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PRIMARY RESEARCH PAPER

Salinity and hydrological barriers have little influence on genetic structure of the mosquitofish in a coastal landscape shaped by climate change

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Abstract Gradients in salinity and vegetation have been dramatically altered along shoreline of the northern Gulf of Mexico by both natural (i.e., erosion, fluctuating sea levels) and anthropogenic (i.e., dredging, habitat restoration) drivers. We gauged the impacts of salinity gradients and open water barriers on the genetic structure of a common marsh resident, western mosquitofish (*Gambusia affinis*) by sampling 15 populations along 440 km of Texas/Louisiana coast. We characterized 602 individuals (~40/population) using seven microsatellite loci and our results reflected significant isolation by distance (IBD) among populations, but without the hypothesized genetic substructure from local adaptation. Large tracts of open water, thought to inhibit mosquitofish movement, were apparently not significant deterrents of dispersal. There was no evidence of a significant relationship between salinity gradients and genetic divergence. Although mosquitofish dispersal is sufficiently limited to result in a strong pattern of IBD, it is high enough to maintain the connectedness of populations. Our results suggest that limited gene flow, combined with large effective size, creates conditions suitable to adaptation to local environment, suggesting that mosquitofish and other marsh residents with similar life histories will be able to adapt to changes occurring in coastal environments.

Keywords Adaptation · Dispersal · Microsatellite DNA · Migration · Salinity

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Introduction

Global climate change is a challenge for ecosystem conservation as it reshuffles environmental conditions and biodiversity gradients as a result of in situ adaptation, distribution shifts, and extirpation (Bellard et al., 2012; Parmesan et al., 2013). As one of most pressing aspects of climate change, sea level rise has manifest consequences for ecological systems (Bordbar et al., 2015). For the past century (i.e., 1900–1990), global mean sea level has elevated 1.2 mm/year (±0.2); however, this rate has increased substantially to 3.0 mm/year (±0.7) over a more recent 13 year span (i.e., 1993–2010) (Hay et al., 2015), and to 4.4 mm/year (±0.5) in the last 3 years (Yi et al., 2015).

Importantly, estimates of absolute sea level rise do not reflect local conditions that can alter the consequences for coastal regions (Bordbar et al., 2015). For example, the coastal wetlands of southeastern Louisiana are recognized as highly susceptible to even small changes in absolute sea level rise (Day et al., 2000). Not as apparent, however, is the role that terrestrial subduction plays in the topography of the region, a process that stems from natural tectonism combined with anthropogenic impacts such as sediment diversions, riverine levees, and dredging (Boesch et al., 1994; Day et al., 2000; Blum & Roberts, 2009). High rates of subsidence result in relative sea level rise that might be 2–3 times the rate of absolute sea level rise (Blum & Roberts, 2009). Furthermore, as relative sea levels increase, saltwater intrusions proliferate, and marshes become fragmented by open water with elevated salinities (Boesch et al., 1994; Day et al., 2000).

How these changes impact Gulf Coast biodiversity is an open question. Most contemporary studies have focused instead on large-scale, ecosystem-level effects that reflect the aftermath of hurricanes (e.g., Chambers et al., 2007; Jeffrey & Martin, 2010) and oil-spills (e.g., Fodrie & Heck, 2011; Williams et al., 2011). Species-specific responses to sea level change have not been examined in detail, nor has connectivity been quantified among populations of species resident to coastal marshes. These are critical knowledge gaps, as sharply defined saline clines, coupled with extensive open water breaks, have considerable potential to fragment populations of marsh-dependent organisms.

Organismal responses to increased salinity in coastal marsh ecosystems are seldom viewed at the population level. Similarly, the role of the coastal landscape in influencing the connectivity among the populations is not clear. For example, the effect of separation of habitats by fresh and saline water barriers has not been adequately evaluated, and it is unclear whether breaks formed by open water represent barriers for marsh-dwelling organisms (Purcell et al., 2012). Yet the presence of such potential barriers might influence adaptations to local conditions (Dobzhansky & Queal, 1938; Taylor, 1991; Fournier-Level et al., 2011). Local adaptation in a heterogeneous environment may arise due to strong selection pressure that outweighs random effect of genetic drift and homogenizing effects of gene flow (Gandon & Michalakis, 2002; Nielson et al., 2005).

Large effective population sizes may enhance the ability of selection to shape genetic makeup of coastal and marine vertebrates minimizing the effects of drift (Hansen et al., 2007; Nielsen et al., 2009). It is not known how these forces affect localized adaptation of marsh fishes to salinity levels.

Considered an invasive species in much of the world, the western mosquitofish *Gambusia affinis* (Baird & Girard, 1853) is a small (17–50 mm SL) livebearing fish native to Central America and southern United States (Meffe & Snelson, 1989). Although typically considered an inhabitant of freshwater, it is one of the most common fishes in brackish coastal marshes in Louisiana (Hitch et al., 2011). It can tolerate wide fluctuation of water temperature, dissolved oxygen, and salinity (Pyke, 2005). In coastal Louisiana, important life history traits including body condition, gonadosomatic index, and number of offspring vary among fresh, intermediate, and brackish water habitats (Martin et al., 2009). There is also evidence for a genetic basis of salinity tolerance in mosquitofish (Purcell et al., 2008). High salinity tolerance of decedents of fish from brackish marshes, relative to those from freshwater marshes, suggests the presence of genetic adaptations for surviving salinity stress in populations with histories of exposure (Purcell et al., 2008).

Coastal marsh provides good connectivity of habitats for small fishes such as *G. affinis*, but large bodies of open water may inhibit movement for marsh residents that are largely confined to shallow vegetated habitats (Hitch et al., 2011). Purcell et al. (2012) found the evidence of high level of gene flow across the salinity gradient in the populations within coastal basin; however, there was considerably more genetic differentiation observed in fish from two marsh basins in Louisiana. The genetic divergence between the central and western portions of Louisiana coast was suspected due to Atchafalaya River restricting the gene flow (Purcell et al., 2012), but limited sampling over large spatial scales created the possibility that the observed differentiation was due to distance alone.

