Origins of the invasive red swamp crayfish (*Procambarus clarkii*) in the Santa Monica Mountains

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Abstract

Although native to the southeastern United States, the red swamp crayfish (*Procambarus clarkii*) has become established worldwide through accidental and intentional actions by humans. In the Santa Monica Mountains of southern California, the presence of the omnivorous crayfish is associated with the absence or reduced abundance of native amphibians. The original source of *P. clarkii* in southern California is unknown; however genetic analysis can be used to determine sources of invasion. We sequenced 16S rRNA subunit and cytochrome oxidase I (COI) mitochondrial genes to trace the origins of *P. clarkii* in the Santa Monica Mountains. The resulting haplotype network of the combined COI and 16S rRNA subunit genes showed 19 distinct haplotypes and suggested multiple introductions of crayfish to the Santa Monica Mountains from possible source locations in Texas, Florida and Louisiana. Identifying original sources and mechanisms of introduction can slow and prevent further expansion of *P. clarkii*.

Key words: biological invasion, genetic, invasive species, source population

Introduction

Invasive species can threaten native biodiversity and damage commercially-important natural resources (Pimentel et al. 2000). Identification of introduction pathways and source populations of invasive species can facilitate the development of techniques to control existing invasions and prevent further introductions. Historical records often provide insufficient documentation of the arrival and expansion of non-native species. However, molecular genetic analysis can provide accurate identification of source populations, determination of number of introduction events and estimation of genetic diversity in introduced populations (Miura 2007). Genetic variation can be used to reconstruct phylogenies, retrace possible invasion pathways and estimate the arrival and frequency of introductions (Crandall and Fitzpatrick 1996; Mathews et al. 2008; Hanfling et al. 2002). Mitochondrial genes can be particularly useful in tracing ancestral relationships among individuals because these genes are passed down maternally and tend to have a higher mutation rate than nuclear DNA (Pfluegl 2009). Previously, the origins of an invasive barnacle, *Chthamalus proteus*, in the Pacific were determined by analysis of the cytochrome oxidase I mitochondrial gene (Zardus and Hadfield 2005).

Although native to the southeastern United States, the red swamp crayfish, *Procambarus clarkii*, has successfully colonized most of the United States (ISSG 2011) and other regions, including Mexico, Europe, Africa and Asia (Barbaresi et al. 2003; Barbaresi and Gherardi 2000; Hernandez et al. 2008; Holdich 1999; Zhu et al. 2013). The spread of *P. clarkii* across the United States is most likely due to mass aquaculture and subsequent transport in the southeastern United States, where the crayfish are served as a popular specialty dish (Hernandez et al. 2008). Mechanisms of dispersion include the release or escape of crayfish used as fishing bait,
kept as domestic pets or used for educational purposes. Tolerance of a wide range of abiotic water conditions, such as low dissolved oxygen concentrations, high salinity and high acidity, facilitate the rapid colonization of *P. clarkii* in freshwater habitat (Barbaresi and Gherardi 2000; Hernandez et al. 2008). Once established, red swamp crayfish can alter ecological processes in invaded freshwater habitats, threatening native species both directly and indirectly. *Procambarus clarkii* feed on a variety of plants, animals and organic detritus (Gutierrez-Yurrita et al. 1998). The red swamp crayfish has been implicated in the decline of amphibians through predation (Gamradt and Kats 1996; Cruz et al. 2006) and recent surveys in the Santa Monica Mountains have shown reduced egg mass and larval density of Pacific treefrogs (*Pseudacris regilla*) in streams where crayfish are present (Riley et al. 2005). *Procambarus clarkii* was first documented in southern California in 1924 (Holmes 1924). However, little else is known about the origins of *P. clarkii* in the Santa Monica Mountains.

