

Discerning invasion history in an ephemerally connected system: landscape genetics of *Procambarus clarkii* in Ash Meadows, Nevada

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Abstract The delimitation of dispersal routes used by individuals moving between populations has the potential to direct management efforts aimed at limiting the spread of invasive species. Red swamp crayfish (*Procambarus clarkii*) have successfully invaded aquatic ecosystems across much of the globe, causing loss of biodiversity and habitat destruction. Our research focuses on a Mojave Desert spring system in Ash Meadows National Wildlife Refuge, where a combination of anthropogenic habitat degradation and the establishment of invasive species have caused extinctions, extirpations, and severe population declines of endangered fishes. To infer the invasion history of hydrologically isolated springs, we tested alternative hypotheses of *P. clarkii* dispersal routes and colonization events throughout Ash Meadows using a landscape genetics approach that combined analysis of microsatellite and mitochondrial DNA genotypes with geographic information system (GIS) mapping. Modeled historic outflows, in conjunction with waterway mapping based on aerial imagery and LiDAR data, show variable drainage routes across the

flat topography of Ash Meadows. Estimates of gene flow between *P. clarkii* populations revealed the drainages utilized by crayfish to move from central to peripheral springs. Additionally, analyses of mtDNA haplotype diversity and distribution suggest isolated springs were colonized by few individuals, and subsequent emigration has been rare. These results will inform ecological restoration in Ash Meadows by directing the placement of barriers to prevent reinvasion of distal springs after eradication of *P. clarkii* populations. Finally, this research provides a case study for elucidating the functional connectivity of ephemerally connected landscapes, using the integration of genetic and GIS techniques.

Keywords Landscape genetics · Invasive crayfish · *Procambarus clarkii* · Microsatellites · Mitochondrial DNA · Ephemeral landscape connectivity

Introduction

Invasive species are a major driver of global change, causing loss of biodiversity (Vitousek et al. 1996), altered community assemblages (Sanders et al. 2003), and subsequent degradation to ecosystem functions and services (Pejchar and Mooney 2009). The ecological and economic damage resulting from biological invasions provides a compelling stimulus for research that informs restoration or mitigation of

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invaded systems, as well as the prevention of invasions.

A recently developed field of study that is well suited for ascertaining the history of a biological invasion is landscape genetics [reviewed by Estoup and Guillemaud (2010)]. Landscape genetics employs population genetic data in conjunction with landscape connectivity metrics to infer ecological processes such as dispersal and colonization. It allows inference of geographical locations of source populations, introduction points, and dispersal corridors, as well as the frequency and magnitude of colonization events, reintroductions, and immigration (e.g. Marrs et al. 2008; Rollins et al. 2009; Estoup et al. 2010), thus lending insight into the management of biological invasions.

We used a landscape genetics approach to study the invasion of a desert spring system in Ash Meadows National Wildlife Refuge (AMNWR), Nevada, by red swamp crayfish (*Procambarus clarkii*, Girard 1852). *P. clarkii* threatens the persistence of native and endemic biota directly through predation, and indirectly by competing for food resources (Miller 1948; Soltz and Naiman 1978; Kennedy et al. 2005). The native range of *P. clarkii* extends from the Gulf Coast of northern Mexico throughout the southeastern United States (Hobbs 1972), but this species has been introduced across the globe primarily as an aquaculture product (Huner 1977; Huner and Avault 1979). The life history traits of *P. clarkii* that make it commercially favorable have also facilitated its success as an invasive species: tolerance of a wide range of environmental conditions (Huner and Lindqvist 1995; Payne 1997), short time to reproductive maturity (Paglianti and Gherardi 2004), high fecundity (Reynolds 2002), and generalist and opportunistic feeding (Gherardi et al. 1999). Additional characteristics that enhance the ability of *P. clarkii* to colonize and invade new habitats include life cycle plasticity (Gutierrez-Yurrita and Montes 1999), high dispersal ability (Gherardi and Barbaresi 2000), and aggressive behavior (Figler et al. 2005). The ecological impacts of this species outside its native range include increased water turbidity (Rodriguez et al. 2003), decreased macrophyte cover and biomass (Lodge and Lorman 1987; Nystrom and Strand 1996), collapsed banks from burrowing activities (Barbaresi et al. 2004), and loss of biodiversity across trophic levels (Rodriguez et al. 2005). Ecosystem engineering behaviors by invasive *P. clarkii*, such as burrowing

and macrophyte clipping, can make habitat unsuitable for many native species (Matsuzaki et al. 2009).

The goal of this research was to inform ecological restoration in AMNWR by delimiting dispersal corridors utilized by crayfish to invade beyond initial introduction points, identifying source populations, and inferring the frequency of dispersal between springs. Although there is continuous hydrologic connectivity between some springs and reservoirs at lower elevations, many of the outflows from higher-elevation springs do not have perennial connections to other spring outflows, effectively isolating the springs. However, rare flooding events during periods of exceptional precipitation extend these outflows and create ephemeral connections with other waterways, allowing aquatic organisms to disperse to the otherwise isolated springs. Although methods such as mark-recapture and radio telemetry have been used to successfully locate movement corridors used by animals, these tools are not appropriate when such movement occurs rarely and unpredictably across the landscape. Genetic variation, on the other hand, can provide a posteriori information about introduction and colonization events, dispersal, and the source of dispersing individuals. The invasion of Ash Meadows by red swamp crayfish provides a case study for the use of landscape genetics to understand invasion processes, and inform management, in a system that experiences rare pulses of ephemeral and geographically variable connectivity.

The study system

Ash Meadows National Wildlife Refuge comprises over 30 springs and seeps, and associated riparian and wetland habitats that extend across 23,000 acres of the arid Mojave Desert landscape, in southwestern Nevada. This unique desert springs ecosystem supports at least 24 endemic plant and animal species (Stevens and Bailowitz 2008), including four fishes currently listed as federally endangered: Devil's Hole pupfish (*Cyprinodon diabolis*), Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*), Warm Springs pupfish (*Cyprinodon nevadensis pectoralis*), and Ash Meadows speckled dace (*Rhinichthys osculus nevadensis*).

The source of the Ash Meadows outflows is a large aquifer, which accumulates drainage from an area of 11,600 km². Collectively, the springs in Ash Meadows

