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Genetic structure and historic demography of endangered unarmoured threespine stickleback at southern latitudes signals a potential new management approach

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Abstract

Habitat loss, flood control infrastructure, and drought have left most of southern California and northern Baja California's native freshwater fish near extinction, including the endangered unarmoured threespine stickleback (*Gasterosteus aculeatus williamsoni*). This subspecies, an unusual morph lacking the typical lateral bony plates of the *G. aculeatus* complex, occurs at arid southern latitudes in the eastern Pacific Ocean and survives in only three inland locations. Managers have lacked molecular data to answer basic questions about the ancestry and genetic distinctiveness of unarmoured populations. These data could be used to prioritize conservation efforts. We sampled *G. aculeatus* from 36 localities and used microsatellites and whole genome data to place unarmoured populations within the broader evolutionary context of *G. aculeatus* across southern California/northern Baja California. We identified three genetic groups with none consisting solely of unarmoured populations. Unlike *G. aculeatus* at northern latitudes, where Pleistocene glaciation has produced similar historical demographic profiles across populations, we found markedly different demographics depending on sampling location, with inland unarmoured populations showing steeper population declines and lower heterozygosity compared to low armoured populations in coastal lagoons. One exception involved the only high elevation population in the region, where the demography and alleles of unarmoured fish were similar to low armoured populations near the coast, exposing one of several cases of artificial translocation. Our results suggest that the current "management-by-phenotype" approach, based on lateral plates, is incidentally protecting the most imperilled populations; however, redirecting efforts toward evolutionary units, regardless of phenotype, may more effectively preserve adaptive potential.

KEYWORDS
conservation, historical demography, lagoon, population structure, sequentially Markovian coalescent, Southern California Bight

1 | INTRODUCTION

Some of the most highly modified habitats in southwestern North America include rivers and streams (Benke, 1990; Minckley & Marsh, 2009; Pringle et al., 2000; Propst et al., 2021; Ricciardi & Rasmussen, 1999), particularly in the southern California-northern Baja California coast ecoregion, where lowland reaches of major rivers are now channelized in concrete and flows are regulated via dam releases from artificial lakes and reservoirs (Gumprecht, 1999; Orsi, 2004). This infrastructure was developed to protect the rapidly growing human population of the mid-20th century from flood damage and to store and divert water in a landscape that was largely devoid of perennial water (Stephenson & Calcarone, 1999; Van Wormer, 1991). However, the effects on the native freshwater fish fauna have been dramatic, reducing the amounts of suitable habitat (Faber et al., 1989; Stephenson & Calcarone, 1999; Swift et al., 1993), eliminating the capacity for gene exchange within and between drainage basins (Benjamin et al., 2016; Richmond et al., 2018), and altering the dynamics of an ecosystem with historically ephemeral and intermittent surface water (Levick et al., 2008; Richmond et al., 2018). Climatic stressors that promote oscillating demography (e.g., strong seasonal and multiyear variation in amounts of precipitation and duration of drought: Levick et al., 2008; Mount, 1995; Stephenson & Calcarone, 1999) and other factors may be accelerating losses of local genetic diversity and pushing these species closer to the brink of extinction (Moyle et al., 2015). Because most of the region's freshwater fish species lack genetic data to guide management (Leidy & Moyle, 2021), more studies are needed to characterize their ancestry, population structure, and historical demography to provide better snapshots of population dynamics prior to 20th century urbanization.

One of the most iconic taxa of this fauna is the unarmoured threespine stickleback, which lacks lateral bony plates, or "body armour", that typically line the flanks of the *G. aculeatus* species complex. The complex comprises marine, anadromous, and freshwater populations, and is distributed across the mid to high latitude in the Northern Hemisphere along the margins of the Pacific, Arctic and Atlantic oceans (Bell & Foster, 1994). Morphology varies widely across this distribution, with the unarmoured morph being the rarest of four recognized plate morphs (i.e., fully armoured, partial, low, and unarmoured: Bell, 1976; Miller & Hubbs, 1969). Fully armoured morphs have a continuous row of 30+ plates per side and predominantly occur in marine environments; partially armoured morphs have a discontinuous row of 11+ plates per side and occur in brackish water; and low and unarmoured morphs occur exclusively in freshwater and have fewer than 11 plates per side or no plates at all (Bell & Foster, 1994). Diversification of the plate phenotypes is often adaptive (reviewed in Bell, 2001; Colosimo et al., 2005; Hagen & Gilbertson, 1973; Reimchen, 1994, 2000). Plates are associated with greater piscivorous predation, while their absence is hypothesized to confer advantages in mobility, buoyancy, calcium availability and faster growth rates (possibly in avoidance of insect predators) (Barrett et al., 2008; Bell et al., 1993; Bell & Foster, 1994; Bergstrom, 2002).

Unarmoured populations were historically more widespread in riverine settings of coastal southern California, but most have become extirpated since the 1940s (Bell, 1978; Miller, 1961; Miller & Hubbs, 1969; Swift et al., 1993). Currently, native populations of unarmoured stickleback remain in three upstream areas: San Antonio Creek on Vandenberg Space Force Base (VSFB, Santa Barbara County); the upper Santa Clara River (Los Angeles County); and the upper Santa Ana River and Baldwin Lake drainage basin in the San Bernardino mountains (San Bernardino County) (USFWS, 2021; Figure 1). *Gasterosteus aculeatus williamsoni* (Girard, 1854) was described on the basis of specimens collected in the upper Santa Clara River in Soledad Canyon (Figure 1: nos. 25–26, type-locality). It was listed as endangered by the U.S. federal government in 1970 (35 Federal Register 16,047) and the state of California (https://wildlife.ca.gov/Conservation/CESA) in 1971. Limited habitat and continued population decline are described in a recovery plan for *G. a. williamsoni*, with status updates conducted every five years to describe current research, management efforts, and progress toward recovery of this endangered species.

Most conservation management efforts in southern California have focused on the unarmoured stickleback population occurring near the type locality in the upper Santa Clara River (USFWS, 2021). However, such emphasis on the phenotype may be misguided, as *G. aculeatus* is well-known for parallel evolution of different plate morphs. This phenomenon was poorly understood at the time the taxonomy was developed, but genetic data have since shown that transitions from fully armoured marine morphs (with over 30 plates) to partially or low armoured freshwater morphs (typically between 30 and one plate) have occurred repeatedly and independently following deglaciation at northern latitudes over the last ~15,000 years (Deagle et al., 2013; Hohenlohe & Magalhaes, 2020; Jones et al., 2012; Reimchen, 1994; Schluter, 2000). Parallel evolution is widely viewed as a phylogenetic signature of adaptive trait evolution (Schluter & Nagel, 1995), as standing genetic variation in closely related populations increases the probability that traits will evolve in the same way in similar environments (Hendry, 2013; Morris et al., 2018; Peichel & Marques, 2017).