Our objective was to examine the effects of potential aquatic barriers and salinity gradients on gene flow among mosquitofish populations. To that end, we examined the following set of null hypotheses: *Mosquitofish populations exhibit no population structure with regard to presumably neutral molecular markers.* This hypothesis would be rejected if there

were differences in genetic diversity among populations or if measures of differentiation among populations were significantly greater than zero. Failure to reject this hypothesis would suggest populations are panmictic and selection would have to be quite strong to produce local adaptations to salinity (Purcell et al., 2012).

Mosquitofish populations do not exhibit a pattern of isolation by distance (IBD). This hypothesis would be rejected if genetic differentiation among sites increased with distance. IBD would be expected if dispersal is too weak to result in panmixia, but strong enough to increase the genetic similarity of spatially proximate populations.

Hydrologic barriers separating expanses of marsh do not inhibit gene flow in mosquitofish. This hypothesis would be rejected if genetic differentiation was greater between populations separated by major rivers than those in more contiguous coastal marshes. Because mosquitofish are dependent on shallow, vegetated environments, major rivers have been hypothesized to reduce gene flow (Purcell et al., 2012).

Salinity gradients do not structure mosquitofish populations. This hypothesis would be rejected if genetic differentiation is stronger between sites with different salinities than between sites with similar salinities. Likewise, we would reject this hypothesis if the salinity of a potential hydrological barrier affected gene flow differentially depending on the local salinities experienced by pairs of populations. Salinity gradients would result in genetic structure of neutral markers if adaptation to local salinity imparts strong survival or reproductive benefits, limiting dispersal and associated gene flow between sites, or across a barrier, with differing salinity.

Our study provides a framework for evaluating the role of coastal landscapes in shaping the population structure of marsh organisms. These data, along with past work on these populations, enhance our understanding the roles of both gene flow and local adaptation in the face of rapid wetland loss and sea level rise.

Materials and methods

Study area

Louisiana contains approximately 40% of the coastal wetlands of United States, and has experienced over 80% of the U.S. losses of this habitat type (Boesch et al., 1994), making it an appropriate area to study the effects of wetland change on coastal organisms. We sampled 15 sites along the eastern Texas/Louisiana coast (Fig. 1; Table 1). This area of extensive coastal marsh is bisected by many canals and natural rivers. We hypothesized that three of them might have been persistent (>200 years in the same basic course) and wide enough (>500 m) to have a large potential effect on the genetic structure of mosquitofish. These included one brackish waterway (the Sabine River) and two freshwater rivers (the Atchafalaya and Mississippi). We identified the marshlands separated by these rivers (west to east) as east Texas (ETX), western Louisiana (WLA), central Louisiana (CLA), and eastern Louisiana (ELA) (Fig. 1; Table 1).

In an attempt to capture the breadth of salinity gradients inhabited by mosquitofish, seven sites were sampled in close proximity to the coast, and eight sites ∼30 to 35 km further inland (Fig. 1). This provided seven pairs of sites, with one member that was coastal (C) and another that was inland (I); only inland site WLA3I was not paired with a coastal site. While there was a trend for coastal sites to have higher salinities than inland sites (Table 1), the considerable influence of rivers and canals on nearby marshes created exceptions to this expected salinity gradient. We used vegetation type to assign salinity levels to sites because vegetation reflects long-term salinity trends at study sites, whereas the salinity of surface waters can have considerable temporal variation (Visser et al., 1998, 2000; Hitch et al., 2011). We categorized marshes according to field observations and data from the Coastwide Reference Monitoring System [CRMS; U.S. Geological Survey (USGS) 2014; Table 1]. We determined the average salinity for each sampled marsh type from stations in the CRMS network. Salinity concentrations averaged 0.86 ppt in fresh marsh, 3.6 ppt in intermediate marsh, 7.0 ppt in brackish marsh, and 14.4 ppt in saline marsh. Following Hitch et al. (2011) and Purcell et al. (2012), these average salinity values for each marsh type were used in analyses requiring an estimate of site salinity.

DNA extraction and genotyping

Genomic DNA was extracted from tail muscle and eluted in 100 ml of buffer using the DNeasy Blood and Tissue kit (Qiagen) following manufacturer's

instructions. Samples were genotyped across seven polymorphic microsatellite loci, five of which comprised dinucleotide repeats (Gafu2, Gafu3, Gafu4, Gafu6, Gafu7) (Spencer et al., 1999), while two others (Gaaf 10, Gaaf13) were tetranucleotides (Purcell et al., 2011). Genotypes from 32 to 40 individuals were determined for each population sample (Table 1) using an ABI Prism 3130 sequencer (Applied Biosystems). An internal size standard was run with each sample. To insure correct base calls, output was first visually inspected and scored using GENEMAPPER 3.7 and then re-scored using PEAK SCANNER (Applied Biosystems).

Hardy–Weinberg equilibrium (HWE) and genetic diversity

We used MICROCHECKER (Oosterhout et al., 2004) to test for large allele dropout, scoring errors, and presence of null alleles. Deviations from Hardy–Weinberg expectations (HWE) and linkage disequilibrium (LD) were evaluated with GENEPOP (Rousset, 2008). A sequential Bonferroni correction (Rice, 1989) was applied to control Type I error rates in these and other multiple comparisons. Expected (H_e) and observed (H_o) heterozygosities and the inbreeding coefficient (F_{IS}) were estimated for each population using GENEPOP (Rousset, 2008). FSTATv2.9.3.2 (Goudet, 2002) with the rarefaction option was used to estimate allelic richness (A_r).

We used a randomized block ANOVA to test if A_r or H_e differed among populations, where the value of the diversity estimates for each locus is the unit of replication. Tukey's Test evaluated differences among populations, and the Pearson product-moment correlation coefficient (r) was used to assess relationships among salinity and genetic diversity estimates. Negative relationships between salinity with A_r and H_e might be expected if populations were smaller or more isolated in brackish than in freshwater marshes.

