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California roach (*Hesperoleucus symmetricus*) in the Eel River of northwestern California: native or introduced?

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Abstract To explore the history of California roach (*Hesperoleucus symmetricus*) in the Eel River, we compiled mitochondrial DNA data for the putatively introduced Eel River population and comparative collections from throughout the native range. Consistent with an introduction scenario, we found that: 1) one haplotype occurred at high frequency in the Eel River, Russian River and Clear Lake populations, making the Russian River and Clear Lake likely source areas, and 2) the introduced population exhibited reduced haplotype diversity in comparison to populations from the native range. However, we also detected four private haplotypes in the putatively introduced population, despite examining 269 individuals from the likely source areas. Extrapolation of the haplotype richness of the likely source population suggested that even with the large sample size, many haplotypes in the source population remained uncollected. The most parsimonious conclusion of our results is a recent introduction of a small number of California roach to the Eel River of California from a nearby drainage. This result aligns with findings for two other California cyprinids introduced into the Eel River from adjacent drainages, Sacramento pikeminnow (*Ptychocheilus grandis*) and speckled dace (*Rhinichthys osculus*).

Keywords Invasion genetics · Mitochondrial DNA · Genetic diversity · Introduced species · California roach · *Hesperoleucus symmetricus*

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Introduction

Efforts to slow the rate of fish introductions would benefit from better understanding of invasion pathways. Much effort has focused on intercontinental introductions. However, particularly for drainages with relatively few species, the possibility of introductions from vicinal sources also deserves consideration. Even in cases of likely introduction from nearby sources, determining if a species is native or introduced, and identification of source populations, can be surprisingly difficult. Historic field surveys can inform questions about species' introductions, but these are subject to the problem of imperfect detection (e.g., MacKenzie et al. 2003). Modern genetic methods have greatly facilitated the identification of introduced species, but in some cases their application has left substantial uncertainty about whether a species is native or introduced (e.g., Scott et al. 2009; Rezansoff et al. 2015; see also Carlton 1996).

The presence of California roach (*Heperoleucus symmetricus*) in the Eel River of northwestern

California represents the challenges that can arise in efforts to explain current freshwater fish distributions from observational data. The species is currently abundant and broadly distributed in the Eel Drainage. But California roach were not detected by a foundational study of fish distributions in coastal Oregon and northern California at the end of the nineteenth Century (Snyder 1908), nor by a variety of surveys conducted in the 1930s and 1950s in many portions of the Eel River drainage with water temperature suitable for California roach (e.g., CDFG 1934, 1959a). In 1959, California Department of Fish and Game personnel recorded "unidentified cyprinids" in the Eel River between Cape Horn Dam and Van Arsdale Dam, the first documentation of cyprinids in the basin (CDFG 1959b). The first record of California roach in the Eel River drainage comes from an October 1967 collection in Yager Creek analyzed by W. I. Follett, then the Curator of Fishes at the California Academy of Sciences (Follett 1968). California roach were not found at this site in a survey conducted three years earlier (CDFG 1964). In 1968, cyprinids were recorded from Bull Creek, a tributary of the lower South Fork Eel River (California Department of Fish and Game (CDFG) Field Note 1968); 1973 and 1974 surveys of the same tributary specifically identify California roach (CDFG Field Note 1973, 1974). Additional surveys in the 1970's suggest a broadening distribution of California roach in the Eel River. The species was apparently encountered at multiple sites in the Van Duzen and South Fork Eel rivers in 1971 and was abundant at South Fork Eel River sites in 1972 (Fite 1973). However, California roach apparently remained absent in the early 1970s from parts of the drainage where they are now abundant, such as the North Fork of the Eel River (e.g., CDFG 1972a, b). Also, as might be expected for an introduced population, California roach in the Eel River are not found in reaches above a barrier to upstream migration on the Van Duzen River that support Sacramento sucker (*Catostomus occidentalis*), an obligate freshwater species apparently native to the basin. A recent range-wide genomics study of California roach has concluded that California roach in the Eel Drainage were introduced (Baumsteiger et al. 2017).