In this study, we used microsatellite markers and low coverage whole-genome sequencing (lcWGS) to characterize the genetic structure and historical demography of *G. aculeatus* in southern California, with emphasis on a geographic area known as the Southern California Bight (SCB; Figure 1). The SCB is formed by a 685 km arc of coastline along the west coast of the United States and Mexico, from Point Conception in California south to Punta Colonet in Baja California. Many upstream and now isolated populations of *G. aculeatus* occur within the SBC, including extant unarmoured populations in the upper Santa Clara (Figure 1: nos. 20–22, 25–26) and in the upper Santa Ana drainage in the San Bernardino Mountains (Figure 1: no. 28), while the third native unarmoured population occurs immediately to the north of the SCB in San Antonio Creek (no. 8) at VSFB. However, questions about the relationship between these unarmoured populations and the low armoured populations in the region remain unanswered. In addition, unarmoured
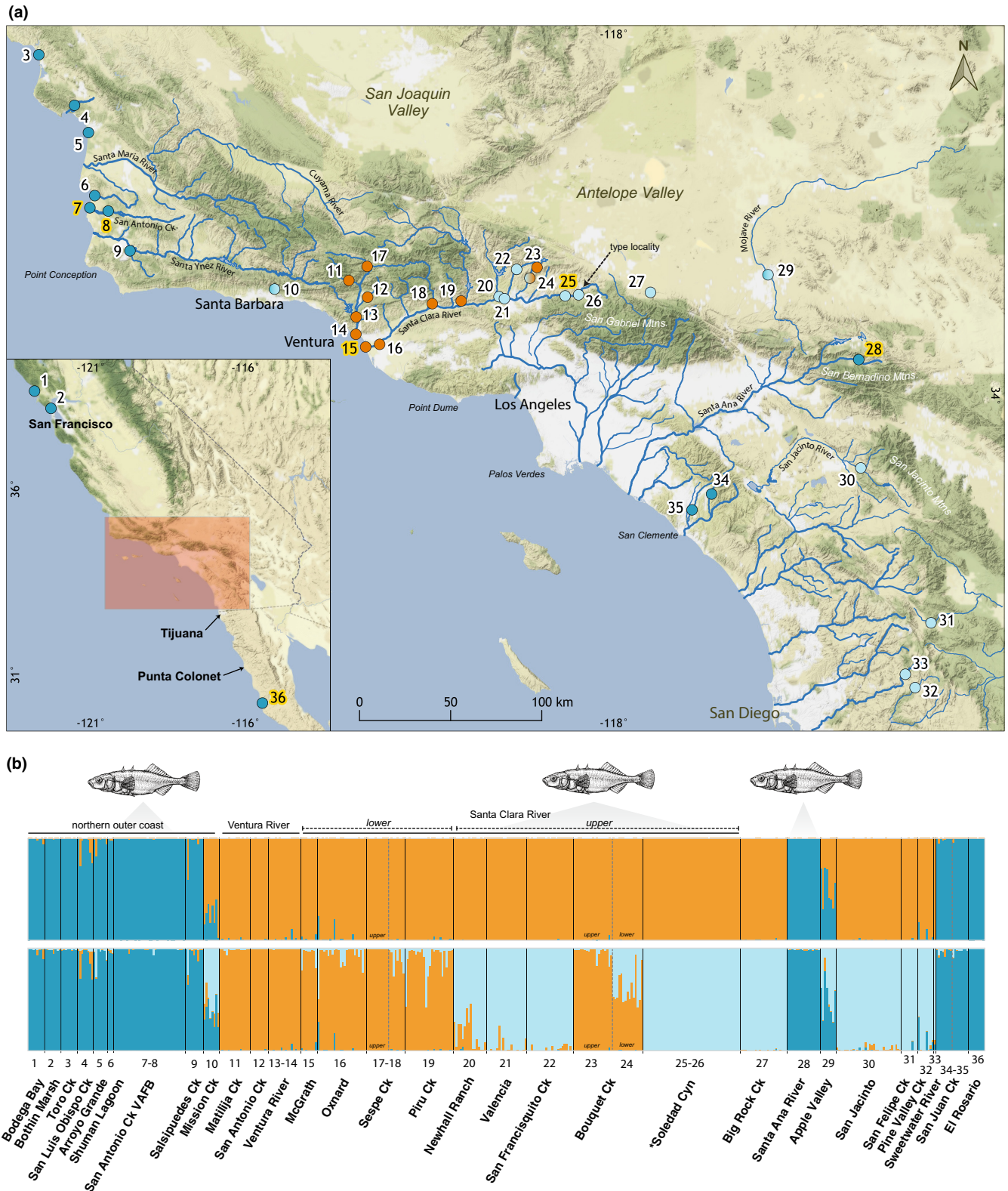
FIGURE 1 Map showing (a) sampling localities and (b) estimates of individual assignments (*K* = 2–3). (a) Red square on the map inset highlights the Southern California Bight (SCB). Sample locations (circles) are colour coded according to the assignments at *K* = 3, and numbers highlighted in yellow indicate the sites used for lcWGS. Locations of each sampling point are provided in Table 1. (b) (lnPD|*K*) and Δ*K* identified three as the best number of clusters, which roughly align with geography: Light blue, upstream Santa Clara River; orange, downstream Santa Clara and Ventura Rivers; dark blue, outer coast (OC) group, including the upper Santa Ana River. Cartoon fish lacking body armour denote the three extant populations of unarmoured *G. aculeatus*, which does not form a single cohesive group at any *K* (Figure S1). Lighter dashed lines in the assignment plots indicate upper and lower tributary reaches. Map was made on QGIS 3.16.2 and edited in Adobe Illustrator. River data set from the National Weather Service (NOAA)

fish have been transplanted from the type locality to other areas to protect against ecological instability and safeguard the genetic integrity of this population. Numerous inadvertent transplants have also occurred during trout stocking and many are not well documented. Given this complex history, comprehensive genetic data can help focus conservation efforts where they are needed most.

The broader goal of this work is to use modern genetic approaches to suggest a management strategy that more accurately reflects evolutionary units. Genomic data in this study are part of a larger, ongoing whole genome sequencing project on *G. aculeatus* throughout the region that will include more samples with higher sequencing coverage and address additional questions related to adaptive evolution. However, these current data are sufficient to address the following key questions: (1) What is the genetic structure of *G. aculeatus* at multiple geographic scales in southern California?; (2) Do unarmoured populations of *G. aculeatus* form a cohesive group when compared to other populations of *G. aculeatus* across the region?; (3) Do temporal trends in historical demography vary between up- and downstream reaches, and are the same trends mirrored across drainages?

2 | MATERIALS AND METHODS

2.1 | Sampling

We obtained tissue samples from monitoring surveys conducted by the U.S. Geological Survey (U.S. Federal Recovery Permits TE-045994, TE 793644–6 & 7; California State Scientific Collecting Permits SCP-2679, SCP-90); Mexican federal collecting permit (Permiso de Pesca de Fomento) DGOPA 14253.101005.6950, and its extension DGOPA 06435.210606.2640 issued by the Comisión Nacional de Acuacultura y Pesca of the Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA); professional colleagues (federal recovery and state scientific collecting permits available on request); and museum collections. Fish were captured using minnow traps, seines, and or dip nets and muscle tissue samples were stored in 95% ethanol. The data set included low and unarmoured *G. aculeatus* from 34 localities across southern California to what used to be the southernmost extent of the species range at Bocana El Rosario in northern Baja California, Mexico. We also included representatives of two fully plated populations collected in the San Francisco Bay area (Figure 1, Table 1).

