RAMPANT DRIFT IN THE ENDANGERED TIDEWATER GOBY (*EUCYCLOGOBIUS NEWBERRYI*): COMPARING GENETIC VARIATION OF NATURALLY AND ARTIFICIALLY FRAGMENTED POPULATIONS

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SUMMARY

The objective of this project was to estimate levels of genetic differentiation, genetic diversity, and migration among geographically isolated North Coast tidewater goby (*Eucyclogobius newberryi*) populations. The data set consisted of 621 tidewater goby sampled from 13 populations including eight Humboldt Bay populations and five coastal lagoon populations. All individuals were genotyped at nine microsatellite loci and a subset of 103 individuals was sequenced at the mitochondrial control region.

Based on the genetic data, natural and artificial habitat fragmentation has caused marked divergence among North Coast tidewater goby. Thus all populations warrant conservation because they may contain unique genetic material not replicated elsewhere within the species. Additionally, the genetic structure in Humboldt Bay versus coastal lagoon populations is very different and we recommend different management approaches at the two scales.

The Humboldt Bay populations exhibited very high levels of among population genetic differentiation, extremely low levels of within population genetic diversity, and no among population migration making them vulnerable to extirpation. We recommend habitat restoration activities that would increase the potential for between population migration among Humboldt Bay populations. Migration would likely erase existing among population genetic differentiation which would potentially restore Humboldt Bay tidewater goby to the presumptive historical population structure for this system. Restoration of among population migration would also allow for re-colonization and (or) colonization of suitable habitats. Lastly, migration should also increase within

population genetic diversity which could potentially increase fitness of the Humboldt Bay populations.

Coastal lagoon populations also exhibited very high levels of among population genetic differentiation, but in contrast, contained substantial levels of within population genetic diversity with infrequent migration among lagoons. All coastal lagoon populations appear to be stable and genetically healthy with the exception of Lake Earl, which exhibited reduced levels of genetic diversity in comparison to similar coastal lagoon populations. The reduced genetic diversity observed within Lake Earl is consistent with repeated population bottlenecking. In Lake Earl population bottlenecks are most likely caused by artificial breaching. We recommend institution of breaching methods in Lake Earl that do not cause mass mortality of tidewater goby.

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INTRODUCTION

Habitat fragmentation is an important process in both evolutionary and conservation biology (Freeman and Herron 2007, Meffe and Carrol 1997). Natural fragmentation often occurs over geologic time scales, increases genetic differentiation of populations, leads to phylogeographic structure, and can result in speciation through a combination of evolutionary mechanisms (Barton and 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 adaptive potential of a population (Frankham 2003, Allendorf and Luikart 2007, Johansson et al. 2007).

While the effects of natural fragmentation and limited dispersal on phylogeographic structure and evolution are well known (Avise 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 and 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 and Beebee 1998, Clark et al. 1999, Fumagalli et al. 2002). Evaluating these consequences is important to the conservation of estuarine species due to the natural fragmentation of their habitats combined with increasing artificial fragmentation from recent, widespread, and

continuing habitat destruction from coastal development (Helfman 2007). Efforts to delineate marine reserves may also benefit from research that compares population genetics among naturally and artificially fragmented coastal habitats (Palumbi 2003). Due to inherent fragmentation from the discrete distribution of habitat combined with recent artificial fragmentation from coastal development, the 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 endemic to naturally fragmented lagoon environments along the entire coast of California, USA (Swift et al. 1989, United States Fish and Wildlife Service 1994). The tidewater goby is unique among eastern Pacific bay gobies because it lacks an explicit marine dispersal stage and spends its entire life cycle within discrete coastal wetlands naturally fragmented 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 one to two times a year during periods of high surf and freshwater input resulting in rapid draining of the estuary (Kraus et al. 2002). Thus, successful migration between lagoon habitats requires coordination of the breaching events. Further, populations are separated by one to 20 km of inhospitable coastline, and although the species is tolerant of full strength seawater, migration between lagoons is thought to be very rare (Crabtree 1985, Swift et al. 1989, Lafferty et al. 1999, Swenson 1999, Dawson et al. 2001, Dawson et al. 2002).

In addition to natural fragmentation, habitat destruction from agriculture and coastal development resulted in a drastic decline in the number of known populations, resulting in the listing of tidewater goby as endangered under the United States Endangered Species Act (United States Fish and Wildlife Service 1994). Presently, about 21 percent of the 135 historically documented populations are extirpated, and about 50 percent of the remaining populations are considered vulnerable to extinction due to severe habitat degradation (United States Fish and Wildlife Service 2005, 2007). This study focuses on the 13 extant populations of tidewater goby inhabiting the North Coast region of California (United States Fish and Wildlife Service 2005) (Figure 1).

North Coast tidewater goby represent a distinct mitochondrial DNA clade divergent from all other populations (Dawson et al. 2001). They also possess a fully developed cephalic lateral line canal system - a morphological adaptation thought to improve sensory ability in the generally wetter climate of the North Coast region (Ahnelt et al. 2004). In addition, tidewater goby have remained relatively abundant in the North Coast region, 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 (Figure 1). The bay scale consists of eight artificially fragmented populations within Humboldt Bay, California's second largest estuary (Barnhart et al. 1992). These populations inhabit the lower reaches of streams flowing into Humboldt

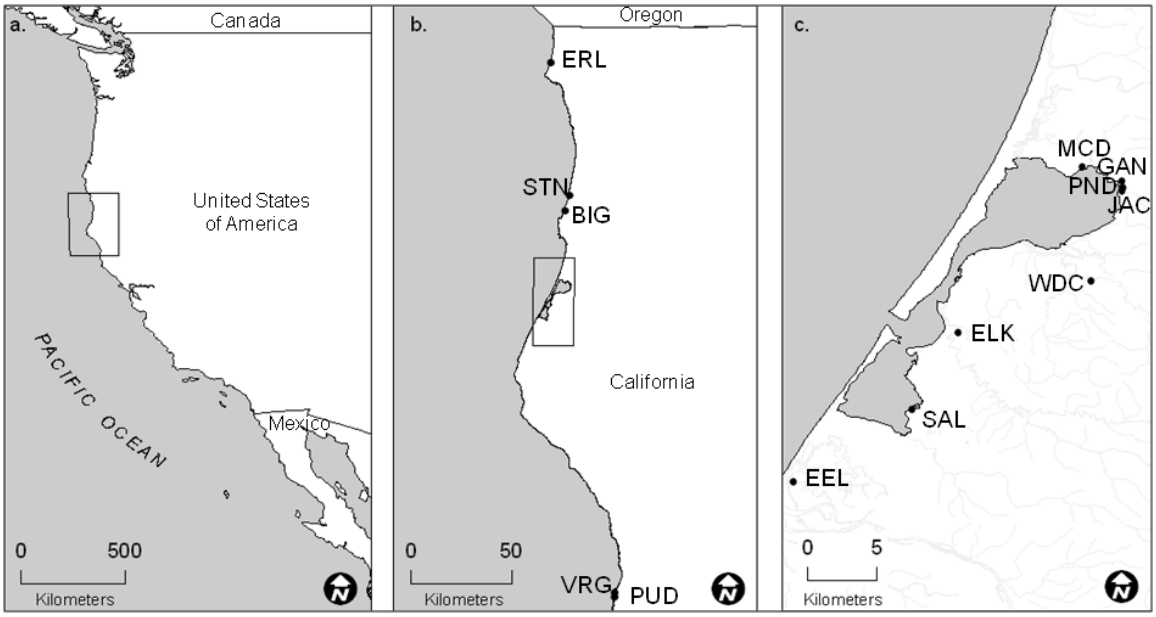

Figure 1. Tidewater goby occur in isolated lagoons along the California coast in western North America. (a.) Rectangle depicts the North Coast region of California, (b.) five sample locations at the coast scale [Lake Earl (ERL), Stone Lagoon (STN), Big Lagoon (BIG), Virgin Creek (VRG), Pudding Creek (PUD)], and (c.) eight collection 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)].

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). A combination of tide gates and levees mute tidal exchange within bay habitats (Chamberlain 2006), and all populations are isolated from each other by reclaimed wetlands modified for human uses. An average pairwise distance of 12.9 km separates bay populations, with a range of 0.3 to 8.4 km between sites (United States Fish and Wildlife Service 2008). The coast scale is comprised of five populations in Del Norte, Humboldt, and Mendocino counties, 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 populations are isolated by sand bars that restrict tidal exchange between the Pacific Ocean and the lagoon (Chamberlain 2006).

