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RESEARCH ARTICLE

Local-scale invasion pathways and small founder numbers in introduced Sacramento pikeminnow (*Ptychocheilus grandis*)

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Abstract Given the general pattern of invasions with severe ecological consequences commonly resulting from multiple introductions of large numbers of individuals on the intercontinental scale, we explored an example of a highly successful, ecologically significant invader introduced over a short distance, possibly via minimal propagule pressure. The Sacramento pikeminnow (*Ptychocheilus grandis*) has been introduced to two coastal rivers in northern California where it poses a risk to threatened and endangered fishes. We assayed variation in seven microsatellite loci and one mitochondrial DNA gene to identify the source populations and estimate founder numbers for these introductions. Our analysis suggests that successful invasion of the Eel River was likely the result of a single transfer of 3–4 effective founders from nearby within the species' native range: Clear Lake or its outflow Cache Creek. The other introduced population (Elk River), known from only seven individuals, likely represents secondary expansion from the introduced Eel River population. Our findings highlight the threat posed by close-range invaders and the ability of some fishes to rapidly invade ecologically suitable areas despite small effective founding numbers.

Keywords Invasion genetics · Effective founder number · Genetic diversity · Exotic species · Sacramento pikeminnow · *Ptychocheilus grandis*

Introduction

Invasive species with strong ecological effects are commonly established via substantial propagule pressure on the intercontinental scale. While propagule pressure is clearly an important parameter for predicting invasion success (Colautti et al. 2006; Drake and Lodge 2006; García-Berthou 2007; Lockwood et al. 2005; Marchetti et al. 2004), examples of ecologically significant invasions from single introductions and very small founding populations are available (Ross and Shoemaker 2008; Kalinowski et al. 2010). Similarly, while intercontinental invasions offer many examples of severe ecological effects, analysis of the effects of inter- versus intra-continental fish invasions suggests that intracontinental translocations pose similar ecological risks (Ricciardi and Simberloff 2009). Understanding the probability of establishment of invasive species from small numbers of individuals from vicinal populations has important implications for the management of biological invasions. This issue might be of particular concern for invasive freshwater fishes because: (1) their limited dispersal abilities can result in large amounts of geographically proximate habitat containing ecologically suitable conditions, and (2) fish can exhibit high population growth even from small founding populations (e.g. Kalinowski et al. 2010; Kinziger et al. 2011). Thus it might be expected that intracontinental invasions comprise a significant proportion of successful introductions of fishes (e.g. Moyle 2002).

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We studied invasion genetics of two introduced populations of Sacramento pikeminnow (*Ptychocheilus grandis*) in northern California. The Sacramento pikeminnow is a large (usually 20–60 cm standard length) piscivorous cyprinid native to the Sacramento-San Joaquin drainage and several smaller coastal rivers in California (Moyle 2002). Sacramento pikeminnow were introduced into the Eel River in 1979 or 1980; field surveys revealed rapid spread to almost all suitable habitats in the basin within about 10 years of introduction (Brown and Moyle 1997). In 2008 a second population of Sacramento pikeminnow was detected a short distance from the Eel River in the Elk River, a tributary to Humboldt Bay (Martin Slough; pers. comm. M. Wallace, California Department of Fish and Game, Arcata, CA). To date only seven Sacramento pikeminnow have been captured from this site and all were sacrificed or sterilized to prevent establishment and spread. To date, no successful reproduction of Sacramento pikeminnow has been documented in the Elk River.

At both introduction sites, Sacramento pikeminnow pose a predation risk to threatened coho salmon (*Oncorhynchus kisutch*) and endangered tidewater goby (*Eucyclogobius newberryi*), both of which are listed under the federal Endangered Species Act in the United States. Sacramento pikeminnow can reach lengths of about 1 m (Moyle 2002); in the Eel River, Sacramento pikeminnow over 300 mm standard length are mostly piscivorous (Nakamoto and Harvey 2003). Due to the commercial, recreational, and cultural importance of salmonids and concerns over high predation rates, millions of pikeminnow have been eradicated from their native and non-native range using sport fishing-derbies and electrocution devices (Friesen and Ward 1999; Moyle 2002; Petersen 1994).