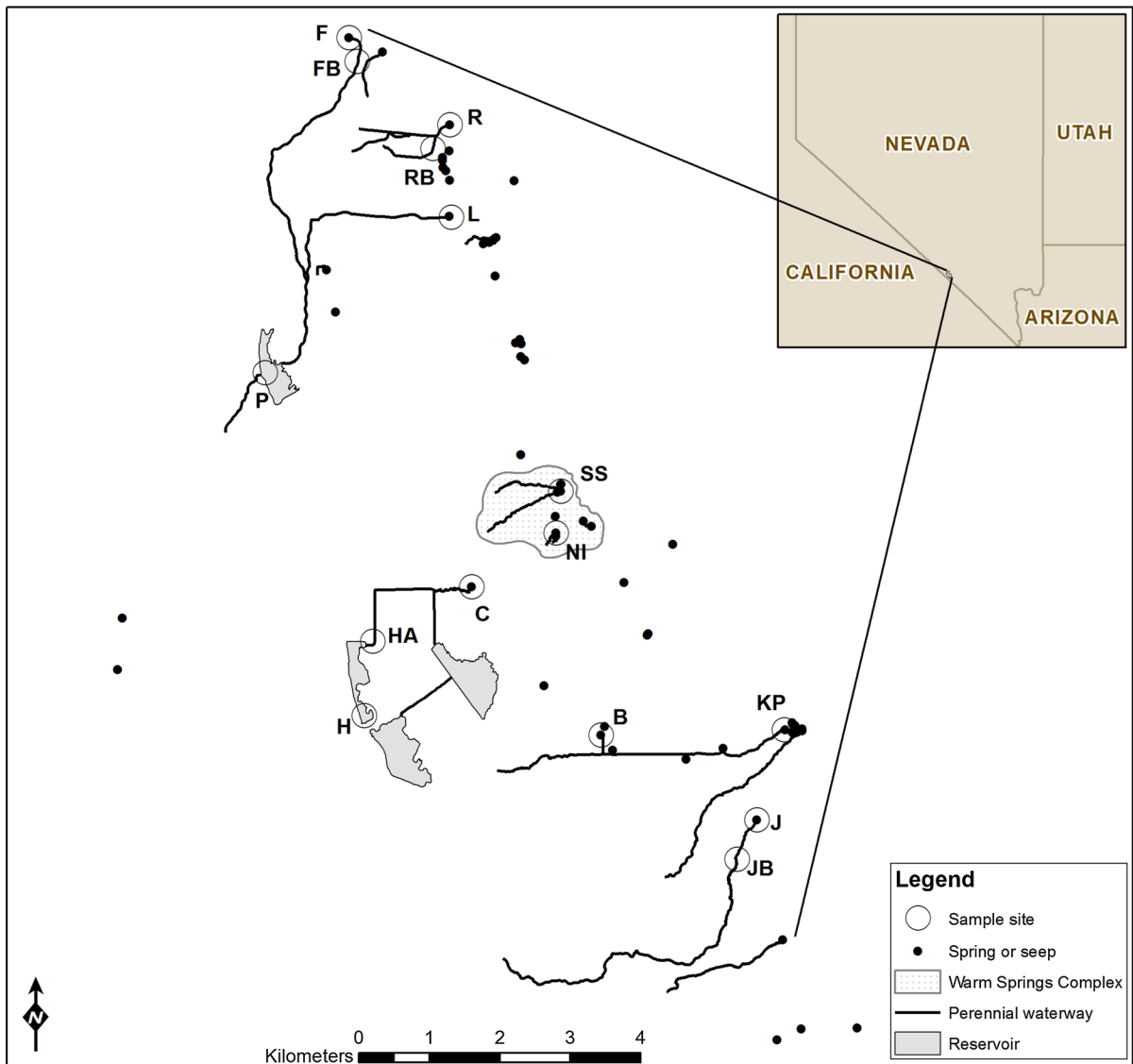


Fig. 1 Perennial waterways, springs, seeps, reservoirs, sampling locations, and the location of the Warm Springs Complex in Ash Meadows National Wildlife Refuge, Nevada. Sample site abbreviations: *B* Bradford, *C* Crystal, *F* Fairbanks, *FB* Below

Fairbanks, *H* Horseshoe, *HA* Above Horseshoe, *J* Jackrabbit, *JB* Below Jackrabbit, *KP* King's Pool, *L* Longstreet, *NI* North Indian, *P* Peterson, *R* Roger's, *RB* Below Roger's, and *SS* South Scruggs

discharge 2,700 hectare-meters of water per year, or about 40,125 l per minute (lpm; Dudley and Larson 1976). Individual springs range in discharge rates from 3 to 10,600 lpm. Although hydrologic connectivity is continual between some springs and reservoirs, many of the outflows from low-flow springs are reabsorbed below the land surface before connecting to other waterways (Fig. 1). The Ash Meadows basin drains through the marshes of the Carson Slough to the upper

Amargosa River, which flows into the southern end of Death Valley, though it is dry most of the year (Soltz and Naiman 1978). The area receives less than 7 cm of rain annually, and annual evaporation is greater than 2.5 m (Sada 1990).

Over the last century, human activities in Ash Meadows have resulted in the introduction of exotic plants and animals, and modification of many of the springs and outflow channels, with corresponding

detrimental effects on native biota (Dudley and Larson 1976; Soltz and Naiman 1978). Red swamp crayfish were first documented in AMNWR in 1942, in Crystal and Fairbanks springs, and were most likely relicts from an attempted aquaculture operation in the 1930s (Miller 1948; Deacon and Williams 1991). Subsequent reintroductions likely occurred in the following decades in the reservoirs, by fishermen who used crayfish as bait and dumped their bait buckets at the end of the day. By the 1990s, red swamp crayfish had dispersed from these initial points of introduction to many of the Ash Meadows springs and waterways.

The hydrological and ecological characteristics of AMNWR have been improved over the last three decades, as a result of dedicated restoration efforts that began when the Refuge was established in 1984. Current restoration work is focused on eradicating invasive crayfish from the low-flow springs that support the endemic Warm Springs pupfish (*C. n. pectoralis*). The entire range of *C. n. pectoralis* is restricted to six springs: School, North Indian, South Indian, Marsh, South Scruggs, and North Scruggs. Accordingly, it is essential that we infer crayfish dispersal routes from source populations to these isolated springs, in order to inform the placement of barriers to prevent reinvasion of the Warm Springs Complex from source areas where eradication is impossible.

Methods

GIS hydrology

Possible routes of aquatic connectivity for crayfish were mapped using ArcGIS 10 (ESRI, Redlands, CA, USA). Three layers of data were assembled to create a map of all possible waterways. Ash Meadows National Wildlife Refuge personnel mapped the first layer, which shows perennial waterways and major drainages in Ash Meadows. The second layer depicts historic outflows, and was modeled by Scopettone et al. (2011) from a 3-m digital elevation model (DEM) generated from light detection and ranging (LiDAR) data using ArcHydro Tools (ArcGIS 9.3). We mapped the third layer, which describes potential waterways and areas of inundation based on vegetation patterns observed on aerial images, and drainages from the LiDAR data. Vegetation patterns in desert landscapes are an excellent indicator of

waterways, as shrubs and trees tend to grow more densely along washes and in areas where inundation occurs during precipitation events. The qualitative compilation of these three layers represents all possible drainages for surface water during high precipitation or flooding events, and thus describes possible movement routes for aquatic organisms. Because the springs of Ash Meadows are fed by water seeping up through porous rock from the aquifer, subsurface dispersal of crayfish (larvae or adults) between springs was not considered a plausible hypothesis; thus, we solely addressed surface connectivity.

Sampling sites and methods

Sample sites were selected from a subset of *P. clarkii*'s distribution in Ash Meadows to facilitate the testing of alternative hypotheses of movement into the Warm Springs Complex from all possible source populations. Two hundred and seventeen individuals were sampled from springheads, outflows, and reservoirs, at fifteen locations (Fig. 1). Three of the sampled springs—Fairbanks, Jackrabbit, and Roger's—have flumes at the outflow from the spring pool. The flumes have a smooth plastic surface, and sufficiently narrow the outflow so as to create a strong current. The combination of slippery substrate and high flow likely impedes the movement of crayfish from the outflow into the spring pool (unless they travel up the bank). Because these flumes may act as barriers to upstream crayfish dispersal, we treated the crayfish above and below the flumes as distinct populations. Crayfish were captured using minnow traps baited with cat food for at least 3 h. Tissue was collected from the cheliped or cephalothorax and immediately placed in 100 % ethanol for storage until DNA extractions were performed.

DNA processing, fragment analysis, and sequencing

DNA was extracted using QIAGEN DNeasy blood and tissue kits (QIAGEN, Valencia, CA, USA). The mitochondrial DNA (mtDNA) gene region cytochrome oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR) using the forward primer 5'-TTTCG GGTG GAGT TAGG TCAA-3', and the reverse primer 5'-TACT TGCG TTCC CTGA AGAGT-3', for 192 individuals. Amplified DNA was

sequenced by Functional Biosciences (Madison, WI, USA) using the same forward and reverse primers. Consensus sequences for all individuals were trimmed and edited in Sequencher 4.0 (Gene Codes Corporation, Ann Arbor, MI, USA) using forward and reverse sequences aligned to a reference sequence published by Taylor and Knouft (2006). Amplification and fragment analysis of nine variable microsatellite markers, described by Belfiore and May (2000), were performed by the Nevada Genomics Center (Reno, NV, USA). The markers used for this study were: PclG4, PclG7, PclG15, PclG16, PclG17, PclG27, PclG28, PclG29, and PclG48. Individuals were genotyped in GeneMapper (Applied Biosystems, Foster City, CA, USA).

