

Rampant drift in artificially fragmented populations of the endangered tidewater goby (*Eucyclogobius newberryi*)

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Abstract

Habitat fragmentation and its genetic consequences are a critically important issue in evaluating the evolutionary penalties of human habitat modification. Here, we examine the genetic structure and diversity in naturally subdivided and artificially fragmented populations of the endangered tidewater goby (*Eucyclogobius newberryi*), a small fish restricted to discrete coastal lagoons and estuaries in California, USA. We use five naturally fragmented coastal populations from a 300-km spatial scale as a standard to assess migration and drift relative to eight artificially fragmented bay populations from a 30-km spatial scale. Using nine microsatellite loci in 621 individuals, and a 522-base fragment of mitochondrial DNA control region from 103 individuals, we found striking differences in the relative influences of migration and drift on genetic variation at these two scales. Overall, the artificially fragmented populations exhibited a consistent pattern of higher genetic differentiation and significantly lower genetic diversity relative to the naturally fragmented populations. Thus, even in a species characterized by habitat isolation and subdivision, further artificial fragmentation appears to result in substantial population genetic consequences and may not be sustainable.

Keywords: conservation genetics, endangered species, fish, habitat fragmentation

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Introduction

Habitat fragmentation is an important process in both evolutionary and conservation biology (Meffe & Carroll 1997; Freeman & Herron 2007). Natural fragmentation often occurs over geologic time scales, increases genetic differentiation of populations, leads to phylogeographical structure, and can result in speciation through a combination of evolutionary mechanisms (Barton & Charlesworth 1984; Dawson *et al.* 2002). In contrast to these natural processes, artificial fragmentation occurs over recent time scales and typically results in more extreme outcomes, including increases in genetic differentiation and loss of genetic diversity in remnant habitat patches (Templeton *et al.* 1990). The final outcomes of artificial fragmentation are reduced fitness and adap-

tive potential of a population (Frankham 2003; Allendorf & Luikart 2007; Johansson *et al.* 2007).

While the effects of natural fragmentation and limited dispersal on phylogeographical structure and evolution are well known (Avice *et al.* 1987; Dawson *et al.* 2001; Gysels *et al.* 2004), and many studies have shown that artificial fragmentation results in drastic changes to the genetic variation of historically continuous populations (Frankham 1995; Keller & Largiader 2003; Johnson *et al.* 2004), artificial fragmentation of a species that is already naturally fragmented can lead to the most severe genetic consequences (Templeton *et al.* 1990; Hitchings & Beebee 1998; Clark *et al.* 1999; Fumagalli *et al.* 2002). Evaluating these consequences is important to the conservation of estuarine species because of the natural fragmentation of their habitats combined with increasing habitat loss and destruction from coastal development (Helfman 2007). Because of inherent fragmentation from the discrete distribution of habitat

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combined with recent artificial fragmentation from coastal development, the lagoons and estuaries of western North America provide a model system to study conservation genetics of a species among different forms of fragmentation.

The endangered tidewater goby (*Eucyclogobius newberryi*) is subject to both natural and artificial fragmentation. It is a small (<55 mm total length) annual teleost fish endemic to naturally isolated lagoon environments along the entire coast of California, USA (Swift *et al.* 1989; United States Fish and Wildlife Service 1994, 2005). The tidewater goby is unique among eastern Pacific bay gobies because it lacks an explicit marine dispersal stage and spends its entire life (approximately 1 year) within discrete coastal wetlands naturally separated by the presence of sand bars that restrict access to the Pacific Ocean (Swift *et al.* 1989; Swenson 1999; Dawson *et al.* 2002). These sand bars generally breach 1–2 times a year during periods of high surf and freshwater input resulting in rapid draining of the estuary (Krauss *et al.* 2002). Thus, successful migration between lagoon habitats requires coordination of the breaching events. Further, populations are separated by 1–20 km of inhospitable coastline, and although the species is tolerant of full strength seawater, migration between lagoons is thought to be rare (Crabtree 1985; Swift *et al.* 1989; Lafferty *et al.* 1999; Swenson 1999; Dawson *et al.* 2001, 2002).

In addition to the typical lagoon-type habitats, populations of tidewater gobies were historically found in habitats on the margin of California's larger tidal bays including Humboldt Bay, Bodega Harbor, Tomales Bay and San Francisco Bay. The historical habitats in these settings are poorly characterized, but tidewater goby habitats comparable to those in lagoons likely formed where bodies of water were isolated in marsh top ponds, in ponds formed on the landward edges of marshes, and where stream and marsh channels were closed by wave action. Further work characterizing these historical habitats is important but beyond the scope of this study.

Habitat destruction from agriculture and coastal development resulted in a drastic decline in the number of known tidewater goby populations, resulting in the listing of the species as 'endangered' under the United States Endangered Species Act (United States Fish and Wildlife Service 1994). Presently, about 21% of the 135 historically documented populations are extirpated, and approximately 50% of the remaining populations are considered vulnerable to extinction because of severe habitat degradation (United States Fish and Wildlife Service 2005, 2007). The most severe destruction of tidewater goby habitats has been focused within tidal bays and urbanized areas. All tidewater gobies have been extirpated from San Francisco Bay and Bodega Harbor

while those in Tomales Bay appear to have been bottlenecked (United States Fish and Wildlife Service 2005). Thus, Humboldt Bay is unique because it represents one of the last remaining tidal bay settings to support tidewater goby populations.

This study focuses on the 13 extant populations of tidewater goby inhabiting the North Coast region of California, including Humboldt Bay (United States Fish and Wildlife Service 2005) (Fig. 1). These tidewater goby populations are recognized as a distinct mitochondrial DNA clade (Dawson *et al.* 2001), are part of a regional distinct radiation in microsatellite phylogeography (Earl *et al.* 2010), and possess a fully developed cephalic lateral line canal system – a morphological adaptation thought to improve sensory ability in the wetter climate of the North Coast region (Ahnelt *et al.* 2004). In addition, tidewater goby have remained relatively abundant in the northern extent of their range, with only two well-documented population extirpations within the last 60 years (United States Fish and Wildlife Service 2008). Taken together, these features make North Coast tidewater goby ideal for studying conservation genetics of an endangered species within the broader ecological context of habitat fragmentation.