We performed DNA extractions using a QIAGEN DNeasy Blood & Tissue Kit on muscle tissue and genotyped 470 individuals for 12 microsatellite loci known to be polymorphic in populations of *G. aculeatus* in southern California following the approach of Richmond et al. (2015). We then performed low coverage whole genome sequencing (lcWGS) on a subset of these samples from six locations (two individuals per location; $N = 12$). Three of the six locations involved each of the upstream, unarmoured populations, and the remaining three were from low armoured lagoonal populations (Figure 1; yellow highlighted dots). For two drainages, San Antonio

Creek-VSFB and the Santa Clara River, we could directly compare upstream unarmoured fish to low armoured lagoonal fish at the mouth. However, because unarmoured stickleback have been extirpated from the lower Santa Ana River, we compared the third unarmoured cohort from the upper part of this drainage at Sugarloaf Meadow (elevation 2055 m) to all lagoonal fish, including those from El Rosario in Baja California. Sugarloaf Meadow is one of three isolated ponds in the San Bernardino Mountains at ~2000 m elevation occupied by unarmoured morphs that are collectively referred to as "Shay Creek stickleback" (Haglund & Buth, 1988; Malcolm, 1992; Moyle, 2002).

2.2 | Microsatellites

We amplified microsatellites in sets of 10 µl multiplex reactions using a Qiagen multiplex PCR kit and 50–100 ng of DNA. We used negative controls in each PCR and ran a subset of the samples as repeats to verify the genotyping calls (i.e., positive controls). We performed genotyping runs on an ABI 3100S automated sequencer (Applied Biosystems) at the San Diego State University Microbiology Core Facility using a LIZ600 size standard (Applied Biosystems) and scored alleles using GeneMarker version 1.85 (Softgenetics LLC). We obtained a total of 261 alleles with 0.39% missing data.

2.3 | Population structure

We used STRUCTURE version 2.3.1 (Pritchard et al., 2000) on the microsatellite data to assign individuals to natural groups, test for admixture across populations, and identify populations that were established through artificial transplantation. We performed assignment tests using the uncorrelated allele frequencies setting for $K = 2$ through $K = 10$, running 25 iterations at each K for 500,000 replicates after a burnin of 500,000 iterations. We used the ΔK method (Evanno et al., 2005) to select the optimal K, but report the assignments at other K values in Supporting Information. To summarize the results, we generated alignments of the assignment coefficient matrices at each K using CLUMPAK (Kopelman et al., 2015).

We also used principal component analysis (PCA) as an exploratory tool to complement the results of the STRUCTURE analyses. We used the function dudi.pca in the adegenet package (Jombart & Ahmed, 2011) and ggplot2 in R version 3.6.2 (R Core Team, 2018) using RStudio version 2022.02.3 (RStudio Team, 2020) to generate the final PCA plot. We replaced missing data using the "mean" method and retained all axes.

2.4 | Genome sequencing

We used the sparQ DNA Frag & Library Prep Kit (Quanta Bio) to generate the libraries from the purified, extracted DNAs. From

TABLE 1 Location and plate phenotype data

Map ID	Population	Drainage	County	Genetic group	Armour phenotype	Lat	Long
1	Bodega Bay	Bodega Bay	Sonoma	OC	Fully armoured	38.327	−123.055
2	Bothin Marsh	Mill Valley	Marin	OC	Low armoured	37.888	−122.524
3	Toro Creek	Toro Creek	San Luis Obispo	OC	Low armoured	35.413	−120.872
4	San Luis Obispo Creek	San Luis Obispo Creek	San Luis Obispo	OC	Low armoured	35.209	−120.696
5	Arroyo Grande Creek	Arroyo Grande Creek	San Luis Obispo	OC	Low armoured	35.099	−120.628
6	Shuman Lagoon	Shuman Canyon	Santa Barbara	OC	Low armoured	34.845	−120.597
7*	San Antonio Creek Lagoon	San Antonio Creek	Santa Barbara	OC	Low armoured	34.795	−120.621
8*	San Antonio Creek-VAFB	San Antonio Creek	Santa Barbara	OC	Unarmoured	34.782	−120.530
9	Salsispuedes Creek	Santa Ynez River	Santa Barbara	OC	Low armoured	34.621	−120.423
10	Mission Creek	Mission Creek	Santa Barbara	OC-Ad	Low armoured	34.466	−119.710
11	Matilija Creek	Ventura River	Ventura	SCB-L	Low armoured	34.501	−119.344
12	San Antonio Creek	Ventura River	Ventura	SCB-L	Low armoured	34.433	−119.251
13	Seaside Park	Ventura River main stem	Ventura	SCB-L	Low armoured	34.282	−119.309
14	Foster Park	Ventura River main stem	Ventura	SCB-L	Low armoured	34.352	−119.307
15*	McGrath	Santa Clara River Lagoon	Ventura	SCB-L	Low armoured	34.230	−119.261
16	Oxnard	Santa Clara River main stem	Ventura	SCB-L	Low armoured	34.241	−119.192
17	Upper Sespe Creek	Santa Clara River	Ventura	SCB-L	Low armoured	34.558	−119.253
18	Lower Sespe Creek	Santa Clara River	Ventura	SCB-L	Low armoured	34.406	−118.932
19	Piru Creek	Santa Clara River	Ventura	SCB-L	Low armoured	34.417	−118.790
20	Newhall Ranch	Santa Clara River main stem	Los Angeles	SCB-U	Low armoured	34.435	−118.603
21	Valencia	Santa Clara River main stem	Los Angeles	SCB-U	Low armoured	34.427	−118.577
22	San Francisquito Creek	Santa Clara River	Los Angeles	SCB-U	Unarmoured	34.546	−118.516
23	Upper Bouquet Creek	Santa Clara River	Los Angeles	SCB-L-Ad	Low armoured	34.554	−118.416
24	Lower Bouquet Creek	Santa Clara River	Los Angeles	SCB-L-Ad	Low armoured	34.510	−118.451
25*	Soledad Canyon, Robin's Nest	Santa Clara River main stem	Los Angeles	SCB-U	Unarmoured	34.438	−118.277
26	Soledad Canyon, Thousand Trails	Santa Clara River main stem	Los Angeles	SCB-U	Unarmoured	34.442	−118.211
27	Big Rock Creek	Mojave River	Los Angeles	SCB-U	Low armoured	34.452	−117.856
28*	Santa Ana River, Sugarloaf Meadow	Santa Ana River	San Bernardino	OC	Unarmoured	34.178	−116.830
29	Apple Valley	Mojave River	San Bernardino	OC-Ad	Low armoured	34.524	−117.277
30	San Jacinto River	Lake Elsinore	Riverside	SCB-U	Low armoured	33.736	−116.819
31	San Felipe Creek	Salton Sea	San Diego	SCB-U	Unarmoured	33.098	−116.473
32	Pine Valley Creek	Otay River	San Diego	SCB-U	Unarmoured	32.830	−116.552
33	Sweetwater River	Sweetwater River	San Diego	SCB-U	Low armoured	32.885	−116.600
34	Trabuco Creek	San Juan River	Orange	OC	Low armoured	33.563	−117.651
35	Bell Creek	San Juan Creek	Orange	OC	Low armoured	33.629	−117.555
36*	Bocana El Rosario	El Rosario River	San Quintin, Baja California	OC	Low armoured	30.041	−115.788

Note: Map ID = map number in Figure 1a. Numbers with asterisk (*) represent the 12 individuals selected for the lcWGS analysis. Genetic Groups (K = 3).