Phylogenetics and population genetics

Although the invasion of AMNWR by *P. clarkii* happened relatively recently, estimates of population genetic structuring were assumed to be the result of dispersal and colonization that occurred after the initial introduction of crayfish into Ash Meadows, based on our understanding of when and where individuals were first introduced. This assumption is further supported by hydrologic regimes, which sufficiently explain patterns of population genetic structuring (see “Discussion”).

The representation of mitochondrial haplotypes among individuals was determined in MacClade 4 (Maddison and Maddison 2005), and the relationships between Ash Meadows mitochondrial haplotypes and those sampled from additional native and invasive localities (Supplemental Information) were visualized in TCS 1.21 (Clement et al. 2000). Descriptive population genetic statistics—genetic diversity within populations and genetic distance between populations—were estimated in GenoDive (Meirmans and Van Tienderen 2004).

Bayesian clustering analysis was used to assess population genetic structure across Ash Meadows with the program STRUCTURE (Pritchard et al. 2000). Individuals were assigned to populations using a log-likelihood method (Paetkau et al. 1995) based on microsatellite allele frequencies within each population. Bayesian clustering was used to infer population clusters (K), independent from prior population designations, as well as the assignment of individuals to one or more clusters, also based on microsatellite

allele frequencies. Prior values of K were assumed to be between 1 and 15 (the total number of sites sampled), and the probability of the data for each number of clusters was calculated over 500,000 Monte Carlo Markov Chain (MCMC) iterations after a 200,000 iteration burn-in period. The burn-in period runs the simulation without collecting data in order to minimize the effect of the starting configuration; thus, recorded MCMC iterations begin with key parameters such as F_{ST} close to equilibrium (Pritchard et al. 2000).

The microsatellite data were also used to conduct assignment tests in GenoDive, based on the method described by Paetkau et al. (1995, 2004). The ratio between the likelihood of an individual coming from the population where it was found, and the maximum likelihood for all sample sites, was used to infer the population of origin for all individuals.

Phylogenetic relationships between individuals were inferred using a neighbor-joining tree in PAUP (Swofford 2003), based on a distance matrix generated from microsatellite allele frequencies. The matrix comprised squared Euclidean distances, and was calculated in GenoDive using the method of Smouse and Peakall (1999).

Results

GIS hydrology analysis

GIS mapping of current, historic, and potential waterways suggests that connectivity increases greatly during high precipitation or flooding events (Fig. 2). Outflows from the Warm Springs Complex may connect to the southern waterways by way of Crystal spring and its associated outflow. The outflow from South Scruggs spring may connect to the Crystal outflow further downstream, which connects directly to the northern waterways by way of an outflow from Peterson Reservoir. However, even with high flooding events, hydrologic isolation seems to be maintained between the springs within the Warm Springs Complex: the outflows from the Scruggs and Indian springs may extend to flow into other waterways at lower elevations, but do not connect directly with each other. Even if inundation occurred around these springs, which could enable extensive terrestrial movement by crayfish, vegetation patterns and topography suggest that these flooded areas remain distinct between the

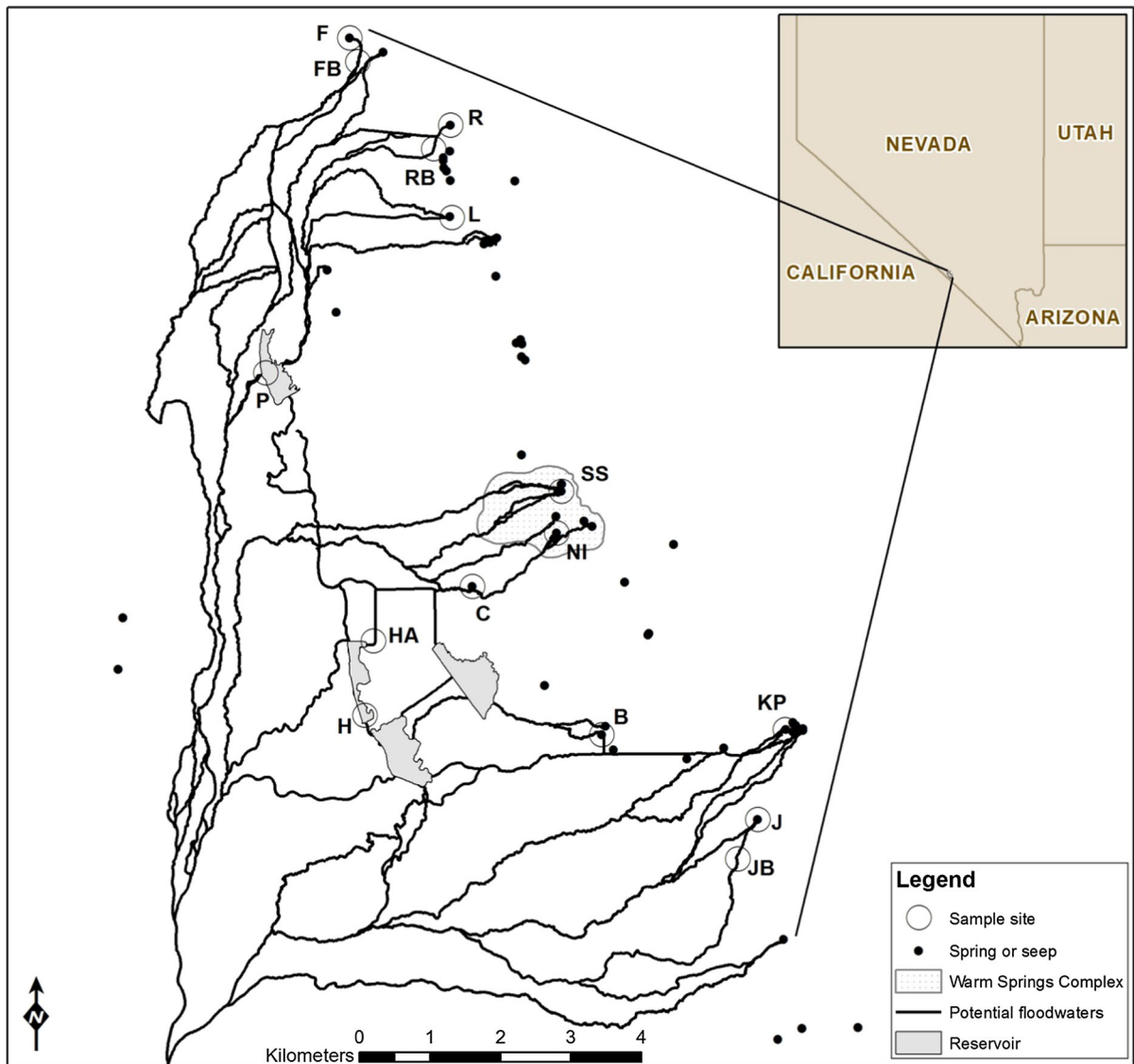


Fig. 2 Hydrologic connectivity in Ash Meadows during flooding events inferred from mapped perennial waterways, modeled historic outflows (Scoppetone et al. 2011), and drainages and vegetation patterns on aerial imagery and LiDAR data. Sample site abbreviations: *B* Bradford, *C* Crystal,

F Fairbanks, *FB* Below Fairbanks, *H* Horseshoe, *HA* Above Horseshoe, *J* Jackrabbit, *JB* Below Jackrabbit, *KP* King's Pool, *L* Longstreet, *NI* North Indian, *P* Peterson, *R* Roger's, *RB* Below Roger's, and *SS* South Scruggs

northern and southern Warm Springs (i.e. between South Scruggs and North Indian).