Several other observations make the apparent nonnative status of California roach in the Eel River intriguing. Geologic evidence indicates that approximately 2 million years ago, portions of the current Eel River drainage flowed southward into the Russian River (Lock et al. 2006), providing a possible colonization opportunity. Also, the native range of California roach includes several smaller coastal drainages south of the Eel River and north of the Russian River (Moyle 2002).

We studied mitochondrial DNA variation in California roach from the Eel River and surrounding native populations in an attempt to clarify the origin of the Eel River population. First, we estimated genetic distances and constructed statistical parsimony networks to resolve genetic relationships and identify the likely source populations. Second, we compared the genetic diversity of the Eel River population to surrounding native populations to determine if there were reductions in genetic diversity consistent with expectations for introduced populations. Population genetics predicts that introduced species should experience founder effects as indicated by shifts in haplotype frequency and reductions in diversity in comparison to the source population (Dlugosch and Parker 2008). We included collections from the entire geographic range of California roach because the accuracy of assignment of sources populations depends upon having sampled all potential sources as well as the degree of genetic differentiation among them (Muirhead et al. 2008).

Materials and methods

We compiled mitochondrial DNA sequence data from a total of 541 individuals, combining 164 individuals from Aguilar and Jones (2009) with an additional 377 individuals collected and sequenced for this study (Fig. 1, Table 1). Samples used in this study were collected across a 12 year span, ranging from 2004 to 2016. Aguilar and Jones (2009) conducted a phylogenetic analysis of samples from throughout the range of California roach, providing a description of mitochondrial variation in eight putative subspecies. However, Aguilar and Jones (2009) did not include the Eel River in their study. We analyzed 108 individuals from the Eel River and bolstered samples sizes from possible source populations by adding 249 individuals from the Russian River (259 total), 12 individuals from the Sacramento River (67 total), and eight individuals from the Navarro River (18 total). We collected specimens for this study by seining or backpack electrofishing. Whole specimens or tissue were preserved in 95% ethanol. All whole specimens were deposited into the Humboldt State University Fish Collection.