Population structure

Fisher's Exact Test in GENEPOP was used to determine if allele frequencies differed significantly between pairs of sites. Population divergence was estimated with F_{ST} in GENEPOP (Weir & Cockerham, 1984), and with Hedrick's standardized G'_{ST} (Hedrick, 2005) in GENALEX v6.5 (Peakall & Smouse, 2012).

The Bayesian clustering algorithm in STRUCTURE v2.3.4 (Pritchard et al., 2000) was also employed to examine population substructure, with an estimate of
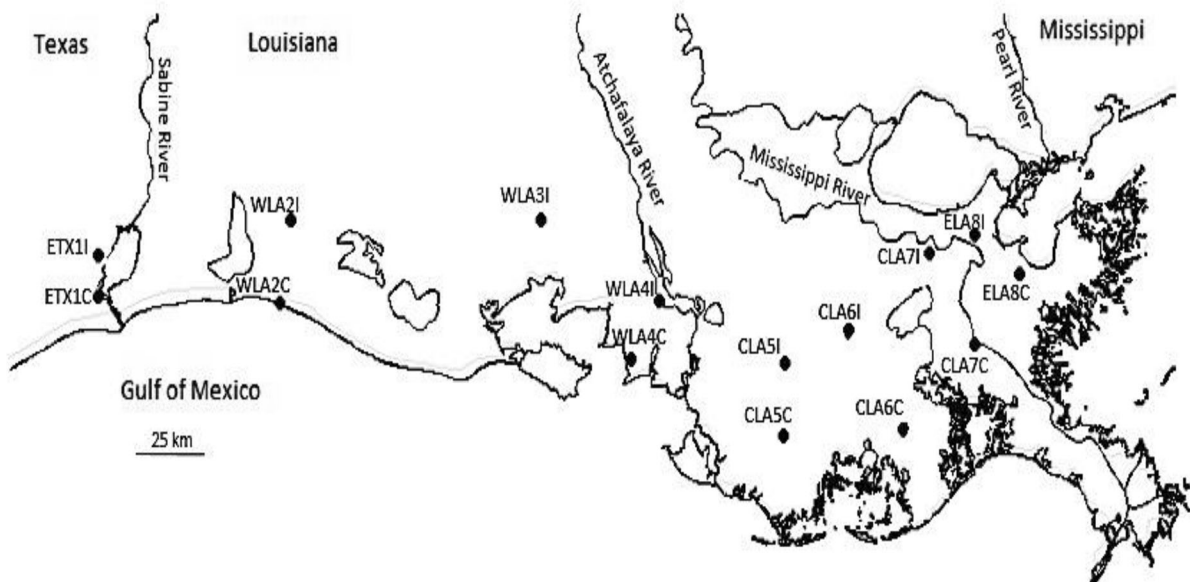

Fig. 1 Sampling sites and major rivers along the northern Gulf of Mexico shoreline evaluated as putative barriers to gene flow in western mosquitofish. Site identifiers indicate the region of the coast from which samples were collected (*ETX* eastern TX,

WLA western LA, *CLA* central LA, *ELA* eastern LA), a site number which increases from west to east, and a letter indicating whether the site was located near the coast (C) or further inland (I)

Table 1 Sample site characteristics, sample size (N), average allelic richness ($A_r \pm$ SE), average expected heterozygosity ($H_e \pm$ SE), observed heterozygosity ($H_o \pm$ SE), and fixation index (F_{IS}) for western mosquitofish sampled at 15 sites along the northern Gulf of Mexico and analyzed across 7 microsatellite loci. Site identifiers (Fig. 1) indicate the region of the coast from which samples were collected

Site	Marsh type	Longitude	Latitude	N	A_r	H_e	H_o	F_{IS}
ETX1I	Fresh	93.948W	29.902N	41	17.2 ± 1.8[b]	0.88 ± 0.02	0.83 ± 0.04	0.07
ETX1C	Brackish	93.948W	29.778N	41	17.7 ± 2.4[b]	0.90 ± 0.02	0.90 ± 0.02	0.03
WLA2I	Fresh	93.070W	30.003N	40	19.1 ± 2.6[ab]	0.89 ± 0.03	0.85 ± 0.02	0.05
WLA2C	Saline	93.121W	29.759N	31	19.9 ± 2.6[ab]	0.91 ± 0.02	0.86 ± 0.03	0.07
WLA3I	Fresh	91.928W	30.003N	38	18.6 ± 2.2[ab]	0.87 ± 0.03	0.83 ± 0.02	0.06
WLA4I	Fresh	91.393W	29.764N	40	18.4 ± 2.5[ab]	0.85 ± 0.06	0.82 ± 0.04	0.05
WLA4C	Fresh	91.518W	29.592N	41	19.4 ± 2.5[ab]	0.87 ± 0.05	0.86 ± 0.04	0.02
CLA5I	Fresh	90.815W	29.582N	39	20.3 ± 1.7[ab]	0.90 ± 0.02	0.86 ± 0.03	0.05
CLA5C	Intermediate	90.823W	29.367N	42	18.3 ± 2.4[ab]	0.89 ± 0.04	0.88 ± 0.05	0.06
CLA6I	Fresh	90.528W	29.681N	31	17.7 ± 2.0[b]	0.87 ± 0.03	0.85 ± 0.04	0.03
CLA6C	Saline	90.282W	29.383N	42	19.4 ± 2.7[ab]	0.88 ± 0.03	0.89 ± 0.03	0.01
CLA7I	Fresh	90.156W	29.905N	40	18.8 ± 2.6[ab]	0.88 ± 0.03	0.88 ± 0.01	0.02
CLA7C	Brackish	89.952W	29.636N	40	19.1 ± 2.6[ab]	0.85 ± 0.05	0.84 ± 0.04	0.03
ELA8I	Brackish	89.955W	29.961N	42	21.7 ± 2.1[a]	0.90 ± 0.03	0.90 ± 0.03	0.02
ELA8C	Brackish	89.748W	29.845N	43	19.1 ± 2.8[ab]	0.89 ± 0.02	0.92 ± 0.02	0.01