Mitochondrial DNA

Four mtDNA COI haplotypes were identified across all sampled individuals (Fig. 3a; GenBank accession numbers KF732701-KF732704). Haplotypes A and B

were distributed in both the northern and southern springs, while haplotype C was only in the northern springs and D was found in two individuals in Horseshoe marsh. The populations sampled from Peterson Reservoir, and the outflows below Roger's and Fairbanks springs had haplotypes A, B, and C, while all other populations had only one or two haplotypes. Two haplotypes were found in the Warm

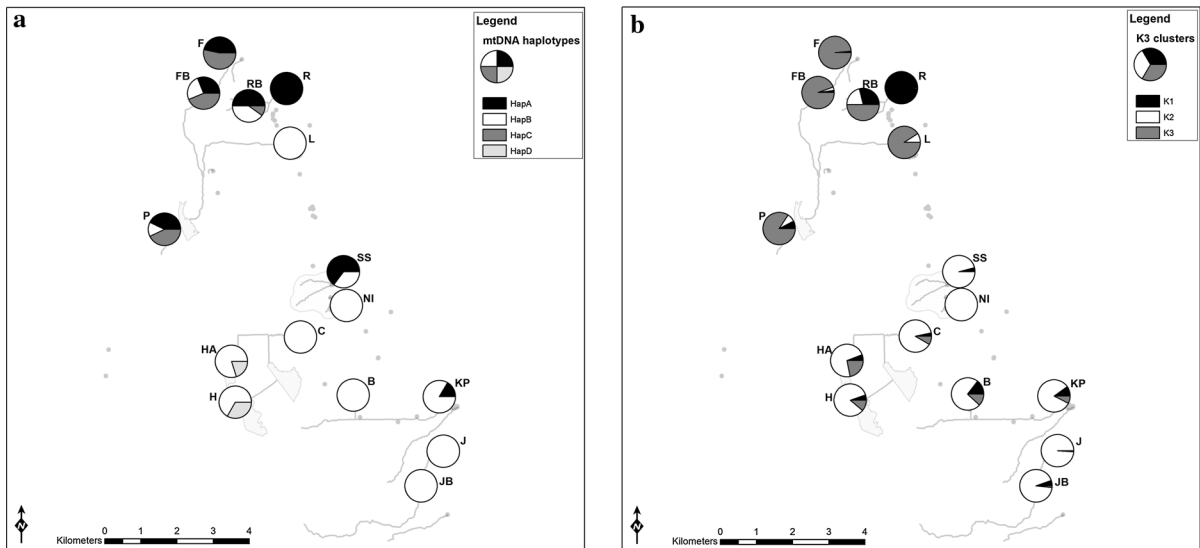


Fig. 3 **a** The distribution and relative abundances of the four mtDNA COI haplotypes in Ash Meadows. Sample site abbreviations: *B* Bradford, *C* Crystal, *F* Fairbanks, *FB* Below Fairbanks, *H* Horseshoe, *HA* Above Horseshoe, *J* Jackrabbit, *JB* Below Jackrabbit, *KP* King's Pool, *L* Longstreet, *NI* North Indian, *P* Peterson, *R* Roger's, *RB* Below Roger's, and *SS* South Scruggs. **b** Bayesian clustering of all sampled *P. clarkii*

individuals based on microsatellite allele frequencies, assuming three population clusters. Sample site abbreviations: *B* Bradford, *C* Crystal, *F* Fairbanks, *FB* Below Fairbanks, *H* Horseshoe, *HA* Above Horseshoe, *J* Jackrabbit, *JB* Below Jackrabbit, *KP* King's Pool, *L* Longstreet, *NI* North Indian, *P* Peterson, *R* Roger's, *RB* Below Roger's, and *SS* South Scruggs

Springs Complex: both A and B occurred in the South Scruggs population, and all individuals sampled from the North Indian population had haplotype B.

A network of mtDNA COI haplotypes (Fig. 4) shows that Ash Meadows haplotypes A, C, and D are most closely related to a haplotype sampled from the native range in Illinois, USA (which is identical to haplotypes found in invasive populations in China, Japan, and France). Ash Meadows haplotype B is identical to a haplotype found in New Orleans, Louisiana (USA).

Microsatellites

Two hundred and five individuals were genotyped for nine microsatellite loci. We identified 97 unique alleles summed across all loci, with 8–15 alleles per locus. The effective number of alleles per locus was between 2.05 and 2.84. The average number of alleles per population was between 2.11 (North Indian) and 7.67 (Crystal), with the effective number of alleles ranging from 1.62 (South Scruggs) to 4.97 (Crystal; Table 1). Gene diversity (H_E) was between 0.35 and 0.84, with North Indian having the lowest diversity

and Horseshoe having the highest. Populations from springs and waterways with perennial connectivity exhibited the highest heterozygosities, whereas the most hydrologically isolated springs—South Scruggs and North Indian—had the lowest.

Estimates of genetic distance (F_{ST}) showed most pairs of populations to be significantly different (Table 2). There was not significant genetic differentiation between Crystal and Horseshoe, or Crystal and Above Horseshoe. These populations are perennially connected by a slow-flowing waterway that would be easily navigable by crayfish. In addition, the population sampled from Horseshoe marsh was not significantly differentiated from the Above Horseshoe population just upstream. Although they are not directly connected by a waterway, the King's Pool crayfish population was not significantly different than the population sampled from below Jackrabbit spring. Interestingly, the populations sampled from Fairbanks and below its flume, and Roger's and below its flume, showed significant population structure. The greatest genetic differentiation between populations was between the pair of springs separated by the shortest Euclidean distance: South Scruggs and North Indian.

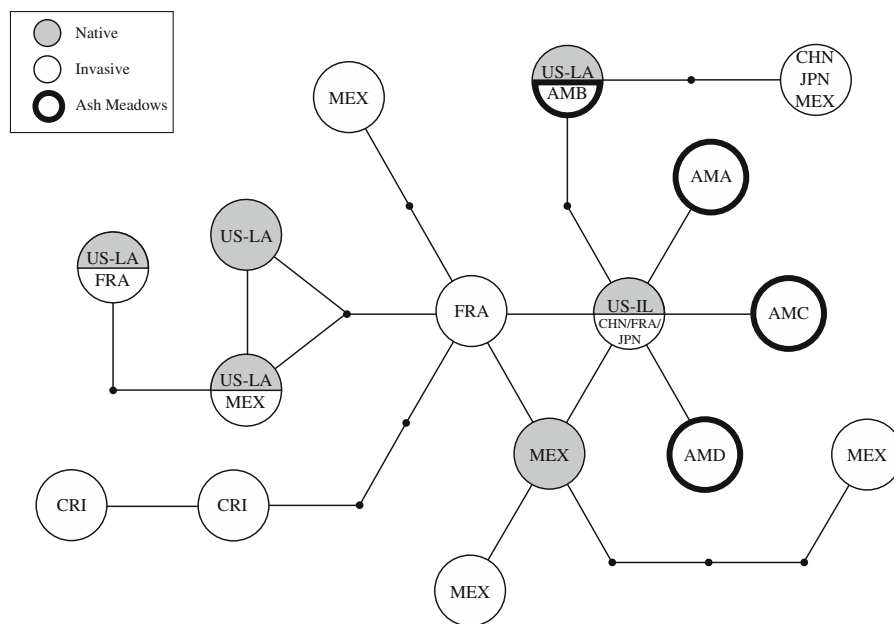


Fig. 4 A network of mtDNA COI haplotypes found in native and invasive *P. clarkii* populations: Ash Meadows (AM) haplotypes A, B, C, and D; native haplotypes from the United States: Illinois (US-IL) and New Orleans, Louisiana (US-LA); native and invasive haplotypes from Mexico (MEX); and invasive haplotypes from China (CHN), Costa Rica (CRI), France (FRA), and Japan (JPN). Native haplotypes are shown in

grey, invasive are white, and Ash Meadows are bold. Ash Meadows haplotype B is identical to a haplotype sampled from New Orleans, LA, and the other three Ash Meadows haplotypes are most closely related to a haplotype found in China, France, Japan, and Illinois. Two other native haplotypes from Louisiana are identical to haplotypes found in France and Mexico