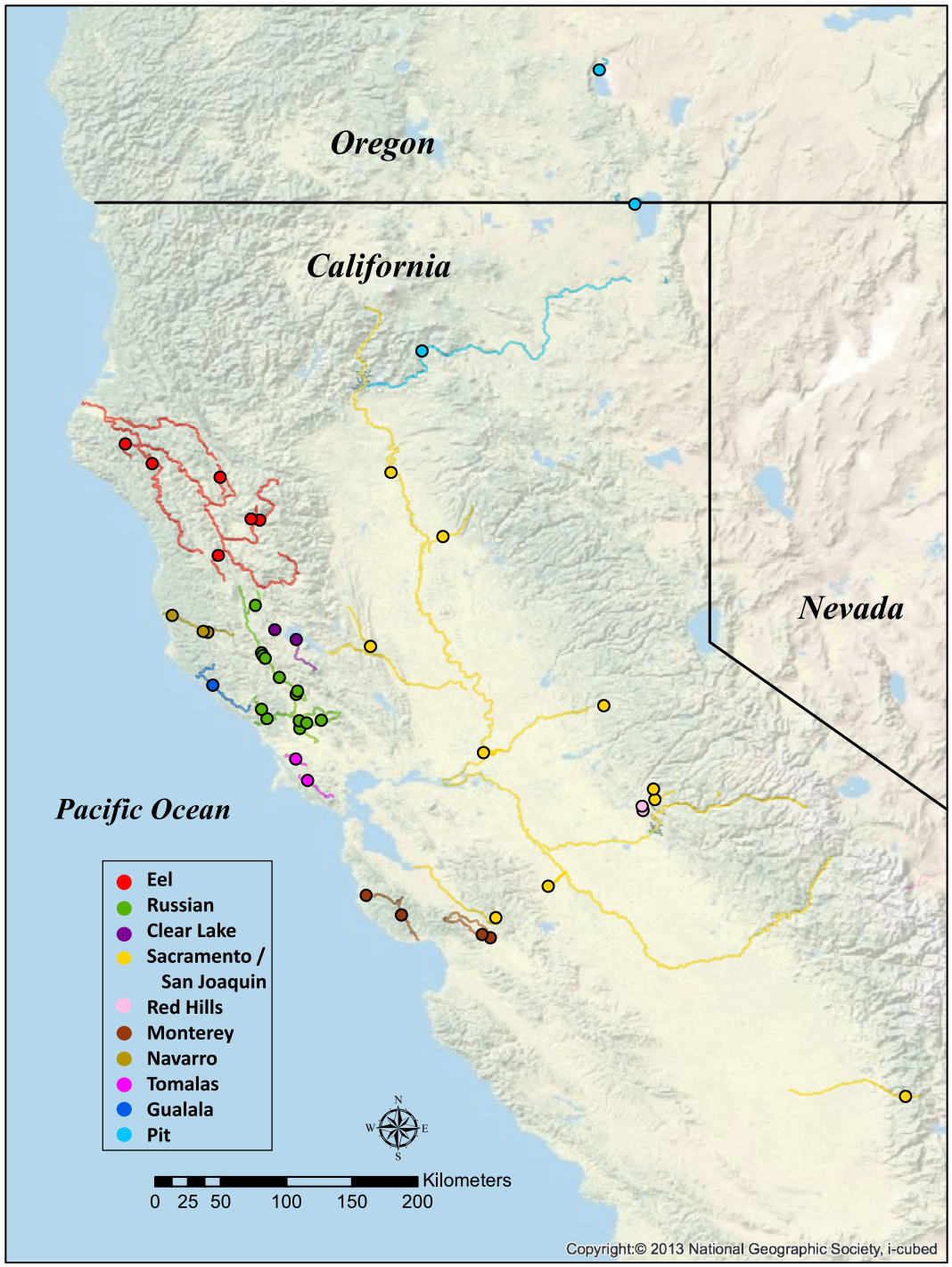

Fig. 1 The distribution of collection locations used for mitochondrial DNA analysis of California roach

Whole genomic DNA was extracted from fin tissue using chelex methods (Walsh et al. 1991). We sequenced a 615-base-pair fragment of mitochondrial DNA data that included 322 base pairs of the NADH-2 subunit and 293 base pairs of control region. Amplification primers, thermocycling temperatures and times,

Table 1 Group, sample location, number of fish sequenced, longitude, latitude and accession numbers for Humboldt State University (HSU) Fish Collection when available for California roach used for mitochondrial DNA analysis

Group	Location	N	Longitude	Latitude	Accession number
Eel	Bull Creek	8	−124.014	40.35151	HSU 5408
Eel	East Branch South Fork	8	−123.781	40.07403	HSU 5402
Eel	South Fork Eel River	21	−123.814	40.21778	HSU 5404
Eel	Van Duzen River	18	−123.963	40.48444	HSU 5403
Eel	Williams Creek	5	−123.144	39.83133	
Eel	Black Butte River	6	−123.084	39.82355	
Eel	Outlet Creek	19	−123.381	39.59460	HSU 5400
Eel	North Fork Eel River	19	−123.350	39.93772	HSU 5405
Eel	Salt Creek	4	−123.366	40.13139	
Russian	East Fork Russian River	1	−123.123	39.24722	HSU 5410
Russian	Russian River	6	−123.055	38.88477	HSU 5411
Russian	Russian River	52	−123.057	38.89805	HSU 5373
Russian	Russian River	10	−123.056	38.91484	HSU 5372
Russian	Russian River	15	−122.830	38.65822	HSU 5407
Russian	Russian River	10	−122.842	38.62405	
Russian	Russian River	2	−122.968	38.76487	HSU 5401
Russian	Santa Rosa Creek	36	−3122.777	38.44583	HSU 5375
Russian	Santa Rosa Creek	34	−122.776	38.44517	HSU 5377
Russian	Austin Creek	19	−123.047	38.47125	HSU 5409
Russian	Austin Creek	22	−123.088	38.52375	HSU 5639
Russian	Mark West Creek	5	−122.823	38.48544	HSU 5371
Russian	Brush Creek	45	−122.677	38.46359	HSU 5393
Russian	Laguna de Santa Rosa	2	−122.818	38.4025	HSU 5406
Clear Lake	Kelsey Creek	5	−122.835	39.01102	
Clear Lake	Hendricks Creek	5	−122.988	39.08031	
Sacramento/San Joaquin	Sacramento River	8	−122.200	40.15292	
Sacramento/San Joaquin	Little Chico Creek	10	−121.839	39.72041	
Sacramento/San Joaquin	Bear Creek	4	−122.341	38.96389	
Sacramento/San Joaquin	Sacramento River	5	−121.557	38.23321	
Sacramento/San Joaquin	Cosumnes River	10	−120.741	38.54807	
Sacramento/San Joaquin	Woods Creek	5	−120.390	37.98152	
Sacramento/San Joaquin	Curtis Creek	5	−120.388	37.91531	
Sacramento/San Joaquin	Deer Creek	10	−118.673	35.87990	
Sacramento/San Joaquin	Orestimba Creek	10	−121.118	37.32199	
Sacramento/San Joaquin	Coyote Creek	5	−121.477	37.10890	