ETX eastern TX, *WLA* western LA, *CLA* central LA, *ELA* eastern LA, a site number which increases from west to east, and a letter indicating whether the site was located near the coast (C) or further inland (I). Estimates of A_r which share a letter are not significantly different based on a Tukey multiple comparison test

possible clusters (=K) ranging from one to 15, burnin = 50,000, and MCMC = 500,000. We used an admixture model with and without correlated alleles frequencies and information on sampling locations. STRUCTURE HARVESTER (Earl & vonHoldt, 2012) was used to determine optimal numbers of gene pools using the Evanno method (Evanno et al., 2005), and STRUCTURE output visualized using CLUMPP (Jakobsson & Rosenberg, 2007). Because STRUCTURE performs poorly in cases where genetic variation is structured by distance or along an environmental gradient, we also used principal coordinate analysis (PCoA) to visualize genetic similarities among sample sites. This analysis was conducted on distance matrixes based on both F_{ST} and G'_{ST} using GENALEX v6.5 (Peakall & Smouse, 2012).

Associations between genetic distances and environmental features

We applied Mantel tests (Mantel, 1967; Douglas & Endler, 1982) to evaluate associations between genetic divergence estimates (F_{ST}, and G'_{ST}), geographic distance, and several measures of environmental distance. The geographic distance matrix was calculated from the coordinates of each sampling location (Table 1) using GENALEX. We assumed that gene flow occurred both longitudinally (i.e., along the coast), as well as from more brackish sites near the coast into the freshwater interior. An estimate of distances along dispersal routes was not possible due to the large number of shifting connections within continuous coastal marshes. Therefore, we employed Euclidian distances among sites. We applied linearized F_{ST} [$=F_{ST}/(1 - F_{ST})$] and a similarly transformed G'_{ST}, along with log-transformed geographic distances, to test for the presence of IBD (Kawecki & Ebert, 2004). These transformations are appropriate when genetic divergence and geographic distances are assessed in two-dimensional habitats, such as those in our coastal marsh study system.

We created a salinity-difference matrix, SALINITY, to represent the gradient in salinities among sites based on dominate vegetation and average salinity for that vegetation type [CRMS; U.S. Geological Survey (USGS) 2014] (Table 1). We also derived two pairwise matrices that reflect potential barriers among sites. The first, RIVER_EQUAL, was

derived by assigning a value of 1 as the "distance" between pairs of populations in the same wetland region (without intervening aquatic barriers), whereas a value of 10 was assigned to population-pairs separated by one or more putative barriers. Other weights (i.e., 2,100) were also employed to represent amounts of resistance of barriers to dispersal, but all yielded similar results so only those based on a weight of 10 are presented. To produce a second matrix (RIVER$_{SALINITY}$), we modified RIVER$_{EQUAL}$ to account for reduced resistance to gene flow if adjacent marshes and barriers had similar salinities. To do so, we reduced assigned values by 50% (weight = 5) to population-pairs with similar salinities to an intervening river barrier.

A Mantel test was performed with XL STAT PROFESSIONAL (Addinsoft, New York, NY) to examine the associations between genetic divergences (F_{ST} and G'_{ST}) versus matrices based on geographic distances, salinity, and barriers. We employed two approaches to control for the effects of geographic distance, with the first being a partial Mantel test that held constant the effects of geographic distance (Smouse et al., 1986). Although often used in landscape analyses, there has been debate concerning biases in P values associated with partial Mantel tests (Raufaste & Rousset, 2001; Castellano & Balletto, 2002). Therefore, we also explored the associations between the genetic and the environmental distance matrices in the presence or absence of geographic distance, using Multiple Matrix Regression with Randomization (MMRR, Wang, 2013). Here, the focus shifted from a test of matrix correlations to whether specific regression coefficients were significantly different from zero. Because each matrix is standardized, the size of the coefficients can be used to compare the importance of each to model fit as long as the predictor matrices are not highly correlated (Wang, 2013). Statistical significance of regression coefficients was determined using 9,999 permutations (Wang, 2013).

Small values of discontinuity could be swamped by IBD or local selection. We used program BARRIER 2.2 (Manni et al., 2004) to derive a graphic representation of such genetic discontinuities among samples. The value of this approach is that discontinuities that are often swamped by IBD or, conversely, local selection, can often be identified. Geographic coordinates for each sample were connected via Delaunay triangulation to derive a network connecting all samples. Next, F_{ST} estimates were calculated between neighboring samples, and potential barriers identified using Monmonier's maximum distance algorithm. This process was initiated at the edge of the network with the largest F_{ST} estimate between samples, and a perpendicular line is drawn to the edges of the network with the next largest F_{ST} value. This was repeated until the expanding boundary either met another boundary or reached the edge of the network. The analysis was performed separately for each locus, as well as for estimates of F_{ST} averaged across loci. To investigate if there was any evidence of asymmetrical gene flow between paired inland and coastal marsh sites, a parallel version of MIGRATE (Beerli, 2009) was run in computer cluster (number of processors = number of loci) with constant mutation rate, a single long chain, 10,000 burnin without heating chain. F_{ST} type used was theta (θ). We used Brownian motion approximation to the ladder model, the widely used model for microsatellite data.

Results

HWE and genetic diversity

Following Bonferroni correction, all loci were in HWE in all populations except Gafu 4 for WLA2I (Online Appendix 1). Because no consistent pattern of deviation from HWE was found for any locus or population, all were retained for subsequent analyses. Neither was there evidence of significant LD after correction for Type I error rates. Observed ($H_o = 0.85 \pm 0.01$) and unbiased expected ($H_e = 0.89 \pm 0.01$) heterozygosities were quite high for all populations over all loci. Similarly, average number of alleles at a locus was also relatively large (=30.9; max = 42; min = 21). The ANOVA, blocked by locus, reflected no significant inter-sample variation in H_e, but did indicate significant differences for estimates of A_r, with ELA8L having significantly larger vales than the two Texas samples, as well as CLA6I (Table 1). We found no significant correlations between salinity and either H_e ($r = 0.33$, $P > 0.20$) or A_r ($r = 0.30$, $P > 0.25$). F_{IS} values had a tendency to be positive, but were small, as would be expected given that the lack of significant deviations from HWE.