Abbreviations: Ad, Admixed; L, lower Santa Clara/Ventura; OC, outer coast; SCB, Southern California Bight; U, upper Santa Clara.

these libraries we generated single-end 100 base-pair sequences on an Illumina Hiseq4000 at the Broad Stem Cell Research Center (BSCRC) at UCLA. We aligned the sequences to the Bear Paw Lake threespine stickleback reference genome (Jones et al., 2012) using BWA-MEM version 0.7.12 (Li, 2013), and performed variant discovery using HaplotypeCaller and GenotypeGVCFs from GATK (McKenna et al., 2010) using default parameters (Table S1 contains the summary quality report of the BAM files). The final VCF file was filtered using the GATK standard thresholds (for SNPs: QD<2.0, MQ<40.0, FS>60.0, MQRankSum < −12.5, ReadPosRankSum < −8.0; for INDELs: QD<2.0, FS>200.0, ReadPosRankSum < −20.0, InbreedingCoeff < −0.8).

2.5 | Inferring historical demography

We used the multiple sequentially Markovian coalescent method (MSMC: Schiffels & Durbin, 2014; an extension of PSMC: Li & Durbin, 2011) to reconstruct the historical demography for a subset of populations in the data set. This method tracks coalescence across genomic regions and estimates how many coalescent events occur within certain time frames across the genome. The inverse of the rate of these events within each time frame, the inverse instantaneous coalescence rate (IICR: Mazet et al., 2016) serves as a proxy for N_e, provided that certain conditions are met. If not, other factors influencing the IICR (e.g., nonrandom mating, inbreeding, admixture) need to be accounted for when interpreting demography through time.

We performed our analyses using the unphased sequences of single individuals. Simulations have shown that MSMC on single genomes (also referred to as PSMC) performs equally well or better than multiple genomes under certain conditions and is preferable for lower coverage sequencing (Beichman et al., 2017).

We filtered VCF files for indels, missing data, multiallelic loci and read depth (DP) before running MSMC using default parameters (number of iterations = 20; recombination rate/mutation rate = 0.25; pattern of fixed time segments = $10^*1 + 15^*2$; fixed recombination rate = NO). Filtering for read depth improves the ability to characterize demographic change when mean coverage is ≤10 (Nadachowska-Brzyska et al., 2016). Therefore, we filtered out any SNPs with coverage below DP≤8 (GATK: -SelectVariants -select "DP≥8") based on the average coverage of our samples and the percentage of missing data after filtering (see Table S3 for number of SNPs retained and percentage of missing data for different filtering thresholds). To remove INDELs, multiallelic loci and missing data from our VCF files, we used VCFtools (−remove-indels −max−allele 2 −min-allele 2 −max-missing 1; Danecek et al., 2011).

We used bootstrap replicates (n = 100) to estimate the variance around the estimates of IICR. We also explored results using two different mutation rates, 3.7×10^{-8} and 6.6×10^{-8} (Liu et al., 2016), and generation times of 1 and 2 years (Liu et al., 2016; Rollins, 2017), as neither parameter is known with certainty for populations in southern California or whether they differ between inland and coastal localities due to environmental heterogeneity (e.g., higher summer

temperatures and greater UV exposure at inland sites). These parameters convert the values calculated by the model to estimates of time and population size. Shorter generation time and higher mutation rates yield younger dates and lower population size estimates (i.e., curve shifted down and to the left), while the reverse is true for longer generation times and lower mutation rates (i.e., curve shifted up and to the right). We plotted the results using the R packages ggplot2, scales and ggpubr.

As a verification step for our MSMC approach, we reanalysed two individuals from our sample from Santa Clara River (lagoon/upstream) and two from Alaska (river/lake comparison) from the work of Liu et al. (2016). Because their genomes had higher coverage than ours (26.7x and 30.2x), we filtered out SNPs with coverage below eight and higher than 20, and reran the analysis using 20 autosomal chromosomes following Liu et al. (2016).

Lastly, we generated PCA plots using the R package SNPrelate (Zheng et al., 2012) to detect population structure. We filtered the data set to include only biallelic SNPs (snpgdsVCF2GDS, method = "biallelic.only") and reduced linkage among sites (snpgdsLDpruning, method = "corr", ld.threshold = sqrt[0.1]).

2.6 | Comparisons of genetic diversity

For the microsatellite data set, we calculated expected (H_s) and observed (H_o) heterozygosity for each population using GenoDive version 3.06 (Meirmans, 2020) (except those that had <8 samples: Arroyo Grande, Shuman Lagoon, Sweetwater River) and report values in Supporting Information (Table S4).

For the lcWGS data, we compared heterozygosity between pairs of samples from different sites, with each pair involving one of the three unarmoured populations and a corresponding low armoured, lagoonal population near the coast (Figure 1). To perform this analysis, we used BCFtools version 1.10.2 (Danecek et al., 2021) to calculate numbers of heterozygous and homozygous sites based on SNP data only, and then calculated the heterozygosity ratio by dividing nHets (RA) / nHom (AA) (R = reference allele; A = alternative/non-reference allele). We used this index because it is less sensitive to density of genotyping (Samuels et al., 2016). The ratio vary between 0–2, with 2 representing the highest level of genetic diversity based on Hardy–Weinberg equilibrium (Guo et al., 2014). We also calculated the number of singletons (i.e., unique variants) for each individual (Table 2).

3 | RESULTS

3.1 | Microsatellites

Unarmoured stickleback populations do not form an exclusive group at any K, and plots of the estimated ln (log-normal) probability of the data (D) at different K values (lnPD|K) and ΔK identified three clusters that roughly align with geography (Figure 1a,b). We report

TABLE 2 Heterozygosity ratio and proportion of singletons for the 12 lcWGS

Map ID	Locality	PCA ID	HetRatio	Number of singletons
7	San Antonio Ck (lagoon)	SAC_L	0.69/0.83	224,330/505,449
8	San Antonio Ck VSFB (upstream)	SAC_U	0.19/0.29	47,5461/56,900
15	Santa Clara River (lagoon)	SCR_L	0.51/0.55	115,463/157,823
25	Santa Clara River (upstream)	SCR_U	0.12/0.15	34,392/40,937
28	Santa Ana River (upstream)	SAR_U	0.43/0.51	255,408/323,017
36	Bocana El Rosario (lagoon)	ERO_L	0.40/0.44	133,055/121,347

Note: Column shows values for both individuals analysed at each location (lower/higher value).

the assignments for $K = 2–7$ in Supporting Information (Figure S1), as informative clustering patterns emerged at $K > 3$.

At $K = 2$ (north to south), one cluster corresponds to fish from Bodega Bay south along the central coast to Salsispuedes Creek (a tributary to the lower Santa Ynez River in Santa Barbara County), the upper Santa Ana River in the San Bernardino Mountains, San Juan Creek (Trabuco Creek and Bell Canyon tributaries), and Bocana El Rosario in Baja California. Except for the upper Santa Ana River, these coastal populations form what we refer to hereafter as the Outer Coast (OC) group. The second cluster consists exclusively of populations within the SCB, and includes the Ventura, Santa Clara, and San Jacinto rivers, Big Rock Creek, and three drainages in San Diego County (San Felipe Creek, Pine Valley Creek, and the Sweetwater River).