Our objective was to determine the source populations and estimate founder numbers for the two Sacramento pikeminnow populations introduced to northern California using genetic methods. The spatial resolution to which source populations can be pinpointed in species invasions is directly related to the degree of genetic differentiation among source populations in their native range (Muirhead et al. 2008). Sacramento pikeminnow exhibit long distance dispersal ability within basins (Harvey and Nakamoto 1999) but exchange between basins is unlikely, so we expected our analysis to provide accuracy of assignment of the source population at the basin scale. Source populations identified during our analysis were then used as standards to evaluate genetic diversity loss in the introduced populations and as a basis for implementing a coalescent-based maximum likelihood approach for estimating the effective number individuals founding the introduced populations (Anderson and Slatkin 2007).

Materials and methods

Sample collection and molecular methods

Sacramento pikeminnow were sampled from the two introduced populations (Eel River and Elk River) and from likely source locations in the native range (Fig. 1; Table S1). To ensure adequate coverage, multiple sites within major river drainages were sampled. In preliminary tests all sites within major river drainages exhibited nonsignificant genetic differentiation and were grouped for analysis with the exception of two locations from the Sacramento River basin, Cache Creek and Clear Lake, which were analyzed separately. Sacramento pikeminnow were collected using a boat or backpack electrofisher. Smaller pikeminnow were preserved in 95 % ethanol and nonlethal caudal fin clips were collected from larger individuals. Fin clips were either dried on filter paper or preserved in 95 % ethanol. Whole genomic DNA was extracted from fin tissue using chelex methods.

A total of 314 Sacramento pikeminnow were genotyped at seven microsatellite loci (Table S1). Microsatellite loci amplification was performed using Master Mix (Promega, Madison, WI, USA) in an MJ Research (Waltham, MA, USA) PTC-100 thermal cycler using 10 or 12.5 uL volumes (Table S2). PCR products were visualized and allele size established using the Beckman-Coulter CEQ 8000 Genetic Analysis System. Allele scores were determined twice and discrepancies were either resolved or no score was assigned.

Each locus in each population was tested for conformance to Hardy–Weinberg equilibrium using GENEPOP (Raymond and Rousset 1995; Rousset 2008) (500 batches of 1,000 iterations). Tests for linkage disequilibrium between locus pairs were conducted in GENEPOP (800 batches of 1,000 iterations). We corrected for multiple tests using Bonferroni methods (Rice 1989).

Introduction source

Pairwise estimates of population differentiation (F_{ST}) and tests of their significance were generated using GenoDive 2.0b22 (Meirmans and Van Tienderen 2004). An unrooted neighbor-joining tree of pairwise population differentiation (F_{ST}) was generated with TreeFit (Kalinowsi 2009) and rendered in FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Discriminant analysis of principal components (DAPC) was used to visually depict genetic relationships among populations (Jombart et al. 2010). DAPC is a two-step process: the first transforms genotypic data using Principal Component Analysis and the second uses Discriminant Analysis to maximize differentiation between previously defined groups. DAPC was conducted using the adegenet