Table 2 Matrix of pairwise F_{ST} values (below diagonal) and P values (above the diagonal) for the test of allele frequency differences (above the diagonal) derived from the test of allele frequency differences for samples of western mosquitofish, collected from 15 sites along the northern Gulf of Mexico and analyzed at 7 microsatellite loci

Sites	ETX1I	ETX1C	WLA2I	WLA2C	WLA3I	WLA4I	WLA4C	CLA5I	CLA5C	CLA6I	CLA6C	CLA7I	CLA7C	ELA8I	ELA8C
ETX1I	0	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
ETX1C	0.0179	0	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
WLA2I	0.0189	0.0081	0	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
WLA2C	0.0175	0.0036	0.009	0	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
WLA3I	0.0352	0.0224	0.009	0.0203	0	**0.0002**	**<0.0001**	**0.0024**	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**0.0002**	**0.0022**	**<0.0001**
WLA4I	0.0416	0.0313	0.0165	0.0303	0.0078	0	0.0251	0.0225	0.0251	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
WLA4C	0.0349	0.0241	0.0161	0.0151	0.0109	0.0073	0	0.0088	0.0066	**0.0043**	**0.0007**	**<0.0001**	**0.0013**	**0.0013**	**<0.0001**
CLA5I	0.0308	0.0157	0.0099	0.014	0.0054	0.0064	0.0066	0	0.0166	**<0.0001**	**0.0001**	**<0.0001**	0.0395	0.0089	**<0.0001**
CLA5C	0.0392	0.0242	0.0236	0.0199	0.0257	0.024	0.0074	0.0159	0	0.0102	**0.0003**	**<0.0001**	0.019	**<0.0001**	**<0.0001**
CLA6I	0.0333	0.0202	0.0196	0.0199	0.0137	0.0129	0.0051	0.0087	0.0114	0	0.1041	0.0156	**0.0015**	**<0.0001**	**<0.0001**
CLA6C	0.0296	0.0146	0.0109	0.011	0.0097	0.0109	0.0029	0.0029	0.0113	0.0095	0	**<0.0001**	0.0948	**0.0026**	**<0.0001**
CLA7I	0.0425	0.0242	0.0193	0.0197	0.0173	0.0225	0.0123	0.0052	0.0114	0.0159	0.0084	0	**0.0015**	**0.0016**	**<0.0001**
CLA7C	0.0413	0.0272	0.0183	0.0263	0.0118	0.0087	0.0027	0.0085	0.0165	0.0079	0.0058	0.012	0	**0.0164**	**<0.0001**
ELA8I	0.0287	0.0161	0.0093	0.0131	0.0066	0.0078	0.0075	0.0003	0.0165	0.0112	0.0056	0.0079	0.0078	0	**0.0001**
ELA8C	0.0252	0.013	0.0129	0.0126	0.0147	0.0225	0.0176	0.0135	0.0284	0.0172	0.0135	0.0196	0.023	0.0074	0

Site designations (Fig. 1) indicate the region of the coast from which samples were collected

ETX eastern TX, *WLA* western LA, *CLA* central LA, *ELA* eastern LA, a site number which increases from west to east, and a letter indicating whether the site was located near the coast (C) or further inland (I)

P values in bold represent tests of allele frequency differences were significant at the 0.05 level, following a sequential Bonferroni correction for multiple comparisons

Population structure

Following Bonferroni corrections, allele frequencies were significantly different between most pairs of populations (Table 2). F_{ST} values suggested large-scale geographic patterns (Table 2), leading to a rejection of the hypothesis of no genetic structure. In general, most of the largest pairwise estimates of F_{ST} were associated with comparisons where at least one of the populations was from the western portion of the study area (ETX, WLA). Most non-significant values between pairs located within or between marshes in central (CLA) and eastern (ELA) Louisiana (Table 2). Estimates of standardized G'_{ST} (Online Appendix 2) were higher than F_{ST} values (i.e., 0.15 ± 0.029 vs. 0.03 ± 0.02), but the two measures of divergence were highly correlated ($r = 0.95$, $P < 0.0001$). Given this strong correlation, we only present results based on F_{ST}.

The PCoA of F_{ST} values accommodated 81% of the variation among samples (first axis = 62.2%; second = 18.8%), with populations in geographic proximity clustering within the ordination space (Fig. 2). The distribution of populations along PC1 generally reflected relative locations along the coast, with higher scores for Texas (TX) and far western Louisiana (WLA) than for more eastern sites, with the exception of ELA8C. There was no evidence for clustering of sites on the basis of river barriers or salinity. Similarly, the Bayesian Assignment Tests conducted in STRUCTURE did not detect distinct spatial clusters, even when location as a prior (results not shown). There was a sharp decline in the likelihood in the number of clusters at higher values of K, and no concordance of cluster assignments with the geographic proximity of sampled individuals.