Our objectives are to survey genetic variation and population structure of *P. clarkii* in the Santa Monica Mountains, identify source populations and determine whether multiple introductions have occurred in the area. We sampled from streams in the Santa Monica Mountains where *P. clarkii* are known to exist (Kerby et al. 2005; Riley et al. 2005) as well as sites in the native range. To identify possible local source populations, we also sampled botanical gardens, pet shops and grocery stores outside of the Santa Monica Mountains. High genetic variation in the mitochondrial genes of introduced populations would indicate multiple introductions to the region while low variance would suggest that a single founder event was more likely. Related haplotypes in the non-native and native regions would indicate a likely source population. By identifying source populations of *P. clarkii* in the Santa Monica Mountains, possible introduction pathways can be reconstructed and used to prevent additional expansion and slow present invasions.

**Materials and methods**

*Collection and processing of specimens*

From 2007 to 2011, a total of 113 samples were collected (Table 1, Figure 1). Fifty-six live crayfish were collected from ten streams in the Santa Monica Mountains. We obtained twenty crayfish from possible, local source populations including several streams, botanical gardens, pet shops and grocery stores surrounding the Santa Monica Mountains. A total of 37 samples were obtained from native regions in Louisiana, Florida, New Mexico, Texas and Arkansas.

Permits for collection were granted by the California Department of Fish and Game and crayfish were handled for in accordance to the protocol of the Chancellor’s Animal Research Committee (ARC) of the University of California, Los Angeles.

**DNA isolation and PCR methods**

DNA was extracted from tissue samples using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s protocol. Polymerase chain reaction (PCR) was performed to amplify fragments of two mitochondrial genes, cytochrome oxidase I (COI) and 16S rRNA ribosomal subunit. The primer pair LCO 1490 and HCO 2198 was used to amplify a 627 base pair fragment of the COI mtDNA gene (Wiklund et al. 2005). The primer pair 1471 and 1472 was used to amplify a 506 base pair fragment of the 16S rRNA mtDNA gene (Crandall and Fitzpatrick 1996). PCR amplification was performed at annealing temperatures of 46.5°C and 49.8°C for COI and 16S, respectively. PCR products were purified with EXOSAP (Usb Corporation), sequencing reactions were performed with ABI Big Dye 3.1 and products were sequenced at MCLAB (Molecular Cloning Laboratory) and Cornell University Life Sciences Core Laboratories Center.

**Data analysis**

Due to low heterogeneity, 16S and COI sequences were combined and analyzed together. Concatenation may improve phylogenetic resolution if sequence variation is minimal (Huelsenbeck et al. 1996). Combined sequence fragments of COI and 16S (both forward and reverse sequences) were edited, trimmed and aligned with Geneious version 4.6 (Drummond et al. 2009) to a uniform length of 622 base pairs including gaps (Table 2S). COI pseudogenes are commonly found in crayfish (Song 2008), therefore to identify possible pseudo-genes, the COI gene fragments were translated and examined for stop codons and, if found, were removed from the data set.

TCS1.21 (Clement M 2000) was used to identify the number of unique haplotypes and generate a haplotype network, with gaps in sequences treated as a 5th state during analysis. DNASP version 5.0
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Figure 1. Sampling locations of crayfish in the Santa Monica Mountains. Crayfish samples were collected from ten locations shown. GPS locations of sampled areas are marked with red dots.

(Librado 2009) was used to estimate mtDNA haplotype diversity (κ) and nucleotide diversity (π) within populations. Arlequin version 3.11 (Excoffier 2005) was used to calculate pairwise FST measures and variance measures to compare genetic diversity across geographical ranges. Three geographic groupings were used to analyze population structure with AMOVA: (i) native and non-native, (ii) Texas, Florida, Louisiana and Santa Monica Mountains, and (iii) Texas, Florida and Louisiana (Table 2).

Results

Haplotype network

A total of 19 unique haplotypes were identified for 113 samples of P. clarkii from introduced and native regions. A total of thirteen haplotypes were present among samples from native regions and a total of ten haplotypes were present among samples from the Santa Monica Mountains (Figure 2). Multiple haplotypes exist in most streams in the Santa Monica Mountains, however, only one haplotype was present in Malibu Creek, Topanga Canyon, Trancas Canyon, Triunfo Canyon, Descanso Gardens, UCLA Mathias Botanical Gardens and Chicarita Creek in San Diego, CA. Four haplotypes unique to the Santa Monica Mountains were identified in Medea Creek, Bell Canyon and Las Virgenes Creek. A single, dominant haplotype was shared by 49.4% of crayfish collected from the Santa Monica Mountains and surrounding areas. No native samples exhibited this haplotype.

