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 8,900 km² in parts of three California counties including San Bernardino, Riverside, and Los Angeles (SAVA 2009; SAWPA 2004). (Figure 2) Watersheds, as a collective drainage system for multiple small tributaries, provide a large part of the water resources to heavily populated areas. The U.S. Environmental Protection Agency lists 153 watersheds 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 watersheds. To this end, studying the evolution of species within these watersheds is necessary. One such species is the small native minnow Rhinichthys osculus, the speckled dace. The Santa Ana Speckled Dace is a small, cyprinid fish approximately 80mm (Figure 1) to length and inhabits environments containing shallow cobbles, 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 fleeing 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 divides (SAWPA 2004). The populations of the Santa Ana Speckled Dace are highly affected 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. Thus 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).

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 Pinne Brook Creek, City Creek, TwinStrawberry Creek, Cajon Creek, Lytle Creek, and San Gabriel Watershed. R. osculus samples were also acquired for the San Gabriel Watershed, Central Coast, Oregon 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 by agarose gel electrophoresis. Each sample underwent amplification using PCR and the corresponding L1-COR labeled primer for mtDNA cytochrome-b and 16S rRNA. PCR amplicons were then sequenced and analyzed on a LI-COR model 4300 automated DNA analyzer.

RESULTS

In October 2003, two major fires affected the five northernmost tributaries of the Santa Ana River Watershed. Following these fires were times of heavy pluvial rains which resulted in flooding within these tributaries. Prior to the fires and floods of 2003 and 2004, the Santa Ana Speckled Dace had reported populations in Lytle Creek, Cajon Creek, TwinStrawberry Creek, City Creek and Pinne Brook. Following the events of 2003-2004, the populations within TwinStrawberry and City Creek were extirpated (Figure 3). Upon phylogenetic analysis of mtDNA D-loop marker (Table 1, Figure 4), the greatest amount of genetic variation as well as unique haplotypes occurred within these extirpated populations.

REFERENCES


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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).

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

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

1. The genetic distance between Santa Ana Speckled Dace and the above drainages is approximately 6-7%, whereas the genetic distances within regions ranges from 0.4% - 0.3%

2. Each of the three geographic locations are reciprocally monophyletic for mitochondrial d-loop and cyt-b sequence suggesting independent evolutionary trajectories.

3. Therefore, the Santa Ana Speckled Dace is a good indicator species for the long term conservation and maintenance of the Santa Ana Watershed.

1. The taxonomic relationship of the Santa Ana Speckled Dace to other Speckled Dace in the Central Coast and Oregon River Drainages.
   • To further address this question nuclear intron sequences will be analyzed.

2. The relationship among Santa Ana Speckled Dace populations within the Santa Ana Watershed is important for current 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.

3. Entering all genetic data into GIS for landscape level analyses of the Santa Ana Watershed.

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

<table>
<thead>
<tr>
<th>Drainage</th>
<th>Sample Size sequenced for d-loop amplifying</th>
<th>Number of haplotypes (b-d)</th>
<th>Number of haplotypes unique to the drainage</th>
<th>Pre-Fire</th>
<th>Post-Fire Flood</th>
<th>Post-Fire Flood Persistence 2007</th>
<th>Loss of native haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lytle</td>
<td>111</td>
<td>35</td>
<td>8</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Cajon</td>
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<td>7</td>
<td>0</td>
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<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>E Twinkle Creek</td>
<td>21</td>
<td>5</td>
<td>1</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Strawberry</td>
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<td>4</td>
<td>0</td>
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<tr>
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<tr>
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<td>0</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Two issues that are proposed by the mtDNA work are:

1. The taxonomic relationship of the Santa Ana Speckled Dace to other Speckled Dace in the Central Coast and Oregon River Drainages.
   • To further address this question nuclear intron sequences will be analyzed.

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