

# Climate-driven habitat change causes evolution in Threespine Stickleback

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## Abstract

Climate change can shape evolution directly by altering abiotic conditions or indirectly by modifying habitats, yet few studies have investigated the effects of climate-driven habitat change on contemporary evolution. We resampled populations of Threespine Stickleback (*Gasterosteus aculeatus*) along a latitudinal gradient in California bar-built estuaries to examine their evolution in response to changing climate and habitat. We took advantage of the strong association between stickleback lateral plate phenotypes and Ectodysplasin A (*Eda*) genotypes to infer changes in allele frequencies over time. Our results show that over time the frequency of low-plated alleles has generally increased and heterozygosity has decreased. Latitudinal patterns in stickleback plate phenotypes suggest that evolution at *Eda* is a response to climate-driven habitat transformation rather than a direct consequence of climate. As climate change has reduced precipitation and increased temperature and drought, bar-built estuaries have transitioned from lotic (flowing-water) to lentic (still-water) habitats, where the low-plated allele is favoured. The low-plated allele has achieved fixation at the driest, hottest southernmost sites, a trend that is progressing northward with climate change. Climate-driven habitat change is therefore causing a reduction in genetic variation that may hinder future adaptation for populations facing multiple threats.

## KEYWORDS

adaptation, climate change, *Gasterosteus aculeatus*, intraspecific variation, latitudinal gradient, rapid evolution, resurvey, space-for-time

## 1 | INTRODUCTION

Climate change has drastically altered the ecology and evolution of natural populations. Most research has focused on the direct effects of abiotic climate drivers on demography, including range shifts, migration, interspecific interactions, and local extinction (Cahill et al., 2012; Caruso, Sears, Adams, & Lips, 2014; Willis, Ruhfel, Primack, Miller-Rushing, & Davis, 2008); yet, climate change may also indirectly influence evolution in populations by modifying their habitats. In addition to research showing eco-evolutionary effects of climate-driven habitat change within experiments (Barrett et al., 2011; Grant & Grant, 1989), field surveys have demonstrated broader biological responses in the context of multidimensional natural systems (Holmes

et al., 2016; Moritz et al., 2006; Tingley, Monahan, Beissinger, & Moritz, 2009; Wogan & Wang, 2018). Historical data and museum collections may be compared to contemporary samples to study recent phenotypic change. When combined with space-for-time substitutions (Wogan & Wang, 2018)—the inference of temporal biological trajectories from current spatial patterns in the field—these resurveys increase our understanding of the relationship among climate, habitat, and evolution. Although resurveys have revealed potential evolution over periods of climate and habitat change (Holmes et al., 2016), space-for-time substitutions have illustrated local adaptation based on phenotypic variation across spatial habitat (Dobzhansky, 1947) and climate gradients (Bradshaw & Holzapfel, 2006; Merilä, 2012; Wogan & Wang, 2018).

Recent studies have documented genetic and phenotypic responses to climate and habitat change; however, few have measured both simultaneously by recording variation in traits with a well-established genetic basis (Bay et al., 2018; Franks & Hoffmann, 2012; Gienapp, Teplitsky, Alho, Mills, & Merilä, 2008). First, researchers have identified genes potentially involved in adaptation to climate change by discerning regions of the genome showing signatures of selection (Franks & Hoffmann, 2012), yet the phenotypic traits associated with these genes are usually unknown. Second, researchers have recorded numerous cases of trait change across spatial and temporal habitat and climate gradients (e.g. Alho et al., 2010; Berner, Grandchamp, & Hendry, 2009; Des Roches, Sollmann, Calhoun, Rothstein, & Rosenblum, 2017; Kingsolver & Buckley, 2017). Though the heritability of these traits may be established through common rearing experiments, deciphering their specific genetic basis remain challenging (Gienapp et al., 2008). Studies that do present molecular genetic data are often correlative (Bay et al., 2018; Franks & Hoffmann, 2012), rarely showing how long-term climate change effects genetically based phenotypic traits (but see Karell, Ahola, Karstinen, Valkama, & Brommer, 2011). Understanding the degree to which phenotypic change is plastic or genetic is important for predicting future responses to climate change (Hoffmann & Sgrò, 2011). For example, diminishing genetic diversity can reduce the potential for future adaptation from standing genetic variation (Hoffmann & Sgrò, 2011). A promising approach, therefore, is to focus on traits that are likely to respond to climate change both genetically and phenotypically.

Threespine Stickleback (*Gasterosteus aculeatus*) is a model system for studying rapid adaptation and provides an opportunity to explore the evolutionary responses to climate change. These small, widely distributed fish are polymorphic for defensive lateral bony plate number, a largely Mendelian trait that responds rapidly to habitat-based selection (Barrett, Rogers, & Schluter, 2008; Colosimo et al., 2004; Kingsley & Peichel, 2007; Münzing, 1963; Swift, Haglund, Ruiz, & Fisher, 1993). Plate number is strongly influenced by allelic variation at the Ectodysplasin A (*Eda*) gene (Barrett et al., 2008; Marchinko, 2009). Completely plated stickleback are typically homozygous for the *Eda* 'complete' allele (*Eda<sub>C</sub>*), whereas low plated stickleback are homozygous for the *Eda* 'low' allele (*Eda<sub>L</sub>*). Heterozygotes have variable phenotypes, but are typically partially plated (Barrett et al., 2008; Colosimo et al., 2004, 2005; Kingsley & Peichel, 2007). Although populations of ancestral anadromous stickleback are usually completely plated (and usually homozygous for *Eda<sub>C</sub>*), not all completely plated stickleback are anadromous: nonmigrating freshwater and estuarine stickleback populations can be polymorphic or monomorphic for plate phenotype. This variation is likely a result of selection on lateral plates and other phenotypes influenced by *Eda* (Barrett et al., 2008), although other minor alleles may be responsible for variation in plate number, plate size, and the presence of keel plates (Colosimo et al., 2004; Lucek, Haesler, & Sivasundar, 2012).