Haplotype network analysis revealed shared haplotypes between introduced populations in the Santa Monica Mountains and native populations from Texas, Florida and Louisiana. Crayfish sampled from a local pet shop and local supermarket also shared haplotypes with Santa Monica Mountain crayfish, however, the original sources of these samples are unknown.

Sequence variability

High haplotype diversity and nucleotide diversity were found in both the Santa Monica Mountains and native ranges. Overall sequence variability was lower in introduced regions than in native regions (Table 1). High haplotype and nucleotide diversity were also observed within individual locations, such as Medea Creek, Baton Rouge and New Orleans. However, several populations in
Table 1. Haplotype (h) and nucleotide diversity (Π) for COI-16S sequences of P. clarkii.

<table>
<thead>
<tr>
<th>Sample Area</th>
<th>N sequenced</th>
<th>Total unique haplotypes</th>
<th>Haplotype diversity (h)</th>
<th>Nucleotide diversity (Π)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduced (Santa Monica Mountains)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medea Creek</td>
<td>6</td>
<td>3</td>
<td>0.733</td>
<td>0.003</td>
</tr>
<tr>
<td>Las Virgenes Creek</td>
<td>8</td>
<td>2</td>
<td>0.250</td>
<td>0.001</td>
</tr>
<tr>
<td>Malibu Creek</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Lindero Canyon</td>
<td>3</td>
<td>2</td>
<td>0.667</td>
<td>0.002</td>
</tr>
<tr>
<td>Topanga Canyon</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Erbes</td>
<td>6</td>
<td>2</td>
<td>0.333</td>
<td>0.002</td>
</tr>
<tr>
<td>Trancas Canyon</td>
<td>5</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Bell Canyon</td>
<td>6</td>
<td>2</td>
<td>0.533</td>
<td>0.002</td>
</tr>
<tr>
<td>Conejo</td>
<td>7</td>
<td>2</td>
<td>0.476</td>
<td>0.002</td>
</tr>
<tr>
<td>Triunfo Canyon</td>
<td>11</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Possible Local Sources</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unnamed pet shop (Venice, CA)</td>
<td>2</td>
<td>2</td>
<td>1.000</td>
<td>0.005</td>
</tr>
<tr>
<td>Unnamed supermarket (San Gabriel, CA)</td>
<td>3</td>
<td>2</td>
<td>0.667</td>
<td>0.001</td>
</tr>
<tr>
<td>Descanso Botanical Gardens</td>
<td>7</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>UCLA Botanical Gardens</td>
<td>4</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Chicarita Creek (San Diego)</td>
<td>4</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Native</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas Totals</td>
<td>19</td>
<td>6</td>
<td>0.579</td>
<td>0.002</td>
</tr>
<tr>
<td>Comal, TX</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fort Bend, TX</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Uvalde, TX</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Kendall, TX</td>
<td>8</td>
<td>3</td>
<td>0.464</td>
<td>0.001</td>
</tr>
<tr>
<td>Travis, TX</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Bell, TX</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McIennan, TX</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Williamson, TX</td>
<td>2</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Black River, NM</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cache River, AR</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escambia, FL</td>
<td>5</td>
<td>2</td>
<td>0.600</td>
<td>0.001</td>
</tr>
<tr>
<td>Louisiana Totals</td>
<td>11</td>
<td>5</td>
<td>0.764</td>
<td>0.004</td>
</tr>
<tr>
<td>Baton Rouge, LA</td>
<td>4</td>
<td>4</td>
<td>0.833</td>
<td>0.004</td>
</tr>
<tr>
<td>New Orleans, LA</td>
<td>7</td>
<td>4</td>
<td>0.810</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>113</td>
<td>19</td>
<td>0.854</td>
<td>0.013</td>
</tr>
</tbody>
</table>

introduced regions (Trancas Canyon, Triunfo Canyon and Descanso Gardens) showed no haplotype and nucleotide diversity (Table 1).