Further distinction of the upper Santa Clara River, San Jacinto River, Big Rock Creek and the three San Diego County drainages emerged at $K = 3$. The population in Bouquet Creek, a tributary to the upper Santa Clara River, is admixed with alleles from both the upper and lower sections of the Santa Clara mainstem due to contamination during trout stocking (see Discussion). At $K = 3$, we also detected that admixture in Mission Creek (no. 10; Figure 1) and Apple Valley (no. 20; Figure 1) reflects shared ancestry between the Santa Clara River (upper and lower reaches) and the OC group.

Successive increases in K continued to show strong association between low armoured *G. aculeatus* in the lower Santa Clara and Ventura rivers; an affinity between the upstream Santa Ana River and San Juan Creek tributaries appeared at $K ≥ 4$; and distinction of the unarmoured population in San Antonio Creek-VSFB from all others became evident at $K ≥ 6$ (Figure S1).

Results of the PCA were consistent with the output from STRUCTURE (Figure 1b), with the percent variance explained by the first 10 principal components (PC) ranging from 14.64 to 2.15 (Figure 2). The OC group (plus upstream Santa Ana River) forms its own cluster, but with some distinction for San Antonio Creek-VSFB. Individuals in the SCB group are distributed along PC1, with the Ventura and lower Santa Clara river samples clustered on the lower left, and upstream Santa Clara samples clustered in the upper right. Admixed fish from Mission Creek and Apple Valley were intermediate between the OC and SCB clusters, and individuals from the upper part of the Bouquet Creek tributary were associated with fish from the lower Santa Clara River (i.e., Figure 2: no. 23).

3.2 | Genomes

Individuals had an average of ~8x coverage, ranging from 6.2–10x (Table S2). We recovered a total of 8,340,853 SNPs; missing data varied from 3.2%–7.0% per individual. We reduced the data set to 10,308 biallelic SNPs after removing linked loci, with the percent of variance explained ranging from 13.14 to 5.07 for the first 11 PCs. While the sampling was limited for lcWGS data set, we still detected clear separation of the three unarmoured stickleback populations, and a general affinity between up- and downstream fish in the same drainage regardless of plate morphology (Figure 3). Fish collected at the same locations tended to cluster in similar PCA space, with partially armoured fish from the lagoon at San Antonio Creek-VSFB showing more divergence than all other pairs. The only drainage that did not involve a direct up- versus downstream comparison was the upper Santa Ana River and Bocana El Rosario (Baja California). Paired samples from these two locations were broadly separated along PC2, which contrasts with the PCA using microsatellites, where both were clustered in the OC group.

3.3 | Historical demography under the MSMC

We present results for two combinations of mutation rate and generation time: $\mu = 3.7 \times 10^{-8}$; age = two years (Figure 4) and $\mu = 6.6 \times 10^{-8}$; age = one year (Figure S2) and show that the shape of the IICR curve remains unchanged regardless of the parameter values used in the analysis. For clarity, populations referred to as "upstream" are unarmoured, whereas those from "lagoons" are low armoured.

Plots show a general congruence in curve shape between fish from the same sampling location, except for the upper Santa Clara River, which we attribute to differences in the amount of coverage (a more stringent filtering threshold produced curves with less overlap between the samples due to a higher percentage of missing data for one of the individuals; Figure S3). Bootstrapped data showed little variation from the observed data, although there was slightly more uncertainty in the IICR curves at the deeper time intervals for upstream fish.

Initial population sizes appear larger at upstream sites compared to the lagoons, although estimates of both time and population size for the upstream Santa Ana River are more similar to
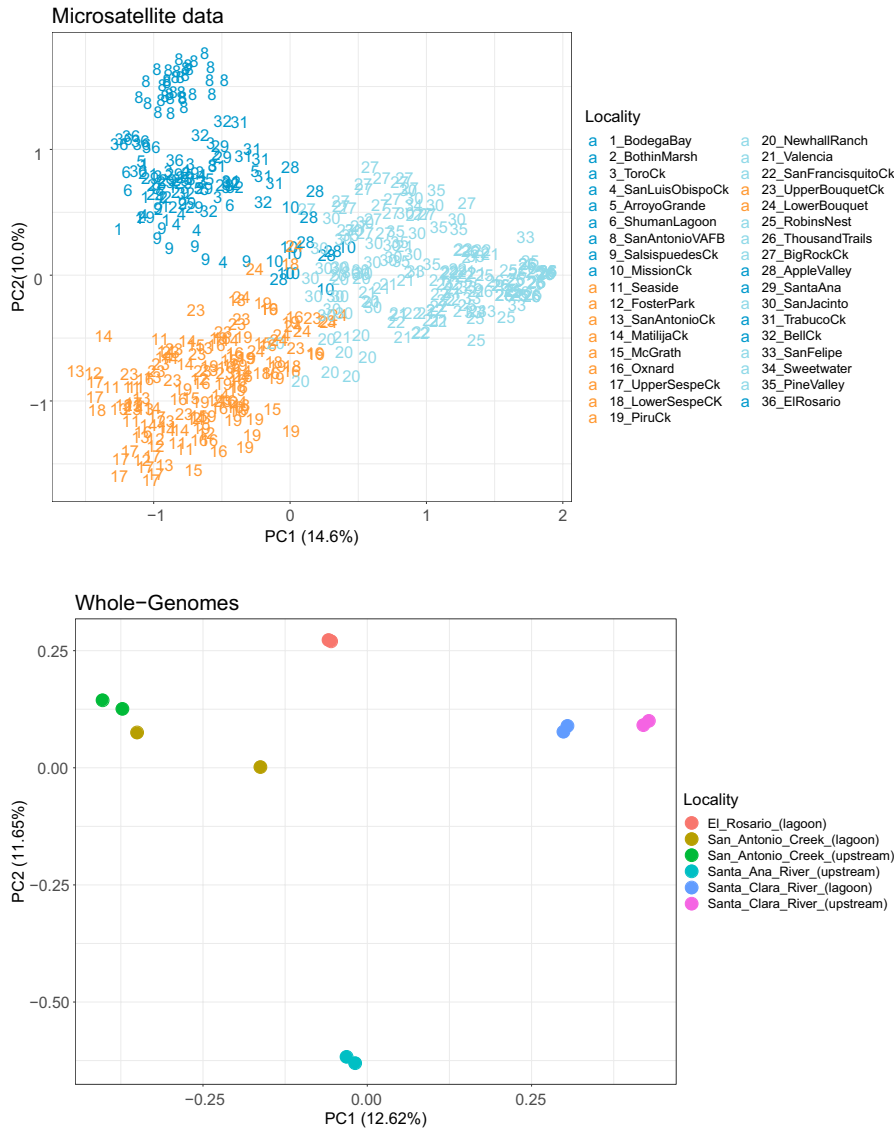
lagoonal populations than to either of the upstream populations further north.