Tidewater goby populations are found at two spatial scales in the North Coast region; bay and coast (Fig. 1). The bay scale consists of eight sampled localities within Humboldt Bay, California's second largest estuary (Barnhart *et al.* 1992). These populations inhabit the diked sloughs and lower reaches of streams flowing into Humboldt Bay, and habitat area available for tidewater goby in these sites varies from approximately 0.2 to 396.9 ha (United States Fish and Wildlife Service 2008) (Table 1). Bay populations are separated by an average pairwise distance of 12.9 km with a range of 0.3–8.4 km between sites (United States Fish and Wildlife Service 2008). A combination of tidegates and levees mute tidal exchange within bay habitats (Chamberlain 2006), and all these sampling sites are currently isolated from each other by reclaimed wetlands modified for human uses or by the tidal-modifying features. Thus, an important premise of this study is that these populations are far more isolated from one another than they were historically, when populations were not confined by levees but were capable of migration across marsh habitats during high tidal or stream flow conditions.

The coast scale is comprised of five naturally fragmented populations covering the northernmost 300 km of coastline in the species range. These populations occupy lagoons that range in size from 4.5 to 1085.4 ha and are isolated from each other by 1.9 to 190.5 km of inhospitable coastline (United States Fish and Wildlife Service 2008) (Table 1). An average pairwise distance of 146.8 km separates coast populations. All coast scale

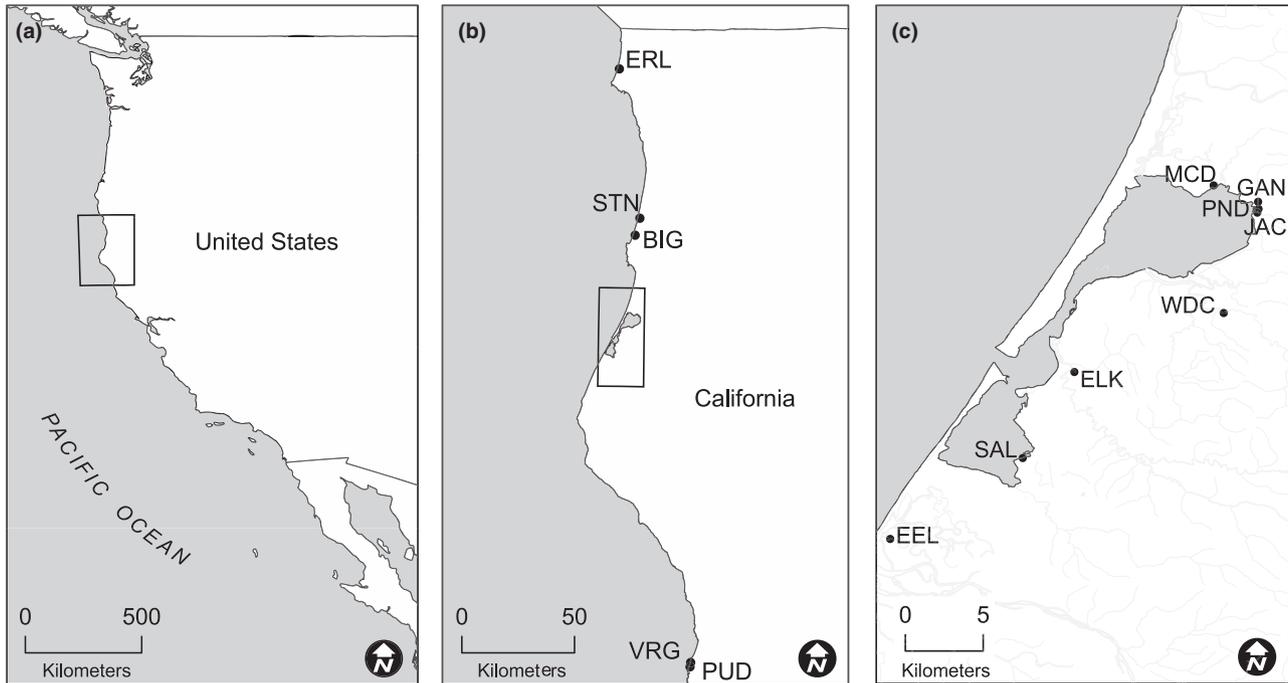


Fig. 1 Tidewater goby occur in isolated lagoons along California’s coast. (a) Rectangle depicts the North Coast region of California, (b) five naturally fragmented populations in the coast scale [Lake Earl (ERL), Stone Lagoon (STN), Big Lagoon (BIG), Virgin Creek (VRG), Pudding Creek (PUD)] with a rectangle around Humboldt Bay, and (c) eight artificially fragmented sites in Humboldt Bay [McDaniel Slough (MCD), Gannon Slough (GAN), Gannon Pond (PND), Jacoby Creek (JAC), Wood Creek (WDC), Elk River (ELK), Salmon Creek (SAL), Eel River (EEL)].

Table 1 Populations, sample ID, habitat area, microsatellite DNA results (sample size (*n*), proportion of polymorphic loci (*P*), number of private alleles (*A_p*), genetic diversity [mean ± standard error: allelic richness (*A*), expected heterozygosity (*H_E*), observed heterozygosity (*H_O*)], and mitochondrial DNA results {samples size (*n*), number of haplotypes (*n_H*), number of private haplotypes (*n_{pH}*), sequence diversity [mean ± standard error: haplotype diversity (*h*), nucleotide diversity (*π*)] in North Coast tidewater goby