The objective of this study was to evaluate how fragmentation has influenced neutral genetic variation of the endangered tidewater goby in the North Coast region. Specifically, we asked the following questions:

1. *How do levels of genetic differentiation compare among artificially and naturally fragmented populations?* Despite the lack of an explicit marine dispersal phase in the life history of the tidewater goby, both abundance survey and genetic evidence suggest that periodic migration between neighboring populations must sometimes occur (Lafferty et al. 1999, Swenson 1999, Dawson et al. 2001). While this

Table 1. North Coast tidewater goby study populations included in the bay and coast scales (listed from north to south), with sample identification codes, habitat area, and geographic coordinates, California, 2006.

Population	Sample ID	Habitat Area (ha)	Latitude	Longitude
Bay Scale				
McDaniel Slough	MCD	34.8	N 40 51 20.14	W 124 07 20.89
Gannon Slough	GAN	18.2	N 40 51 02.31	W 124 04 43.17
Gannon Pond	PND	0.2	N 40 50 37.59	W 124 04 48.23
Jacoby Creek	JAC	6.2	N 40 50 29.04	W 124 04 46.21
Wood Creek	WDC	0.4	N 40 47 00.95	W 124 05 57.38
Elk River	ELK	35.1	N 40 44 51.17	W 124 11 14.20
Salmon Creek	SAL	396.9	N 40 41 57.72	W 124 12 58.71
Eel River	EEL	108.5	N 40 39 09.45	W 124 17 34.77
Coast Scale				
Lake Earl	ERL	1085.4	N 41 50 30.48	W 124 12 25.20
Stone Lagoon	STN	236.7	N 41 13 58.44	W 124 05 03.48
Big Lagoon	BIG	612.5	N 41 09 51.48	W 124 07 50.16
Virgin Creek	VRG	4.5	N 39 28 11.29	W 123 48 10.49
Pudding Creek	PUD	9.5	N 39 27 10.81	W 123 48 18.62

species appears to maintain some gene flow among populations that are naturally fragmented, we propose that migration will be reduced among artificially fragmented populations, resulting in a pattern of increased genetic differentiation at smaller spatial scales (Bohonak 1999).

2. *Can a species that is well-suited to natural fragmentation maintain genetic diversity in an artificially fragmented situation?* The tidewater goby possesses several adaptations to living in the naturally fragmented estuaries of California, including an annual life history that is completed entirely within lagoons, early sexual maturation, multiple spawning periods, and tolerance to a broad range of environmental conditions (Goldberg 1977, Swift et al. 1989, Swenson 1999, Dawson et al. 2002, McGourty et al. 2008). While the species persists and is often abundant in these seasonally dynamic and isolated environments, we predict that reductions in available habitat from artificial fragmentation will lead to lower levels of genetic diversity.

MATERIALS AND METHODS

Sample Collections

Tissue samples were collected August and September 2006 from 706 individuals representing all known populations in the North Coast region at the time of this study (United States Fish and Wildlife Service 2005, Figure 2, Table 1). Approximately equal numbers of individuals were gathered from each scale with beach seine or dip net. Tissue samples were obtained non-lethally by dissection of a small (one mm^2) piece of the pelvic disc and were either dried or preserved in 95 percent ethanol. Individuals were held in a recovery tank to ensure survival before release. Mortalities were stored as voucher specimens in the Humboldt State University Fish Collection (Catalog Numbers: HSU 4498-4512). Genomic DNA was extracted using spin columns lined with a silica membrane (Qiagen DNeasy® Blood and Tissue Kit) following the manufacturers protocols.

Microsatellite DNA Analysis

Variation was assayed at eight microsatellite loci recently developed from samples collected throughout the species' range (primer sequences provided by D. K. Jacobs, Department of Ecology and Evolutionary Biology, University of California Los Angeles, Los Angeles, CA 90095), and one microsatellite locus previously developed from central California populations (Mendonca et al. 2001). Genotypes were assayed by polymerase chain reaction with fluorescent labeling of the forward primer and automated capillary gel electrophoresis. Reactions were performed using Master Mix® (Promega, Madison, WI) in an MJ Research (Waltham, MA) PTC-100 thermal cycler with 12.5 µl

volumes. Cycling conditions were standardized over all loci as follows: 95°C for 15 min, 12 cycles of 94°C 30 s, 60°C 90 s -0.5°C per cycle, 72°C 60 s, 33 cycles of 89°C 30 s, 55°C 90 s, 72°C 60 s, followed by 60°C for 30 min and then cooled to 4°C. 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.

Test of Assumptions

We estimated the microsatellite scoring error rate in my dataset by randomly re-sampling 10 percent of the individuals assayed 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 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 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 9,360 permutations.

Population Structure

We used the Bayesian assignment algorithm implemented in the computer program STRUCTURE 2.2 (Pritchard et al. 2000, Falush et al. 2003) to estimate the number of genetically distinct clusters (*K*) of individuals based on their multi-locus genotypes. The default options were utilized in the program. To do this we used the admixture model, ignoring sample collection locality, and assuming allele frequencies were correlated. We began with a series of pilot runs to estimate the likelihood of *K* based upon the data at each *K* from one to 13 (i.e., the total number of distinct geographic sampling localities) by running four independent runs of 1,000,000 iterations each preceded by a burn in period of 100,000 iterations. From these pilot runs we determined that the $L(K)$ increased in a step-wise manner up to nine clusters. We focused on the range of one to 10 clusters and ran 16 more independent runs at each of these *K* for a total of 20 replicates. We evaluated the hierarchical patterns of population clustering by plotting the proportional membership coefficients (*Q*) of individuals based on admixture analyses at several levels of population structure in DISTRUCT 1.1 (Rosenberg 2004).

In the documentation for STRUCTURE 2.2, the authors suggest caution in determining the number of genetically distinct clusters of individuals in situations when the $L(K)$ increases in a step-wise manner with increasing parameter (*K*) values (Pritchard and Wen 2003). To avoid overestimating *K*, we relied on ΔK (Evanno et al. 2005), which takes into account the rate of change and variance in the probability between replicate runs at each value of *K*, to infer the most biologically meaningful amount of population structure.

We constructed a phenogram of North Coast tidewater goby populations using PHYLIP 3.67 (Felsenstein 1993). Cavalli-Sforza and Edwards (1967) chord distances were estimated between population pairs and an unrooted neighbor-joining tree was generated. Support for branches was estimated by building 1,000 bootstrap pseudoreplicates and a majority-rule consensus tree.

FSTAT was used to estimate genetic differentiation (F_{ST}) between population pairs and test for their significance (Weir and Cockerham 1984). We assessed relative influences of migration and drift on population structure at each scale by correlating F_{ST} with geographic distance and testing for statistical significance with 20,000 permutations of Mantel's (1967) test in FSTAT (Hutchinson and Templeton 1999). A significant linear relationship of increased genetic differentiation at greater geographic 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 and Snell 2008). In contrast, a non-significant linear relationship between genetic differentiation and geographic distance combined with large variance in population divergence is expected under the scenario of drift in extreme isolation (Hutchinson and Templeton 1999).

Genetic Diversity

We used HP-RARE 1.0 (Kalinowski 2005) and GENALEX 6.1 (Peakall and Smouse 2001) to estimate mean number of alleles per locus, rarefied allelic richness, and number of private alleles per population. Rarefied allelic richness provides a better estimate of the average number of alleles in a population and reduces bias when comparing samples of different sizes (Kalinowski 2004). ARLEQUIN was used to estimate observed (H_O) and Nei's (1978) unbiased expected heterozygosity (H_E). Tests

for significant differences in estimates of rarefied allelic richness and observed heterozygosity between bay and coast scales were performed with 15,000 permutations of the populations 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 rarefied allelic richness as a function of Log_{10} 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 and Snell 2008). A significant relationship is expected between these two variables when reductions in available habitat area have resulted in population bottlenecks. Second, to evaluate the role of fluctuating population sizes on genetic differentiation, we correlated F_{ST} with the average of observed heterozygosity for each population pair at inter and intra-scale levels (Hedrick 1999, Goodman et al. 2001, Jordan and Snell 2008). A significant negative correlation between F_{ST} and mean pairwise H_O is expected when drift in isolation 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® (Promega, Madison, WI) in a MJ Research (Waltham,

MA) PTC-100 thermal cycler with 25 µl 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 and Maddison 2008) was used to manually edit the aligned sequences.

We used ARLEQUIN 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 and Harpending 1992). Sequence variation was assessed by estimating haplotype diversity (h) and nucleotide diversity (π) for each population (Nei 1987).

RESULTS

Tests of Assumptions

The microsatellite loci assayed exhibited varying levels of polymorphism that ranged from three to 30 alleles per locus (Table 2). On average, less than five percent of the microsatellite genotypes were missing from the final dataset, and levels of missing data are reported per locus (Table 2). We checked for microsatellite scoring errors in the dataset by re-analyzing a random subset of individuals. The results indicated that some mistakes were present due to large allele dropout and technical sizing errors of the automated capillary gel electrophoresis procedure. Average error rates were within the generally accepted range of five percent of alleles and two percent 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 re-inspected 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 (*CATG2* in Lake Earl, *CATG2* and *TAGA11* in McDaniel Slough, and *B113* in Eel River), but after strict Bonferroni corrections for multiple comparisons (P=0.0006, for an experiment-wide significance at α=0.05) all loci in all populations conformed. None of the tests for genotypic disequilibrium were significant

after 9360 permutations at the adjusted five percent nominal significance level of $P \leq 0.0001$.