In California, stickleback plate phenotype has a strong climate signal (Baumgartner & Bell, 1984; Paccard et al., 2018). In a 40-year-old survey, Baumgartner and Bell (1984) showed that the frequency

of low-plated stickleback in bar-built estuaries—highly seasonal fresh- to brackish-water habitats with periodic ocean connection (Heady et al., 2015)—increased with decreasing latitude along the coast of California. Correspondingly, the frequency of low plated fish also increased with decreasing precipitation and increasing temperature. In the 1970s and 1980s, completely plated fish were common as far south as Big Sur River (~4,015,850 mN) and found south to San Luis Obispo Creek (~3,895,350 mN), whereas southern California populations were virtually monomorphic for the low-plated phenotype, indicating potential near fixation of *Eda<sub>L</sub>*.

The substantial effects of climate on bar-built estuary habitat may drive the observed variation in stickleback plate phenotype in California (Jacobs, Stein, & Longcore, 2011). In warm, dry weather, low streamflow permits tidally built sandbars to form between rivers and the ocean (Rich & Keller, 2013), resulting in completely or partially isolated pond-like (lentic) estuaries (Table S2; Richards, Moal, & Pallud, 2018). Primary production from phytoplankton and macroalgae may increase with higher temperature and lower precipitation, which further promote slowed streamflow and more habitat structure (Heady et al., 2015). In these structured, vegetated habitats, selection on stickleback may favour the improved maneuverability enabled by fewer lateral plates (Dalziel, Vines, & Schulte, 2011; Leinonen, McCairns, Herczeg, & Merilä, 2012; Reimchen, 1983; Taylor & McPhail, 1986). In contrast, high precipitation leading to increased streamflow causes estuaries to swell with water, breaching sandbars and connecting streams to the ocean (Rich & Keller, 2013; Richards et al., 2018). The clearer, faster water, lower productivity, and reduced habitat structure of these riverine estuaries may facilitate stronger selection for increased plate number by visual predators in just a few generations (Kitano et al., 2008). Due to the significant effects of precipitation, temperature, and drought on their surroundings, stickleback in bar-built stream estuaries may be particularly subject to climate-influenced habitat variation.

We used temporal changes in Threespine Stickleback lateral plate phenotype to infer the evolutionary consequences of several decades of climate change across a latitudinal gradient. Though we primarily focused our resurvey on Californian populations previously sampled in the 1970s–1980s by Baumgartner and Bell (1984), we were also able to include stickleback collections from as early as the 1890s for several sites (Miller & Hubbs, 1969; Rutter, 1896; Swift et al., 1993). Because we were unable to obtain DNA from historically preserved specimens, our results are based on the frequency of the low allele (*Eda<sub>L</sub>*) inferred from plate morph phenotype. We predicted that the *Eda<sub>L</sub>* allele has increased in stickleback populations across California as a result of decreasing precipitation, increasing temperature, and drought severity, promoting more lentic (pond-like) estuaries. We further predicted that the latitudinal pattern found by Baumgartner and Bell (1984) would remain, but will have shifted northward. Specifically, we expected the shift towards populations dominated by *Eda<sub>L</sub>* allele to occur in central, rather than southern California. Finally, we predicted intraspecific variation to decline between historic and contemporary populations if either drift or directional selection on plate phenotype resulted in the fixation of *Eda<sub>L</sub>*.

## 2 | MATERIALS AND METHODS

### 2.1 | Sampling

We sampled stickleback from estuaries and streams along the coast of California (for list of sites and their latitude; see Table S1) using a 10 foot seine, and occasionally dip-nets and minnow traps (see also Baumgartner & Bell, 1984). We categorized sites as 'true lagoons' (little to no upstream fluvial inputs, rare ocean connection), 'bar-built stream estuaries' (upstream fluvial inputs, changes in ocean connectivity), or 'upstream' (Table S2; definitions from Heady et al., 2015). At each site, we collected and euthanized up to 40 fish with an overdose of MS222 prior to storage in a 95% ethanol solution. Samples from the 1970s were fixed in 10% formalin without anaesthesia. We estimated sampling locations using inferred coordinates from original topographic maps where sample sites used in Baumgartner and Bell (1984) had been marked and latitude, longitude values were available (for older samples). In all cases, we sampled no farther than 500 m from historical sampling sites. All estuary sites were located less than 1.5 km along the stream course from the ocean, whereas upstream sites were up to 14 km from the ocean (Table S1). We collected fish from 54 of the 144 sites originally sampled by Baumgartner and Bell, excluding eight sites where we recovered no more than one adult fish (Los Osos Creek, Oak Knoll Creek, Oso Flaco Lake, in San Luis Obispo County, and the two farthest upstream sites of Big Sur River in Monterey County and San Luis Obispo Creek in San Luis Obispo County). We have complied with all relevant ethical regulations and fish handling and collection protocols were approved by the University of California Santa Cruz Institutional Animal Care and Use Committee (IACUC) valid as of 21 November 2016. Collection permits were granted by California State Parks, the California Department of Fish and Wildlife, municipal and private land owners.