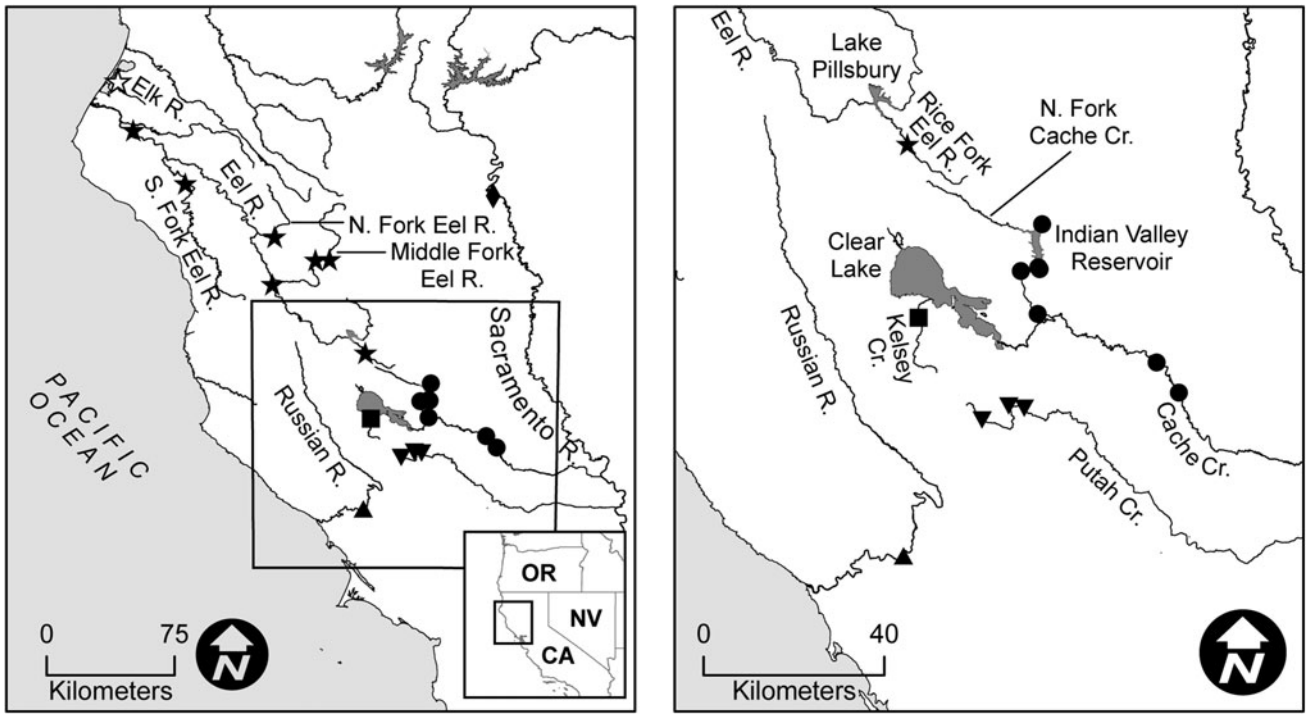

Fig. 1 Distribution of sampling sites for Sacramento pikeminnow in northern California, USA. Filled stars indicate the introduced Eel River collections and the open star indicates the introduced Elk River site. Populations from the native range include: Clear Lake (*square*), Cache Creek (*circle*), Sacramento River (*diamond*), Putah Creek (inverted *triangle*), and Russian River (*triangle*)

package (Jombart 2008) for the R software (R Development Core Team 2011). Lastly, STRUCTURE 2.3.3 (Falush et al. 2003; Pritchard et al. 2000) was used to evaluate genetic groupings, generate individual assignments and evaluate the extent of admixture among genetically distinct groups. Analyses were conducted using the default parameters. Simulations were run for 20,000 steps (with 10,000 discarded as burn-in) and 20 independent runs were conducted assuming our data consisted of 1–6 clusters (K). Estimates of the number of genetic clusters present in the data was evaluated by calculating the log probability of the data (ln Pr(X|K)) and ΔK using STRUCTURE HARVESTER (Earl and vonHoldt 2012; Evanno et al. 2005). STRUCTURE runs were aligned using the LargeKGreedy algorithm (with 10,000 random input orders) using CLUMPP (Jakobsson and Rosenberg 2007) and graphical depictions of CLUMPP results were generated using DISTRUCT (Rosenberg 2004).

Effective founder number

We used a coalescent-based maximum likelihood approach to estimate the effective founder size of Sacramento pikeminnow introduced to the Eel River (Anderson and Slatkin 2007). The exact value of many input parameters was unknown so we explored a range of reasonable values to evaluate their effects on estimates of effective founder number. As indicated by our analysis we used three populations as sources (CL, CAC, and SAC). The number of generations since introduction and our field collections was set to 6 and 11. These values seemed reasonable given that the duration of a single generation in Sacramento pikeminnow is 3–4 years (Moyle 2002) and 25–33 years passed between the introduction (1979–1980) and our field collections (2004–2012). We assumed carrying capacities for pikeminnow in the Eel River of 30,000, 60,000, and 90,000. These values were derived from personal observations by BCH and RJN of the abundance of Sacramento pikeminnow in the Eel River. Given the high fecundity of Sacramento pikeminnow (15,000–40,000 eggs per female, for fish measuring 31–65 cm standard length) and the rapid spread of this species following introduction (Brown and Moyle 1997; Moyle 2002), we tried intrinsic rates of increase of 3 and 4. Estimates of founder number were generated using all 36 combinations of effective carrying capacity (30,000, 60,000, and 90,000), intrinsic rate of increase (3 and 4), generations since founding (6 and 9), and source population (CL, CAC, and SAC). We did not generate effective founder number estimates for the Elk River introduction because our sample size for this population was too small ($N = 6$) and there is no evidence that this population has successfully reproduced since introduction. Analyses were conducted using the software COALIT and NFCONE (Anderson and Slatkin 2007).