Table 2. North Coast tidewater goby microsatellite information, including primer sequences, repeat lengths, number of alleles, size ranges, and quality control results (percent missing data, percent errors per allele, percent errors per reaction), California, 2006.

Locus	Primer sequences (5'-3')	Nucleotide Repeat	Number of Alleles	Size Range	Missing Data (%)	Errors per Allele (%)	Errors per Reaction (%)
AO18	GCTTGTGCAGTATGGGATCTC CTCGGAGCGTTCATTTATCTC	Tetra	4	291-301	5.6	0.0	0.0
AO19	TCAGGTTTGTGCTAAAATGATG TCCGATGACCACTTGTCC	Di	8	218-232	8.7	0.0	0.0
ATG16.1	GAGGAAGGCGAGCTGATTA CGGAGAGAAGGTGTTGAGAG	Tri	12	127-181	7.3	0.0	0.0
ATG17	CCTTCATTTTTCCATCAGAAGCG CCTTATTTACATCTTCCCTCCA	Tri	30	116-218	5.2	7.3	8.3
B113	CTGGGATTGTCTTGGAACAG GGGTGTGTGTGAGAGAGTGG	Tri	11	187-220	1.9	0.0	0.0
B117	TGAAGCATCTTTGGGTGTC GTTTGAAATGGTCACTGTGTG	Tri	3	244-250	3.6	2.6	2.1
CATG2	GTCGCCTTGATTTTATTGTGA CTCAGCGTGGTTTCATTAT	Tetra	6	158-178	2.9	7.7	8.3
ENE2	GTCGACTGGCAGTATGGGAT AGACTCAAATATGTGCACACCAC	Hex	4	143-161	4.2	2.8	2.1
TAGA11	GGAGAACGAGAGAGAAAGA GGCTGGTRTTTGATACATC	Tetra	6	110-126	5.0	0.0	0.0
All Loci					4.9	5.2	2.3

Population Structure

The Bayesian cluster analysis performed in STRUCTURE returned the highest likelihood at nine clusters of genetically distinct individuals (Figure 2). After examining the membership coefficient plots at nine clusters it was apparent that the *L(K)* alone was overestimating the number of genetically distinct clusters of individuals because all of the genotypes within some populations were split in half between groups (Figure 3). With consideration to the author's warnings on the potential for overestimating *K* based on *L(K)* alone (Pritchard and Wen 2003), we relied on ΔK to infer the most biologically meaningful grouping of populations. The highest ΔK was at two groups of populations (Figure 2). One group consisted of all bay scale populations and the other group consisted of all coast scale populations (Figure 3).

The neighbor-joining phenogram that we constructed using Cavalli-Sforza and Edwards (1967) chord distances was consistent with the Bayesian results of population structure (Figure 4). The longest branch separated bay samples from coast samples with 100 percent bootstrap support. Within the coast scale, populations south of Humboldt Bay were strongly divergent (100 percent bootstrap support) from populations north of Humboldt Bay (82 percent bootstrap support).

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 estimates of F_{ST} were significant after 1,560 permutations (adjusted five percent significance level for multiple comparisons was at $P \leq 0.0006$) with exception to the test

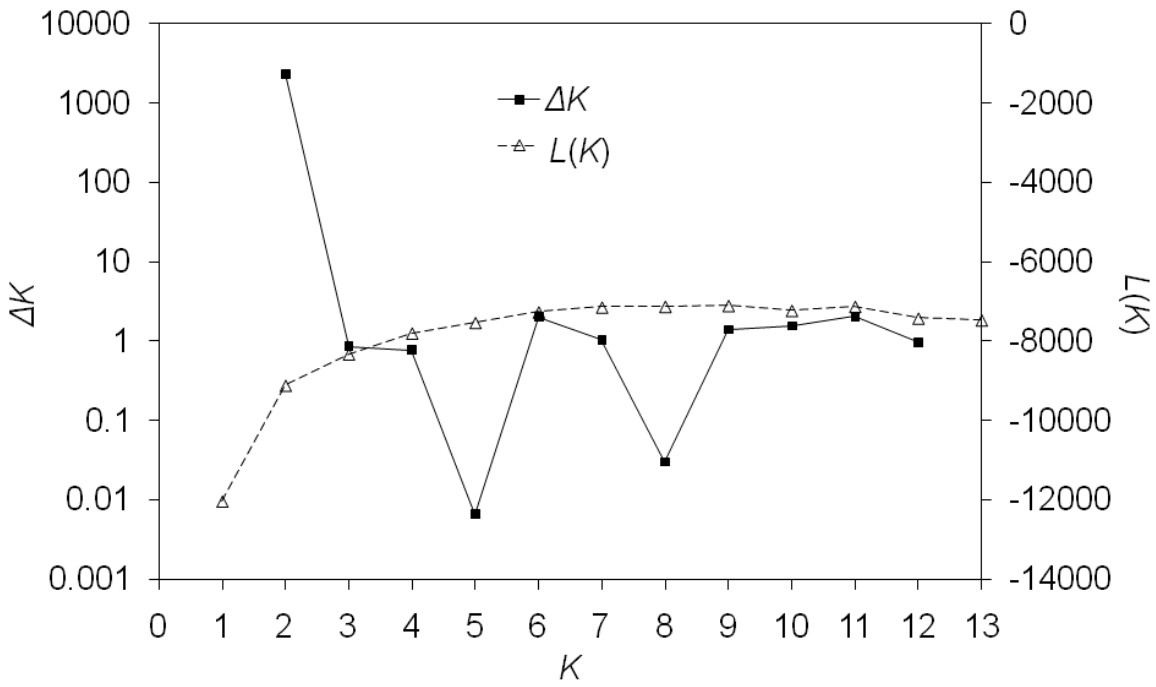

Figure 2. *ΔK* and *L*(*K*) values as a function of *K*, the number of genetically distinct population clusters of North Coast tidewater goby, California, 2006.

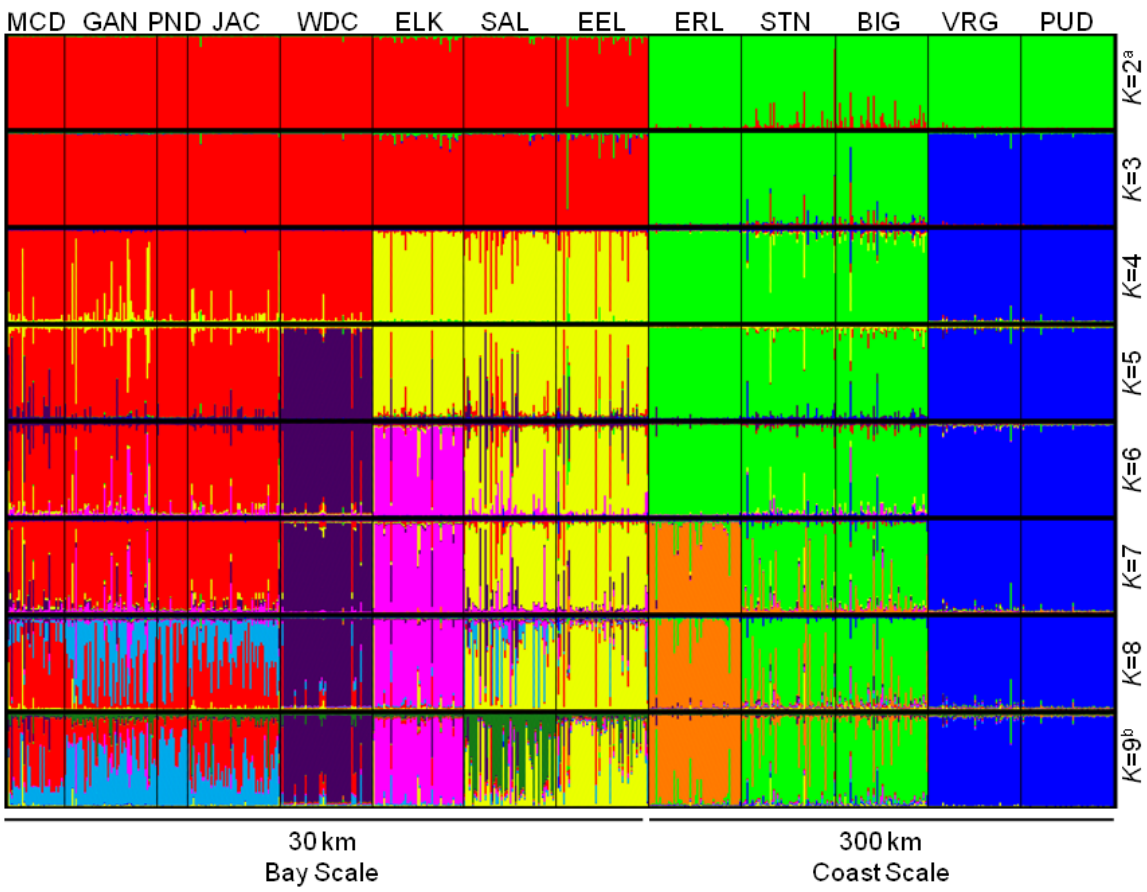

Figure 3. Proportional membership coefficient (*Q*) plots of North Coast tidewater goby (California, 2006) for two to nine groups (*K*) of genetically distinct individuals. Collection localities are blocked by vertical black lines, population names are listed above the plots, and approximate geographic distance separating populations in each scale are below the plots. [a] The greatest ΔK was at K=2 groups and [b], the $L(K)$ was highest at K=9 groups.