### 2.2 | *Eda* genotyping

To estimate the accuracy of inferred *Eda* frequencies, we genotyped 482 fish from 20 different Californian estuaries (median  $N = 15$ /estuary) collected between 2016 and 2018; approximately half of these fish were from 14 of the estuaries used in the current study. We took tissue samples for Ectodysplasin-*a* (*Eda*) genotyping from the right pectoral fin of each individual, which we preserved in a 95% ethanol solution. We extracted DNA using sodium form Bio-Rad Chelex<sup>®</sup> 100 Molecular Biology Grade Resin, 200–400 mesh, and amplified the *Eda* gene in specimens using polymerase chain reactions (PCR) with the diagnostic indel locus, Stn381, to identify low and complete alleles (Colosimo et al., 2005). We electrophoresed *Eda* PCR amplification products through 2% agarose gels with an 100 bp Invitrogen<sup>®</sup> DNA ladder and visualized bands using Biotium<sup>®</sup> GelRed nucleic acid stain. We characterized PCR fragments by length to determine genotype as described by Colosimo et al. (2005).

### 2.3 | Plate morphology and inference of *Eda* genotype

We uniquely labeled individual fish prior to removal of the pectoral fin (for DNA extraction) and subsequent fixation in a 10% formalin solution. We used the following alizarin red staining protocol: after formalin fixation, we submerged fish in the following solutions (in order): 12 hr of deionized (DI) water, at least 5 hr of 0.5% KOH, at least 5 hr of alizarin red solution, and at least 5 hr of KOH solution. After staining, we rinsed fish in DI water and stored them in an 80% ethanol solution. We counted plates and categorized fish into the three plate morph categories (low, partial, complete) as described in Baumgartner and Bell (1984). 'Low' morphs had 10 or fewer continuous plates starting at the head; 'Complete' morphs had an unbroken row of 28–36 plates from the head to the caudal peduncle; 'Partial' morphs had broken rows of typically 10–20 plates that either did or did not include keel plates.

We inferred  $Eda_L$  from plate phenotype because genetic data were not available for historic collections and we could accurately predict populations'  $Eda_L$  frequency from phenotypic data. To evaluate the accuracy of our genotypic categorizations, we compared inferred genotypes of the 482 contemporary Californian stickleback to actual genotypes obtained through DNA analysis (described above). We first calculated the Goodman–Kruskal Correlation (described in Marchinko, Matthews, Arnegard, Rogers, & Schluter, 2014), which demonstrated high association between inferred and actual genotype ( $\gamma = .9937 \pm .006$ , where 1 = perfect association). We found *Eda* to explain 80% of the variation in plate number and 85% of the variation in plate morph (using ANOVA analysis as described in Colosimo et al., 2004; Kitano et al., 2008). Though the degree of *Eda* dominance may vary by population (Cresko et al., 2004), other studies in California show a similarly high degree of matching between genotype and plate morph (Morris, Kaufman, & Rogers, 2019) indicating that  $Eda_C$  may show weaker dominance effects in Californian populations. To test this assumption, we used the R package 'SNPassoc' (Gonzalez et al., 2007) and Akaike information criteria to compare dominance, recessive, codominance, and additive genotype–phenotype association models using our sample of stickleback with known genotypes. Analyses using either morph category or plate number showed support for the codominance model over the dominance model, providing support for our *Eda* genotype assignment based on plate morph, particularly for heterozygotes. Due to some dominance effects of  $Eda_C$ , miscategorization of heterozygotes as homozygous for  $Eda_C$  may have caused us to underestimate the true frequency of  $Eda_L$ ; still, of the 482 genotyped fish, we inferred the correct genotype over 93% of the time and only 4% of these fish were heterozygotes incorrectly categorized as homozygous  $Eda_C$ . Furthermore, we have no reason to expect this rate to differ among populations or between historical and contemporary samples.

For each sample, we calculated the inferred frequency of the low allele using the formula:

$$\text{Freq}(Eda_L) = \frac{(2 \times L) + P}{2N},$$

where  $L$  is the frequency of the low morph,  $P$  is the frequency of the partial morph, and  $N$  is the total number of alleles in the sample ( $2 \times$  sample

size). For samples from MA Bell, we used data of morph counts from Baumgartner and Bell (1984) Supplemental Material, verifying accuracy by comparing counts and categorizations for a subset of samples from various Californian populations (>15 individuals per plate morph). For older historical samples, we performed morph categorizations ourselves from preserved collections at the California Academy of Sciences in San Francisco and the Natural History Museum of Los Angeles County or used historically reported data only when unambiguously reported.

## 2.4 | Climate and habitat variables

We collected measurements of habitat and climate variables from publicly available online resources. First, we downloaded and calculated contemporary habitat data for seven parameters that described the estuary and watershed habitat on a spectrum of lentic (pond-like) to lotic (river-like): (a) California rapid assessment method (CRAM) Index of human impact and hydro-modification (Heady et al., 2015); (b) percent riverine wetland habitat at the estuary; (c) maximum streamflow averaged over the last 5 years; (d) length of the longest continuous river flow in the watershed; (e) proportion forested habitat in the watershed; and (f) proportion of impervious surfaces in the watershed. We downloaded all habitat data from EcoAtlas<sup>TM</sup> (California Wetlands Monitoring Workgroup, 2019). We calculated percent riverine wetland habitat from spatial wetland data gathered by the US Geological Survey using the 'geojson' package v 0.3.2 (Chamberlain & Ooms, 2017) in R (R Core Team, 2019); we calculated the area of each wetland type within a 500 m radius of the sampling point using the R package 'sp' v 1.3-1 (Bivand, Pebesma, & Gomez-Rubio, 2013), then estimated the percentage of 'riverine' wetland habitat compared to overall wetland area. Second, we downloaded climate data from the closest upstream National Weather Service station (Table S1) for three variables averaged over the 4 years preceding both historical (1970s) and contemporary (2017–2018) samples: (a) average temperature; (b) Palmer Drought Index (Palmer, 1965); and (c) total annual precipitation.