Associations among genetic distances and environmental features

Using Mantel tests, we found strong associations between the log of geographic distance and F_{ST} among populations ($r = 0.509$, $P < 0.001$; Online Appendix 3). There was no association between F_{ST} and average
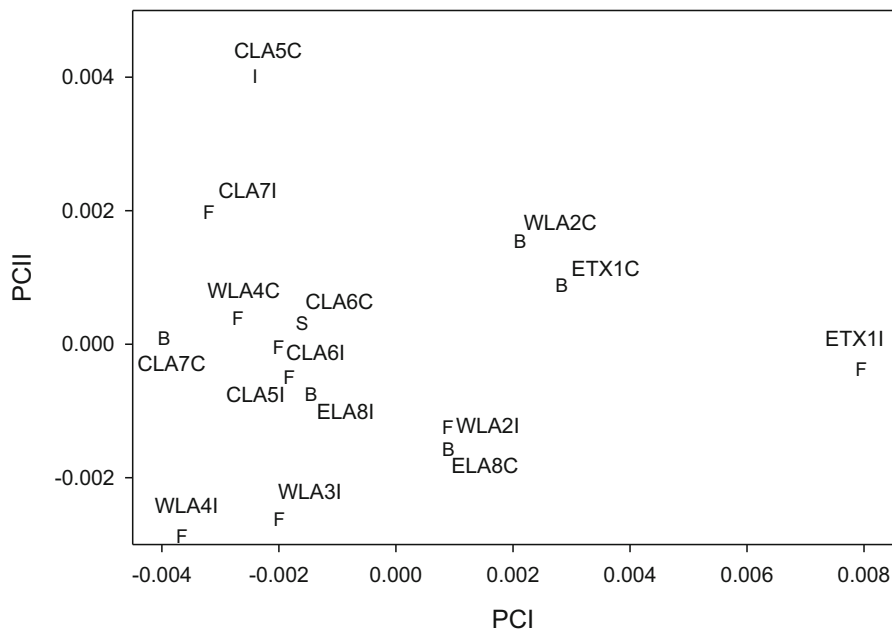

Fig. 2 Principal coordinates analysis of population-level F_{ST} values derived from 602 individuals of western mosquitofish, collected from 15 sites along the northern Gulf of Mexico and analyzed across 7 microsatellite DNA loci. Site identifiers (Fig. 1) consist of the region of the coastal marsh from which samples were collected [i.e., *ETX* eastern TX (*red*), *WLA* western LA (*blue*), *CLA* central LA (*green*), *ELA* eastern LA (*brown*)]. A letter following the site number in the above acronyms (i.e., I and C, respectively) indicates whether the site was found inland or near the coast. The symbol for each site in the graph [fresh (F), intermediate (I), brackish (B), or saline (S)] indicates the classification of aquatic habitat based on marsh vegetation
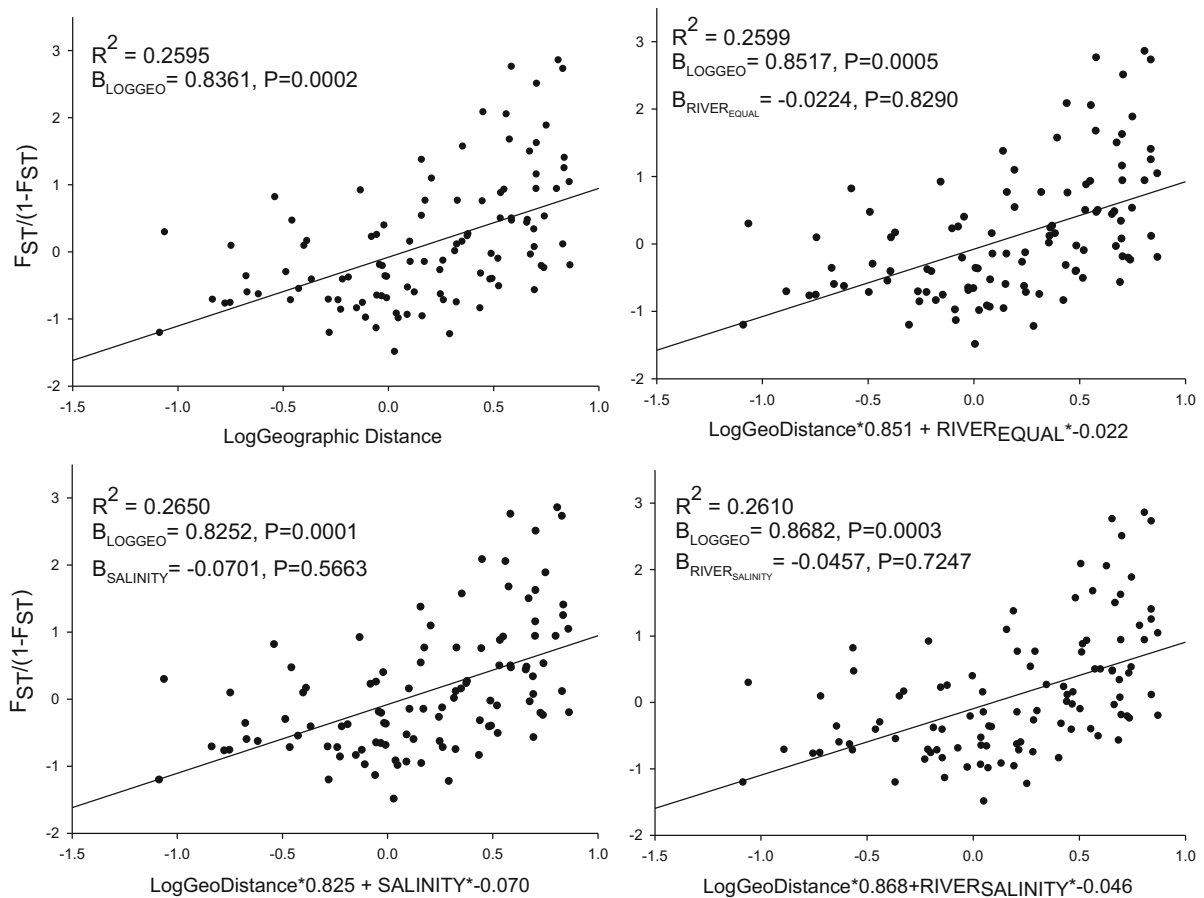
Fig. 3 Regression predictions of genetic distance ($F_{ST}/1 − F_{ST}$) with models with log geographic distance (*top left*), in addition to models with geographic distance in combination with salinity (*bottom left*), the presence of barriers (RIVER$_{EQUAL}$) (*top right*), and the salinity of barriers (RIVER$_{SALINITY}$) (*bottom right*) between pairs of sites. Standardized matrices are used in MMRR so that axes are centered on 0 and units represent the number of standard deviations from the mean

salinity ($r < 0.01$, $P > 0.05$). A significant association was apparent between F_{ST} and the matrix representing the occurrence of aquatic barriers (RIVER$_{EQUAL}$) among sites ($r = 0.272$, $P = 0.004$). Likewise, the RIVER$_{SALINITY}$ matrix was also significantly associated with F_{ST} ($r = 0.295$, $P = 0.004$).