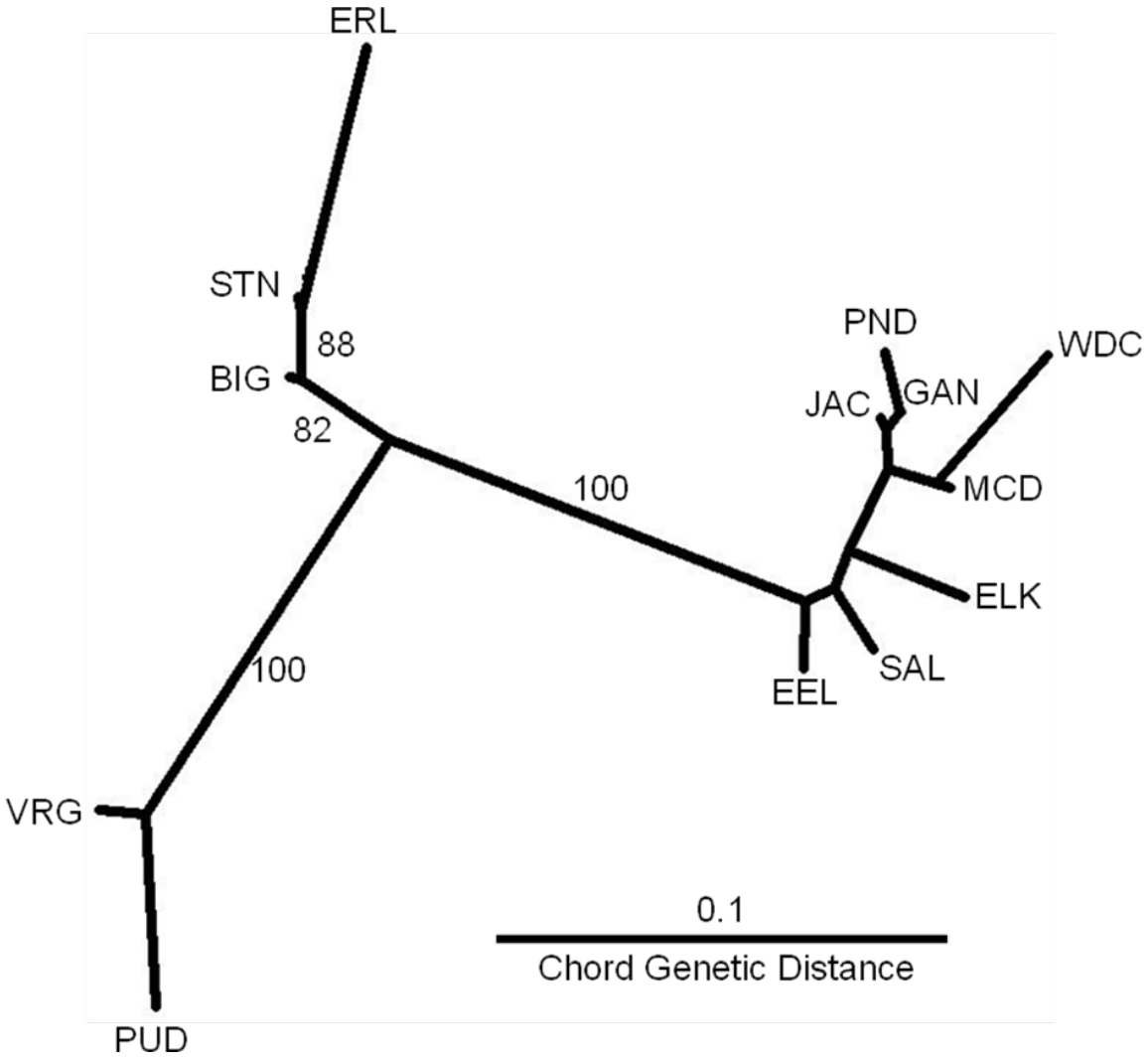

Figure 4. Neighbor-joining phenogram of North Coast tidewater goby populations (California, 2006) constructed using Cavalli-Sforza and Edwards (1967) chord genetic distances. Support on branches was estimated with 1,000 bootstrap pseudoreplicates and a consensus tree.

Table 3. Pairwise F_{ST} estimates among all North Coast tidewater goby populations, California, 2006. All estimates were statistically significant ($P \leq 0.0006$) at the five percent level with exception to Stone Lagoon - Big Lagoon ($P = 0.003$; marked with an asterisk).

	MCD	GAN	PND	JAC	WDC	ELK	SAL	EEL	ERL	STN	BIG	VRG
GAN	0.127											
PND	0.231	0.105										
JAC	0.093	0.063	0.279									
WDC	0.353	0.414	0.544	0.458								
ELK	0.318	0.263	0.382	0.325	0.445							
SAL	0.197	0.194	0.336	0.198	0.347	0.238						
EEL	0.216	0.289	0.337	0.308	0.296	0.295	0.131					
ERL	0.691	0.643	0.650	0.706	0.740	0.572	0.612	0.578				
STN	0.460	0.413	0.394	0.488	0.529	0.349	0.396	0.370	0.159			
BIG	0.415	0.373	0.353	0.443	0.489	0.314	0.364	0.337	0.188	0.009*		
VRG	0.484	0.453	0.410	0.515	0.564	0.428	0.449	0.417	0.407	0.192	0.165	
PUD	0.610	0.579	0.545	0.636	0.679	0.556	0.582	0.535	0.501	0.322	0.284	0.102

between Stone and Big Lagoons (P=0.003). Mean F_{ST} was 0.28 in the bay scale and 0.23 in the coast scale. The highest levels of genetic differentiation were detected between the two scales, where average F_{ST} was 0.47. The relationship between genetic differentiation and geographic distance was not significant in the bay scale (R^2=0.0241; P=0.4325), with large amounts variance in population divergence apparent at all pairwise geographic distances (Figure 5). The model of isolation-by-distance in the coast scale was highly significant (P=0.0006), with geographic distance explaining 75 percent of the variation in genetic differentiation between populations (Figure 5).

Genetic Diversity

The mean number of alleles per locus within populations ranged from 1.4 to 3.0 at the bay scale and 3.2 to 6.8 at the coast scale (Table 4). Levels of rarified allelic richness were 1.4 to 2.7 at the bay scale and 2.9 to 5.0 at the coast scale. Private alleles were detected within one bay population and in four coast populations. Observed heterozygosity ranged from 0.10 to 0.28 at the bay scale and from 0.27 to 0.59 at the coast scale. Unbiased expected heterozygosity in the bay populations ranged from 0.12 to 0.31, and in the coast populations ranged from 0.27 to 0.57 (Table 4). Permutation tests indicated that the bay scale populations contained significantly lower levels of rarefied allelic richness (P=0.0009) and observed heterozygosity (P=0.0046) than the coast scale populations.