Genetic diversity

Allelic richness (A), observed heterozygosity (H_O) and Hardy–Weinberg expected heterozygosity (H_E) were calculated using GenoDive 2.0b22 (Meirmans and Van Tienderen 2004). Estimates of private allelic richness (A_p), and allelic richness (A_R) were standardized to a sample size of 30 genes using rarefaction methods implemented in HP-RARE 1.0 (Kalinowski 2005). We compared standardized allelic richness, standardized private allelic richness and heterozygosity using a two-way analysis of variance (ANOVA) with population and locus as fixed factors. Standardized allelic richness (log transformation) and standardized private allelic richness (square root transformation) were transformed to improve model fit and the normality of residuals, using transformation selection methods in Draper and Smith (1998). We determined the significance of pairwise contrasts between populations using Tukey's tests ($P < 0.05$) in SAS Proc Mixed. The introduced ELK population was excluded because the sample size for this population was too small ($N = 6$) to make meaningful comparisons.

Mitochondrial DNA

We sequenced a total of 281 individuals from the two introduced and five putative source populations for a 718 bp fragment of the mitochondrial cytochrome *b* gene (Table S1). Amplifications were conducted using primers L14724 and H15915 under the following conditions (Irwin et al. 1991): 35 cycles of 94 °C for 60 s, 48 °C for 60 s, and 72 °C for 120 s. Sequences were generated using L14724 at High-Throughput Sequencing Solutions (University of Washington, Department of Genome Sciences). Sequences were aligned in CLUSTALX2 (Larkin et al. 2007) and haplotype frequencies, average number of nucleotide differences between population pairs, haplotype diversity (h), and nucleotide diversity (π) were estimated using ARLEQUIN 3.1 (Schneider et al. 2000). An estimate of the genetically effective number of individuals founding the introduced Eel River population was generated using simulation procedures (Kinziger et al. 2011; Ross and Shoemaker 2008). This approach uses haplotype distributions in the source and introduced populations to model the effects of the founding event on haplotype richness.

Preliminary analysis indicated the presence of hardhead (*Mylopharodon conocephalus*) haplotypes in individuals diagnosed as Sacramento pikeminnow using morphological traits and nuclear microsatellite analysis. These findings are consistent with transfer of hardhead mitochondrial DNA into Sacramento pikeminnow (see also Duvernell and Aspinwall 1995; Keck and Near 2010; Rognon and Guyomard 2003). All such individuals were removed from both the microsatellite and mitochondrial DNA data sets prior to analysis.

Results

All loci were highly polymorphic, ranging from 10 to 43 alleles, with an average of 20.4 alleles per locus. Missing data were not characteristic of loci or populations and each individual multilocus genotype contained at least six of the seven assayed loci. Of the 42 tests for conformance to Hardy–Weinberg proportions (6 populations at 7 loci), one was significant following Bonferroni correction for multiple tests (critical value = 0.0012). A total of two of 126 tests for linkage disequilibrium were significant following Bonferroni correction for multiple tests (critical value = 0.0004). The maximum number of alleles observed at a locus in the introduced populations was 9 in EEL and 5 in ELK.

Introduction source

The introduced populations (EEL and ELK) exhibited nonsignificant genetic differentiation (F_{ST}) from one another and significant genetic differentiation from all native populations (Table 1). In the neighbor-joining tree the two introduced populations (EEL and ELK) clustered together and were most similar to CL, followed by CAC (Fig. 2). In DAPC 88.9 % of the total genetic variation could be explained by the first two axes and plot of the first two axes showed that the introduced populations (ELK and EEL) occupied the same multivariate space and clustered closely with CL, followed by CAC (Fig. 3). In the STRUCTURE analysis the ad hoc statistic ΔK suggested three clusters and the log probability of the data indicated the data was best described by four clusters (Figs. S1, S2). Hierarchical inspection of STRUCTURE results starting at

Table 1 Pairwise estimates of genetic differentiation among Sacramento pikeminnow populations. Microsatellite (F_{ST}) is below diagonal and mitochondrial DNA (average number of nucleotide differences between populations) is above diagonal