Population	ID	Area (ha)	Microsatellite DNA						Mitochondrial DNA				
			<i>n</i>	<i>P</i>	<i>A_p</i>	<i>A</i> (±SE)	<i>H_E</i> (±SE)	<i>H_O</i> (±SE)	<i>n</i>	<i>n_H</i>	<i>n_{pH}</i>	<i>h</i> (±SE)	<i>π</i> (±SE)
Artificially fragmented bay scale													
McDaniel Slough	MCD	34.8	32	0.56	0	1.7 (0.3)	0.18 (0.07)	0.18 (0.08)	4	1	0	0.00 (0.00)	0.0000 (0.0000)
Gannon Slough	GAN	18.2	52	0.67	0	1.9 (0.3)	0.22 (0.09)	0.22 (0.09)	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Gannon Pond	PND	0.2	17	0.44	0	1.6 (0.2)	0.21 (0.09)	0.23 (0.10)	4	1	0	0.00 (0.00)	0.0000 (0.0000)
Jacoby Creek	JAC	6.2	52	0.56	0	1.7 (0.2)	0.15 (0.06)	0.16 (0.07)	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Wood Creek	WDC	0.4	52	0.33	0	1.4 (0.2)	0.12 (0.06)	0.10 (0.06)	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Elk River	ELK	35.1	51	0.78	0	1.8 (0.2)	0.27 (0.07)	0.28 (0.08)	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Salmon Creek	SAL	396.9	52	0.67	1	2.2 (0.6)	0.24 (0.09)	0.23 (0.09)	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Eel River	EEL	108.5	52	0.89	0	2.7 (0.5)	0.31 (0.09)	0.28 (0.09)	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Naturally fragmented coast scale													
Lake Earl	ERL	1085.4	52	0.67	2	3.2 (1.0)	0.27 (0.10)	0.27 (0.10)	8	2	1	0.25 (0.06)	0.0005 (0.0020)
Stone Lagoon	STN	236.7	53	0.89	4	4.9 (1.4)	0.52 (0.10)	0.52 (0.09)	8	4	2	0.75 (0.05)	0.0018 (0.0006)
Big Lagoon	BIG	612.5	52	0.89	6	4.9 (1.3)	0.56 (0.09)	0.59 (0.09)	8	3	1	0.68 (0.04)	0.0015 (0.0005)
Virgin Creek	VRG	4.5	52	1.00	3	3.7 (0.6)	0.57 (0.07)	0.58 (0.07)	9	3	1	0.56 (0.06)	0.0016 (0.0005)
Pudding Creek	PUD	9.5	52	1.00	0	2.9 (0.7)	0.43 (0.08)	0.45 (0.09)	8	2	1	0.43 (0.06)	0.0008 (0.0003)

populations are isolated by sand bars that restrict tidal exchange between the Pacific Ocean and the lagoon (Chamberlain 2006).

Our objective in this study was to describe how fragmentation has influenced neutral genetic variation of the endangered tidewater goby in the North Coast

region. We predicted that artificially fragmented bay populations would exhibit extreme population structure at small spatial scales because of the levees and tidegates that isolate populations and presumably restrict migration. Also, despite this species adaptations for residence in the naturally fragmented estuaries of California, including an annual life history within lagoons, early sexual maturation, multiple spawning periods and broad environmental tolerance (Goldberg 1977; Swift *et al.* 1989; Swenson 1999; McGourty *et al.* 2008), we expected that artificially fragmented populations would suffer impoverished genetic diversity from habitat loss and reduced population sizes. To confirm these two predictions, we compared population structure and genetic diversity of artificially fragmented bay and naturally fragmented coast samples.

Materials and methods

Sample collection and tests of assumptions

Tissue samples were collected between August and September 2006 from 621 individuals representing all known populations in the North Coast region at the time of this study (Fig. 1, Table 1). Approximately equal numbers of individuals were gathered from each scale with beach seine or dip net. Tissue samples were obtained nonlethally by dissection of a small (1 mm²) piece of the pelvic disc and were either dried or preserved in 95% ethanol. Genomic DNA was extracted using spin columns lined with a silica membrane (Qia-gen DNeasy[®] Blood and Tissue kit) following the manufacturers protocols.

Variation was assayed at nine microsatellite loci specifically developed for tidewater goby (Mendonca *et al.* 2001; Earl *et al.* 2010) (Table 2). Genotypes were assayed by polymerase chain reaction with fluorescent labelling of the forward primer and automated capillary gel electrophoresis. Reactions were performed using Master Mix[®] (Promega, Madison, WI, USA) in an MJ Research (Waltham, MA, USA) PTC-100 thermal cycler with 12.5 µL volumes following cycling conditions in Mendonca *et al.* (2001) and Jacobs *et al.* (2005). All microsatellite genotypes were read and scored using the Beckman-Coulter CEQ 8000 Genetic Analysis System. We verified all fragment sizes estimated by Beckman-Coulter Genetic Fragment Analysis software by visual inspection of the electropherograms.

We estimated the microsatellite scoring error rate in the dataset by randomly resampling 10% of the individuals from each population and re-genotyping them. The original electropherograms were compared to the test electropherograms to evaluate levels of large allele dropout and technical sizing errors. We calculated the

Table 2 North Coast tidewater goby microsatellite information, including the number of alleles (*k*), and quality control results (per cent missing data, per cent errors per allele, per cent errors per reaction)

Locus	<i>k</i>	Missing data (%)	Errors per allele (%)	Errors per reaction (%)
ENE2	4	4.2	2.8	2.1
ENE5	4	5.6	0.0	0.0
ENE6	8	8.7	0.0	0.0
ENE8	12	7.3	0.0	0.0
ENE9	30	5.2	7.3	8.3
ENE12	11	1.9	0.0	0.0
ENE13	3	3.6	2.6	2.1
ENE16	6	2.9	7.7	8.3
ENE18	6	5.0	0.0	0.0
Overall		4.9	2.3	2.3

error rate per allele and per reaction for each locus and then averaged the rate over all loci (Bonin *et al.* 2004; DeWoody *et al.* 2006). ARLEQUIN 3.1 (Schneider *et al.* 2000) was used to test microsatellite genotypes for deviations from Hardy–Weinberg Equilibrium at each locus within each population with a Markov Chain Monte Carlo (MCMC) procedure of Fisher's exact test. Strict Bonferroni corrections were applied to critical significance levels to adjust for multiple comparisons (Rice 1989). We used FSTAT 2.9.3.2 (Goudet 1995) to test for genotypic disequilibrium on each locus pair across all populations with 9360 permutations.