Table 1 Microsatellite genetic diversity indices for all populations: number of alleles (A), effective number of alleles (AE), observed heterozygosity (HO), and expected heterozygosity (HE)

Population	N	A	AE	HO	HE
Bradford	8	3.667	2.498	0.519	0.644
Crystal	24	7.667	4.972	0.720	0.808
Fairbanks	19	3.667	2.137	0.415	0.478
Below Fairbanks	17	4.222	2.524	0.550	0.569
Horseshoe	3	4.000	3.441	0.741	0.843
Above Horseshoe	8	5.667	4.159	0.694	0.794
Jackrabbit	25	4.444	2.855	0.596	0.632
Below Jackrabbit	4	4.000	3.285	0.722	0.796
King's Pool	6	4.333	3.204	0.648	0.731
Longstreet	4	2.444	2.077	0.444	0.523
North Indian	9	2.111	1.663	0.420	0.349
Peterson	17	5.667	3.698	0.673	0.726
Roger's	34	3.444	2.164	0.459	0.530
Below Roger's	10	4.333	2.995	0.586	0.653
South Scruggs	17	2.333	1.618	0.340	0.356

N number of individuals sampled

As discussed previously, these springs seem to maintain hydrologic isolation from each other, even during flooding events (though they are likely to experience indirect connectivity through the outflow from Crystal spring).

The results of the assignment tests showed that individuals not assigned to their own populations were often inferred to belong to populations with high hydrologic connectivity: Peterson Reservoir, the inflow to Horseshoe Marsh (Above Horseshoe), the waterway below Fairbanks, and Crystal spring (Table 3). One individual sampled from Bradford spring was assigned to an upstream spring (King's Pool), indicating downstream movement from King's Pool to Bradford. Similarly, four individuals sampled from the waterway below Fairbanks were assigned to the Fairbanks spring pool population. However, four individuals sampled from the Fairbanks spring pool were assigned to the outflow population—potentially indicating upstream movement. The Warm Springs individuals mostly assigned to their original sample populations. All individuals from North Indian were

Table 2 Pairwise genetic distances (F_{ST}) between all sample sites

Population	BF	C	F	FB	H	HA	J	JB	KP	L	NI	P	R	RB
Crystal	0.170													
Fairbanks	0.352	0.247												
Fairbanks, below	0.239	0.165	0.092											
Horseshoe	0.187	0.005 ^{NS}	0.281	0.187										
Horseshoe, above	0.146	0.012 ^{NS}	0.241	0.134	-0.003 ^{NS}									
Jackrabbit	0.246	0.140	0.396	0.325	0.221	0.196								
Jackrabbit, below	0.142	0.041	0.330	0.248	0.092	0.068	0.015 ^{NS}							
King's Pool	0.140	0.074	0.340	0.262	0.110	0.101	0.190	0.037 ^{NS}						
Longstreet	0.248	0.164	0.284	0.200	0.164	0.096	0.309	0.208	0.232					
North Indian	0.432	0.288	0.516	0.437	0.388	0.357	0.382	0.415	0.409	0.508				
Peterson	0.155	0.081	0.149	0.045	0.097	0.057	0.231	0.126	0.141	0.127	0.360			
Roger's	0.280	0.243	0.325	0.308	0.266	0.227	0.373	0.268	0.281	0.325	0.512	0.227		
Roger's, below	0.199	0.156	0.251	0.193	0.159	0.136	0.239	0.149	0.204	0.214	0.433	0.074	0.220	
South Scruggs	0.422	0.259	0.517	0.437	0.411	0.345	0.383	0.398	0.396	0.508	0.536	0.360	0.447	0.431

Abbreviations along the top of the table begin with Bradford, and subsequently correspond to sample sites listed at the left side of the table. F_{ST} values marked with NS are not significant

Table 3 Results of log-likelihood population assignments for individuals assigned to locations other than where they were sampled

Exporters	B	C	F	FB	HA	KP	P	RB	SS
C					8	3	1		1
F				4					
FB			4				2		
HA		3							
KP	1								
P	1		1	2				2	

Sampled locations are listed along the top, and assigned locations are along the left. In other words, inferred exporters of *P. clarkii* individuals are listed along the left, and receiving locations are along the top. Site abbreviations: Bradford (B), Crystal (C), Fairbanks (F), Below Fairbanks (FB), Above Horseshoe (HA), King's Pool (KP), Peterson (P), Below Roger's (RB), and South Scruggs (SS). Individuals sampled from all other sites were assigned to their sampling location

inferred to be from that population. One individual from South Scruggs was assigned to the Crystal population, and the other 16 sampled individuals were assigned to South Scruggs. Three populations had too few samples for assignment: Horseshoe ($n = 3$), Below Jackrabbit ($n = 4$), and Longstreet ($n = 4$).

The results of the Bayesian clustering analysis indicated $K = 2$ as the most likely number of population clusters. One cluster was primarily composed of individuals from the northern sites (Rogers, Fairbanks, Longstreet, and Peterson Reservoir), and the other included individuals from the southern and central sites (Jackrabbit, Bradford, Crystal, Horseshoe Marsh, King's Pool, North Indian, and South Scruggs). However, when three population clusters were assumed, individuals were divided similarly between northern, and southern and central sites, except Roger's spring had its own population cluster (Fig. 3b). This provides further evidence for the efficacy of the flume in preventing dispersal from lower waterways into Roger's spring. The proportion of individuals within populations assigned to each cluster generally reflects the distribution of mtDNA haplotypes, with K2 dominating the southern springs, and K1 and K3 occurring mostly in the northern springs. However, the clustering analysis showed more genetic connectivity between the Crystal-Horseshoe-Bradford system and the northern springs than is represented by the mtDNA haplotypes. Also, fewer individuals from South Scruggs were assigned to the northern drainages than would be expected based on the mtDNA haplotype distribution.

A phylogeny of all sampled individuals inferred from the microsatellite data shows general regional clustering, and two monophyletic clades: North Indian

and South Scruggs (Fig. 5). Clusters were organized generally as (1) the Warm Springs and Crystal, (2) the Jackrabbit system and Crystal, (3) the Roger's system and Peterson, and (4) the Fairbanks system. The nearest relatives to the South Scruggs clade were from Crystal and Horseshoe, with North Indian as the closest sister clade. Principal components analysis reflects similar regional clustering (Supplemental Information).

Discussion

Introductions and colonization events

The level of genetic diversity in Ash Meadows indicates multiple, separate introductions of crayfish into the system. This is congruent with our knowledge of an initial introduction during the 1930s for aquaculture, and subsequent reintroductions by fishermen dumping their bait buckets. The analysis of mtDNA COI haplotypes is also indicative of at least two introductions, from source populations in southern Illinois and New Orleans, Louisiana. Two studies in other systems found comparable mtDNA haplotype richness. First, in an invaded system in China, only two mitochondrial COI haplotypes were identified from 313 individuals across 35 sites (Li et al. 2012). Additionally, a study of three crayfish populations in the native range of Mexico and the southern USA, and six invasive populations in Mexico and Costa Rica showed one to two COI haplotypes per sampled population, though only one to five individuals were sequenced per site (Torres and Alvarez 2012).