We ran separate principal component analyses (PCA) for each of the seven (contemporary) habitat variables three (historical and contemporary) climate variables using the R package 'PCAmethods' (Stacklies, Redestig, Scholz, Walther, & Selbig, 2007). We used the first principal component of each PCA for subsequent analyses. The first principal component for habitat (hereafter,  $PC1_{\text{habitat}}$ ) described 40% of the variation and corresponded to decreasing CRAM index (=increasing human impact, loading = -0.09), and increasing riverine wetland (+0.39), log-transformed streamflow (+0.62), log-transformed longest river flow (+0.56), forested habitat (+0.37), and impervious surfaces (+0.06). The first principal component for climate (hereafter,  $PC1_{\text{climate}}$ ) described 74% of the variation and corresponded to increasing average annual temperature (+0.647), decreasing log-transformed total annual precipitation (-0.56), and decreasing drought index (=increasing drought severity, -0.52).

## 2.5 | Analysis

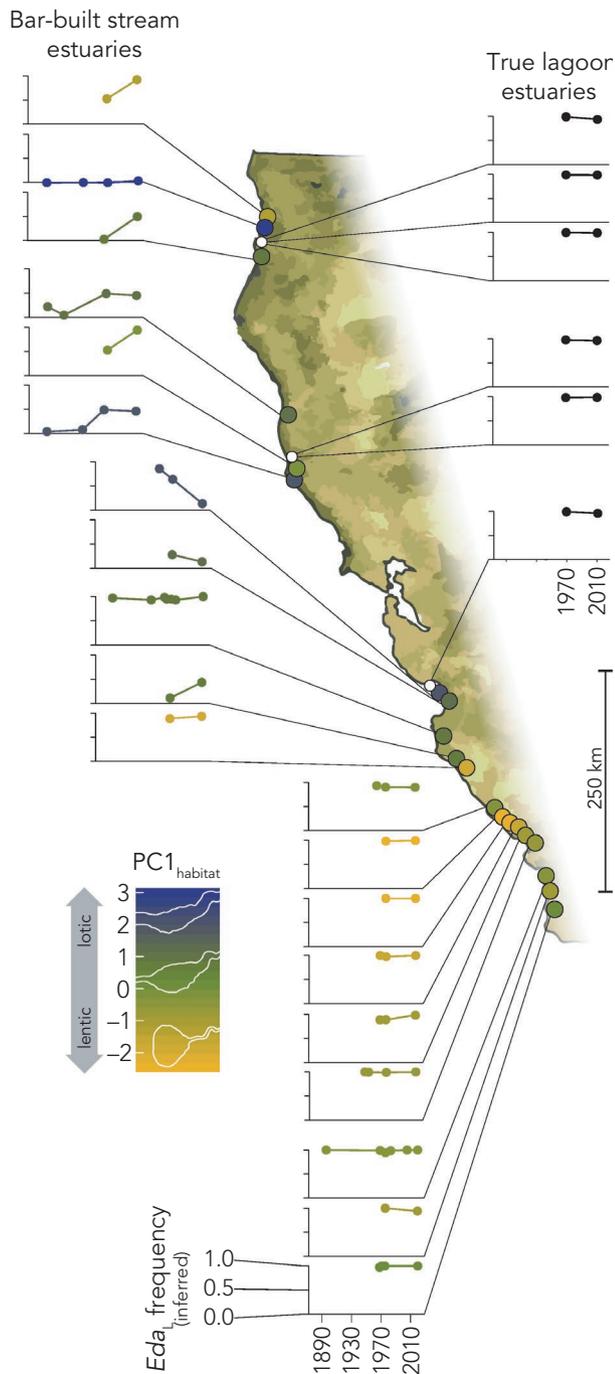
We first examined the relationship between  $PC1_{\text{habitat}}$  and  $PC1_{\text{climate}}$  and between change in  $PC1_{\text{climate}}$  (1970s to contemporary) and  $PC1_{\text{habitat}}$ , using a linear fixed-effects model. We compared the results of these models for both bar-built stream estuaries and true lagoons. We performed bootstrap analysis (comparing the actual  $t$ -value for lagoon and stream estuary comparison to a distribution of  $t$  values for six randomly drawn sites) to estimate the likelihood of the differences in inferred allele frequencies between these two ecosystem types. We then explored relationships between the inferred frequency of  $Eda_L$  and latitude, sample year (all historical samples), and their interaction. We next used structural equation models (with the R package 'piecewiseSEM'; Lefcheck, 2016) to determine the interrelationships among contemporary inferred  $Eda_L$  frequency,  $PC1_{\text{habitat}}$ , and  $PC1_{\text{climate}}$ . We tested the effects of  $PC1_{\text{habitat}}$  and change in  $PC1_{\text{climate}}$  on relationship between contemporary and historical frequency of inferred  $Eda_L$ . For all models with inferred  $Eda_L$  as a response variable, we used generalized linear fixed-effects models fitted with a binomial distribution employing a logit link function. We cross-validated these models using a 'leave-one-out' analysis to confirm that the results were not driven by any single site. Because permits restricted our collection numbers, contemporary sample sizes were necessarily smaller than historical sample sizes (Table S3); in order to account for this discrepancy, we weighted all generalized linear-models by sample size. Finally, we evaluated change in heterozygosity (frequency of the partial morph) between historical and contemporary samples using a Welch's paired  $t$  test, and determined whether the slope of the relationship between historical and contemporary samples differed significantly from one using a linear hypothesis test. We used quantile regression to evaluate whether contemporary plate number variance was related to  $PC1_{\text{climate}}$  and  $PC1_{\text{habitat}}$ . We evaluated the significance of main effects and their interactions using Type III ANOVA tests implemented in the R package 'car' (Fox & Weisberg, 2011) and performed all other analysis in base R (R Core Team, 2019).