Population structure

We examined population structure among North Coast tidewater goby with principal components analysis (PCA) of populations and Bayesian clustering of individuals. The primary objective of these analyses was to verify our assumption that the artificially fragmented bay scale and the naturally fragmented coast scale comprised separate groups – a critical premise for subsequent comparisons of genetic variation over these two scales. The computer program PCA-GEN 1.2 (Goudet 1999) was used to ordinate samples and test the first three principal components axes for significance with 15 000 randomizations. Bayesian clustering of individuals was performed in the computer program STRUCTURE 2.3.2 (Pritchard *et al.* 2000; Falush *et al.* 2003) for 1–13 clusters (*K*) of individuals. From exploratory analyses, we determined that burn in and MCMC lengths of 10 000 iterations each were sufficient for convergence. We principally relied on the default parameter set (i.e., admixture model, allele frequencies correlated) to cluster individuals but also conducted a second analysis using sampling locations as priors under the admixture

model. Twenty independent runs at each K were simulated to account for variation in the posterior probability of the data [$L(K)$] from different runs, and following the method of Evanno *et al.* (2005), we estimated ΔK to infer the strongest level of population structure. Proportional membership coefficients (Q) of individuals from the default parameter simulations were plotted in DISTRICT 1.1 (Rosenberg 2004) to visualize hierarchical patterns in clustering of predefined populations.

FSTAT was used to estimate genetic differentiation (F_{ST}) between population pairs (Weir & Cockerham 1984) and test for pairwise differentiation (Goudet *et al.* 1996). We evaluated the relative influences of migration and drift on population structure at each scale by correlating F_{ST} with geographical distance and testing for statistical significance with 20 000 permutations of Mantel's (1967) test in FSTAT (Hutchinson & Templeton 1999). A significant linear relationship of increased genetic differentiation at greater geographical distances is expected under the stepping-stone model of gene flow when the opposing forces of migration and drift are in equilibrium (Johnson *et al.* 2003; Jordan & Snell 2008). In contrast, a nonsignificant linear relationship between genetic differentiation and geographical distance combined with large variance in pairwise F_{ST} is expected under the scenario of drift in extreme isolation (Hutchinson & Templeton 1999).

Genetic diversity

The proportion of polymorphic loci (P) was calculated for each population by averaging the number of loci with frequencies of the most common allele below 0.99. We used FSTAT to estimate allelic richness based on a minimum sample size of 15 diploid individuals. ARLEQUIN was used to estimate observed (H_O) and Nei's (1978) unbiased expected heterozygosity (H_E). Tests for significant differences in estimates of allelic richness, observed and unbiased expected heterozygosity between bay and coast populations were performed with 15 000 permutations of the samples between scales in FSTAT.

We evaluated the influence of demography on the genetic diversity and differentiation of North Coast tidewater goby in two ways. First, to investigate the effects of habitat area on genetic diversity within populations, we plotted allelic richness as a function of Log10 habitat area (ha) for each population scale, and tested the null hypothesis that levels of genetic diversity are independent of population size (with available habitat area assumed to be a correlate of population size) (Jordan & Snell 2008). A significant linear relationship is expected between these two variables when reductions in available habitat area have resulted in popula-

tion bottlenecks. Second, to evaluate the role of population bottlenecks on genetic differentiation, we correlated F_{ST} with the average of observed heterozygosity for each population pair at inter- and intrascale levels (Hedrick 1999; Goodman *et al.* 2001; Jordan & Snell 2008). A significant negative correlation between F_{ST} and mean pairwise H_O is expected when drift because of extreme isolation and/or small population size has reduced genetic diversity and inflated estimates of population differentiation. All tests for statistical significance were conducted with 20 000 permutations of the Mantel test in FSTAT.

Mitochondrial DNA

In addition to the microsatellite assays, we also sequenced a total of 103 individuals from the 13 populations at the D-loop region of the mitochondrial control region using primers CR-A and CR-M (Lee *et al.* 1995). Polymerase chain reactions were performed using Master Mix[®] in a MJ Research PTC-100 thermal cycler with 25 μ L volumes following cycling conditions as in Dawson *et al.* (2001). Template was sequenced using the forward primer CR-A at High-Throughput Sequencing Solutions (University of Washington, Department of Genome Sciences). We visually inspected sequences using the computer program FINCH TV 1.4 (Geospiza, Inc.) and aligned them in CLUSTALX2 (Larkin *et al.* 2007). MACCLADE 4.06 (Maddison & Maddison 2008) was used to manually edit the aligned sequences.

ARLEQUIN was used to calculate a mismatch distribution for North Coast tidewater goby and to test the observed distribution for goodness-of-fit to the expected distribution of a rapidly expanding population with 10 000 bootstrap pseudoreplicates (Rogers & Harpending 1992). Sequence variation was assessed by estimating haplotype diversity (h) and nucleotide diversity (π) for each population (Nei 1987).

Results

Sample collection and tests of assumptions

The microsatellite loci assayed exhibited varying levels of polymorphism that ranged from 3 to 30 alleles per locus (Table 2). On average, <5% of the microsatellite genotypes were missing from the final dataset, and missing data was not symptomatic of particular loci or populations (Table 2). Our microsatellite error checking results indicated that some mistakes were present because of large allele dropout and technical sizing errors of the automated capillary gel electrophoresis procedure. Average error rates were within the generally accepted range of 2% of alleles and 2% of reactions

(DeWoody *et al.* 2006) and are reported for each locus (Table 2). The errors discovered were corrected, and all of the electropherograms were carefully reinspected for evidence of similar microsatellite scoring errors.

Testing for Hardy–Weinberg Equilibrium at each locus in each population gave a possibility of 117 tests. All populations except Lake Earl, Virgin Creek and Pudding Creek contained at least one monomorphic locus, which could not be tested. Excluding the monomorphic loci gave a total of 89 possible tests. Four tests showed departure from Hardy–Weinberg Equilibrium (ENE16 and ENE18 in McDaniel Slough, ENE12 in Eel River, and ENE16 in Lake Earl), but after standard Bonferroni corrections for multiple comparisons ($P = 0.0006$ for an experiment-wide significance at $\alpha = 0.05$) all loci in all populations conformed. No tests for genotypic disequilibrium were significant after 9360 permutations at an adjusted 5% significance level of $P \leq 0.0001$.