In the Warm Springs Complex, the existence of two mtDNA haplotypes in South Scruggs suggests

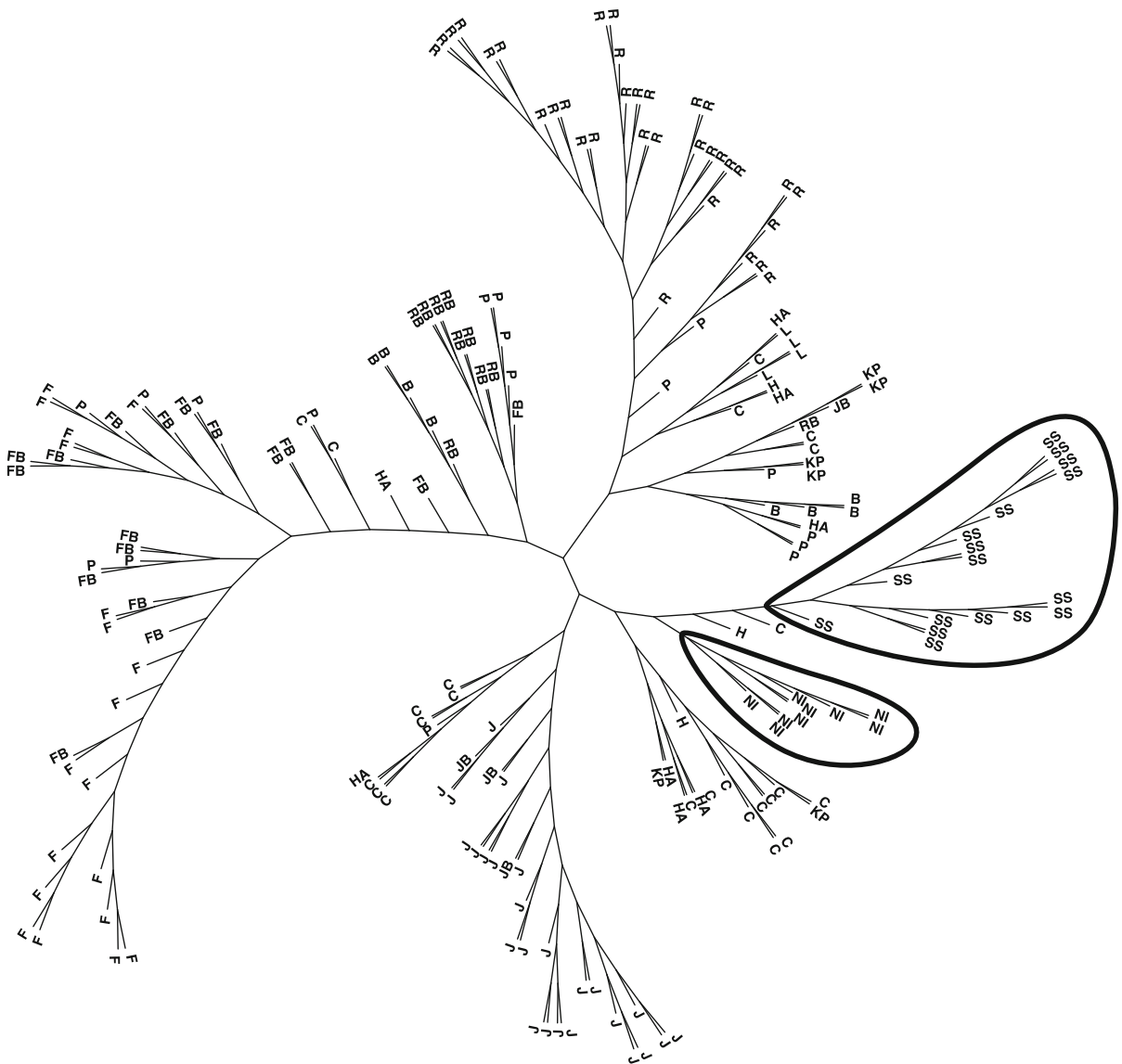


Fig. 5 A phylogeny of all sampled *P. clarkii* individuals based on the microsatellite data show two monophyletic clades from South Scruggs (SS) and North Indian (NI) springs (see circled clusters). The closest relatives to these clades are individuals sampled from Crystal (C) and Horseshoe (H). Other sample site

abbreviations: *B* Bradford, *F* Fairbanks, *FB* Below Fairbanks, *HA* Above Horseshoe, *J* Jackrabbit, *JB* Below Jackrabbit, *KP* King’s Pool, *L* Longstreet, *P* Peterson, *R* Roger’s, and *RB* Below Roger’s

colonization from both the northern springs, by way of Peterson Reservoir, as well as Crystal spring. The single haplotype in North Indian spring indicates a single colonization event most likely originating from Crystal spring. The hypothesis that Crystal spring was the source for the colonization of the Warm Springs is also supported by the microsatellite data. Furthermore, low genetic diversity and relatively high F_{ST} values indicate

a small number of colonists and infrequent immigration: perhaps two independent colonization events into South Scruggs and only one into North Indian.

Invasion patterns

The results of the genetic analyses indicate that movement of *P. clarkii* between springs in Ash

Meadows is congruent with hydrologic regimes. Populations in springs and reservoirs that experience a high degree of hydrologic connectivity through perennially flowing waterways—Fairbanks, Crystal, Horseshoe, Bradford, and King’s Pool—also exhibit high levels of genetic diversity and low F_{ST} values, indicating substantial gene flow. In contrast, *P. clarkii* populations sampled from North Indian and South Scruggs springs have low genetic diversity and high F_{ST} values, and these are highly isolated springs that rarely experience connectivity with the rest of the system.

The most common source of invading crayfish into the Warm Springs Complex is likely Crystal spring and its outflow, based on the results of the assignment tests, the phylogenetic analyses, and low F_{ST} values between Crystal Spring and South Scruggs (0.259) and Crystal Spring and North Indian (0.288), in conjunction with our understanding of drainage patterns. However, the mitochondrial DNA haplotype data suggest dispersal to South Scruggs from the northern waterways as well, by way of the outflow from Peterson Reservoir. This could explain the presence of Haplotype A in South Scruggs, which is predominantly absent from the southern springs and waterways. Low genetic diversity of microsatellite genotypes in South Scruggs, and especially North Indian, suggests that these springs were colonized by a small number of individuals, and subsequent emigration from lower waterways has been rare.

Similar patterns of connectivity between springs have been demonstrated for the Warm Springs pupfish (*C. n. pectoralis*) and the Ash Meadows pupfish (*C. n. mionectes*; Martin 2010). Evidence from microsatellite analyses of *C. n. pectoralis* suggests that connectivity within the Warm Springs (School to North Indian, South Scruggs to Marsh) has occurred at least once in the past 15 years, allowing movement of pupfish between springs. Based on the analysis of potential waterways draining from the Warm Springs Complex, connectivity between South Scruggs and Marsh springs likely happened indirectly through the outflow from Crystal spring—which would also indicate an opportunity for crayfish dispersal from Crystal to South Scruggs at least once in the past 15 years. Because crayfish were first documented in the Warm Springs Complex as early as 1983 (Weissenfluh 2010), we estimate there have been at least two dispersal events of crayfish into these isolated springs.

Dispersal of crayfish into the Warm Springs Complex probably happened during brief periods of extraordinary precipitation. Precipitation records from the Community Environmental Monitoring Program (CEMP) weather station in the Amargosa Valley indicate a handful of high precipitation events since 1999. Flooding was documented in Ash Meadows on 22 December 2010, a day when the refuge received 2.26 cm of rain. Other storms that yielded at least this much precipitation may have also caused flooding in Ash Meadows, facilitating connectivity between the Warm Springs and lower elevation springs and waterways, and providing dispersal opportunities for crayfish and other aquatic organisms. However, the height of the water table—which varies seasonally—likely influences the probability of flooding as well, with flooding being more likely during the winter months when the water table is higher. If so, CEMP precipitation data indicate flooding in Ash Meadows has occurred five times between 1999 and spring of 2012.

Implications for restoration

Restoration efforts in Ash Meadows have succeeded in eradicating *P. clarkii* and other invasive aquatic species from School spring, and North and South Indian springs. These springs, and their pupfish populations, were temporarily diverted into large tanks in order to destroy crayfish habitat and eradicate their populations through desiccation. The School spring outflow was dried out for 1 month in the spring of 2008, and the Indian springs were desiccated for 21 months from June 2009 to March 2011. Crayfish have not been observed in the School or Indian springs since their restoration (Weissenfluh 2008; Darrick Weissenfluh, USFWS, pers. comm.). Crayfish eradication from South Scruggs spring commenced in November 2012. In order to prevent reinvasion of these springs, further efforts must be taken to eliminate either connectivity or source populations at lower elevations. Due to the significant cost, time, labor, and potential damage to native and endemic biota caused by the restoration process, the eradication of crayfish from all source populations in Ash Meadows does not seem feasible. Therefore, a reduction in connectivity is the best method to prevent crayfish dispersal into the Warm Springs Complex from source populations.