## 3 | RESULTS

### 3.1 | Change in frequency of the low allele across time and latitude

Inferred frequency of low  $Eda$  allele ( $Eda_L$ ) in stickleback populations increases with decreasing latitude, and has generally increased over time; however, the magnitude of this trend is strongly dependent on ecosystem type, with the most extreme changes occurring in bar-built stream estuaries. Our results are consistent with  $Eda_L$  remaining near fixation for populations from isolated 'true lagoons' (little to no upstream fluvial inputs, rare ocean connection; Heady et al., 2015), remaining unchanged over time and across latitudes (Figure 1: binomial GLM: lagoon  $Eda_L \sim (\text{latitude} + \text{time})^2$ ; all  $p > .05$ ). Bootstrap analysis

showed a 2.5% chance of randomly sampling sites with as little change in  $Eda_L$  frequency as that displayed in the true lagoons. In contrast, populations from bar-built stream estuaries show significant changes

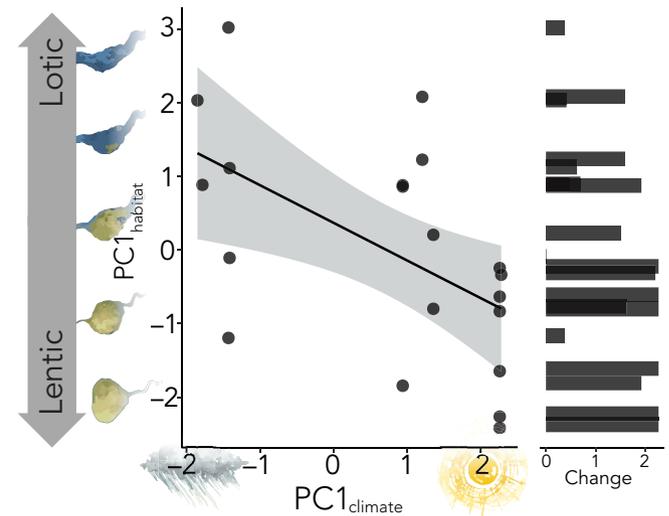


**FIGURE 1** Change in the frequency of  $Eda_L$  (Ectodysplasin A low allele) inferred from plate phenotype over the last century (1896–2018) for bar-built stream (left) and lagoon (right) estuaries across a latitudinal gradient in California. Lagoon estuaries represented by the same point on the map are within 20 km of one another. Bar-built stream estuaries are coloured by  $PC1_{habitat}$ . Bar-built stream estuaries become more lotic and less lentic with increasing  $PC1_{habitat}$ , which corresponds to increased: human modification, riverine wetland, streamflow, longest river flow, forested habitat, and impervious surfaces

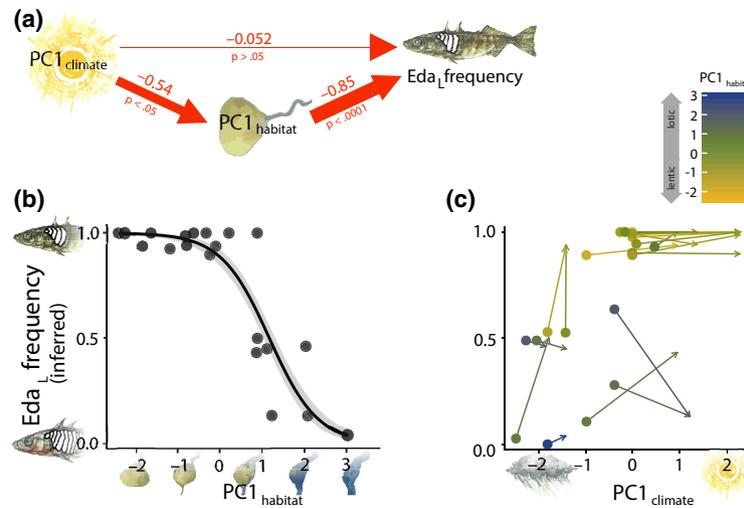
in inferred  $Eda_L$  frequencies with time, latitude, and their interaction; specifically, increase in inferred  $Eda_L$  frequency through time is most drastic at higher latitudes (Figure 1; Binomial GLM:  $Eda_L \sim (\text{latitude} + \text{time})^2$ ; all main effects and two-way interactions  $p \ll .0001$ ). Inferred frequency of  $Eda_L$  in bar-built stream estuaries generally predicts  $Eda_L$  frequency upstream; a pattern that is more prominent at low latitudes and for historical samples from the 1970s (binomial GLM: upstream  $Eda_L \sim (\text{estuary } Eda_L + \text{latitude})^2$ ; all main effects and interactions  $p \ll .0001$ ). At northern latitudes, shifts in inferred  $Eda_L$  frequency between contemporary and historical samples are more drastic at estuaries compared to upstream sites (Figure S1), where inferred  $Eda_L$  generally decreases with increasing distance from the ocean (binomial GLM:  $Eda_L \sim (\text{time} + \text{distance} + \text{latitude})^2$ ; distance, latitude, year\*latitude, distance\*latitude:  $p \ll .0001$ ).