Crayfish obtained from the local pet shop and supermarket, which shared haplotypes with Santa Monica Mountain crayfish, showed relatively high haplotype diversity compared to other potential source populations.

Population genetic structure

There was significant genetic differentiation at all levels examined (among groups, among locations within groups and within locations) in the AMOVA analysis (Table 2). When partitioning samples as “Native and Non-native” and “Texas, Florida, Louisiana, and Santa Monica Mountains,” significant population structure was present at both the group level and locality level (Table 2). However, when native populations were analyzed separately, population structure was only evident at the group level.

Pairwise Fst values between native and non-native populations were generally high and statistically significant (Table 1S). The Texas population generally showed large, significant differentiation from non-native populations, and is most genetically divergent from the Triunfo Canyon, Topanga Canyon and Descanso Gardens populations. There was minimal differentiation, as seen in pairwise Fst values (Table 1S), between the native Louisiana population and the introduced Bell Canyon, Lindero Canyon, UCLA Botanical Garden and San Diego populations.

Pairwise comparisons revealed significant genetic divergence between stream populations in the
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Table 2. AMOVA test results comparing COI-16S sequence variation of *P. clarkii*.

<table>
<thead>
<tr>
<th>Geographic groupings</th>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>% of variation</th>
<th>F&lt;sub&gt;ST&lt;/sub&gt;</th>
<th>F&lt;sub&gt;SC&lt;/sub&gt;</th>
<th>F&lt;sub&gt;CT&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texas vs. Florida vs. Louisiana vs. Santa Monica Mountains</td>
<td>Among groups</td>
<td>3</td>
<td>47.704</td>
<td>0.54086</td>
<td>28.53</td>
<td>0.285**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Among populations w/in groups</td>
<td>15</td>
<td>54.713</td>
<td>0.56764</td>
<td>29.94</td>
<td>0.419**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>93</td>
<td>73.208</td>
<td>0.78719</td>
<td>41.53</td>
<td>0.585*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Among groups</td>
<td>1</td>
<td>23.374</td>
<td>0.29676</td>
<td>16.21</td>
<td>0.162*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native vs. Nonnative</td>
<td>Among groups</td>
<td>19</td>
<td>86.102</td>
<td>0.74693</td>
<td>40.80</td>
<td>0.487**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>93</td>
<td>73.208</td>
<td>0.78719</td>
<td>43.00</td>
<td>0.570**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Among groups</td>
<td>2</td>
<td>27.244</td>
<td>1.39720</td>
<td>52.69</td>
<td>0.527</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = P<0.05; ** = P<0.001; statistical probabilities derived from 1023 permutations.

Figure 2. Mitochondrial haplotype network created by analysis of combined cytochrome oxidase I and 16S rRNA subunit sequences (622 bp). Area of circle is proportional to the number of sampled individuals present in each haplotype. Each connecting line represents a single base change in the sequence.
Santa Monica Mountains (Table 1S). Nonsignificant pairwise $F_{st}$ values between samples collected from the local pet shop and other populations were most likely due to inadequate sample size. In general, these results from population analysis were consistent with the haplotype network analysis.

Discussion

Our analysis of *P. clarkii* in the Santa Monica Mountains revealed no single, clear source population. Instead, our results suggest multiple introductions of the invasive crayfish from various populations in the native range, including Texas, Florida and Louisiana. Native regions showed high haplotype diversity. Variability in native regions combined with successful dispersal ability and rapid reproduction rates, are characteristic of successful invasive species (Duda 1994).

Despite exhibiting less genetic variability than native populations, Santa Monica Mountain crayfish also showed surprisingly high levels of diversity. Non-native populations are expected to be less diverse than native regions due to genetic bottlenecks and founder effects (Wong et al. 2011). High levels of genetic diversity found in introduced populations can be attributed to either a single introduction event involving a large number of effective founders or multiple, independent introductions (Kolbe et al. 2004). The presence of multiple haplotypes within streams in the Santa Monica Mountains supports the multiple introduction hypothesis. Multiple introductions from native regions explain the high levels of genetic diversity of crayfish in the Santa Monica Mountains and reduced bottleneck and founder effects (Rius et al. 2008). Shared haplotypes between native samples from Texas, Florida and Louisiana and samples collected from the Santa Monica Mountains indicate that these regions may be possible source populations.