Table 1 (continued)

Group	Location	N	Longitude	Latitude	Accession number
Red Hills	Horton Creek	5	−120.454	37.85752	
Red Hills	Roach Creek	5	−120.459	37.84785	
Monterey	San Lorenzo River	10	−122.121	37.12762	
Monterey	Pescadero Creek	1	−121.604	36.92075	
Monterey	Uvas Creek	5	−121.571	36.99166	
Monterey	Llagas Creek	5	−121.512	36.97641	
Navarro	Navarro River	5	−123.694	39.17838	
Navarro	Navarro River	8	−123.464	39.07484	
Navarro	Indian Creek	5	−123.442	39.05795	
Tomalas	Walker Creek	5	−122.849	38.19636	
Tomalas	Lagunitas Creek	5	−122.776	38.05036	
Gualala	Gualala River	10	−123.415	38.69994	
Pit River	Ana River	10	−120.769	42.90320	
Pit River	Dry Creek	10	−120.517	41.99502	
Pit River	Pit River	3	−121.971	40.99311	

and sequencing primers followed Aguilar and Jones (2009). DNA sequence data were generated at High-Throughput Sequencing Solutions (University of Washington, Department of Genome Sciences) or MCLAB (San Francisco, California). The DNA fragments generated herein were aligned with sequences from Aguilar and Jones (2009) using the software CLUSTALX2 (Larkin et al. 2007).

For data analysis we placed samples into 10 groups, including the putatively introduced Eel River collection, the eight subspecies recognized in Aguilar and Jones (2009), and one additional group separating samples from the Russian River and Clear Lake. The latter division was made to provide finer geographic resolution for pinpointing the likely source area(s). While Aguilar and Jones (2009) did not resolve a genetic distinction between these areas, Baumsteiger and Moyle (2019) recently concluded from a genomics study that Russian River roach should be placed in a new species, Coastal Roach (*Hesperoleucus venustus*), and that Clear Lake populations are introgressed between Coastal Roach and California Roach. To examine patterns of genetic differentiation and identify the likely source population(s), we calculated the average number of nucleotide differences between pairs of sites using ARLEQUIN 3.5.1.3 (Excoffier and Lischer 2010) and examined the evolutionary relationships among haplotypes by constructing a statistical parsimony network using the software PopART v1.7 (Clement et al. 2000; Leigh and Bryant 2015). The number of haplotypes, number of private haplotypes, haplotype diversity, nucleotide diversity and mean number of pairwise differences within each site were calculated using the software ARLEQUIN 3.5.1.3.