The relationship between habitat area and rarefied allelic richness was significant at the bay scale (P=0.0261; Mantel Test) with habitat area, a surrogate for population size, explaining 59 percent of the variation in allelic richness (Figure 6). In contrast,

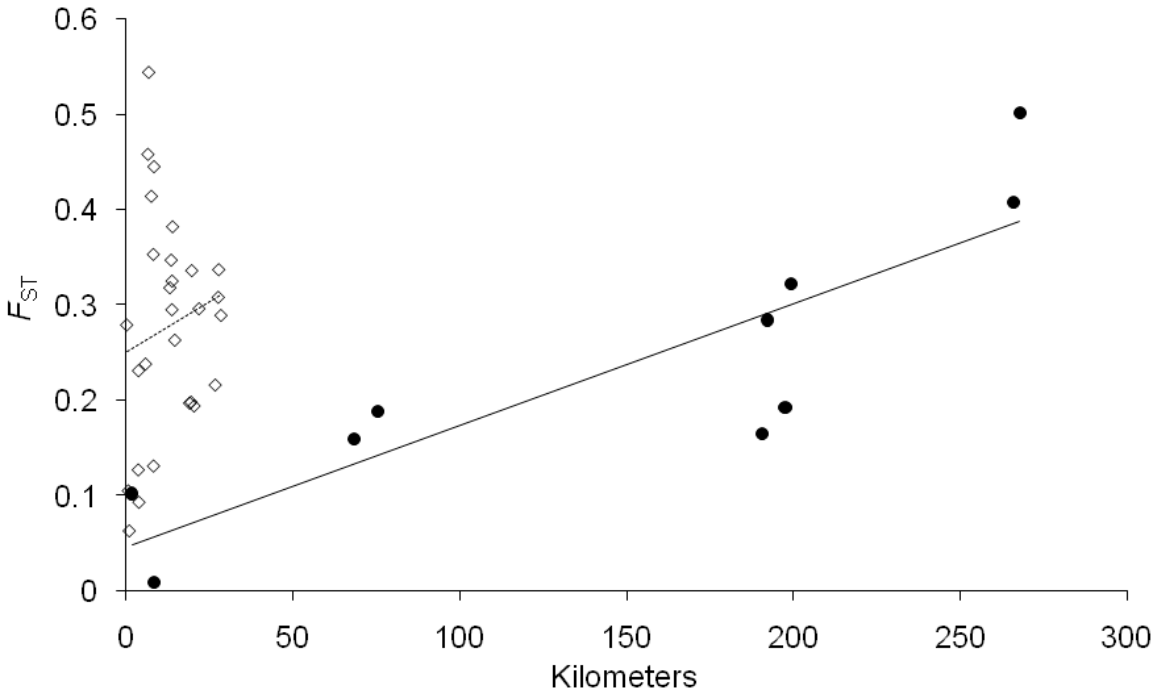

Figure 5. The relationships between pairwise genetic differentiation (F_{ST}) and geographic distance for the bay scale (white squares, dotted line) and the coast scale (black circles, solid line) of North Coast tidewater goby, California, 2006. The only significant relationship was in the coast scale (R^2=0.7504, P=0.0006; Mantel Test).

Table 4. Populations samples size (n), percent missing data, number of monomorphic loci (n_{ML}), number of private alleles (A_p), and measures of genetic diversity [mean ± standard error: alleles per locus (A), rarified allelic richness (A_r), expected heterozygosity (H_E), observed heterozygosity (H_O)] in North Coast tidewater goby, California, 2006.

Population	n	Missing Data (%)	n_{ML}	A_p	A (±SE) [A_r (±SE)]	H_E (±SE)	H_O (±SE)
Bay Scale							
MCD	32	2.1	4	0	1.9 (0.3) [1.8 (0.3)]	0.18 (0.07)	0.18 (0.08)
GAN	52	6.4	3	0	2.1 (0.4) [1.9 (0.3)]	0.22 (0.09)	0.22 (0.09)
PND	17	2.6	5	0	1.6 (0.2) [1.6 (0.2)]	0.21 (0.09)	0.23 (0.10)
JAC	52	5.3	3	0	1.9 (0.3) [1.7 (0.2)]	0.15 (0.06)	0.16 (0.07)
WDC	52	5.6	5	0	1.4 (0.2) [1.4 (0.2)]	0.12 (0.06)	0.10 (0.06)
ELK	51	7.8	2	0	2.0 (0.2) [1.8 (0.2)]	0.27 (0.07)	0.28 (0.08)
SAL	52	3.0	3	1	2.7 (0.7) [2.2 (0.6)]	0.25 (0.09)	0.23 (0.09)
EEL	52	6.6	1	0	3.0 (0.7) [2.7 (0.5)]	0.31 (0.09)	0.28 (0.09)
Coast Scale							
ERL	52	3.8	0	2	5.0 (1.6) [3.2 (1.0)]	0.27 (0.10)	0.27 (0.10)
STN	53	9.4	1	4	6.6 (2.2) [4.9 (1.4)]	0.52 (0.09)	0.52 (0.09)
BIG	52	6.2	1	6	6.8 (2.3) [5.0 (1.3)]	0.57 (0.09)	0.59 (0.09)
VRG	52	2.6	0	3	4.3 (0.8) [3.7 (0.6)]	0.57 (0.07)	0.58 (0.07)
PUD	52	2.6	0	0	3.2 (0.9) [2.9 (0.7)]	0.43 (0.08)	0.45 (0.09)

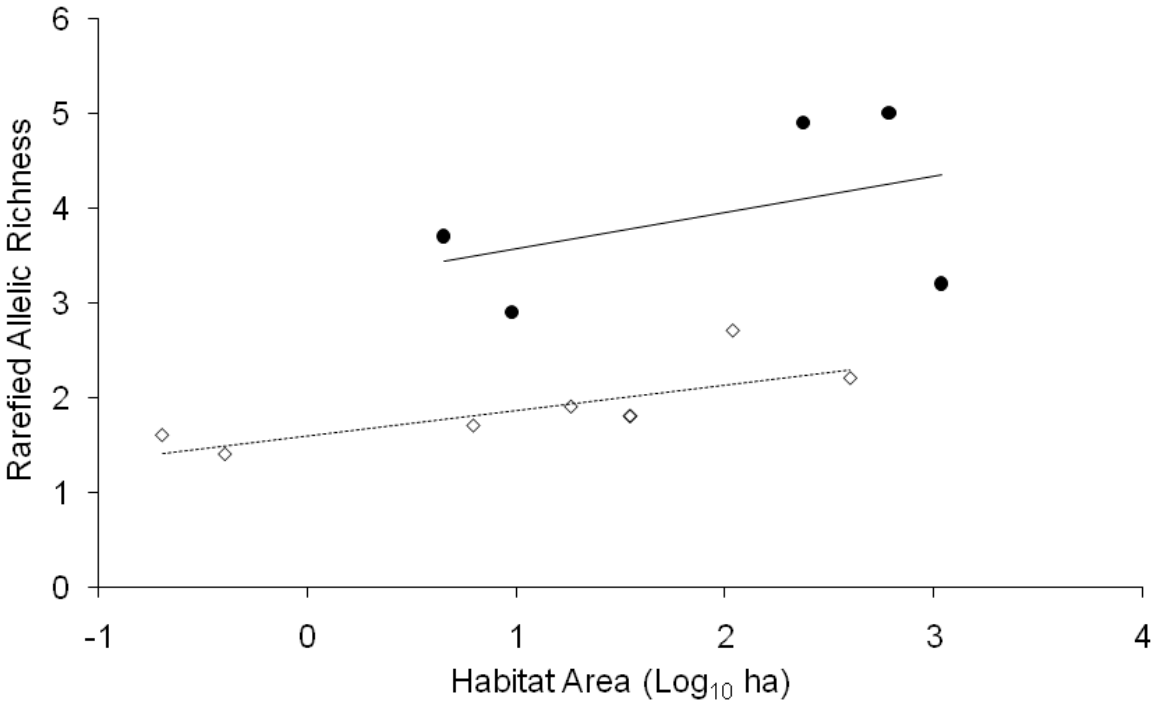

Figure 6. The relationships between rarefied allelic richness and habitat area for the bay scale (white squares, dotted line) and the coast scale (black circles, solid line) of North Coast tidewater goby populations, California, 2006. The only significant relationship was in the bay scale (R^2=0.5873, P=0.0261; Mantel Test).

rarefied allelic richness was not related to population size in the coast scale (R^2=0.1881, P=0.4054; Mantel Test). Inter-scale 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 percent of the variation in F_{ST} between the bay and coast scales (Figure 7).

Mitochondrial DNA

Five hundred and twenty-two bases of mitochondrial DNA control region from 103 tidewater goby were aligned. We detected a total of nine different haplotypes from the 13 populations assayed, of which one haplotype (H1) occurred at a high frequency in both bay and coast scales, occurring in 78 percent of individuals sampled (Table 5). The remaining eight haplotypes were restricted to the coast scale and occurred at low frequencies (one to eight percent). Each coast scale population contained at least one private haplotype (Table 6).

Pairwise sequence differences were distributed exponentially, with an average of 0.59 differences, ranging from zero to four mismatches. The mismatch distribution was not significantly different than the distribution expected under the model of rapid expansion (P = 0.8688). Haplotype diversity was absent throughout the bay scale but ranged from 0.25 to 0.75 in the coast scale (Table 6). Nucleotide diversity was not detected in any bay scale population but ranged from 0.0005 to 0.0018 in the coast scale.

