawberry	N=12	C(1),G(3),H(3), I(1),J(1),K(1), L(1),M(1)	I,J,K,L,M(1)	Y	Y	N	Y
City	N=4	B(1),D(1),E(1),F(1)	D,E(1)	Y	Y	N	Y
Plunge	N = 8	A(4),B(2),C(2)	None	Y	Y	Y	?

RESULTS

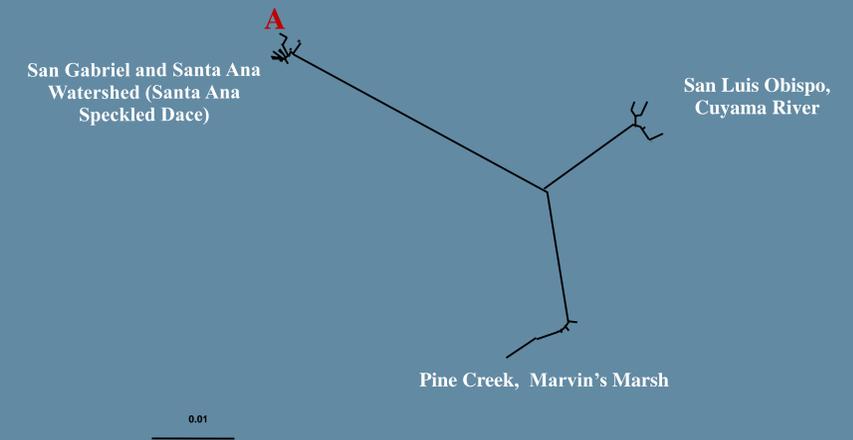


Figure 4: Phylogenetic relationships among Speckled Dace in Southern California. The fifteen haplotypes described in Table 1 are all found within location "A."

- The genetic distance between Santa Ana Speckled Dace and the above drainages is approximately 6-7%, whereas the genetic distances within regions ranges from 0.0%- 0.3%
- Each of the three geographic locations are reciprocally monophyletic for mitochondrial d-loop and cyt-b sequence suggesting independent evolutionary trajectories.
- Therefore, the Santa Ana Speckled Dace is a good indicator species for the long term conservation and maintenance of the Santa Ana Watershed.

CURRENT STUDY QUESTIONS

Two issues that are proposed by the mtDNA work are:

- The taxonomic relationship of the Santa Ana Speckled Dace to other Speckled Dace in the Central Coast and Owens River Drainages.
 - To further address this question nuclear intron sequences will be analyzed.
- The relationship among Santa Ana Speckled Dace populations within the Santa Ana Watersheds is critical for within watershed management strategies.
 - Microsatellites have been characterized and identified for *R.osculus* which will allow us to examine population structure and other population genetic parameters.
- Entering all genetic data into GIS for landscape level analyses of the Santa Ana Watershed.

REFERENCES

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