However, a major consideration in conserving populations of endemic fish—such as the Warm

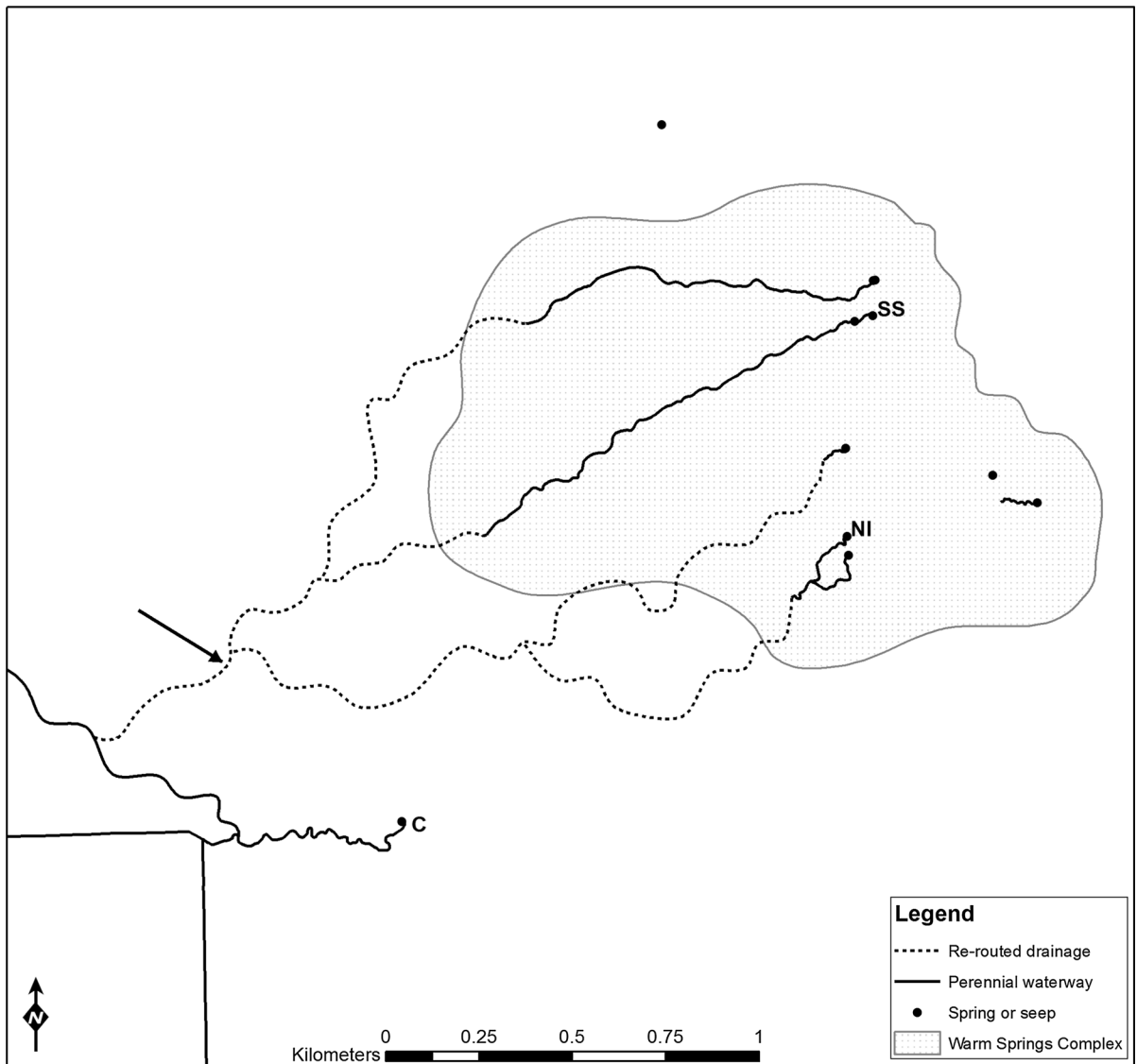


Fig. 6 One possible restoration scenario for the Warm Springs Complex involves redirecting the outflows to connect with each other before flowing into the Crystal spring outflow. A single barrier could then be placed below the confluence of the Warm Springs outflows (see *arrow*) to prevent reinvasion of these restored springs by crayfish from Crystal spring outflow during

flooding. This scenario would improve connectivity between populations of the Warm Springs pupfish while eliminating connectivity for aquatic invasive species outside the Warm Springs Complex. Site abbreviations: *SS* South Scruggs, *NI* North Indian, *C* Crystal

Springs pupfish and Ash Meadows speckled dace—is the maintenance of genetic diversity through gene flow, which requires aquatic connectivity between populations in geographically isolated springs. Accordingly, the most desirable mechanism would simultaneously prevent crayfish movement while enabling dispersal of fish. This could be achieved

through directed geographical placement and the design of barriers. As suggested by Martin (2010), one way to implement this system would be to redirect the outflows from the Warm Springs so that they connect with each other before flowing into the Crystal outflow (Fig. 6). This would increase connectivity for the Warm Springs pupfish, while creating a single

drainage from the Warm Springs Complex into the outflow from Crystal spring. A barrier could then be installed above the junction of the Warm Springs outflow with the Crystal outflow to prevent crayfish dispersal into the Warm Springs Complex during flooding. Barrier design could include mesh to impede adult crayfish movement, and could also prevent larval dispersal by incorporating a vertical drop, or high flow rates across a slippery substrate. Terrestrial circumnavigation by adult crayfish could be prevented through the use of adjacent drift fences.

Landscape genetics provides an informative tool that can be used to facilitate effective ecological restoration of invaded ecosystems. In this study, we demonstrated how measures of genetic distance between populations, genetic diversity within populations, and phylogenetic analyses can be used in conjunction with landscape connectivity metrics to infer the frequency of dispersal as well as the location of movement routes used by an invasive species. This research has direct implications for management of invasive crayfish in Ash Meadows, and also provides a case study that may be applied to other invaded systems where connectivity is rare and ephemeral. We have demonstrated how population genetic data can be used to make a posteriori inferences about ecological processes such as dispersal and colonization when such processes are too infrequent for direct observational research, and when movement corridors are temporary and geographically variable.

The importance of managing invasive species in the Great Basin and Mojave deserts is amplified by continued and accelerating anthropogenic pressures across these landscapes, in the form of water removal. To meet the demands of growing desert population centers such as Las Vegas, the Southern Nevada Water Authority and officials from satellite communities hope to increase regional water withdrawal to 2.2 billion cubic meters per year, which is estimated to exceed perennial regional aquifer yield by 250 % (Deacon et al. 2007). This increase in pumping from wells throughout Nevada is predicted to cause a drop of nearly 3 m in the aquifer that feeds the springs of Ash Meadows (Deacon et al. 2007), which in conjunction with future climate predictions of decreased precipitation in the Mojave and Great Basin deserts (Seager et al. 2007), may result in dramatic alterations to hydrologic connectivity regimes for spring systems in this area. Connectivity that is already rare and ephemeral may occur even less

frequently, and for shorter durations. Although decreased hydrologic connectivity can have the positive consequence of impeding the spread of aquatic invaders, the overall effects will likely be negative: loss of aquatic, wetland, and riparian habitat, and reduced genetic connectivity for small populations of endemic biota could lead to increased rates of extirpations and eventually extinctions.

