Journal of Heredity 2011:102(6):678–687
doi:10.1093/jhered/esr088
Advance Access publication September 1, 2011

Temporal Population Genetic Structure of Eastern Mosquitofish in a Dynamic Aquatic Landscape

Thomas C. McElroy, Karen L. Kandl, and Joel C. Trexler

From the Department of Biology and Physics, Kennesaw State University, Kennesaw, GA 30144 (McElroy); the Department of Biology, Western Carolina University, Cullowhee, NC (Kandl); and the Department of Biological Sciences, Florida International University, Miami, FL (Trexler).

Address correspondence to Thomas C. McElroy at the address above, or e-mail: tmcelro2@kennesaw.edu.

Data deposited at Dryad:doi:10.5061/dryad.72j1j

Abstract

We analyzed the effect of periodic drying in the Florida Everglades on spatiotemporal population genetic structure of eastern mosquitofish (*Gambusia holbrooki*). Severe periodic drying events force individuals from disparate sources to mix in dry season relatively deep-water refuges. In 1996 (a wet year) and 1999 (a dry year), we sampled mosquitofish at 20 dry-season refuges distributed in 3 water management regions and characterized genetic variation for 10 allozyme and 3 microsatellite loci. In 1996, most of the ecosystem did not dry, whereas in 1999, many of our sampling locations were isolated by expanses of dried marsh surface. In 1996, most spatial genetic variation was attributed to heterogeneity within regions. In 1999, spatial genetic variation within regions was not significant. In both years, a small but significant amount of variation (less than 1% of the total variation) was partitioned among regions. Variance was consistently greater than zero among long-hydroperiod sites within a region, but not among short-hydroperiod sites within a region, where hydroperiod was measured as time since last marsh surface dry-down forcing fishes into local refuges. In 1996, all sites were in Hardy–Weinberg equilibrium. In 1999, we observed fewer heterozygotes than expected for most loci and sites suggesting a Wahlund effect arising from fish leaving areas that dried and mixing in deep-water refuges.

Key words: *aquatic landscape, colonization, extirpation, Florida Everglades, genetic structure, temporal sampling*

The filtering effect of dispersal to refuges, survival in them, and movement back out leading to recolonization may shape population dynamics in temporally dynamic environments (Pulliam 1996; Chesson and Huntly 1997). Following extirpation, the spatial scale of sources of colonists can affect the spatial genetic structure of regional populations (averaged over all sampled sites) and the pairwise genetic structure among local sites in metapopulations (Wade and McCauley 1988; Harrison and Hastings 1996). Thus, nonequilibrium spatial genetic structure may provide a signal of environmental factors (e.g., loss or gain of habitat, dispersal barriers, or corridors) driving demographic patterns that underlie metapopulation dynamics (Castric et al. 2001; Charbonnel et al. 2002; Hansen et al. 2002). Reading these indirect signals of migration is potentially an important tool for ecologists, particularly in systems where directly tracking the origins and endpoints of dispersing organisms is difficult (Slatkin 1985). However, interpretation of population genetic structure often assumes that observed spatial patterns are, from a practical standpoint, stable over time (Tessier and Bernatchez 1999). Temporally unstable or nonequilibrium genetic structure may be typical of species inhabiting environmentally fluctuating landscapes. Analysis of temporal change in spatial population genetic structure can provide insight to the landscape features, ecological processes, and scale of migration that creates it (Hedrick and Gilpin 1997; Manel et al. 2003).

The Florida Everglades is a large wetland ecosystem extending from the southern shores of Lake Okeechobee to Florida Bay in Florida, USA. Historically, water flowed southward across a broad expanse of wet prairies interspersed by deeper sloughs. The central sloughs are bounded by wetlands that dry annually (short hydroperiod). In years with little rainfall, these wetlands experience seasonal drying events (typically November to May) that force aquatic organisms to concentrate in deep-water refuges, such as alligator ponds (Loftus and Kushlan 1987; Trexler et al. 2001). Seasonal drying causes the local

extirpation of populations, with recolonization coming from these deep-water refuges. Drying may force population mixing by long-range movement of fishes into refuge habitats. In particular, populations of fish that inhabit short-hydroperiod wetlands (those drying annually) persist in a state of numerical flux; fishes that inhabit long-hydroperiod areas, such as the central sloughs, experience similar drying events but at intervals extending from a few to many years (Trexler et al. 2001; Ruetz et al. 2005; Trexler et al. 2005). However, some fraction of the total population of Everglade's fishes is subjected to seasonal mixing and turnover each year.