### 3.2 | The effect of habitat and climate on $Eda_L$ frequency

The contemporary habitat of Californian stream estuaries is significantly related to climate. Increasing  $PC1_{habitat}$  generally corresponds with the transition from a primarily lentic (pond-like) to a primarily lotic (river-like) habitat. Specifically,  $PC1_{habitat}$ , which describes increases in river wetland, maximum stream flow, and modification at the estuary, and increases in impervious surfaces, forested habitat, and continuous river flow in the watershed (see Section 2 for detailed description) is negatively correlated with  $PC1_{climate}$ , which corresponds to increasing temperature and drought, and decreasing



**FIGURE 2** Relationship between  $PC1_{habitat}$  and  $PC1_{climate}$  (left) and change in  $PC1_{climate}$  over the last 40 years (right) for bar-built stream estuaries only. Increasing  $PC1_{climate}$  corresponds to decreasing precipitation, and increasing temperature and drought. Bar-built stream estuaries become more lotic and less lentic with increasing  $PC1_{habitat}$ , which corresponds to increased: human modification, riverine wetland, streamflow, longest river flow, forested habitat, and impervious surfaces



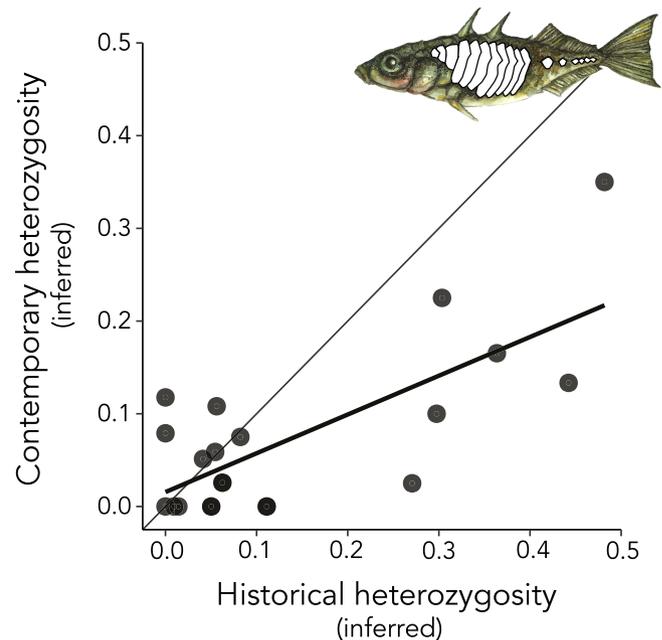
**FIGURE 3** (a) Path diagram for structural equation model demonstrating support for the indirect effects of PC1<sub>climate</sub> on *Eda<sub>L</sub>* (Ectodysplasin A low allele) through PC1<sub>habitat</sub>. (b) PC1<sub>habitat</sub> at bar-built stream estuaries (from lentic to lotic habitat) as a predictor of contemporary frequency of *Eda<sub>L</sub>* inferred from plate phenotype. (c) Vectors showing change in the frequency of *Eda<sub>L</sub>* and change in PC1<sub>climate</sub> (increasing temperature and drought, decreasing precipitation) between historical (1970s, 1980s) and contemporary (2017, 2018) samples coloured by PC1<sub>habitat</sub>, which corresponds to increased: human modification, riverine wetland, streamflow, longest river flow, forested habitat, and impervious surfaces

precipitation (LM: PC1<sub>habitat</sub>~PC1<sub>climate</sub>;  $F = 7.67$ ;  $p < .05$ ; adjusted- $R^2 = .26$ , Figure 2). Change in PC1<sub>climate</sub> between historical (1970s) and contemporary samples was also significantly correlated with PC1<sub>habitat</sub>, showing evidence that 'lentic' habitats experienced greater changes in temperature, drought, and precipitation (LM:  $\Delta PC1_{climate} \sim PC1_{habitat}$ ;  $F = 8.54$ ;  $p < .01$ ; adjusted- $R^2 = .28$ ; Figure 2).

Within stream estuaries, contemporary variation in inferred *Eda<sub>L</sub>* frequency is tied to habitat and climate (Figure 2). Results of our structural equation model favour the hypothesis that climate affects *Eda<sub>L</sub>* through habitat (Figure 3a;  $Eda_{L} \sim PC1_{climate}$ : Std. Estimate =  $-0.0516$ ;  $p = .077$ ;  $Eda_{L} \sim PC1_{habitat}$ : Std. Estimate =  $-0.848$ ,  $p \ll .0001$ ;  $PC1_{habitat} \sim PC1_{climate}$ : Std. Estimate =  $-0.547$ ;  $p = .01$ ). Specifically, *Eda<sub>L</sub>* decreases with increasing PC1<sub>habitat</sub> (Figure 3b: binomial GLM:  $Eda_{L} \sim PC1_{habitat}$ ;  $\chi^2 = 955.7$ ;  $p \ll .0001$ , McFadden pseudo- $R^2 = .74$ ), which is significantly correlated with PC1<sub>climate</sub>, as previously described. The relationship between contemporary and historical *Eda<sub>L</sub>* frequency is significantly affected by change in PC1<sub>climate</sub> and by contemporary PC1<sub>habitat</sub> (Figure 3c: binomial GLM:  $Eda_{L} \sim (\Delta PC1_{climate} + PC1_{habitat})^2$ ; all two-way interactions  $p < .01$ ). Leave-one-out cross validation analysis confirmed model results were not sensitive to, and thereby unlikely driven by, the data at any given site (all  $p < .05$ ).