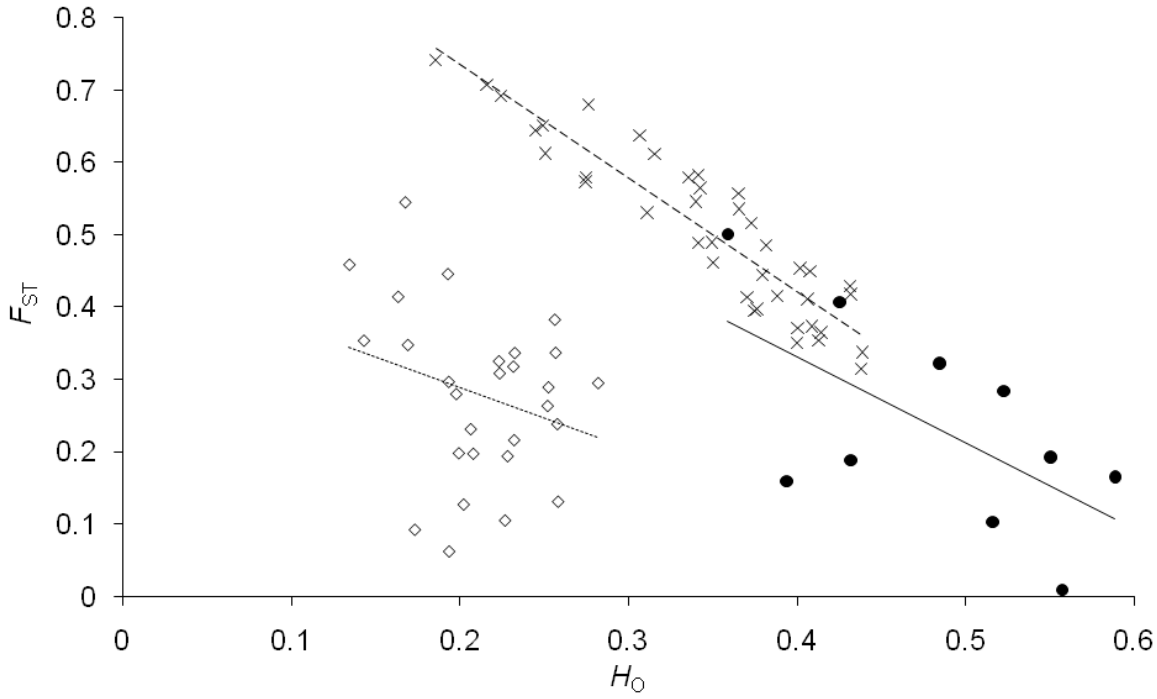

Figure 7. The correlations between pairwise genetic differentiation (F_{ST}) and average of observed heterozygosity (H_O) for the bay scale (white squares, dotted line), the coast scale (black squares, solid line), and between scales (crosses, dashed line) of North Coast tidewater goby, California, 2006. The only significant relationship was between scales (R^2=0.8398, P<0.0001; Mantel Test).

Table 5. Distribution of North Coast tidewater goby haplotypes per population, California, 2006. "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.

	0	0	2	2	3	4	4	Bay Scale								Coast Scale				
	6	9	5	6	7	5	8													
	4	5	3	0	1	8	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	

Table 6. Populations with samples size (n), number of haplotypes (n_H), number of private haplotypes (n_{pH}), and measures of mitochondrial DNA control region diversity [mean ± standard error: haplotype diversity (h), nucleotide diversity (π)] in North Coast tidewater goby, California, 2006.

Population	n	n_H	n_{pH}	h (±SE)	π (±SE)
Bay Scale					
McDaniel Slough	4	1	0	0.00 (0.00)	0.0000 (0.0000)
Gannon Slough	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Gannon Pond	4	1	0	0.00 (0.00)	0.0000 (0.0000)
Jacoby Creek	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Wood Creek	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Elk River	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Salmon Creek	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Eel River	9	1	0	0.00 (0.00)	0.0000 (0.0000)
Coast Scale					
Lake Earl	8	2	1	0.25 (0.06)	0.0005 (0.0020)
Stone Lagoon	8	4	2	0.75 (0.05)	0.0018 (0.0006)
Big Lagoon	8	3	1	0.68 (0.04)	0.0015 (0.0005)
Virgin Creek	9	3	1	0.56 (0.06)	0.0016 (0.0005)
Pudding Creek	8	2	1	0.43 (0.06)	0.0008 (0.0003)

DISCUSSION

Historical Context

The coastal lagoons and estuaries that North Coast tidewater goby inhabits were formed when the last rapid rise of sea level slowed approximately 7,000 years ago. This allowed sandbars to build and extend across open embayments (Barnhart et al. 1992, Stanley and Warne 1994). Range-wide phylogoegraphic 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, Dawson et al. 2002). The mitochondrial DNA mismatch distribution and haplotype data herein are consistent with this hypothesis, suggesting that colonization of the North Coast region occurred as a single, recent episode of rapid expansion to newly formed habitats (Rogers and Harpending 1992).

We found no haplotype or nucleotide diversity within or between any of the bay scale populations, suggesting either recent drift or a lack of mitochondrial DNA divergence prior to artificial fragmentation (Fumagalli et al. 2002). In agreement with previous studies, the presence of private haplotypes in every coast scale population indicates that migration is rare (Swift et al. 1989, Lafferty et al. 1999, Dawson et al. 2001, Dawson et al. 2002).

Bay Scale

Extreme genetic consequences are expected for artificially fragmented populations (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. My results strongly suggest these patterns are due to recent drift in isolation as shown by:

1. Lack of any mitochondrial DNA sequence variation.
2. No relationship between genetic differentiation and geographic distance.
3. The prevalent fixation of polymorphic microsatellite loci.
4. A strong correlation between habitat area and rarefied allelic richness.

Humboldt Bay has suffered a 90 percent reduction in marsh habitat from anthropogenic manipulation of the estuary beginning 120 years ago (Barnhart et al. 1992). Construction of jetties at the bay entrance, persistent dredging of the sand bar, 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 has been suggested that prior to artificial fragmentation, a large population of tidewater goby was distributed throughout the 4,047 ha of potentially suitable habitat recently lost through these land use practices (United States Fish and Wildlife Service 2008). Human activities around Humboldt Bay have threatened the historically large population in two critical ways. First, destruction of 90 percent of the tidewater goby's habitat reduced the size of the ancestral population. Second, the presence of tide gates and levees around suitable habitat patches has restricted gene flow between the insularized and (or) recently founded populations. These two processes have resulted in a scenario of rampant drift in isolation; driving extreme genetic differentiation at small spatial scales and reducing genetic diversity through demographic stochasticity and inbreeding over the last 120 years.

In most circumstances, artificially fragmented populations are not expected to behave as a metapopulation, as dispersal is often severely restricted and local extinctions are not followed by recolonization (Hanski and 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). Whereas seasonal flooding has been shown to explain a small (but statistically non-significant) portion of tidewater goby recolonization rates in the naturally fragmented coast populations of southern California (Lafferty et al. 1999), all evidence from this study suggests population interaction among the artificially fragmented bay scale is not an important component influencing genetic variation. Thus, while assuming a metapopulation model and treating the bay scale populations as a single unit may be convenient for management purposes (Chamberlain 2006, United States Fish and Wildlife Service 2008), we suspect that the variance in detectability of bay populations may represent an artifact of sampling rather than metapopulation dynamics (Swift et al. 1989, Swenson 1999).

Coast Scale

Natural fragmentation often occurs over geologic timescales and is expected to increase the genetic differentiation of subpopulations when dispersal is limited. The five populations in the coast scale generally exhibited high levels of genetic differentiation combined with substantial amounts of genetic diversity. My results suggest that most of

these populations have remained large and stable since Holocene colonization, and that migration between them occurs on an infrequent basis, as shown by:

1. Mitochondrial DNA sequence variation and private haplotypes.
2. Strong genetic isolation-by-distance.
3. Considerable microsatellite DNA polymorphism.
4. No relationship between habitat area and rarefied allelic richness.

The coast scale populations are found in the largest habitats separated by some of the longest geographic 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 migration among populations, whereby a combination of unlikely events must occur for successful gene flow, including coordination of distinct lagoon breaching and passive dispersal of the small benthic fish into new habitats (Swenson 1999). However, the non-significant estimate of population differentiation between Stone and Big Lagoon populations (8.55 km apart) suggests that there is enough gene flow between these two naturally fragmented populations to prevent genetic divergence. Taken together, this information supports the hypothesis 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 4). This result has been confirmed by comparison of our 2006 heterozygosity estimate to that generated by an analysis of 48 Lake Earl tidewater goby from 1999 (authors, unpublished data) that revealed consistently low levels of heterozygosity (H_o = 0.29 (0.06 SE)) in Lake Earl.