	EEL	ELK	CL	CAC	SAC	PUT	RUS
EEL	–	**1.2**	**2.9**	**2.5**	**2.1**	**4.0**	**6.3**
ELK	**0.0320**	–	**1.8**	**1.7**	**1.1**	**3.0**	**5.1**
CL	**0.1194**[a]	**0.1678**[a]	–	3.0	2.6	3.8	4.3
CAC	**0.0998**[a]	**0.1015**[a]	0.0964[a]	–	2.6	4.1	5.5
SAC	**0.1115**[a]	**0.1043**[a]	0.1231[a]	0.0291[a]	–	3.7	5.3
PUT	–	–	–	–	–	–	5.2
RUS	**0.2292**[a]	**0.2485**[a]	0.2558[a]	0.1697[a]	0.1464[a]	–	–

Introduced populations in bold

[a] Indicates significant differentiation in permutation tests following Bonferroni correction for multiple tests (critical value = 0.0033)

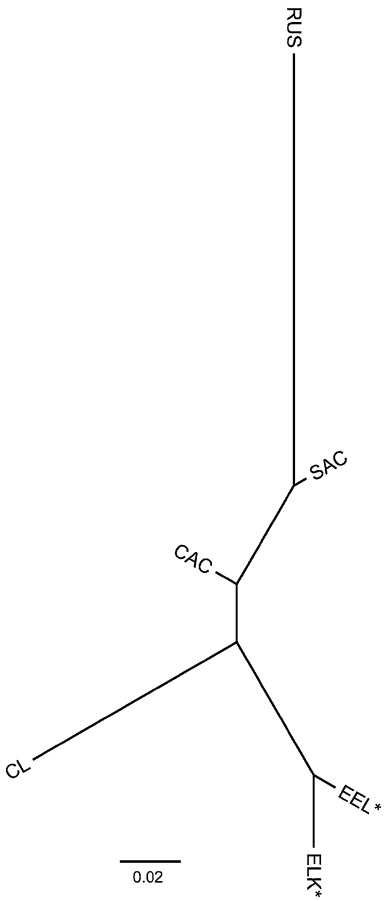

Fig. 2 Unrooted neighbor-joining tree generated using pairwise genetic distances (F_{ST}). Introduced populations (EEL and ELK) indicated by *asterisks*

K = 2 showed one group consisting of the two introduced populations (EEL and ELK) and the other cluster consisting of native populations (CAC, RUS and SAC; Fig. 4). While admixture was suggested by the STRUCTURE analysis, confidence intervals for individual assignments indicated lack of statistical support.

Founder number

Estimates of the effective number of individuals founding the Eel River were only modestly influenced by assumed source population and the broad range of demographic parameters we explored (Table S3). Founder number estimates were 4.3 (support limits 3.3–5.8) when CL was the assumed source, 3.3 (support limits 2.7–4.1) when CAC was the assumed source, and 2.9 (support limits 0–3.5) when SAC was the assumed source.

Genetic diversity

Inspection of the nuclear genetic diversity data suggested two groups of populations: the introduced population (EEL) along with CL, and RUS versus the more diverse CAC and SAC (Table 2). These groupings were statistically supported by the analysis of standardized allelic richness. Private allelic richness was lowest in the introduced population (EEL), although not distinguishable from CL and RUS populations. Expected heterozygosity did not differ statistically among populations.

Mitochondrial DNA

The 281 mitochondrial DNA sequences yielded 22 variable nucleotide positions defining 17 haplotypes (Tables S4, S5). The average number of nucleotide differences in pairwise comparisons among populations ranged from 1.1 to 6.26 (Table 1). A single haplotype (Hap_2) occurred at high frequency and was common to all populations, with the exception of RUS, which shared no haplotypes with the other populations (Fig. 5; Table S5). The introduced EEL population contained two haplotypes, including the common haplotype (Hap_2) and another haplotype that was restricted to one native population (CAC). The simulation procedure indicated that the most likely number of founders containing the two haplotypes detected in the introduced population EEL was four when CAC was the assumed source.