### 3.3 | Changes in intraspecific variation in heterozygosity and phenotype

Inferred heterozygosity (frequency of the partial morph) at *Eda* is significantly lower in contemporary compared to historic samples from the 1970s (paired  $t$  test:  $t = -2.31$ ;  $p = .03$ ), and the slope of the relationship between historic and contemporary heterozygosity is significantly lower than 1 (Figure 4; linear hypothesis test:  $\chi^2 = 28.8$ ,



**FIGURE 4** Contemporary (2017–2018) versus historical (1970s, 1980s) frequency of the 'partial' phenotype (approximate inferred heterozygosity at the Ectodysplasin A locus) plotted with a 1:1 line

$p < 0.0001$ ). Populations with historically higher heterozygosity and with higher PC1<sub>habitat</sub> show the sharpest declines in heterozygosity (Binomial GLM:  $Eda_{CL} \sim (\text{historical } Eda_{CL} + PC1_{habitat})^2$ ; historic heterozygosity, PC1<sub>habitat</sub>, all main effects and two-way interactions:  $p < .01$ ). For contemporary samples, mean and variation in plate phenotype increases with PC1<sub>climate</sub> and decreases with PC1<sub>habitat</sub> (Figure S2: quantile regression ANOVA;  $p \ll .001$ ).

## 4 | DISCUSSION

Our broad resurvey of Threespine Stickleback populations in Californian estuaries provides comprehensive evidence for climate-driven evolution in a heritable phenotypic trait across a latitudinal gradient. First, we show that the inferred frequency of  $Eda_L$  (derived 'low' allele) in contemporary samples increases with decreasing latitude and is strongly correlated with habitat characteristics, generally characterized on the spectrum of lentic to lotic. Next, we show that inferred frequency of  $Eda_L$  has generally increased over the last century, corresponding with decreased precipitation, and increased temperature and drought severity, a pattern also reflected across a spatial climate gradient from drier in the south to wetter in the north. Our results show that contemporary variation in stickleback genotype with climate may be mediated by estuary habitat. This relationship suggests the mechanism by which climate change may be driving evolutionary change in stickleback over time. Finally, we demonstrate that intraspecific diversity, in terms of inferred heterozygosity at  $Eda$  (frequency of partially plated stickleback) has declined since the 1970s, a possible consequence of climate change driving the fixation of  $Eda_L$  and the loss of the complete  $Eda$  allele.

Inferred  $Eda_L$  frequency varies significantly across spatial and temporal gradients. First, the frequency of  $Eda_L$  generally increases from north to south, a pattern consistent with historic results from Californian (Baumgartner & Bell, 1984; Miller & Hubbs, 1969; Rutter, 1896; Swift et al., 1993) and European (Gross, 1977; Münzing, 1963) estuaries. Our results also indicate that the range of the ancestral complete allele may be retracting northward: the most southern estuary where we collected a completely plated stickleback was in San Carpoforo Creek (~3,959,370 mN, zone 10N), whereas previous samples record its presence as far south as the San Simeon Creek estuary in 1977 (~3,940,750 mN; Baumgartner & Bell, 1984) and the Pico Creek estuary in 1969 (~3,942,980 mN, the Natural History Museum of Los Angeles County collections), though further sampling is needed to support its actual absence. Latitudinal distribution of  $Eda_L$  may be at least partially associated with the current and historic absence of the completely plated anadromous morph in southern estuaries, which do not have continuous access between the river and ocean (Baumgartner & Bell, 1984; Jacobs et al., 2011; Swift et al., 1993). Yet, our results are more consistent with the hypothesis that  $Eda_L$  frequency is associated with habitat and climatic variation. Second, our results support increases in  $Eda_L$  frequency in Californian stickleback populations over the span of decades. Directional selection on plate phenotype and genotype is widespread, and researchers have recorded its rapid evolution both in experimental and wild populations of stickleback (reviewed in Bell & Aguirre, 2013). The evolutionary loss or gain of plates in nonanadromous stickleback may be contingent on the presence and type of piscivorous predators, for example, completely plated fish are less vulnerable to predation from sculpin and salmon (Barrett & Schluter, 2008). Yet, there are many accounts (including the current study) of low-plated populations coexisting with these predators in certain habitats (Kitano et al., 2008; Moodie, 1972). For example,

stickleback were primarily low-plated in the presence of predatory salmonids in the turbid, eutrophic water of Lake Washington. Only after de-eutrophication efforts increased water clarity did stickleback 'reverse evolve' the completely plated phenotype, presumably following increased threat from visual predators (Kitano et al., 2008).

The frequency of inferred  $Eda_L$  in stickleback populations is strongly correlated with contemporary habitat characteristics across latitudes. First, both historical and current allele frequencies depend on whether stickleback were from isolated 'true lagoons' (little to no upstream fluvial inputs, rare ocean connection), bar-built stream estuaries (upstream fluvial inputs, changes in ocean connectivity), or upstream habitats. Populations inhabiting bar-built stream estuaries show the most variation in allele frequency across both space and time compared to those from true lagoons, which show consistently high frequencies of the low allele, even at higher latitudes where  $Eda_L$  frequency of populations in stream estuaries have changed dramatically. Bar-built stream estuaries are particularly dynamic, influenced by climatic impacts from both upstream and marine habitats (Heady et al., 2015; Jacobs et al., 2011; Rich & Keller, 2013).