All fish reveal marked demographic declines from initially large population sizes but differed in the general shape of the IICR curves depending on whether the fish were from upstream areas or lagoons. Plots from lagoonal fish have more of a sigmoid shape, with an inflection in the middle part of the curve that reflects a transition from early, rapid decline to a period of mild "growth" (Figure 4). The timing and duration of these "growth" periods is roughly congruent across lagoons. In contrast, none of the upstream fish show evidence of a "growth" phase, although the Santa Ana River fish reveal a long period of stable demography, expressed as a plateau in the IICR plot, that leads to a sigmoid-like curve shape. The chronology of this plateau also overlaps with the "growth" phase in lagoonal fish. In contrast, plots of the other upstream fish from San Antonio Creek-VSFB and the Santa Clara River reveal steeper and more continuous declines across their histories, with the upper Santa Clara River fish showing the steepest trajectory and no inflection in the curves at all.

Our reanalysis of data from *G. aculeatus* sampled in Alaska recovered the same overlap in IICR curves shown in Liu et al. (2016)

(Figure S5). We also note that they look distinctively flatter, a pattern we attribute to lower depth of sequencing coverage (which is known to flatten IICR curve shapes: Nadachowska-Brzyska et al., 2016) and possibly the use of an updated SMC algorithm (Schiffels & Durbin, 2014). These results suggest that the nonoverlapping curves between up- and downstream fish in this study is not an artefact of how the data was processed and analysed, but rather a result of distinct and divergent demographic histories between populations from the same drainage.

3.4 | Genetic diversity

Measurements of heterozygosity based on SNP data show that upstream fish have lower gene diversity (H_o) than lagoonal fish in the cases where within-drainage comparisons are made (e.g., San Antonio Creek-VSFB and in the Santa Clara River). In the comparison involving the upper Santa Ana River, H_o was comparable to lagoonal fish from Bocana El Rosario and the lower Santa Clara River, and considerably higher than either of the other unarmoured stickleback populations (Table 2). Individuals with the highest heterozygosity were from the
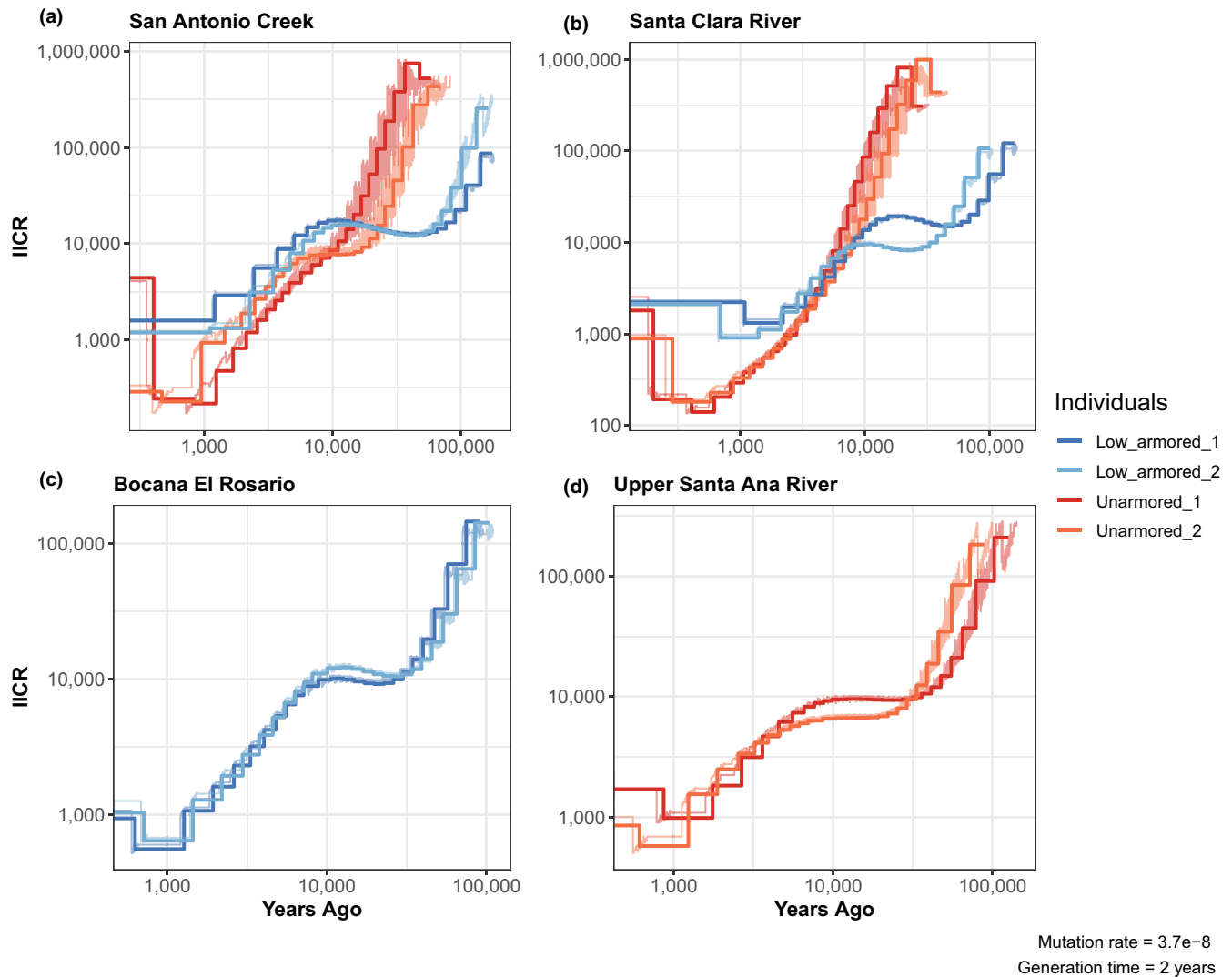
FIGURE 4 MSMC plots of upstream, unarmoured (red) and lagoonal, low armoured (blue) individuals. Thick lines are the point estimates, and faint lines represent the bootstrap replicates. Age and IICR estimates reflect a generation time of 2 years and mutation rate of 3.7×10^{-8}, respectively. Plots for Bocana El Rosario and upper Santa Ana River are shown separately because they occur in different drainage basins (see text for additional details). There is lack of congruence between the curves of fish from the same drainage (lagoonal/upstream), which is the result of divergent demographic history

San Antonio Creek-VSFB lagoon and the lowest from upper Santa Clara River. The same pattern is observed for the number of singletons (Table 2) and is also reflected in the microsatellite data (Table S3).

4 | DISCUSSION

Rapid decline of the freshwater fish fauna of southern California (Moyle et al., 2015) and northern Baja California (Ruiz-Campos et al., 2014) comes as no surprise given the extensive loss of habitat and permanent alteration of the region's natural hydrology over the past century (Swift et al., 1993). Yet, results from this study indicate that demographic decline is more the norm than the exception for *G. aculeatus* in the region, even at the deepest time scales. This may reflect the realities of survival in a climate that has become increasingly more arid over the Holocene, combined with warmer conditions at

subtropical latitudes that reduces the availability of perennial water. We explore these factors below by describing population structure and characterizing demographic histories across different parts of the study area. We then discuss the implications of the current data for management and suggest that the emphasis on plate phenotype has both aided and detracted from conserving the important genetic variation across this part of the species range.