Discussion

Parallel to the result for another introduced cyprinid in the Eel River (Kinziger et al. 2011), the Sacramento pikeminnow population in the Eel River was founded by a small number of fish from a nearby population. Analysis of both nuclear microsatellite and mitochondrial DNA suggested an initial colonizing group of Sacramento pikeminnow in the Eel River of about four individuals. The nuclear coalescent analyses were robust to the broad range of carrying capacities, generations since founding, and source populations that we explored. Lack of variation in effective founder number estimates likely occurred because all demographic models resulted in minimal post-introduction drift owing to rapid population growth following the initial introduction (Anderson and Slatkin 2007). The assumption of rapid population expansion seems reasonable given the high fecundity of Sacramento pikeminnow (15,000–40,000 eggs per female, for fish measuring 31–65 cm standard length) and the rapid spread of this species following introduction (Brown and Moyle 1997). Small founding numbers suggests a single introduction, likely via a baitbucket, and thus Eel River Sacramento pikeminnow contrasts with successful invaders established by multiple founding sources and large founder numbers (Stepien et al. 2005; reviewed in Roman and Darling 2007; Brown and Stepien 2009).

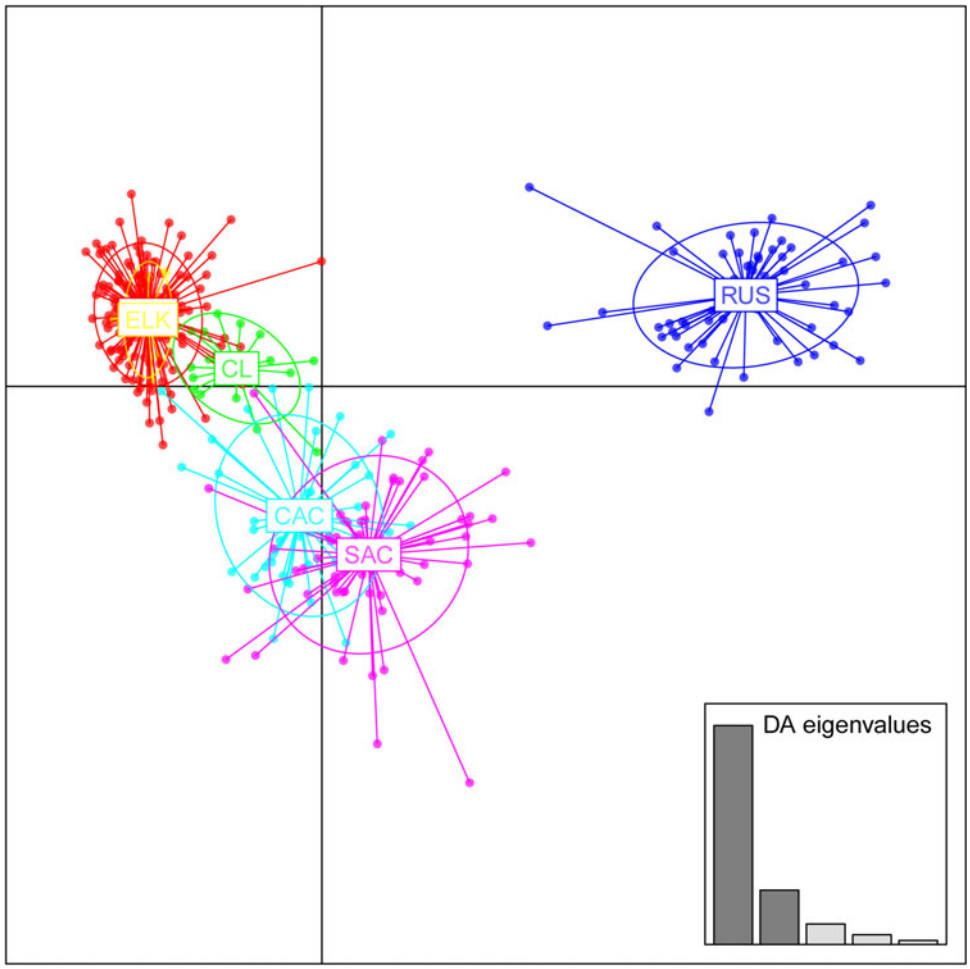

Fig. 3 Scatterplot of the first two principal components from DAPC. Populations are labeled inside their 95 % inertia ellipses and points represent individuals. The inset indicates the eigenvalues of the first five principal components. The introduced EEL population is superimposed by the introduced ELK population

Both nuclear microsatellite and mitochondrial analyses resolved genetic similarity between the introduced Eel River Sacramento pikeminnow and two geographically proximate source populations from the native range: Clear Lake and Cache Creek. Although the two analyses left ambiguity about the precise origin of the Eel River population, it seems clear that the introduced population is derived from an adjacent drainage within the native range. The microsatellite analysis identified Clear Lake fish as those most closely related to the Eel River population, while the mitochondrial DNA analysis pointed to a population from elsewhere in the same drainage, Cache Creek: the Cache Creek population shared a mitochondrial DNA haplotype with the introduced population that was not present in any other population in the native range. The other likely source population, Clear Lake, lacked this haplotype, but this result may be due to the low frequency of this haplotype in native populations and associated sampling errors (Muirhead et al. 2008).