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References

- Barbaresi S, Tricarico E, Gherardi F (2004) Factors inducing the intense burrowing activity by the red swamp crayfish, *Procambarus clarkii*, an invasive species. *Naturwissenschaften* 91:342–345
- Belfiore NM, May B (2000) Variable microsatellite loci in red swamp crayfish, *Procambarus clarkii*, and their characterization in other crayfish taxa. *Mol Ecol* 9:2231–2234
- Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1660
- Deacon JE, Williams CD (1991) Ash Meadows and the legacy of the Devils Hole pupfish. In: Minckley WL, Deacon JE (eds) *Battle against extinction: native fish management in the American West*. The University of Arizona Press, Tucson, pp 69–87
- Deacon JE, Williams AE, Williams CD, Williams JE (2007) Fueling population growth in Las Vegas: how large-scale groundwater withdrawal could burn regional biodiversity. *Bioscience* 57:688–698
- Dudley WW, Larson JD (1976) Effect of irrigation pumping on desert pupfish habitats in Ash Meadows, Nye County, Nevada. U.S. Geological Survey Professional Paper 927, p 153
- Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how, and so what? *Mol Ecol* 19:4113–4130
- Estoup A, Baird SJE, Ray N, Currat M, Cornuet JM, Santos F, Beaumont MA, Excoffier L (2010) Combining genetic, historical and geographical data to reconstruct the dynamics of bioinvasions: application to the cane toad *Bufo marinus*. *Mol Ecol Resour* 10:886–901
- Figler MH, Blank GS, Peeke HVS (2005) Shelter competition between resident male red swamp crayfish *Procambarus clarkii* (Girard) and conspecific intruders varying by sex and reproductive status. *Mar Freshw Behav Phys* 38:237–248

- Gherardi F, Barbaresi S (2000) Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv für Hydrobiologie* 150:153–168
- Gherardi F, Baldaccini GN, Barbaresi S, Ercolini P, De Luise G et al (1999) The situation in Italy. In: Gherardi F, Holdich DM (eds) *Crayfish in Europe as alien species. How to make the best of a bad situation?* A.A. Balkema, Rotterdam, pp 107–128
- Gutierrez-Yurrita PJ, Montes C (1999) Bioenergetics and phenology of reproduction of the introduced red swamp crayfish, *Procambarus clarkii*, in Doñaña National Park, Spain, and implications for species management. *Freshw Biol* 42:561–574
- Hobbs HH (1972) *Biota of freshwater ecosystems, identification manual 9: crayfishes (Astacidae) of North and Middle America*. Water Pollution Control Research Series. U.S. Environmental Protection Agency, reprinted in 1976
- Huner JV (1977) Introductions of the Louisiana red swamp crayfish, *Procambarus clarkii* (Girard): an update. *Freshw Crayfish* 3:193–202
- Huner JV, Avault JW (1979) Introductions of *Procambarus* spp. *Freshw Crayfish* 4:191–194
- Huner JV, Lindqvist OV (1995) Physiological adaptations of freshwater crayfishes that permit successful aquacultural enterprises. *Am Zool* 35:12–19
- Kennedy TA, Finlay JC, Hobbie SE (2005) Eradication of invasive *Tamarix ramosissima* along a desert stream increases native fish density. *Ecol Appl* 15:2072–2083
- Li Y, Gou X, Cao X, Deng W, Luo W, Wang W (2012) Population genetic structure and post-establishment dispersal patterns of the red swamp crayfish *Procambarus clarkii* in China. *PLoS ONE* 7:e40652
- Lodge DM, Lorman JG (1987) Reductions in submersed macrophyte biomass and species richness by the crayfish *Oreonectes rusticus*. *Can J Fish Aquat Sci* 44:591–597
- Maddison DR, Maddison WP (2005) *MacClade 4: analysis of phylogeny and character evolution*. Version 4.08a. <http://macclade.org>
- Marrs RA, Sforza R, Hufbauer RA (2008) Evidence for multiple introductions of *Centaurea stoebe* (spotted knapweed, Asteraceae) to North America. *Mol Ecol* 17:4197–4208
- Martin AP (2010) The conservation genetics of Ash Meadows pupfish populations. I. The Warm Springs pupfish *Cyprinodon nevadensis pectoralis*. *Conserv Genet* 11:1847–1857
- Matsuzaki SS, Usio N, Takamura N, Washitani I (2009) Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia* 158:673–686
- Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analyses of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794
- Miller RR (1948) *The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada*. Miscellaneous Publications, Museum of Zoology, Univ of Michigan 68, pp 1–155, Pl. 1–15
- Nystrom P, Strand JA (1996) Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshw Biol* 36:673–682
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol Ecol* 4:347–354
- Paetkau D, Slade R, Burdens M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol Ecol* 13:55–65
- Paglianti A, Gherardi F (2004) Combined effects of temperature and diet on growth and survival of young-of-year crayfish: a comparison between indigenous and invasive species. *J Crustac Biol* 24:140–148
- Payne JF (1997) Adaptive success within the cambarid life cycle. *Freshw Crayfish* 11:1–12
- Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. *Trends Ecol Evol* 24:497–504
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Reynolds JD (2002) Growth and reproduction. In: Holdich DM (ed) *Biology of freshwater crayfish*. Blackwell Science, Oxford, pp 152–191
- Rodriguez CF, Becares E, Fernandez-Alaez M (2003) Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (*Procambarus clarkii*). *Hydrobiologia* 506:421–426
- Rodriguez CF, Becares E, Fernandez-Alaez M, Fernandez-Alaez C (2005) Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biol Invasions* 7:75–85
- Rollins LA, Woolnough AP, Wilton AN, Sinclair R, Sherwin WB (2009) Invasive species can't cover their tracks: using microsatellites to assist management of starling (*Sturnus vulgaris*) populations in Western Australia. *Mol Ecol* 18:1560–1573
- Sada DW (1990) Recovery plan for the endangered and threatened species of Ash Meadows, Nevada. U.S. Fish and Wildlife Service, Portland, Oregon, p 123
- Sanders NJ, Gotelli NJ, Heller NE, Gordon DM (2003) Community disassembly by an invasive species. *Proc Natl Acad Sci USA* 100:2474–2477
- Scoppettone GG, Rissler P, Johnson D, Hereford M (2011) Relative abundance and distribution of fishes and crayfish at Ash Meadows National Wildlife Refuge, Nye County, Nevada, 2007–2008. U.S. Geological Survey Open-File Report 2011-1017, p 56
- Seager R, Ting M, Held I, Kushnir Y, Lu J, Vecchi G, Huang H, Harnik N, Leetmaa A, Lau N, Li C, Velez J, Naik N (2007) Model projections of an imminent transition to a more arid climate in southwestern North America. *Science* 316:1181–1184
- Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* 82:561–573
- Soltz KL, Naiman RJ (1978) The natural history of native fishes in the Death Valley system. Natural History Museum of Los Angeles County, California. Science Series v. 30, p 76
- Stevens LE, Bailowitz RA (2008) Odonata of Ash Meadows National Wildlife Refuge, southern Nevada, USA. *J Arizona-Nevada Acad Sci* 40:128–135
- Swofford DL (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts
- Taylor CA, Knouft JH (2006) Historical influences on genital morphology among sympatric species: gonopod evolution and reproductive isolation in the crayfish genus *Oreonectes* (Cambaridae). *Biol J Linn Soc* 89:1–12

- Torres E, Alvarez F (2012) Genetic variation in native and introduced populations of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea, Decapoda, Cambaridae) in Mexico and Costa Rica. *Aquat Invasions* 7:235–241
- Vitousek PM, D'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. *Am Sci* 84:218–228
- Weissenfluh DS (2008) School Spring Refugium restoration accomplishment report. US Fish and Wildlife Service Report, p 9
- Weissenfluh DS (2010) Conservation of *Cyprinodon nevadensis pectoralis* and three endemic aquatic invertebrates in an artificial desert spring refuge located in Ash Meadows National Wildlife Refuge, Nevada. M.S. Thesis, Texas Tech University