The effect of climate on contemporary  $Eda_L$  frequency appears to be mediated by habitat characteristics, with the strongest associations between climate and habitat, and between habitat and  $Eda_L$  frequency. The relationship between climate and estuary habitat is multifaceted. Climate may directly affect estuaries by altering streamflow and the amount of river habitat, and its effects may depend on habitat characteristics such as the extent of human hydro-modification and extent of surrounding impervious surface (Jacobs et al., 2011; Richards et al., 2018). Generally,  $PC1_{\text{climate}}$  is negatively related to  $PC1_{\text{habitat}}$ : drier, warmer, and more drought-prone estuaries tend to be more lentic and have more low-plated fish (higher  $Eda_L$  frequency), whereas wetter, cooler, and less drought-prone estuaries tend to be more lotic with more completely plated fish (lower  $Eda_L$  frequency). Our results are consistent with previous studies showing that the low-plated phenotype is typically associated with slower streamflow and more habitat structure (Baumgartner & Bell, 1984; Bell, 1982; Hagen, 1967; Taylor & McPhail, 1986). For example, several studies show that selection favours fewer plates to increase maneuverability and fast starts in slow-water habitats with more complex structure (Dalziel et al., 2011; Klepaker, 1993; Reimchen, 2000; Taylor & McPhail, 1986).

The relationships among climate, habitat, and  $Eda_L$  across space indicate that similar processes may be operating through time. Our results show that both contemporary habitat and shifts in climate are related to the change in  $Eda_L$  frequency. Climate change had the largest impact on populations inhabiting bar-built estuaries that were polymorphic in the 1970s. Though the southernmost populations experienced the largest increases in values of  $PC1_{\text{climate}}$  since the 1970s, they did not display the greatest increases in  $Eda_L$  frequency, likely because the allele was already near fixation. More lotic estuaries (high  $PC1_{\text{habitat}}$ ) showed either little change or decreased  $Eda_L$  frequency, suggesting that the effect of other habitat changes (i.e. human estuary

modification, urban development) might buffer or even override climate impacts. For example, the two sites that showed decreased *Eda<sub>L</sub>* frequency are among of the most managed estuaries in California (DW Alley & Assoc, personal communication) and in the last 40 years have undergone river channelization, dredging, and forced ocean breaching, all leading to a more lotic habitat (Alley, Lyons, Chartrand, & Sherman, 2004; Heady et al., 2015). Although we lack historical estuary habitat data, our results stress the importance of the close relationship between climate change and habitat.

Frequency of the partial morph (an indicator of heterozygosity at *Eda*), has decreased since the 1970s. Decline in genetic diversity is one inevitable consequence of allele fixation through either drift or directional selection (Wright, 1965). Over the last 40 years, the most polymorphic populations in the 1970s showed the greatest evolutionary change and experienced the largest decreases in heterozygosity. No substantial evolution occurred in populations that were at or near 100% frequency of *Eda<sub>L</sub>*, indicating its potential fixation at lower latitudes. Loss of diversity at genes of large effect often foreshadows population extirpation due to the lack of standing genetic variation required for future adaptation (Botero, Weissing, Wright, & Rubenstein, 2015). This issue is further compounded by climate change causing increased estuary isolation, restricting geneflow from introducing new genetic variation (Paccard et al., 2018). Finally, the three estuaries for which we were unable to recover stickleback during contemporary sampling efforts showed *Eda<sub>L</sub>* frequencies that were at or near fixation (inferred heterozygosity = 0–0.003) in the 1970s. These three estuaries have also been subject to introduced fishes, including the Sacramento Pikeminnow (*Ptychocheilus grandis*) and the Largemouth Bass (*Micropterus salmoides*), both known stickleback predators (Bell & Stamps, 2004; Nakamoto & Harvey, 2003). These patterns implicate a possible combination of climate change and predation by an exotic invader in the local extirpation of a native species.

Global climate change is altering habitats and, as a result, shifting the selective pressures that drive adaptation in natural populations. Associations between strongly heritable phenotypes and climate variables across latitudes can signal contemporary and future evolutionary responses to climate and habitat change. In our study, both spatial and temporal patterns suggest that climate change has caused rapid evolution in Threespine Stickleback in California. Patterns of evolved changes in stickleback *Eda<sub>L</sub>* frequency over the last century mirror inferred contemporary and historical allelic distributions across latitudes, predictably increasing with decreased precipitation, and increased temperature and drought severity. Our results show that climate change can impact species through a complex relationship with their habitats. The evolutionary effects of climate may therefore depend strongly on changes in habitat. Globally, excessive human water use is driving the lentification of rivers, causing previously open and dynamic systems to become more structured, stagnant, and pond-like (Sabater, 2008), which has likely selected for low-plated stickleback in Californian estuaries. Climate-related habitat changes may be counteracted by other factors such as direct human habitat modification, which may have caused an evolutionary reversal in

stickleback by creating more riverine, or lotic estuaries. Our results suggest that stickleback may be used as a model indicator species for the evolutionary and ecological effects of climate change, and also that habitat management could be directed at deliberately offsetting the eco-evolutionary effects of climate change and preserving genetic variation and the potential for future adaptation.

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## DATA AVAILABILITY STATEMENT

All data are accessible in the Dryad Digital Depository at <https://doi.org/10.5061/dryad.08kpr4xt>.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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