To aid in interpreting the significance of the haplotypes unique to the Eel drainage, particularly in light of the extreme diversity exhibited by California roach in the Russian River, we sought to estimate the haplotype richness of the likely source population. We used the estimator of Chiu et al. (2014; iChao1), which uses the information in the frequency of rare types (singleton, doubletons, tripletons and quadrupletons) in a sample to estimate total richness. We also used extrapolation methods (Colwell et al. 2012; Chao et al. 2016) to explore the relation between sample size and haplotype richness for California roach in the Russian River. For comparison, we conducted parallel analyses on the Eel River data.

Results

The final data set was composed of mitochondrial DNA sequence data (control region plus NADH-2 genes) for a total of 541 California roach. The data included 118 polymorphic sites, 93 sites that informed the parsimony analysis, and 119 different haplotypes.

Eel River California roach exhibited the fewest average pairwise differences with Clear Lake (1.0) and the Russian River (1.8) and diverged more strongly from the remaining California roach we examined (average pairwise difference ranging from 11.4 to 65.3; Table 2). In the parsimony network, haplotypes from the Eel River, Russian River, and Clear Lake clustered into a group separated by five nucleotide substitutions from all other groups (Fig. 2). All haplotypes resolved from the Eel River and Clear Lake were associated with this group. In contrast, haplotypes from the Russian River also occurred outside of this group.

The 108 individuals examined from the Eel River yielded seven haplotypes: four private haplotypes, two shared with the Russian River, and one haplotype shared with both the Russian River and Clear Lake drainages (Fig. 2). The last haplotype was the most common haplotype in all three drainages (Eel River: 73%; Russian River: 25%; Clear Lake: 30% [a second haplotype also occurred at 30% frequency in Clear Lake]). One haplotype unique to the Eel River occurred at relatively high frequency (21%); it differed from one Russian River haplotype by two substitutions. The Eel River population exhibited markedly reduced mitochondrial DNA diversity, especially in comparison to the likely source areas (e.g., Russian River and Clear Lake; Table 3). The Russian River collections exhibited the highest mitochondrial DNA diversity of all populations included in the dataset.

Estimates of haplotype richness suggested that our relatively robust sample sizes from the Russian and Eel rivers left a high probability of unsampled haplotypes and the extrapolated relationship between sample size and haplotype richness suggested a significant number of unsampled haplotypes would remain under any practical sampling effort (Fig. 3). The 259 individuals from the Russian River yielded 56 haplotypes, with many haplotypes represented by few individuals. The lower bound of the 95% confidence interval for the iChao1 estimate of haplotypes in the Russian River was 97. In contrast, the lower bound of the 95% confidence interval for haplotype richness in the Eel River was 8.

Discussion

Our results for California roach from the Eel River reflect patterns expected for a recent introduction of a small number of individuals from a nearby drainage. We observed both low genetic diversity in the Eel River population and haplotype sharing with populations in nearby drainages, the Russian River and Clear Lake. The minimal level of differentiation between the Eel River and Russian River / Clear Lake contrasted sharply with the highly differentiated populations of California roach in two nearby coastal drainages with apparently native populations, the Navarro and Gualala rivers

Table 2 Average number of pairwise differences between sites (above diagonal), average number of pairwise differences within sites (bolded, diagonal elements) and corrected average pairwise differences (below diagonal) for California roach

Group	EEL	RUS	CL	SAC	RH	MONT	NAV	TOM	GUAL	PIT
Eel River	**1.5**	8.3	4.3	16.8	13.2	17.7	21.8	22.4	42.7	68.2
Russian River	1.8	**11.4**	1.0	4.7	12.1	15.8	20.2	21.2	44.7	66.1
Clear Lake	1.0	9.2	**5.0**	8.2	11.7	16.2	20.7	20.5	43.4	67.7
Sacramento / San Joaquin	11.4	15.0	15.3	**9.2**	8.1	9.5	15.2	16.8	46.7	65.3
Red Hills	11.6	5.5	8.3	2.6	**1.8**	8.6	13.3	15.0	44.0	63.2
Monterey	14.0	7.1	10.7	2.0	4.7	**5.9**	15.6	16.7	43.9	64.2
Navarro	16.5	10.0	13.8	6.1	7.9	8.1	**9.0**	19.3	44.3	71.2
Tomales	20.4	14.2	16.8	11.0	12.9	12.5	13.6	**2.4**	47.4	62.5
Gualala	41.0	38.0	40.0	41.2	42.2	40.0	38.8	45.3	**1.9**	80.9
Pit	65.3	58.2	63.1	58.6	60.1	59.2	64.6	59.2	77.9	**4.2**