Population structure

We found similar groupings of populations from the PCA and Bayesian clustering methods. The first two axes of the PCA explained 79% of the genetic variation, with PC 1 and PC 2 explaining 60% and 19%, respectively (Fig. 2). The artificially fragmented bay scale populations grouped together and were significantly separated from coast scale populations by PC 1 ($P = 0.0118$). The second axis divided northern and southern components of bay and coast scale populations. The highest posterior probability of the data returned by averaging $L(K)$ across 20 independent STRUCTURE runs was at eight clusters ($K = 8$, Fig. 3) using the default parameters and nine clusters ($K = 9$) using

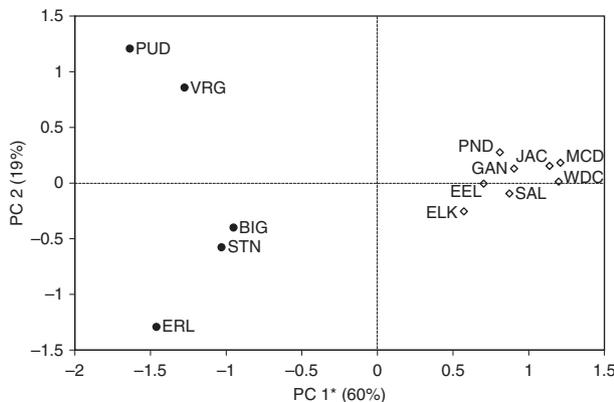


Fig. 2 Plot of the first two principal components axes of North Coast tidewater goby microsatellite variation. The artificially fragmented bay populations are marked with white squares and the naturally fragmented coast populations are marked with black circles. * $P = 0.0118$, randomization test.

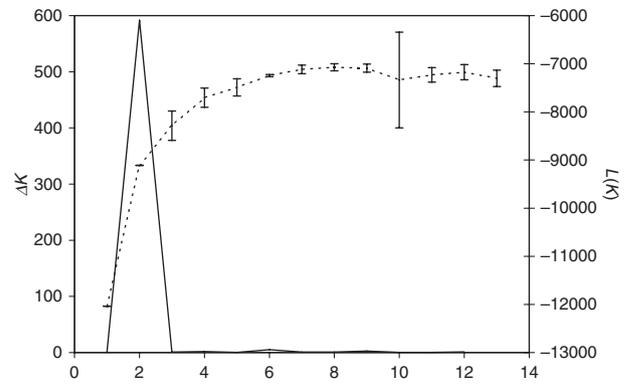


Fig. 3 ΔK (solid line) and $L(K)$ (dotted line, \pm SD) estimates for 1–13 clusters (K) of North Coast tidewater goby.

sampling locations as priors (data not shown). However, we found that two clusters ($K = 2$, Fig. 3) were inferred to capture the strongest level of population structure using the statistic ΔK , regardless of the parameter options selected in the simulations. At two clusters, one group contained all bay scale samples and the other all coast scale samples (Fig. 4), each with high (>0.92) mean population membership proportions. Multimodality was common at $K > 2$ with independent runs at the same K producing substantially different clustering configurations. The number of different clustering arrangements generally increased with increasing K . For example, two different clustering arrangements were produced from the 20 independent runs at $K = 3$, three clustering arrangements resulted at $K = 4$, and at $K = 5$ there were four different outcomes. The most common clustering configurations were selected for plotting proportional membership of individuals for 2–7 groups (Fig. 4). Following the strongest grouping at $K = 2$, northern and southern components of the coast and bay scale populations formed clusters at $K = 3$ and $K = 4$, respectively.

Pairwise estimates of F_{ST} ranged from 0.01 to 0.74, with a mean of 0.39, indicating very high levels of genetic differentiation over all populations (Table 3). All pairwise tests of differentiation were significant after 1560 permutations (adjusted 5% significance level for multiple comparisons was at $P \leq 0.0006$) with exception of the test between Stone and Big Lagoons ($P = 0.003$). Mean pairwise F_{ST} was 0.28 at the bay scale and 0.23 at the coast scale. The highest levels of genetic differentiation were observed between the two scales, where average pairwise F_{ST} was 0.50. The relationship between genetic differentiation and geographical distance was not significant in the bay scale ($R^2 = 0.0241$; $P = 0.4325$), with large variances in population divergence apparent at all pairwise geographical distances (Fig. 5). The model of isolation-by-distance in the coast

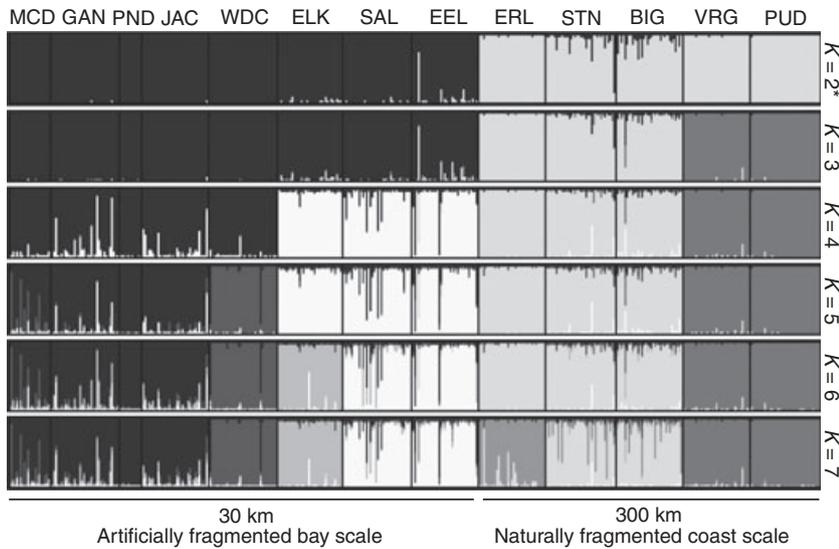


Fig. 4 Proportional membership coefficient (Q) plots of North Coast tidewater goby for 2–7 groups (K) of genetically distinct individuals. Collection localities are blocked by vertical black lines, population names are listed above the plots, and approximate geographic distance separating populations in each scale are below the plots. * The strongest level of population structure inferred by the statistic ΔK was at $K = 2$.