The presence of a dominant haplotype (Figure 2) shared among 49.4% of crayfish collected from the Santa Monica Mountains and surrounding areas may indicate a primary introductory population (Ashton et al. 2008). However, none of our native samples exhibited this haplotype, with the exception of one individual from New Mexico. Due to the high haplotype diversity present in the native range and the low number of sites sampled in native regions, it is likely that this haplotype is present in native populations but was not sampled in this study. Samples from Louisiana exhibited haplotypes most closely related to the dominant haplotype, suggesting a possible source population or related source population. Populations that exhibited a single haplotype that was not the dominant haplotype, such as Trancas Canyon, Topanga Canyon, UCLA Mildred E. Mathis Botanical Gardens and Chicarita Creek (San Diego, CA), may represent relatively new populations established by a recent, single introduction.

Although haplotype network analysis indicated four unique haplotypes present in the Santa Monica Mountains, the introduction of crayfish to this region is too recent to have diverged into a novel haplotype. The average mutation rate of the cytochrome oxidase I gene in decapod crustaceans is about 1.4–2.6% per million years (Projecto-Garcia et al. 2010). It is more likely that these unique haplotypes were not sampled from the native range.

We sampled from pet shops, grocery stores and streams surrounding the Santa Monica Mountains to evaluate possible, local sources of introduction. Although the original sources for these crayfish are unknown, samples obtained from the local pet shop and supermarket exhibited relatively high genetic diversity, indicating a diverse, original source population or the presence of crayfish from multiple sources at these locations. Although the high level of genetic variation present in these locations may be attributable to a low sample size, our results still hold important implications for the invasion of *P. clarkii* to the Santa Monica Mountains. Should these crayfish be introduced to the surrounding areas, high levels of genetic diversity will allow for rapid adaptation, increasing the potential for detrimental colonization of the invaded ecosystem. Haplotype network analysis showed shared haplotypes between crayfish collected from the local supermarket and Erbes Creek, indicating that crayfish from these sources may already have been introduced to the Santa Monica Mountains.

The local pet shop is a particularly interesting sample site. It has been located in Venice, CA for about 40 years and sells at least two different species of crayfish (*Procambarus clarkii* and *Orconectes virilis*) as domestic pets. *Orconectes virilis* has not been documented in the Santa Monica Mountains (Benson 2011). It is unclear when the pet shop began supplying live crayfish. Employees have informed us that these crayfish are sold to the pet shop by two supply groups: private suppliers and an aquatic, reptile, pond supply company located in Ontario, California. The two samples of *P. clarkii* obtained from the
pet shop belonged to different haplotypes. The presence of haplotype diversity in a pet shop near the Santa Monica Mountains can potentially hinder conservation efforts attempting to restrict the spread of invasive crayfish. Although it is possible that private suppliers may be trapping crayfish in the Santa Monica Mountains and selling them to the pet shop privately, these crayfish could be easily reintroduced to the Santa Monica Mountains or introduced to streams that were not previously colonized by crayfish. These crayfish suppliers may act as initial source populations for *Orconectes virilis* invasions in the future.

The initial introduction of species can occur in the three ways: naturally through active dispersal, accidentally by escape or deliberately by humans (Barbaresi et al. 2003). The spread of crayfish throughout the United States and other countries is most likely due to anthropologically mediated dispersal, including the release of recreational fishing bait, the escape of live aquaculture and the release of pet crayfish (Barbaresi et al. 2007). Given the wide range of possible introduction pathways to non-native regions, multiple introductions of *P. clarkii* are very likely.