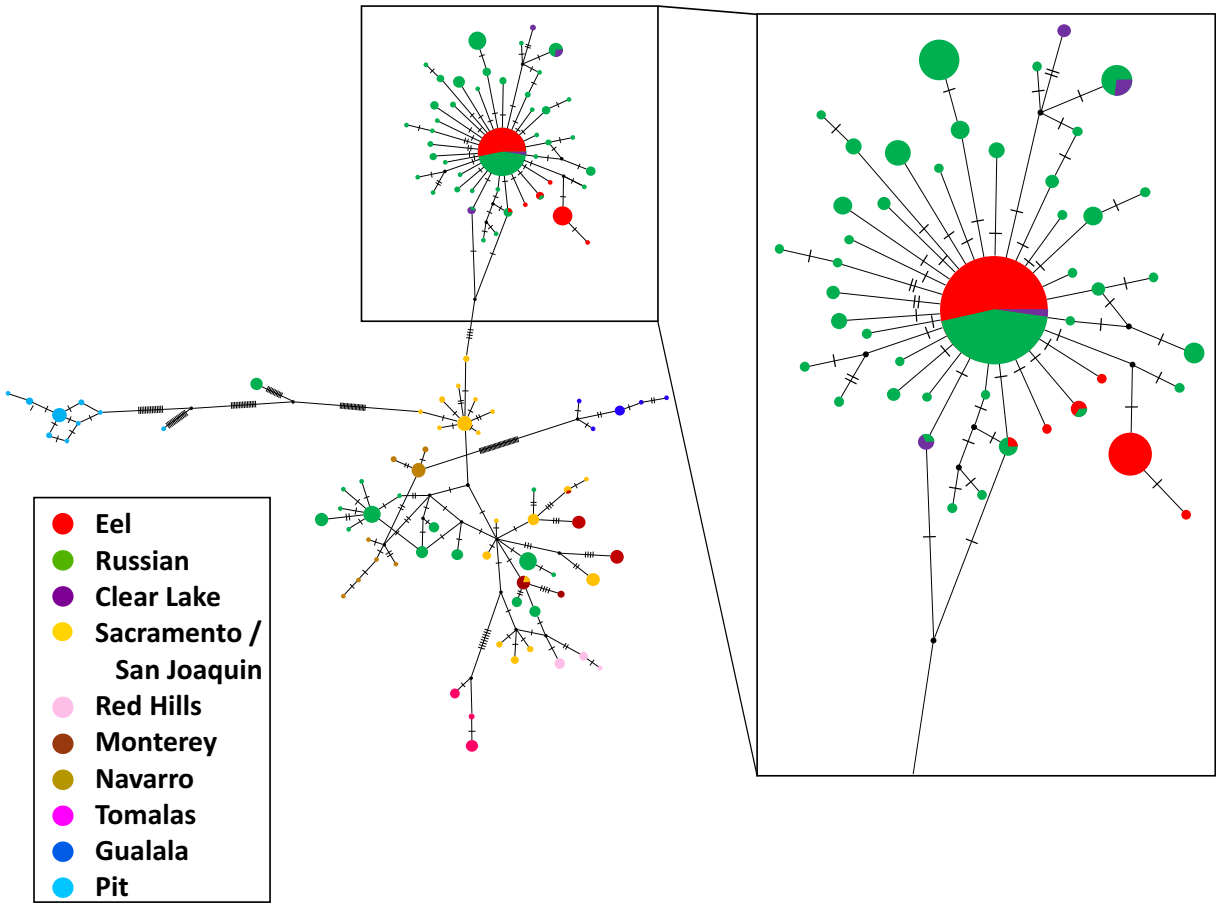

Fig. 2 Statistical parsimony network for California roach constructed using mitochondrial DNA. The slashes indicate nucleotide substitutions and the dots indicate inferred substitutions. The circle diameters are proportional to haplotype frequency. The enlarged cluster (right) includes all haplotypes identified from the introduced Eel River population, and haplotypes from the Russian River and Clear Lake