When effects of geographic distance were controlled for using the partial Mantel test, associations between F_{ST} and SALINITY, RIVER$_{EQUAL}$, or RIVER$_{SALINITY}$ were not significant ($P > 0.10$). Although the partial Mantel test has been shown to have higher than expected type I error rates when there is spatial co-variation within the entries of the distance matrices (Guillot & Rousset, 2013), we failed to find significant partial matrix correlations lending credence

to our results that there is not association with genetic divergence with salinity gradients or the presence of riverine barriers. Furthermore, there was no observable improvement of the ability to predict FST when SALINITY, RIVER$_{EQUAL}$, or RIVER$_{SALINITY}$ were included in MMRR models with log geographic distance (Fig. 3). The regression coefficient for geographic distance versus F_{ST} was significant ($P < 0.002$), but those for SALINITY, RIVER$_{EQUAL}$, and RIVER$_{SALINITY}$ were not ($P > 0.10$) when geographic distance was also included in the model. Furthermore, the coefficients for these terms were over an order of magnitude smaller than the coefficients for geographic distance (Fig. 3). This direct comparison is informative because the matrices were all standardized

prior to conducting the MMRR, and thus the coefficients represent a measure of effect size (Wang, 2013).

Interpretation of correlations between genetic and environmental distance is aided by knowledge of the correlations between the various environmental distance matrices under consideration (Smouse et al., 1986; Wang, 2013). Although geographic distance and the two river matrices were not correlated with SALINITY ($P > 0.10$), the log geographic distance matrix was itself correlated with both $RIVER_{EQUAL}$ ($r = 0.562$) and $RIVER_{SALINITIY}$ ($r = 0.593$). Not surprisingly, the two RIVER matrices were themselves strongly correlated ($r = 0.849$).

We found no areas of restricted gene flow when using pairwise F_{ST} estimates in program BARRIER, indicating little evidence for the presence of relatively indistinct barriers (results not shown). Likewise, we found no evidence of directional gene flow between inland and coastal sites using the program MIGRATE (results not shown). Furthermore, this analysis indicated that gene flow was similar between pairs of nearby sites regardless of salinities or locations (i.e., inland or near the coast).

Discussion

The testable framework we provide can be a valuable tool for evaluating the manner by which coastal landscapes shape the population structure of marsh biodiversity, and is particularly relevant at a global scale given the contemporary nature of ongoing sea level rise. The life history attributes of mosquitofish, and its importance in coastal marsh ecosystems (Hitch et al., 2011), establish it as a reasonable study species for such an endeavor. Understanding how these marsh-dependent fish respond to large expanses of unvegetated rivers and estuaries is important because canalization, subsidence, and fragmentation are causing conversion of marsh to open water (Day et al., 2000). If open water is a barrier to dispersal of marsh fishes, it might be driving genetic differentiation observed among populations (Purcell et al., 2012), enhancing the potential for local adaptation to increasing salinity (Purcell et al., 2008). Additionally, Chapman & Warburton (2006) detected evidence for habitat segregation and population subdivision within *Gambusia*, with few movements occurring between pools separated by only a few meters. As mosquitofish

are almost always found only in shallow, vegetated shorelines, it would not be surprising if open water was an impediment to gene flow.

Population divergence despite gene flow

Levels of allelic diversity varied among populations and there were significant levels of divergence among most of our sample populations, leading us to reject the hypothesis of a panmictic population structure. Yet, genetic differences were small, as measured by F_{ST}, suggesting some level of gene flow among populations. Because of highly polymorphic markers used in this study, the divergence value measured by F_{ST} may have depressed. Therefore, we also examined G'_{ST}, which is an attempt to standardize interpopulation differentiation by the within population diversity (Hedrick, 2005). Although G'_{ST} estimates are quite high compared to estimates of F_{ST}, both present a similar picture of the genetic structure in study populations, with differentiation increasing markedly with geographic distance. We did not find population structure while using program STRUCTURE, which could be due to STRUCTURE's low power in the presence of gene flow and IBD (Evanno et al., 2005; Waples & Gaggiotti, 2006; Chen et al., 2007).

Based on Mantel, MMRR, and ordination analyses, we reject the null hypothesis that mosquitofish populations are not structured by geographic distance. Genetic divergence due to IBD is expected in continuous environments when dispersal is limited by distance (Wright, 1943). A likely explanation for the strong IBD pattern observed by us and others (Smith et al., 1989; Díez-del-Molino et al., 2013) is that *Gambusia* dispersal is restricted by distance. Wetlands of the northern Gulf of Mexico are semi-continuous marsh land habitats interspersed with canals, rivers, and pools of varying salinity. In such an environment, short-distance movements are probably common enough to maintain linkages between nearby populations, but insufficient to result in panmixia at larger spatial scales.

Dispersal and aquatic barriers

None of our analyses indicated that expanses of unvegetated habitat, in the form of major rivers, are major barriers to the movement of mosquitofish, regardless of the salinity of the river system. Although

there was considerable interpopulation differentiation, and while some of that differentiation occurred among samples separated by putative barriers, much of the genetic diversity among sites could be explained by IBD.

We know little about the movement of resident marsh fishes across major barriers. Within marshes, the movement speed of eastern mosquitofish (*G. holbrooki*), which is ecologically and morphologically similar to western mosquitofish, has been recorded as 0.001–0.30 meters per second when search for dry season refuge (Obaza et al., 2011). Furthermore, mosquitofish is a rapid colonizer and efficient emigrant in response to changing environments (Hoch et al., 2015). When invading recently flooded wetlands, *G. holbrooki* occupies habitats rapidly regardless of connection depth (Hohausova et al., 2010). Alemadi & Jenkins (2008) noted that mosquitofish can disperse faster in deeper water (24 mm) as compared to shallow water but they can still disperse through as little as 3-mm-deep corridors. However, no ecological studies have shed much light on movements across major rivers. Purcell et al. (2012) speculated that genetic differences between southwestern and southeastern Louisiana samples might be due to the Atchafalaya River as a barrier. Our more extensive sampling suggests that distance, and not the river, better explains the divergence they observed.