Suggested source populations from Texas, Florida and Louisiana indicated by this study are consistent with hypotheses suggesting that the initial dispersal vector of crayfish in the Santa Monica Mountains was aquaculture, as *P. clarkii* are farmed in these regions. Because initial introductions may be to be due to aquaculture, legislation regarding aquaculture should be improved. The U.S. Environmental Protection Agency’s (EPA) effluent limitations guidelines (ELGs) for concentrated aquatic animal production (CAAP) lack adequate criteria for the housing and enclosure of farmed animals (Johnson et al. 2004). Guidelines for appropriate enclosures focus on maintaining acceptable water conditions with respect to human health, rather than enclosures designed to prevent the escape of farmed animals. Although the negative effects of invasive species on natural ecosystems are detailed and acknowledged in the document, the EPA assigns the regulation of aquaculture to individual states. However, as this study exemplifies, the aquaculture trade is often a cross-state issue and therefore jurisdiction over regulation is difficult to standardize. Farming of crayfish is particularly challenging because the harvesting of crayfish is a fundamental source of economic livelihood in some parts of the United States. Of the annual $1 billion United States aquaculture production income, *P. clarkii* is the only crustacean that is harvested on a large-scale basis (USDA 1995).

Other potential sources of introduced crayfish are the intentional release and unintentional escape of pets and live bait. Release of domestic crayfish kept as pets complicates the undocumented history of crayfish introduction to the Santa Monica Mountain region. Many classrooms utilize crayfish as educational tools and release of these crayfish can exacerbate existing invasions (Mead 2008). Crayfish are also used to bait several species of freshwater fish and can easily escape and colonize new habitats. Several bait shops bordering the Santa Monica Mountains have sold live crayfish as bait in the past but had since discontinued stocking crayfish during our sampling period. It is difficult to monitor and regulate the release of crayfish used for teaching or recreational fishing purposes. However, small and simple steps, such as increased public awareness and education about the effects of invasions on natural biodiversity, can help prevent and slow introductions due to bait bucket release in the future.

Other methods of preventing the expansion of crayfish include the installation of barriers and trapping. Studies in the Santa Monica Mountains have shown that large, natural barriers, such as waterfalls, can restrict the movement of *P. clarkii* both upstream and downstream (Kerby et al. 2005). However, these barriers must be relatively large and steep to be effective and the installation of unnatural barriers would hinder the natural flow of streams, which may result in even greater ecological damage. Further development of adequate barriers, as well as economic and environmental cost-benefit analyses of barriers, must be investigated before this method can be utilized effectively.

Successful control and removal of invasive crayfish usually requires a combination of approaches, such as physical removal, biological control and biocidal methods (Gherardi et al. 2011). Although tedious, trapping significantly reduces the number of crayfish in streams and may be sufficient enough to eliminate the detrimental impact invasive crayfish have on native amphibians (Kerby et al. 2005). Previous studies have also shown that coexistence between crayfish and native organisms is possible provided that crayfish population density is reduced and controlled (Kats et al. 2006). Effective trapping is time-consuming and can be expensive, as it requires adequate enclosures and storage facilities for removed crayfish (Barbaresi and Gherardi 2008).
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However, previous efforts have shown dramatic rebounds of native flora and fauna following the removal of crayfish from invaded areas. Trancas Creek in the Santa Monica Mountains, which contained an abundance of crayfish and no California newts, experienced a return of newt egg masses, larvae and reproducing adults in the spring of 1995 following heavy winter rains that washed crayfish out of the creek (Gamradt and Kats 1996).

In the future, we plan to increase our sample size of red swamp crayfish from native ranges and potential source populations surrounding the Santa Monica Mountains. With these additional samples, we will potentially be able to more accurately identify the source populations of P. clarkii in the Santa Monica Mountains. Nonetheless, our results clearly rule out a limited historic introduction and suggest an ongoing process of introduction and population expansion that threatens native amphibian populations.

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Origins of the invasive red swamp crayfish


Supplementary material

The following supplementary material is available for this article:

Table 1S. Pairwise Fst values for COI-16S sequences of P. clarkii. Dark grey indicates native to native comparisons, white indicates non-native to non-native comparisons, and light grey indicates native to non-native comparisons.

Table 2S. GenBank accession numbers.

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