Lake Earl is California's largest coastal lagoon and is thought to support the most abundant population range-wide of a few million tidewater gobies (Swift et al. 1989, United States Fish and Wildlife Service 2005). Unlike the other four coast scale populations studied, Lake Earl is artificially breached several times a year during the fall and winter months (United States Fish and Wildlife Service 2008). This management practice has occurred for at least 75 years, initially for the purpose of increasing pastureland for livestock grazing, and recently to prevent flooding of private property around the lagoon (California Coastal Commission 1999). Each artificial breach results in rapid draining of the lagoon and reductions in habitat for the tidewater goby (United States Fish and Wildlife Service 2008). Many thousands of individuals are swept into the Pacific Ocean immediately after each breach, and stranding of tidewater gobies within small pools around the perimeter of the lagoon is well documented (United States Fish and Wildlife Service 2005). This problem is so pervasive that special conditions outlined in the permit to breach Lake Earl require the permittees to survey for stranded gobies and return them to the main basin of the lagoon following each breaching event (California Coastal Commission 1999, United States Fish and Wildlife Service 2008). We suspect

that the markedly reduced levels of heterozygosity in Lake Earl may be due to the numerous population bottlenecks resulting from artificial breachings.

Artificial versus Natural Fragmentation

Artificial fragmentation has resulted in extreme genetic consequences to the bay scale populations. The severity of these genetic effects are emphasized by the fact that the artificially fragmented bay populations are separated by merely 0.3 to 28.5 km of reclaimed wetlands, however they contain higher amounts of population structure than the coast populations (Figure 3, Table 3), which are naturally fragmented by up to 267.8 km of inhospitable coastline. This drastic difference in geographic magnitude exemplifies the significance of the increased population structure detected within the artificially fragmented bay scale. Thus, migration among the bay populations has been reduced or eliminated due to the artificial fragmentation, yet the coast populations appear to maintain small amounts of gene flow in their naturally fragmented distribution (Figure 5).

Artificial habitat fragmentation has resulted in a severe reduction of genetic diversity within the bay populations, whereas genetic diversity appears to be maintained in most of the naturally fragmented coast populations (Table 4, Table 6). The artificially fragmented bay populations contained, on average, less than half the amount of genetic diversity as the naturally fragmented coast populations. One concern when comparing genetic diversity among populations of varying size is that large populations are expected to contain more alleles than small populations. Levels of heterozygosity, however are thought to generally remain independent of population size, and can thus be used to

compare genetic diversity among populations found in both large and small habitats (Allendorf and Luikart 2007). The permutation test between scales confirmed that observed heterozygosity was significantly lower in the bay populations. Therefore, the reductions of genetic diversity in the bay scale have been due to the consequences of artificial habitat fragmentation. In contrast, my results 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=0.57), one of the largest tidewater goby habitats range-wide (612.5 ha).

The extreme levels of genetic differentiation (mean pairwise F_{ST} = 0.39) estimated across all North Coast populations were surprising. By hierarchically examining estimates, we found that the average pairwise genetic differentiation between the bay and coast scales was 0.47. This is approximately twice the amount of differentiation estimated within either scale. The significant correlation of mean pairwise observed heterozygosity and genetic differentiation between bay and coast scales explains these extreme F_{ST} estimates (Figure 7). This relationship together with the significant correlation of rarefied allelic richness and habitat area in the bay scale (Figure 6) suggests that rampant drift in bay populations has resulted in the extreme estimates of genetic differentiation between scales.

Conservation Strategies

Due to the extent that both natural and artificial habitat fragmentation have caused marked divergence among North Coast tidewater goby, all populations are of conservation concern because they may contain unique genetic material not replicated

elsewhere within the species. However, the threat of extirpation exists at different intensities at the two scales examined. The Humboldt Bay populations appear to be at an extreme risk of extinction due to low levels of genetic diversity and lack of among population migration. Within Humboldt Bay we recommend habitat restoration activities that would increase the potential for between population migration. Population surveys conducted before and after restoration projects in artificially fragmented terrestrial habitats have shown increases in dispersal, recolonization, and population growth following the return to natural conditions (Brisson et al. 2003). If habitat restoration results in increased migration among the bay scale populations, the amount of genetic diversity within populations should increase. To evaluate the effect of restoring connectivity among bay populations, we determined the amount of genetic diversity within all eight bay populations combined. In this analysis we detected the fixation of only one locus, which is comparable to the level of fixation in robust coast populations Stone and Big Lagoon (Table 4). The reduction in the number of fixed loci would ultimately increase mean heterozygosity in the bay. In contrast, mean allelic richness would remain lower in the bay than in the coast populations (A=3.889 (0.964 SE), A_r= 3.053 (0.686 SE)) (Table 4). Allelic richness increases very slightly because the bay populations all have the same alleles, but at different frequencies, having lost the same rare alleles due to drift (Table 4). Thus, restoring connectivity would likely increase heterozygosity and minimize some of the detrimental effects genetic diversity loss. However, no increase in allelic diversity would be realized until mutations replaced them.

Proposed restoration projects in Humboldt Bay aimed at modifying or removing tide gates to allow passage of threatened anadromous salmonids have not been approved

by managers because of concerns regarding the potentially harmful effects to the tidewater goby (United States Fish and Wildlife Service 2008). These concerns are valid because construction projects may result in the extirpation of some populations (Stillwater Sciences 2006). Nonetheless, managers should consider the long-term consequences of restricted migration and low genetic diversity of these populations, as taking risks to modify or remove tide gates may be acceptable if habitat is restored.

Unfortunately, not all habitat restoration projects result in improved status of imperiled species that have undergone extreme changes in genetic structure. Extended periods of drift and inbreeding can force populations into an 'extinction vortex' requiring *ex situ* conservation strategies (Westemeier et al. 1998). Due to this possibility, research that measures inbreeding depression and other fitness related characters in North Coast tidewater goby populations is essential to future conservation planning (Fumagalli et al. 2002, Johansson et al. 2007).

Professional opinions differ as to what is the best strategy for conservation of an endangered species when populations are highly divergent and may differ in their long-term potential for recovery. Traditionally, conservation priority is given to those populations that have been severely threatened by habitat loss or reductions in census size (Primack 1998). However, some scientists see this strategy as faulty and recommend putting conservation efforts into those populations that have eminent threats but which otherwise remain healthy (Hankin, D. G. 2008. Personal Communication. Department of Fisheries Biology, Humboldt State University, 1 Harpst Street, Arcata, CA 95521). Fortunately, most of the robust coast group populations of tidewater goby are already protected within California State Parks, so preservation of these habitats should continue.

Lake Earl, in contrast, affords some protection through its designation as a California Department of Fish and Game Wildlife Area, however the artificial lagoon breaching activity appears to be reducing genetic diversity. Lake Earl presumably contains the largest population of tidewater goby range-wide and has been assumed to be an important source of genetic diversity in the region (United States Fish and Wildlife 2008). Thus, prioritizing conservation of the Lake Earl population may be the most sensible approach to the long-term recovery of the species in northern California.

CONCLUSIONS

Artificial fragmentation has reduced gene flow in the bay populations, resulting in extreme consequences to the genetic structure of the tidewater goby. The levees and tide gates isolating habitats pose obstacles that this species cannot circumvent. Levees and tide gates will continue to 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 remains stable. The isolation caused by the naturally formed sand bars restricts regular marine dispersal, yet migration does occur on an infrequent basis.

The tidewater goby cannot maintain genetic diversity in artificially fragmented populations. The species' evolutionary history of persistence in the naturally fragmented estuaries of California is not sufficient to mitigate degradation of genetic diversity 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.

LITERATURE CITED

Ahnelt, H., J. Goschl, M. N. Dawson, and D. K. Jacobs. 2004. Geographical variation in the cephalic lateral line canals of *Eucyclogobius newberryi* (Teleostei, Gobiidae) and its comparison with molecular phylogeography. Folia Zoologica 53:385-398.

Allendorf, F. W. and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Malden, Massachusetts.

Avise, J. C., J. Arnold, R M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:498-522.

Barnhart, R. A., M. J. Boyd, and J. E. Pequegnat. 1992. The ecology of Humboldt Bay, California: an estuarine profile. United States Department of the Interior, Fish and Wildlife Service, Biological Report 1, Washington, D. C.

Barton, N. H. and B. Charlesworth. 1984. Genetic revolutions, founder effects and speciation. Annual Review of Ecology and Systematics 15:133-146.

Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. The Quarterly Review of Biology 74:21-45.

Bonin, A., E. Bellemain, P.B. Eidesen, P. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetics studies. Molecular Ecology 13:3261–3273.

Brisson, J. A., J. L. Strasburg, and A. R. Templeton. 2003. Impact on fire management on the ecology of collared lizard (*Crotaphytus collaris*) populations living on the Ozark Plateau. Animal Conservation 6:247-254.