(Aguilar and Jones 2009; this study). The most parsimonious conclusion of our results and historical field surveys is a recent introduction of California roach to the Eel River of California from the adjacent areas (Russian River/Clear Lake). The results of this study concur with Baumsteiger et al. (2017), who concluded from a genomics analysis that the Eel River California roach population is most likely a recent introduction from the Russian River.

Two results superficially conflict with the conclusion that the Eel River population is introduced. The first is the occurrence of four unique mtDNA haplotypes in the Eel Drainage. However, all four of those haplotypes are closely related to those from the Russian River, and our analysis makes clear that we did not approach a complete sampling of Russian River mtDNA haplotypes. Finding unique mtDNA haplotypes in populations certain to be introduced is not unusual (e.g., Hänfling et al. 2002; Kelly et al. 2006; Vidal et al. 2010; Johnson et al. 2011; Marques et al. 2016), particularly when source populations include an abundance of haplotypes (Blakeslee et al. 2008), and sampling in the source populations is insufficient to recover all possible haplotypes. Reliable conclusions about native status using rare haplotypes or alleles requires adequate sample sizes and appropriate statistical approaches (Anderson 1999; Pereira et al. 2004; Spies et al. 2007). The second superficially problematic result is that one of the four unique haplotypes was so well-represented (21%) in our Eel River sample. However, this result also becomes unsurprising given that the population was highly

Table 3 Mitochondrial DNA diversity in California roach

Group	Sample size	Number of Haplotypes	Number of private haplotypes	Haplotype diversity	Nucleotide diversity	Mean number of pairwise differences
Eel River	108	7	4	0.42 ± 0.05	0.0025 ± 0.0017	1.5 ± 0.9
Russian River	259	56	50	0.91 ± 0.01	0.0186 ± 0.0094	11.43 ± 5.2
Clear Lake	10	4	0	0.82 ± 0.07	0.0081 ± 0.0049	5.0 ± 2.6
Sacramento / San Joaquin	67	20	18	0.91 ± 0.02	0.0149 ± 0.0077	9.2± 4.3
Red Hills	10	3	3	0.60 ± 0.13	0.0029 ± 0.0021	1.8 ± 1.1
Monterey	21	4	2	0.67 ± 0.07	0.0096 ± 0.0053	5.9 ± 2.9
Navarro	18	8	8	0.64 ± 0.13	0.0147 ± 0.0079	9.0 ± 4.3
Tomales	15	3	3	0.63 ± 0.09	0.0040 ± 0.0026	2.4 ± 1.4
Gualala	10	5	5	0.67 ± 0.16	0.0030 ± 0.0022	1.9 ± 1.2
Pit	23	9	9	0.76 ± 0.09	0.0069 ± 0.0039	4.2 ± 2.2

Group, sample size, number of haplotypes, number of private haplotypes, haplotype diversity, nucleotide diversity, and mean number of pairwise differences within each site

likely to have had few founders, a process that could have resulted in a rare haplotype drifting to high frequency. The shift in haplotype frequency and overall low genetic diversity in the Eel River sample are consistent with a single introduction of a small number of individuals. Multiple introductions, including repeated events from the same source, are expected to homogenize haplotype frequencies and elevate haplotype diversity in introduced species (Dlugosch and Parker 2008). Two other populations of introduced cyprinids in the Eel River were likely to have been founded by very few individuals (Kinziger et al. 2011, 2014).