Small founding numbers in species introductions are expected to result in shifts in allele frequencies and losses of genetic variation (Dlugosch and Parker 2008; Spencer et al. 2000). Consistent with this hypothesis, the nuclear microsatellite analysis resolved the Eel River as distinct from all other native populations in estimates of pairwise genetic differentiation, neighbor-joining trees, multivariate plots, and Bayesian clustering analyses. Similarly, in the mitochondrial DNA analysis, one haplotype occurred at a higher frequency in the Eel River than the most likely source, Cache Creek (Fisher's Exact Test, $P < 0.05$). However, we did not resolve significant losses in heterozygosity in the introduced population and only one of the two likely source populations (Clear Lake) exhibited less allelic variation than the introduced population. We suspect that accumulation of new genetic diversity in the generations since introduction, the relative insensitivity of heterozygosity to founder effects (Spencer et al. 2000), especially when recovery is rapid (Nei et al. 1975), and possible population bottlenecks in the Clear Lake source population contributed to this result.

Multiple examples of invasive fish populations founded by few individuals (Carvalho et al. 1996; Kalinowski et al. 2010; Kinziger et al. 2011, this study) raise the possibility that freshwater fishes have relatively strong capabilities in this regard. Allee Effects that can limit invasion success (Taylor and Hastings 2005; Drake and Lodge 2006) have

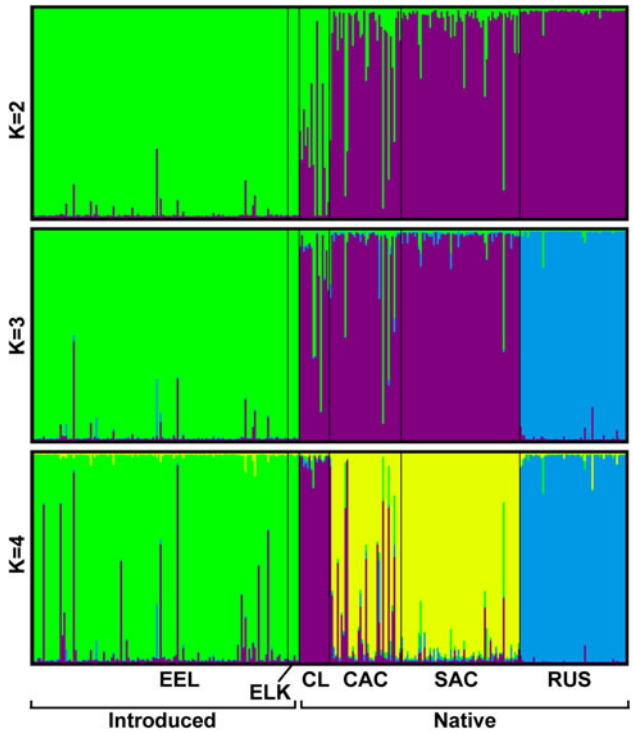

Fig. 4 The proportion of each individual's genome assigned to each of two, three, and four clusters inferred by Bayesian cluster analysis with STRUCTURE. *Vertical black lines* distinguish populations