4.1 | Population structure across southern California

We detected three regional clusters within *G. aculeatus* across southern California and show that unarmoured populations assign to separate groups. The clearest distinction is between populations within the SCB, specifically those from Ventura and Santa Clara

river drainages, and those from the OC group. High admixture between Ventura River and lower Santa Clara River populations has been reported previously and is probably tied to the proximity of the river mouths, which are separated by ≤8 km of coastline and well within the dispersal distance of *G. aculeatus* in the ocean (Richmond et al., 2015). In contrast, the OC group has maintained its cohesion across a much larger expanse of coastline, consistent with long-distance movement in marine environments (Fang et al., 2018; Jones & John, 1978; Mäkinen et al., 2006).

An unexpected finding was the clustering of the upstream Santa Ana River population within the OC group (at $K = 2$–5, Figure 1b) given its inland and isolated location in the San Bernardino Mountains (elevation = 2055 m: Figure 1a). It is the only population that occurs at high elevation, more than ~1200 m higher than any other native *G. aculeatus* in the region. This retention of ancestral polymorphism and connection with the OC group, especially San Juan Creek (Trabuco Creek and Bell Canyon tributaries; $K \geq 4$, Figure S1), suggests that the current isolation of the source population (i.e., Shay Creek) may be recent. However, the absence of any known hydrologic connection between Shay Creek and the coast (Flint et al., 2012; French & Busby, 1974) presents a challenge to this explanation (see next section for further details). Whole genome data now being collected will help shed more light on this issue.

Our results also reveal cases where naturally occurring populations of *G. aculeatus* have been mixed with nonlocal fish, usually through accidental introduction. One example is Bouquet Creek (Figure 1: nos. 23–24), where a resident population of unarmoured *G. aculeatus* was mixed with low armoured individuals from the lower Santa Clara River as a byproduct of trout stocking (Richmond et al., 2015). A second example is Mission Creek (no. 10), which is situated along the Santa Barbara coastline between the OC sites and the mouth of the Santa Clara and Ventura rivers. In this case, admixture could be due to either accidental introduction with trout stocking or natural dispersal from the nearby Ventura or Santa Clara rivers (Figure 1).

Another set of populations are probably the result of pure incidental transport during trout stocking, with no evidence that *G. aculeatus* existed at the site previously. These include San Jacinto Creek (no. 30) and the Sweetwater River (no. 33), which also support populations of hatchery-sourced trout (Abadía-Cardoso et al., 2016). The population at Apple Valley (no. 29) (i.e., Mojave River) also fits this category; however, unarmoured *G. aculeatus* were also purposely introduced to this drainage prior to 1940, but then were eliminated by subsequent incidental transport of hatchery fish sometime thereafter (Buth et al., 1984; Miller & Hubbs, 1969; Swift et al., 1993).

Still, a third set of populations involve cases of purposeful translocations aimed at safeguarding the gene pool at the type locality for *G. a. williamsoni* in Soledad Canyon (USFWS, 2021). The most well-documented example in this category is the San Felipe Creek population at the edge of the Anza Borrego Desert in San Diego County, where translocations occurred in 1972, 1973, and 1981 (Swift et al., 1993). The population in Pine Valley Creek is also a suspected, intentional transplant based on archival memos and hearsay,

and cluster assignments confirm that *G. aculeatus* in this drainage share the same genetic background as those from the upper Santa Clara River.

4.2 | Historical demography varies with geography

The history of glaciation and transitions from wetter to drier climates in southern California makes it challenging to specify points in time that unequivocally coincide with transitions in the IICR curves, especially when mutation rates and generation times are estimated from *G. aculeatus* in other regions. However, qualitative comparison of the demographic trajectories can provide useful information about the approximate timing and location of population expansions and contractions, and some indication of how their contemporary status compares to the backdrop of their deeper history. Moreover, our results show similarities to those in Liu et al. (2016) for *G. aculeatus* from Vancouver Island, a possible glacial refuge near the edge of the unglaciated region at the Last Glacial Maximum (18–20 ka). These fish may represent a set of interacting populations that extended from this area through the large unglaciated region to the south, including southern California and northern Baja California (Bell, 1976; Glover et al., 2021; Heusser, 1998).

A key piece of information provided by the MSMC analysis is that all populations of *G. aculeatus* have declined dramatically from early peaks, and that the patterns of decline show some consistency with respect to location (Figure 4). Lagoonal populations also show a later transient period of mild "growth" that varies slightly in chronology and duration among sites, whereas upstream unarmoured stickleback populations do not. One explanation for these "growth phases" is that they represent admixture with nonlocal migrants along the coast, which could offset declines in the IICR to produce inflections in the estimated trajectories. Other studies document the genetic connectivity of estuarine populations on the Pacific coast as a result of habitat expansion as sea level rose (Dolby et al., 2016, 2018, 2020; Stiller et al., 2021). Sea level rise would also lead to the formation and broad distribution of lagoons following maturation of the coast (Jacobs et al., 2011; Masters, 2006).

Lagoons provide freshwater habitat for most of the year but can open to the ocean during heavy rain events (Jacobs et al., 2011). The complex coastal geomorphology and hydrology of lagoons probably created dispersal opportunities that help explain the cohesion of the OC group, a pattern consistent with *G. aculeatus* worldwide where marine fish tend to show genetic uniformity across large geographic distances (Mäkinen et al., 2006). It may also explain why the lagoonal populations tend to have higher heterozygosity.

That the initial population sizes in the early IICR curves appear larger in upstream areas compared to lagoons could reflect a wetter landscape and cooler climate during the time when *G. aculeatus* first expanded into the region (Glover et al., 2021; Heusser, 1998). However, we interpret these relative population size estimates in the earliest part of the IICR curves with scepticism (see *Caveats to the MSMC below*). Wetter and cooler conditions within the SCB

were prevalent from ~65 ka up to ~14 ka (Faribanks & Carey, 1910; Glover et al., 2017, 2020; Heusser et al., 2015; Kirby et al., 2013; Owen et al., 2003; Sharp et al., 1959) and probably provided more expansive freshwater habitat that could have sustained larger and more interconnected populations of *G. aculeatus* in inland areas. In fact, habitat supporting unarmoured *G. aculeatus* extended well into the Los Angeles Basin as recently as the mid-20th century (Culver & Hubbs, 1917; Mendenhall, 1908; Swift et al., 1993).

In contrast to lagoonal fish, none of the upstream fish show evidence of a reversal in demographic decline, although the IICR for upper Santa Ana River samples reveals an interval of stable size that produces a sigmoid-shaped curve that more closely resembles OC fish. The remaining unarmoured fish in San Antonio Creek-VSFB and the Santa Clara River instead show a steeper, more continuous decline across their full histories, with the latter showing no inflection in the IICR at all. We interpret these steeper, more linear trajectories as evidence of isolation with limited or no gene flow, particularly the upper Santa Clara River, where several large dry gaps separate the population from lower reaches of the main channel (Richmond et al., 2015). Multiple molecular data sources confirm that, while fish occasionally get flushed downstream across these gaps, there is no evidence of genes moving upstream (Buth et al., 1984; Richmond et al., 2015). Susceptibility to recurrent bottlenecks due to fluctuating hydrology and El Niño-related climate events (reviewed in Richmond et al., 2015), and more recently increased fire frequency (Flint et al., 2019), may also be reducing the IICR of these fish at a contemporary time scale. It is likely that this history of isolation between upstream and lagoonal fish and sequential bottlenecking from fluctuating climatic events led to distinct and divergent demographic histories, as captured by the lack of overlap in their IICR curves.