Population structure and salinity

In addition to distance and rivers as potential influences on fish movements, we also examined the role of salinity in structuring genetic diversity. Mosquitofish are typical much less common in brackish than in fresh marshes (Gelwick et al., 2001; Hitch et al., 2011; Ruiz-Navarro et al., 2013). Stress resulting from salinity spikes, expected in coastal marshes due to storm surges, might also reduce local populations. However, we found no evidence for a negative correlation of either allelic richness or heterozygosity with salinity, suggesting that populations in brackish or saline habitats were still large enough, or experienced high enough gene flow, to maintain levels of genetic diversity similar to larger populations in freshwater marshes.

Common garden experiments indicate that mosquitofish populations from both southwestern and southeastern Louisiana are adapted to local salinity conditions (Purcell et al., 2008). It is possible that salinity differences between marshes might serve as a filter to reduce gene flow. We failed to reject the null hypothesis that salinity was not structuring microsatellite variation in mosquitofish populations. This result, based upon sampling along 500 km of the coast, does not confirm the observation of Purcell et al. (2012) that there was an association between salinity and population structure within a local wetland basin. However, this difference in results is marginal, as Purcell et al. (2012) found only a weak association of genetic structure and salinity in one of three analyses; other statistical tests they conducted supported our finding of no pattern in genetic differentiation that could be explained by the presence of salinity gradients. MMRR and partial Mantel analyses also indicate that the salinity of putative hydrologic barriers did not affect genetic divergence. Simply put, the pattern of genetic diversity found among mosquitofish populations was explained by IBD rather than a response to salinity gradients of the salinity of putative riverine barriers.

Our results suggest that gene flow is limited enough to result in a strong correlation between genetic differentiation and space, but is substantial enough to maintain connections among sample sites preventing the creation of distinct local populations. Adaptation can occur in the presence of considerable gene flow, particularly if selection is sufficiently strong (Moore et al., 2007; Nosil, 2008). Although mosquitofish are euryhaline, high salinities can depress growth, reproduction, and survival (Congdon, 1994; Kandl, 2001; Alcaraz & García-Berthou, 2007; Purcell et al., 2008), suggesting elevated salinity is potentially a strong selective force on *Gambusia* populations. In addition to the gradients in salinities we examined in this study, which are shifting in response to relative sea level rise and coastal management, populations near the coast are exposed to spikes in salinity due to storm surges and unusual tides (Nicholls & Cazenave, 2010). Thus, populations of resident marsh fishes might experience sufficient selective pressures to develop local adaptation, even when exchanging genetic material from nearby fresh water sites.

It is important to remember that gene flow is the product of both effective population size and the number of migrants per generation. High effective population sizes aid in the development of local adaptation by minimizing the effects of drift even

when migration is low (Kawecki & Ebert, 2004). Hitch et al. (2011) found average mosquitofish densities of 6.5 fish/m^2 across marsh types. Even in brackish conditions, where the average density was estimated at 3.8 fish/m^2, a square km of continuous marsh habitat probably supports at least hundreds of thousands of individuals. In addition to high abundances, life history characteristics of this species including multiple paternity, sperm storage, and rapid maturation help promote large effective population sizes (Echelle et al., 1989). It is not clear what the dispersal distances are for mosquitofish. Chapman & Warburton (2006) found that *G. holbrooki* occasionally moved 60 m in a thirty-day period when dispersing into unoccupied habitat. Hohausova et al. (2010) also report that individuals (or their progeny) moved approximately 2,300 m over 3–4 months. Allowing that not all dispersal results in gene flow (Slatkin, 1985), and that census population sizes are typically larger than effective population sizes (Frankham, 1995), these observations of movements and densities suggest that the local effective sizes of mosquitofish are probably quite large. Given this, selection to local salinity might be enhanced by the very low rates of drift that might occur in marsh populations (Purcell et al., 2012), even in the face of genetic connections between populations exposed to different levels of salinity. The occurrence of local adaptation at a trait, in situations of large effective population sizes and high apparent gene flow, as measured by neutral molecular markers, has been observed in other coastal and marine vertebrates (Hansen et al., 2007; Nielsen et al., 2009). Such observations appear similar to the situation in mosquitofish, where our observations of the spatial patterns of microsatellite variation contrast with patterns of local salinity adaptations (Purcell et al., 2008). The potentially huge effective population sizes of mosquitofish also have implications for apparent gene flow across rivers, canals, and other barriers. When effective sizes are large, the actual number of migrants per generation can be very low and still maintain genetic similarities of the populations separated by a barrier to most movement.

Conclusions

Our results suggest considerable connectedness among study populations of mosquitofish, despite the rapid fragmentation going on in Gulf Coast marshes (Boesch et al., 1994; Day et al., 2000). This may not be surprising, given success of mosquitofish as an invasive species (Courtenay & Meffe, 1989; Purcell & Stockwell, 2015). Furthermore, combined with the Purcell et al. (2008, 2010, 2012), our study suggests that mosquitofish will adapt to changing conditions in coastal environments. Short generation times (males and females mature in approximately 22 and 31 days of age, respectively, Martin & Leberg, 2011) and large effective population sizes should assist them in adapting to changing conditions (Chevin et al., 2010; Hoffmann & Sgro, 2011). The ability of populations of mosquitofish to adapt to salinity changes and maintain connectedness in the face of fragmentation is important for stability of coastal marshes, given the numeric dominance of the species in wetland nekton (Hitch et al., 2011). However, it remains to be seen if other marsh organisms, which have lower population sizes or different life histories, will exhibit a similar potential to prosper under future conditions, particularly given the negative global prognosis for coastal habitat loss and sea level rise (Craft et al., 2009).

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