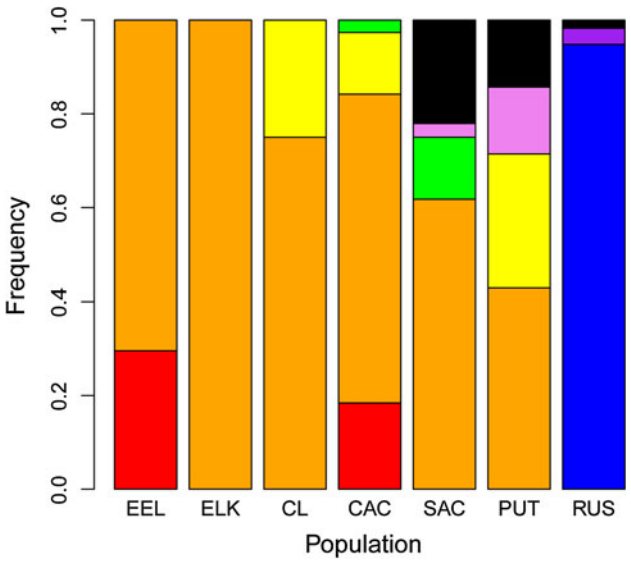

Fig. 5 Mitochondrial haplotype frequencies in introduced and likely source populations of Sacramento pikeminnow. Hap_1 (*red*), Hap_2 (*orange*), Hap_3 (*yellow*), Hap_4 (*green*), Hap_5 (*blue*), Hap_6 (*purple*), Hap_14 (*violet*), unique haplotypes grouped in *black*. Haplotype definitions in Table S4. (Color figure online)

Table 2 Population, sample ID, microsatellite DNA results [sample size (n), rarified number of private alleles (A_P), rarified allelic richness (A_R), allelic richness (A), observed heterozygosity (H_O), and expected heterozygosity (H_E)], and mitochondrial DNA results [sample size (n), number of haplotypes (N_H), number of private haplotypes (N_{pH}), haplotype diversity (h), and nucleotide diversity (π)] in Sacramento pikeminnow

Water body	ID	Microsatellite DNA						Mitochondrial DNA				
		n	A_P	A_R	A	H_O	H_E	n	N_H	N_{pH}	h (±SD)	π (±SD)
Eel River	**EEL**	**135**	**0.25**[a]	**4.88**[a]	**6.4**	**0.64**	**0.66**[a]	**88**	**2**	**0**	**0.4211 (0.0404)**	**0.0766 (0.0502)**
Humboldt Bay	**ELK**	**6**	**–**	**–**	**3.3**	**0.79**	**0.62**[a]	**6**	**1**	**0**	**0**	**0**
Clear Lake	CL	16	0.78[a]	5.24[a]	5.3	0.60	0.62[a]	16	2	0	0.4000 (0.1135)	0.1273 (0.0794)
Cache Creek	CAC	38	1.77[b]	8.94[b]	12.0	0.71	0.77[a]	38	4	0	0.5292 (0.0795)	0.1356 (0.0805)
Sacramento River	SAC	63	2.54[b]	9.75[b]	15.7	0.77	0.79[a]	68	11	8	0.6001 (0.0651)	0.0943 (0.0568)
Putah Creek	PUT	0	–	–	–	–	–	7	4	1	0.8095 (0.1298)	0.2294 (0.1451)
Russian River	RUS	56	0.84[ab]	5.2[a]	7.0	0.67	0.66[a]	58	3	3	0.1010 (0.0535)	0.0076 (0.0115)

Introduced populations in bold

Genetic diversity metrics sharing letters exhibited nonsignificant differences in statistical tests

not been significant in invasions by several freshwater fishes. The mate-finding capabilities of fishes (e.g. Sorenson and Stacey 2004) may raise the probability of success for very small founding populations. It also seems likely that bottlenecks do not necessarily cause reductions in variation in ecologically important traits, so that establishment, spread, and adaptive evolution by invaders founded by small numbers is possible (Dlugosch and Parker 2008).

Our analysis provides a second example of a close-range introduction in that the occurrence of Sacramento pikeminnow in the Elk River likely represents a secondary range expansion from the introduced Eel River population. It seems most likely that Sacramento pikeminnow arrived in the Elk River by human activity, but natural colonization is also a possibility. Radio tracking of a sterilized Elk River Sacramento pikeminnow revealed downstream migration into saline waters (up to 15 ppt) during high streamflows

(pers. comm. Seth Ricker and Mike Wallace, California Department of Fish and Game). Another member of the genus *Ptychocheilus* has exhibited the ability to survive similar salinity (Nelson and Flickinger 1992). These findings suggest pikeminnow could have utilized a low-salinity marine pathway between the mouth of the Eel River and Elk River during extreme flooding.

Our investigation provides additional evidence that intracontinental invasions and local-scale introduction pathways deserve consideration. The establishment of ecologically important invasive populations from small numbers of founders moved short distances represents an important challenge for resource managers. This study also illustrates at least two ways such challenges can be exacerbated: 1) close-range introduction sites are likely to provide suitable habitat conditions facilitating establishment and rapid spread; and 2) the Bridgehead Effect, in which successful introduced populations can become the source for additional introductions (Lombaert et al. 2010).

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