Table 3 Pairwise F_{ST} estimates (above diagonal) and significance of pairwise tests of differentiation (below diagonal) among all North Coast tidewater goby populations

	Artificially fragmented bay scale								Naturally fragmented coast scale				
	MCD	GAN	PND	JAC	WDC	ELK	SAL	EEL	ERL	STN	BIG	VRG	PUD
MCD		0.127	0.231	0.093	0.353	0.318	0.197	0.216	0.691	0.460	0.415	0.484	0.610
GAN	*		0.105	0.063	0.414	0.263	0.194	0.289	0.643	0.413	0.373	0.453	0.578
PND	*	*		0.280	0.544	0.382	0.336	0.337	0.650	0.394	0.353	0.410	0.545
JAC	*	*	*		0.458	0.325	0.198	0.308	0.706	0.488	0.443	0.515	0.636
WDC	*	*	*	*		0.445	0.347	0.296	0.740	0.529	0.489	0.564	0.679
ELK	*	*	*	*	*		0.238	0.295	0.572	0.349	0.314	0.428	0.556
SAL	*	*	*	*	*	*		0.131	0.612	0.396	0.364	0.449	0.582
EEL	*	*	*	*	*	*	*		0.578	0.370	0.337	0.417	0.535
ERL	*	*	*	*	*	*	*	*		0.159	0.188	0.407	0.501
STN	*	*	*	*	*	*	*	*	*		0.009	0.192	0.322
BIG	*	*	*	*	*	*	*	*	*	NS		0.165	0.284
VRG	*	*	*	*	*	*	*	*	*	*	*		0.102
PUD	*	*	*	*	*	*	*	*	*	*	*	*	

* $P < 0.05$ after standard Bonferroni corrections.

scale was highly significant ($P = 0.0006$), with geographical distance explaining 75% of the variation in genetic differentiation between populations (Fig. 5).

Genetic diversity

The proportion of polymorphic microsatellite loci ranged from 0.33 to 0.89 in the bay samples and from 0.67 to 1.00 in the coast samples. Private alleles were detected within one bay population and in four coast populations. Mean levels of allelic richness were 1.9 in the bay samples and 3.9 in the coast samples. Observed heterozygosity ranged from 0.10 to 0.28 at the bay scale and from 0.27 to 0.59 at the coast scale. Mean unbiased

expected heterozygosity was 0.21 in the bay samples and 0.48 in the coast samples (Table 1). Permutation tests indicated that the coast populations contained significantly greater levels of allelic richness ($P = 0.001$), observed ($P = 0.003$) and unbiased expected ($P = 0.003$) heterozygosity than the bay populations.

The relationship between habitat area and allelic richness was significant at the bay scale ($P = 0.0261$; Mantel Test) with habitat area explaining 59% of the variation in allelic richness (Fig. 6). In contrast, allelic richness was not related to population size in the coast scale ($R^2 = 0.1881$, $P = 0.4054$; Mantel Test, Fig. 6), although historic impacts on Lake Earl, the largest habitat studied, may have influenced this result as discussed later.

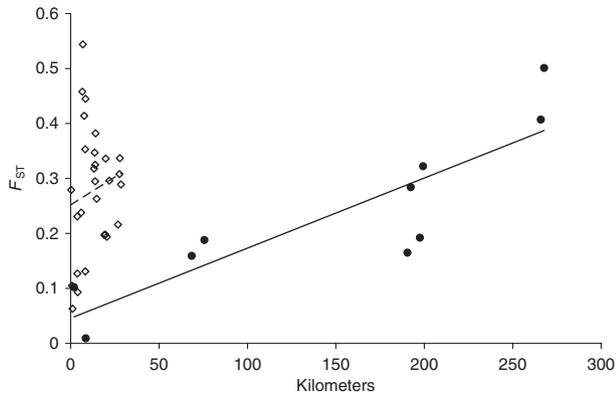


Fig. 5 The relationships between pairwise genetic differentiation (F_{ST}) and geographical distance for the artificially fragmented bay scale (white squares, dashed line) and the naturally fragmented coast scale (black circles, solid line) of North Coast tidewater goby. The relationship was significant in the coast scale ($R^2 = 0.7504$, $P = 0.0006$; Mantel Test).

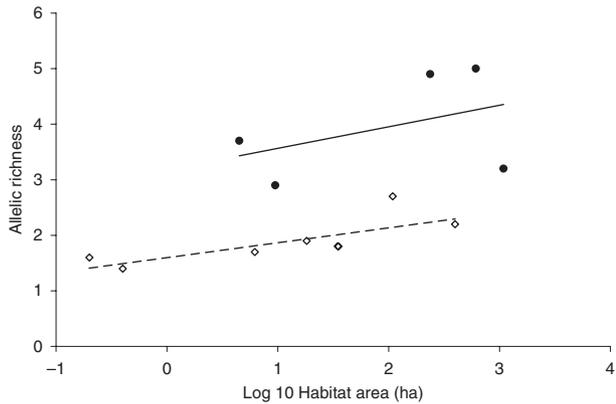


Fig. 6 The relationships between allelic richness and habitat area for the artificially fragmented bay scale (white squares, dashed line) and the naturally fragmented coast scale (black circles, solid line) of North Coast tidewater goby populations. The relationship was significant for the bay scale ($R^2 = 0.5873$, $P = 0.0261$; Mantel Test).

Interscale levels of genetic differentiation were significantly correlated with genetic diversity ($P < 0.00001$; Mantel Test), where the mean observed heterozygosity of population pairs explained 84% of the variation in F_{ST} between the bay and coast scales (Fig. 7).

Mitochondrial DNA

Five hundred and twenty-two bases of mitochondrial DNA control region from 103 tidewater goby were aligned (GenBank accession numbers HM484396–HM484500). A total of nine different haplotypes were re-covered from the 13 populations assayed, of which one haplotype (H1) occurred at a high frequency in

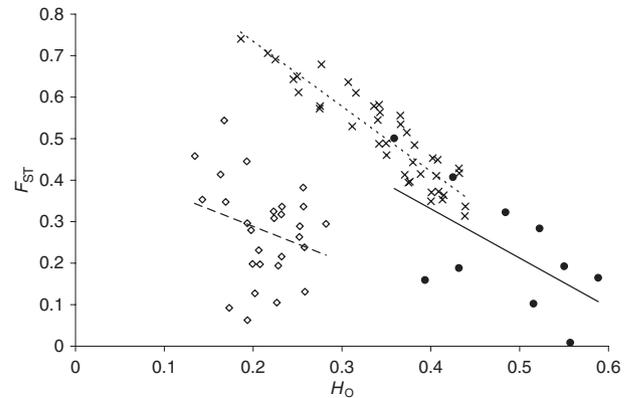


Fig. 7 The correlations between pairwise genetic differentiation (F_{ST}) and average of observed heterozygosity (H_O) for the artificially fragmented bay scale (white squares, dashed line), the naturally fragmented coast scale (black circles, solid line), and between scales (crosses, dotted line) of North Coast tidewater goby. The only significant relationship was between scales ($R^2 = 0.8398$, $P < 0.0001$; Mantel Test).

both bay and coast scales, observed in 78% of individuals sampled (Table 4). The remaining eight haplotypes were restricted to the coast scale and occurred at low frequencies (1–8%). Each coast scale population contained at least one private haplotype (Table 1).