California Coastal Commission. 1999. Staff report regular calendar. Application file number: 1-97-76. San Francisco, California.

Cavalli-Sforza, L. L. and A. F. W. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics 19:233-257.

Chamberlain, C. D. 2006. Environmental variables of northern California lagoons and estuaries and the distribution of tidewater goby (*Eucyclogobius newberryi*). United States Fish and Wildlife Service, Arcata Fisheries Technical Report TR 2006-04, Arcata, California.

Clark, A. M., B. W. Bowen, and L. C. Branch. 1999. Effects of natural habitat fragmentation on an endemic scrub lizard (*Sceloporus woodi*): an historical

perspective based on a mitochondrial DNA gene geneology. Molecular Ecology 8:1093-104.

Crabtree, C. B. 1985. Allozyme variability in the tidewater goby, *Eucyclogobius newberryi* (Pisces: Gobiidea). Isozyme Bulletin 18:70.

Dawson, M. N., K. D. Louie, D. K. Jacobs, and C. C. Swift. 2002. Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California transition zone. Molecular Ecology 11:1065-75.

Dawson, M. N., J. L. Stanton, and D. K. Jacobs. 2001. Phylogeography of the tidewater goby, *Eucyclogobius newberryi* (Teleostei, Gobiidae), in coastal California. Evolution 55:1167-1179.

DeWoody, J., J. D. Nason, and V. D. Hipkins. 2006. Mitigating scoring errors in microsatellite data from wild populations. Molecular Ecology Notes 6:951–957.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Felsenstein, J. 1993. PHYLIP-Phylogenetic Inference Package, Version 3.5c. University of Washington, Seattle, Washington.

Frankham, R. 1995. Conservation genetics. Annual Review of Genetics 29:305-327.

Frankham, R. 2003. Genetics and conservation biology. CR Biologies 326:S22-S29.

Freeman, S. and J. C. Herron. 2007. Evolutionary analysis. 4th edition. Pearson Prentice Hall, Upper Saddle River, New Jersey.

Fumagalli, L., A. Snoj, D. Jesensek, F. Balloux, T. Jug, O. Duron, F. Brossier, A. J. Crivelli, and P. Berrebi. 2002. Extreme genetic differentiation among the remnant populations of marble trout (*Salmo marmoratus*) in Slovenia. Molecular Ecology 11:2711-2716.

Geospiza, Inc. 2006. FINCH TV 1.4. Seattle, Washington.

Goldberg, S. R. 1977. Seasonal ovarian cycle in the tidewater goby, *Eucyclogobius newberryi* (Gobiidae). The Southwestern Naturalist 22:557-559.

Goodman, S. J., H. B. Tamate, R. Wilson, J. Nagata, S. Tatsuzawa, G. M. Swanson, J. M. Pemberton, and D. R. McCullough. 2001. Bottlenecks, drift and differentiation: the population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. Molecular Ecology 10:1357–1370.

Goudet, J. 1995. FSTAT Version 1.2.: a computer program to calculate F-statistics. Journal of Heredity 86:485-486.

Gysels, E. S., B. Hellemans, C. Pampoulie, and F. A. M. Volckaert. 2004. Phylogeography of the common goby, *Pomatoschitus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. Molecular Ecology 13:403-417.

Hanski, I. and M. Gilpin. 1991. Metapopulation dynamics: brief history and conceptual domain. Biological Journal of the Linnean Society 42:3-16.

Hedrick, P. W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53:313–318.

Helfman, G. S. 2007. Fish conservation. Island Press, Washington, D. C.

Hitchings, S. P. and T. J. C. Beebee. 1998. Loss of genetic diversity and fitness in Common Toad (*Bufo bufo*) populations isolated by inimical habitat. Journal of Evolutionary Biology 11:269-283.

Hutchinson, D. W. and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898-1914.

Johansson, M., C. R. Primmer, and J. Merila. 2007. Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporara*). Molecular Ecology 16:2693–2700.

Johnson, J. A., J. E. Toepfer, and P. Dunn. 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. Molecular Ecology 12:3335-3347.

Johnson, J. A., M. R. Bellinger, J. E. Toepfer, and P. Dunn. 2004. Temporal changes in allele frequencies and low effective population size in greater prairie-chickens. Molecular Ecology 13:2617–2630.

Jordan, M. A. and H. L. Snell. 2008. Historical fragmentation of islands and genetic drift in populations of Galapagos lava lizards (*Microlophus albemarlensis* complex). Molecular Ecology 17:1224-1237.

Kalinowski, S. T. 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conservation Genetics 5:539-543.

Kalinowski, S. T. 2005. hp-rare 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5:187–189.

Keller, I. and C. R. Largiader. 2003. Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. Proceeding of the Royal Society of London 270**:**417–423.

Krauss, N. C., A. Militello, and G. Todoroff. 2002. Barrier breaching process and barrier spit breach, Stone Lagoon, California. Shore and Beach 70:21-28.

Lafferty, K. D., C. C. Swift, and R. F. Ambrose. 1999. Extirpation and recolonization in a metapopulation of an endangered fish, the tidewater goby. Conservation Biology 12:1447-1453.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. ClustalW2 and ClustalX version 2. Bioinformatics 21:2947-2948.

Lee, W.-J., J. Conroy, W. H. Howell, and T. D. Kocher. 1995. Structure and evolution of teleost mitochondrial control regions. Journal of Molecular Evolution 41:54-66.

Maddison, W. P. and D. R. Maddison. 2008. MacClade. Sinauer Associates, Sunderland, Massachusetts.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209-220.

McGourty, K., A. Kinziger, and G. Goldsmith. 2008. Spawning time, fecundity, habitat utilization, and parasites of a northern California population of tidewater goby, *Eucyclogobius newberryi*. California Fish and Game 94:18-32.

Meffe, G. K. and C. R. Carroll. 1997. Principles of conservation biology. Sinauer Associates, Sunderland, Massachusetts.

Mendonca, H., J. Smith, and C. Brinegar. 2001. Isolation and characterization of four microsatellite loci in the tidewater goby (*Eucyclogobius newberryi*). Marine Biotechnology 3:91-95.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance for small number of individuals. Genetics 89:583-590.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York.

Palumbi, S. R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications 13:5146-5158.

Peakall, R. E. and P. E. Smouse. 2001. GENALEX V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra. Available at http://www.anu.edu.au/BoZo/GenAIEx.

Primack, R. B. 1998. The essentials of conservation biology. Sinauer Associates, Sunderland, Massachusetts.

Pritchard, J. K., P. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pritchard, J. K. and W. Wen. 2003. Documentation for STRUCTURE software: Version 2. Available at http://pritch.bsd.uchicago.edu.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rogers, A. R. and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

Rosenberg, N. A. 2004. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4:137-138.

Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN, Version 2000: a software for population genetics data analysis. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Stanley. D. J. and A. G. Warne. 1994. Worldwide initiation of Holocene marine deltas by deceleration of sea level rise. Science 265:228-231.

Stillwater Sciences. 2006. Guidelines to evaluate, modify, and develop estuarine restoration projects for tidewater goby habitat. Report of Stillwater Sciences to United States Fish and Wildlife Service, Arcata, California.

Swenson, R. O. 1999. The ecology, behavior, and conservation of the tidewater goby, *Eucyclogobius newberryi*. Environmental Biology of Fishes 55:99-114.

Swift, C. C., J. L. Nelson, C. Maslow, and T. Stein. 1989. Biology and distribution of the tidewater goby, *Eucyclogobius newberryi* (Pisces: Gobiidae) of California.

Contributions in Science 404, Natural History Museum of Los Angeles County, Los Angeles, California.

Templeton, A. R., K. Shaw, E. Routman, and S. K. Davis. 1990. The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden 77:13-27.

United States Fish and Wildlife Service. 1994. Endangered and threatened wildlife and plants: determination of endangered species status for the tidewater goby. Federal Register 59:5494-5498.

United States Fish and Wildlife Service. 2005. Recovery plan for the tidewater goby (*Eucyclogobius newberryi*). United States Fish and Wildlife Service, Portland, Oregon.

United States Fish and Wildlife Service. 2007. Tidewater goby (*Eucyclogobius newberryi*) 5-year review: summary and evaluation, Ventura Fish and Wildlife Office, Ventura, California.

United States Fish and Wildlife Service. 2008. Endangered and threatened wildlife and plants: revised designation of critical habitat for the tidewater goby (*Eucyclogobius newberryi*): final rule. Federal Register 73:5920-6006.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Westemeier, R. L., J. D. Brawn, S. A. Simpson, T. L. Esker, R. W. Jansen, J. W. Walk, E. L. Kershner, J. L. Bouzat, and K. N. Paige. 1998. Tracking the long-term decline and recovery of an isolated population. Science 282:1695-1698.