This study provides additional evidence that small-scale introductions from nearby sources can generate large, widespread introduced populations. In addition to the likely introduction of California roach, the Eel River has gained two other introduced cyprinid populations via introduction of a few individuals from nearby drainages: Sacramento pikeminnow (*Ptychocheilus grandis*), most likely from the Clear Lake / Cache Creek Drainage

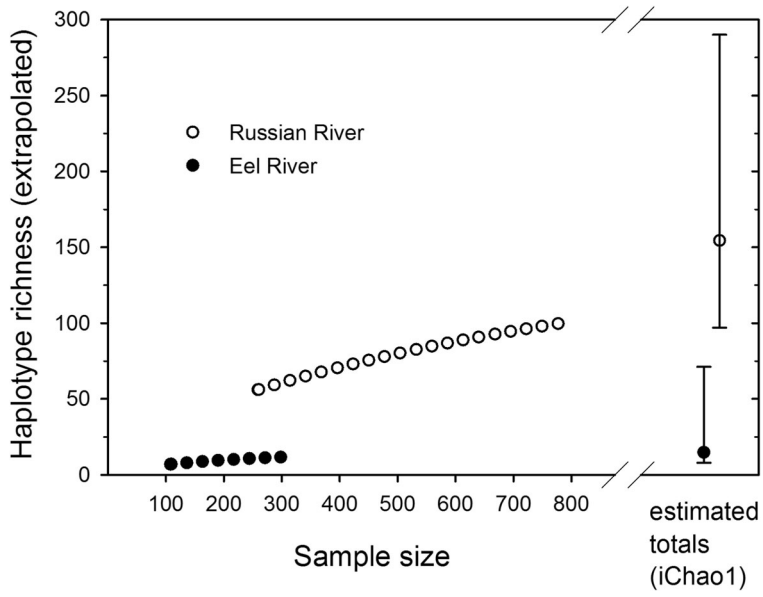

Fig. 3 Extrapolation of California roach mitochondrial DNA haplotype richness and estimation of total haplotype richness (iChao1) in the Eel and Russian rivers, following methods in Colwell et al. (2012) and Chiu et al. (2014)

(Kinziger et al. 2014) in about 1979 (Brown and Moyle 1997); and speckled dace (*Rhinichthys osculus*), from the adjacent South Fork of the Trinity River (Kinziger et al. 2011) before 1986 (Brown and Moyle 1997). All three introduced cyprinids established Eel River populations despite substantial reductions in genetic diversity, supporting the conclusion that genetic diversity among introduced individuals may not be an important predictor of invasion success (Sakai et al. 2001; Lindholm et al. 2005; Yonekura et al. 2007; Dlugosch and Parker 2008; Dlugosch et al. 2015).

Neighboring drainages deserve consideration as source populations for several reasons. The series of cyprinid introductions into the Eel River highlights that neighboring drainages must be included when attempting to identify prospective introductions worthy of attention and when assessing the potential for cryptic introductions. The Eel River example also supports the expectation that introduced fish from neighboring drainages are relatively likely to find suitable physical habitat: both the California roach and Sacramento pikeminnow are abundant and broadly distributed in the Eel Drainage. That all three cyprinids have now inhabited the Eel Drainage for at least several decades suggests they are likely to persist, although the speckled dace apparently remains limited in distribution, perhaps by biotic interactions (Harvey et al. 2004).

In contrast to the recent genomics study by Baumsteiger et al. (2017) that included 40 individuals from the Eel River, we found no evidence of multiple introductions from different source populations. A motivation for multiple introductions is not apparent, given the broad distribution and abundance in the Eel Drainage achieved by fish from apparently one source. While multiple introductions and high levels of propagule pressure can result in high levels of genetic diversity in introduced populations (Roman and Darling 2007), these factors do not appear to be important to the success of introduced cyprinids in the Eel River.

Cyprinids and catostomids are patchily distributed among coastal drainages in California (Moyle 2002). These distributions and the likely successful introductions of three cyprinids to the Eel River suggest high likelihood of future introductions. Additional introductions of Sacramento pikeminnow into coastal drainages could cause significant ecological harm, in that the species can consume (White and Harvey 2001; Nakamoto and Harvey 2003) and compete (Reese and Harvey 2002) with native fishes. The consequences for native fishes of additional introductions of smaller-bodied species such as California roach and speckled dace remain less clear.

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