Pairwise sequence differences were distributed exponentially, with an average of 0.59 differences, ranging from 0 to 4 mismatches. The mismatch distribution was not significantly different than the distribution expected under the model of rapid expansion ($P = 0.8688$). No sequence diversity was detected throughout the bay scale, but within the coast scale haplotype diversity ranged from 0.25 to 0.75 and nucleotide diversity ranged from 0.0005 to 0.0018 (Table 1).

Discussion

Historical context

The coastal lagoons and estuaries that North Coast tidewater goby inhabits in northern California were formed when the last rapid rise of sea level slowed approximately 7000 years ago, allowing sandbars to build and extend across open embayments (Barnhart *et al.* 1992; Stanley & Warne 1994). Range-wide phylogeographical analyses suggest that tidewater goby expanded from their ancestral range in central California and colonized the North Coast region during this Holocene deceleration of sea level rise (Dawson *et al.* 2001, 2002). The mitochondrial DNA mismatch distribution and haplotype data herein are consistent with this hypothesis, suggesting that colonization of the North Coast region occurred in a few recent episodes of rapid expansion to newly formed habitats (Rogers & Harpending 1992).

Table 4 Distribution of North Coast tidewater goby haplotypes per population. 'H' refers to the specific haplotype composed of the nucleotide substitutions shown under numbers corresponding to location in the 522-base sequence of mitochondrial DNA control region

	Artificially fragmented bay scale							Naturally fragmented coast scale												
	0	0	2	2	3	4	4	MCD	GAN	PND	JAC	WDC	ELK	SAL	EEL	ERL	STN	BIG	VRG	PUD
H1	G	A	A	C	A	C	A	4	9	4	9	9	9	9	9	7	4	4	2	
H2	G											1		
H3	T.	.												6	2
H4	G	.	.								1					
H5	.	.	.	T	.	.	.										1			
H6	.	.	G	.	.	T	.													6
H7	.	G										2	3		
H8	.	G	.	T	.	.	.									1				
H9	T	.	.	T	.	T	.												1	

Bay scale

Extreme genetic consequences are expected when populations are artificially fragmented and migration is restricted (Templeton *et al.* 1990; Frankham 1995). The eight populations in the bay exhibited very high levels of genetic differentiation at small spatial scales combined with low genetic diversity. Our results suggest these patterns are because of recent drift in isolation, as shown by the absence of mitochondrial DNA sequence variation, lack of a relationship between genetic differentiation and geographical distance, widespread fixation of polymorphic microsatellite loci, and strong correlation between habitat area and allelic richness.

Humboldt Bay has suffered a 90% reduction in marsh habitat from anthropogenic manipulation of the estuary beginning 120 years ago (Barnhart *et al.* 1992). Dredging and construction of jetties to stabilize the bay entrance, human-induced erosion, and the ubiquitous diking and draining of surrounding wetlands have resulted in drastic changes to the morphology of Humboldt Bay (Barnhart *et al.* 1992). It is thought that prior to artificial fragmentation a large population of tidewater goby was distributed throughout the 4047 ha of potentially suitable habitat recently lost through these land use practices (United States Fish and Wildlife Service 2008), although as noted, above-mentioned characterization of tidewater goby habitat in historic bay settings has yet to be reconstructed in detail. It appears that recent human activities around Humboldt Bay have threatened this species in two ways. First, destruction of a large (estimated at 90%) fraction of the tidewater goby's habitat reduced the size of the ancestral population. Second, the construction of tidegates and levees around the remaining habitat patches has

restricted gene flow between the insularized and (or) recently founded populations. These two processes have presumably resulted in a scenario of drift in isolation; driving genetic differentiation at very small spatial scales and reducing genetic diversity through demographic stochasticity, inbreeding and (or) founder effects over the last 120 years. It is important to note however that this scenario remains speculative because of the absence of any samples or data describing the original genetic variation of the Humboldt Bay population prior to destruction of the natural environment.

In many circumstances, artificially fragmented populations do not necessarily behave in the manner of a classic metapopulation, as dispersal is often severely restricted and local extinctions are not followed by recolonization (Hanski & Gilpin 1991). However, surveys conducted throughout Humboldt Bay have indicated the alternating presence and absence of tidewater goby at certain sites. Further, the observation that flooding during severe winter storms inundates reclaimed wetlands that separate habitat patches has led to speculation that the artificially fragmented bay populations comprise a metapopulation (United States Fish and Wildlife Service 2008). While seasonal flooding has been implicated in tidewater goby recolonization in the naturally fragmented coast populations of southern California (Lafferty *et al.* 1999), the levels of genetic differentiation and diversity from our data suggest that there is no regular migration among the artificially fragmented bay scale. Given the observed presence and absence of populations, it is tempting to infer that drift is a product of bottlenecks associated with intermittent founding of these populations. However, we suspect that the variable detection of bay populations may represent an artefact of sampling (Swift *et al.* 1989;

Swenson 1999), or the abrupt loss of most adults at senescence during certain times of the year (A. P. Kinzinger, unpublished data). Thus, it is yet to be determined whether and in which locations drift between samples is a product of metapopulation processes (i.e., recolonization/founder effect), or whether the observed drift is a function of multidecadal isolation of populations in artificial habitats. A time series of data will likely be required to resolve the differential contributions of these processes. In either case, the artificial fragmentation of the landscape by levee systems and tidegates appears to be significantly implicated.

Coast scale

Natural habitat fragmentation often occurs over geologic timescales and is expected to increase the genetic differentiation of subpopulations when migration is limited. The five populations in the coast scale generally exhibited high levels of genetic differentiation combined with substantial amounts of genetic diversity. Our results suggest that most of these populations have persisted since Holocene colonization, and that migration between them occurs on an infrequent basis, as shown by mitochondrial DNA variation and private haplotypes, the steep slope of the isolation-by-distance model, microsatellite DNA polymorphism, and the lack of a relationship between habitat area and allelic richness.