The greater similarity between unarmoured *G. aculeatus* in the upper Santa Ana River and low armoured fish in the OC group, in terms of the shape of the IICR plot (more sigmoid than linear), age and size of the founder population, chronology of the transition in the IICR, level of heterozygosity, and clustering affinity, is peculiar given the isolation and high elevation of the source population at Shay Creek in the San Bernardino Mountains. Two hypotheses explain this conundrum. The first is that Shay Creek supports a naturally occurring, relictual population that became isolated during uplift of the San Bernardino Mountains, and that suitable freshwater habitat has potentially persisted in the upland plateau as far back as the Last Glacial Maximum (18–20 ka; Owen et al., 2003; Sharp et al., 1959). However, Shay Creek drains northward into Baldwin Lake instead of toward the coast via the Santa Ana River drainage basin, challenging this view of natural genetic connectivity with outer coast fish.

A second, more plausible hypothesis, is that the Shay Creek population is introduced. This better explains the presence of outer coast alleles in these small, isolated ponds, and why it is the only population to occur at high elevation. Sticklebacks have also been introduced to the Big Bear Reservoir just 3 km to the west of Baldwin Lake and there has been at least one accidental and several known introductions of Shay Creek fish to other high elevation ponds in

the area (Swift et al., 1993). These introductions further attest to the ease and regularity that this species has been moved artificially, although a definitive outer coast source(s) for the Shay Creek population has yet to be identified.

4.3 | Caveats to the MSMC

There are limits as to how far back in time the MSMC can reliably infer demographic parameters, as alleles with deep coalescence become increasingly rare at older time scales (Beichman et al., 2017; Mazet et al., 2016; Takahata & Nei, 1985). This explains the greater noise in the bootstrapped data, and perhaps the differences in IICR between upstream and lagoonal populations at the oldest time intervals. For this reason, IICR estimates for early population history are often viewed with scepticism (Beichman et al., 2017). Natural selection for specific mutations and any linked neutral variants can also distort the IICR, particularly if the type of selection reduces polymorphism (Ewing & Jensen, 2016; Schrider et al., 2016), and there is good reason to assume that selection is a factor in this system. Phenomena that contribute to population structure in mating/migration also act as confounding factors, and may be expected in this case, given the known demographic fluctuation of landlocked populations in upstream areas (Moyle, 2002). For example, inbreeding (as a nonrandom mating process) can affect interpretation of the IICR curve because it increases the rate of coalescence and leads to a reduction in N_e. Sequences from the larger ongoing WGS study will allow us to screen for long runs of homozygous sequence, in which case we can remove such runs and repeat the analyses to test the sensitivity of our results (Freedman et al., 2014; Mather et al., 2020).

4.4 | Rethinking management

Our results are consistent with separate origins for unarmoured populations in southern California, although phylogenetic analyses are needed to polarize the relationships. Nonetheless, the current data provide evidence that the morphological entity now classified as *G. a. williamsoni* consists of distinctive genetic units, and that managing according to these units may be prudent.

A number of partners have focused management on unarmoured fish from the type locality in Soledad Canyon (upper Santa Clara River) (USFWS, 2021), and until now there were questions as to whether this made sense from a genetics perspective. The answer is yes, given that the population has low genetic diversity, is geographically isolated, and the habitat is ecologically unstable and subject to persistent human disturbance (e.g., fires, garbage dumps, recreational vehicle abandonment, illegal water diversions, homeless encampments, off-highway vehicle activity, etc.). Climate change may also be impacting the population as perennial water no longer occurs at the type locality of *G. a. williamsoni* (Girard, 1854). Whether low genetic diversity is cause for alarm

requires further study, as the population may have survived for much of its history with low diversity, and deleterious alleles that were potentially hidden from selection in the heterozygous state could have been purged over time due to drift (Robinson et al., 2018). If it can be shown that specific polymorphism is responsible for parallel loss of body armour, managing the three unarmoured populations as separate units may provide greater assurance that those alleles will be preserved.

Increased knowledge about the demographic history of *G. aculeatus* in southern California also lays the groundwork for understanding how these fish have adaptively responded to selection, as the evolutionary potential of populations may rely on their pre-existing genetic variation (Barrett et al., 2008; Jones et al., 2012; Lai et al., 2019). Pre-existing variation may be key to understanding the complete loss of body armour, a rare condition in *G. aculeatus* worldwide that could be related to the absence of predators, as it has been extensively documented in the transition from armoured to low armoured morphs (e.g., Eriksson et al., 2021; Paccard et al., 2018; Reimchen, 1994; Wasserman et al., 2020, and references therein). However, whether the unarmoured condition is driven by predation or lack thereof remains unresolved (Reimchen, 1994).

Considerable knowledge exists on the genetic underpinnings of plate reduction in fully armoured fish (typically marine), where parallel evolution of partially armoured fish in freshwater habitat is largely the product of selection for an allele of the ectodysplasin gene (EDA), a signalling protein that is important for the development of the skeleton, skin and other tissues (Colosimo et al., 2005; Cresko et al., 2004; O'Brown et al., 2015). The allele is recessive and rare in the marine environment and presents a striking example of how standing variation can lead to rapid shifts in phenotype depending on the selective environment (Barrett et al., 2008). Earlier work found no association between EDA polymorphism and unarmouredness, suggesting that other genes or regulatory regions may be involved with the complete loss of plates (Richmond et al., 2015). This subject will be explored in further detail in our ongoing WGS project.

Results of this work also raise the question of whether low armoured stickleback populations might also be the focus of conservation efforts, given the genetic distinctiveness and geographic isolation of some populations in the SCB. For example, low armoured *G. aculeatus* in San Juan Creek (Figure 1: nos. 34–35) share close ancestry with the unarmoured stickleback population now in the San Bernardino Mountains and are the only remaining representatives of the OC group in the Los Angeles Basin. The low armoured stickleback population at Bocana El Rosario is also unique in representing the southernmost tip of the species' distribution in the eastern Pacific Ocean. However, it is currently considered extirpated (Ruiz-Campos & González-Acosta, 2022). The only other extant population occurring in Mexico is in El Descanso lagoon, Rosarito (Ruiz-Campos et al., 2014). This edge population may still harbour critical genetic diversity that is adaptive in transitional environments that define the range edge itself.

These findings highlight the importance of using population genetic and genomic data to re-examine management strategies that were initially developed according to phenotype-based taxonomies. In this case, efforts to manage populations with a rare phenotype have probably helped to preserve important adaptive polymorphism, but emphasis on the unarmoured phenotype alone may exclude other populations in the region that have geographically unique variation that was once widespread across the coastal lagoons and drainages of southern California and northern Baja California.

CONFLICT OF INTEREST
The authors declare no conflict of Interest.

DATA AVAILABILITY STATEMENT
DNA sequences that support the findings of this study are openly available in Dryad at DOI: https://doi.org/10.5068/D15960. Scripts used to run MSMC and R analyses that generated the findings of this

study are openly available in GitLab at gitlab.com/rturba/uts-2022, Project ID 18790157.

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SUPPORTING INFORMATION

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