The coast scale populations are found in the largest habitats separated by some of the longest geographical gaps present throughout the species range (Swift *et al.* 1989; United States Fish and Wildlife Service 2007). The inherent isolation and discrete distribution of lagoon habitats appears to not entirely eliminate migration among these naturally fragmented coast populations. The strong genetic isolation-by-distance reflects the infrequent nature of chance gene flow among populations, whereby a combination of unlikely events must occur for successful migration, including coordination of distinct lagoon breaching and passive dispersal of the small benthic fish into new habitats (Swenson 1999). However, the nonsignificant test of population differentiation between Stone and Big Lagoon populations (8.55 km apart) suggests that there is enough gene flow between these two naturally isolated habitats to prevent population subdivision. Taken together, this information supports previous hypotheses that migration occurs among isolated populations of tidewater goby and is more likely between geographically proximate habitats (Lafferty *et al.* 1999; Dawson *et al.* 2001).

Genetic diversity appeared substantial within most of the coast scale populations. Locus fixation was rare, private alleles were common, and levels of allelic richness

indicated a considerable degree of microsatellite DNA polymorphism within populations. Estimates of heterozygosity were similar among the coast populations with one notable exception: Lake Earl contained markedly reduced levels of heterozygosity (Table 1). Lake Earl is California's largest coastal lagoon and is thought to support the most abundant population range wide of perhaps a million tidewater gobies (Swift *et al.* 1989; United States Fish and Wildlife Service 2005). Unlike the other four coast scale populations, Lake Earl is artificially breached several times a year (United States Fish and Wildlife Service 2008). This management practice has occurred for at least 75 years, initially for the purpose of increasing pastureland, and recently to prevent flooding of private property (California Coastal Commission 1999). Each artificial breach results in rapid draining of the lagoon and stranding of tidewater gobies within small isolated pools that may desiccate and are subject to seabird predation (United States Fish and Wildlife Service 2005). This problem is so pervasive that field survey teams are required to search for and return stranded gobies to the main basin of the lagoon (California Coastal Commission 1999, United States Fish and Wildlife Service 2008). We suspect reduced heterozygosity in Lake Earl is because of the numerous and repeated bottlenecks caused by these artificial breaches.

Natural vs. artificial fragmentation

Artificial fragmentation appears to have resulted in extreme genetic consequences for the bay scale populations. These genetic effects are emphasized by the observation that artificially fragmented bay populations are separated by a mere 0.3–28.5 km of reclaimed wetlands, yet they exhibit comparable or sometimes even higher levels of population structure than coast populations (Fig. 4, Table 3), which are naturally fragmented by up to 267.8 km of inhospitable coastline. While sampling of artificial and natural sites that overlap geographically and span comparable geographical distances would be preferred for robust comparisons, the drastic differences in interpopulation distances from bay and coast scales in this study highlight the importance of the genetic differentiation observed among the artificially fragmented bay populations.

The artificially fragmented bay populations contained, on average, less than half the amount of genetic diversity as the naturally fragmented coast populations. The permutation test between scales confirmed that heterozygosity was significantly lower in the bay populations, suggesting that reductions of genetic diversity in the bay scale may have been because of artificial habitat fragmentation. In contrast, data from the coast scale

suggest that even populations persisting in small habitats have maintained robust levels of genetic diversity. For example, expected heterozygosity in Virgin Creek ($H_E = 0.57$), a population found in an estuary smaller than most of the bay group habitats (4.5 ha), matches that of Big Lagoon ($H_E = 0.56$), one of the largest tidewater goby habitats range wide (612.5 ha).

The substantial levels of genetic differentiation (mean pairwise $F_{ST} = 0.39$) estimated across all North Coast populations were surprising. By hierarchically examining mean estimates, we found that the average pairwise genetic differentiation between the bay and coast scales was 0.50, approximately twice the amount of differentiation estimated within either scale. The highly significant negative correlation of mean pairwise observed heterozygosity and genetic differentiation between bay and coast scales offers an explanation for these extreme F_{ST} estimates (Fig. 7). This relationship together with the significant relationship of allelic richness and habitat area in the bay scale (Fig. 6) provide evidence that rampant drift in bay populations has resulted in the high estimates of genetic differentiation between scales.

Conservation implications

The threats to long-term persistence exist at different intensities among the two scales that we have examined. We provide evidence suggesting that artificial fragmentation has reduced or possibly severed gene flow in the bay populations, resulting in extreme consequences to the genetic structure of the tidewater goby. The levees and tidegates that currently isolate habitats are the putative obstacles that restrict migration and thus may threaten the long-term persistence of the tidewater goby within Humboldt Bay. In contrast, natural fragmentation has not completely eliminated gene flow, and the genetic structure of coastal populations appears stable. The isolation caused by the naturally formed sand bars restricts regular marine dispersal, yet migration does occur on an infrequent basis between some neighbouring populations.

The tidewater goby does not appear to be maintaining natural or historical levels of genetic diversity in artificially fragmented populations. Unfortunately, without any prefragmentation sampling, we cannot reject the argument that the lower genetic diversity within Humboldt Bay existed prior to artificial fragmentation, or that tidewater goby were not present at all. This could have been because of poor quality of historical bay habitats or founding into sites after artificial fragmentation, although this seems unlikely. In either case, the species' evolutionary history of persistence in the naturally fragmented estuaries of California appears insufficient for endurance in an artificially fragmented

situation, and the bay populations may suffer reduced fitness and adaptive potential. Naturally fragmented coast populations, in contrast, should maintain genetic diversity and long-term potential if their habitat is protected.

Conclusion

The population genetic consequences of habitat fragmentation remain a critical issue in both evolutionary and conservation biology. Because of both natural and artificial fragmentation of habitats, estuaries provide a model system to study and compare population genetics among different forms of fragmentation. Here, we have presented evidence that even for a species characterized by natural isolation and limited dispersal, anthropogenic fragmentation appears to result in substantially reduced migration and increased genetic drift, as shown by the elevated differentiation and depauperate genetic diversity of the artificially fragmented populations. Further work that replicates across species inhabiting both artificial and natural habitats should be conducted to rigorously test these hypotheses regarding the genetic consequences from different forms of fragmentation. By evaluating population genetics among different forms of fragmentation, we can improve our understanding of the mechanisms that shape and (or) maintain genetic variation, and can gain insights into the sustainability of rapidly changing environments.

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