Over the past century, more than half of the original Everglades have been lost to drainage and development (Davis et al. 1994). The ecosystem is now divided into regional management units covering hundreds of square kilometers (reviewed in Blake 1980; Light and Dineen 1994). The predominant form of the deep-water refuges has changed from alligator ponds and solution holes to canals, and levees may have reduced the extent of movement across the ecosystem. Previous work with spotted sunfish (*Lepomis punctatus*) detected significant genetic structure among marsh, but not canal, sites (McElroy et al. 2003). These findings supported the hypothesis that the annual cycle of marsh drying events and local population dynamics has a marked effect on population genetic structure of spotted sunfish. There was no evidence that water management structures (levees and canals) superimpose a second level of genetic structure on that species, possibly because canals facilitate gene flow. A continent–island (canal–marsh) population structure best described spotted sunfish genetics, with high gene flow between regions and recurrent mixing in marshes from canal and creek habitats (McElroy et al. 2003). However, the analyses by McElroy et al. (2003) did not address the temporal stability of genetic structure.

We surveyed variability at allozyme and microsatellite loci at 2 sampling times (1996 and 1999) for eastern mosquitofish (*Gambusia holbrooki*), a rapid colonizer in the Everglades marshes (Trexler et al. 2001). After a number of relatively wet years, the Everglades experienced a local drought in 1999 that restricted fish populations to solution holes, alligator ponds, long-hydroperiod marshes, and canals. We resampled sites visited in 1996 to assess the effect of the 1999 drought on population genetic structure.

We applied McCauley et al.'s (1995) hypothesis-testing approach to our data analysis and examined how landscape and environmental features influenced spatiotemporal population genetic structure in eastern mosquitofish. We expected that 1) periodic cycles of local extirpation and recolonization driven by seasonal water level fluctuations would structure mosquitofish population genetic variation. As a result, variation among sites within water management units would swamp regional-scale (water management units) variation. 2) Local deeper water refuges are filled by fish from nearby sites that do not spread far between dry-down disturbances, which would lead to greater genetic disequilibrium among regularly disturbed (short hydroperiod, regular extirpations) sites relative to stable ones (long hydroperiod, no recent extirpations). Thus, 3) local patterns in short-hydroperiod areas would not be stable through time because of recurrent disturbance events.

Materials and Methods

Field Collections

We made 2 field collections (March 1996 and March 1999) of eastern mosquitofish from 20 sites throughout Water Conservation Area 3A (WCA 3A), Shark River Slough (SRS), and Taylor Slough (TS) to document their population genetic structure (Figure 1). The Everglades is a spatially structured habitat with sloughs separated by ridges covered by dense sawgrass and that extend parallel to the prevailing direction of flow (Figure 1, insert) and that impede movement of fishes (Trexler et al. 2001). Six or more generations of mosquitofish passed between our sampling events and local densities in marshes dropped to zero for one or more months at sites that dried during the study (Figure 2, see Trexler et al. 2001; Ruetz et al. 2005). Depending on availability of fish, up to 50 adult eastern mosquitofish were collected with dip nets from each location. The sites were distributed among 3 water management areas to permit comparisons within and among areas separated by water-control structures and canals. The second collection was made during a severe drying event at the end of the 1999 dry season. At that time, the marsh surface at several sites was dry, including all in TS, forcing us to sample fish in refuge habitats nearby the 1996 collection sites. Between 15 and 25 individuals from each site were surveyed for genetic variation at both marker types for each site and sampling year. We used data from automated water depth gages to estimate the number of days that had passed before each collection site had last dried (days since dry; see Ruetz et al. 2005).

Allozyme Analysis

We used starch gel electrophoresis to document patterns of allozyme variation in the study species. On collection, specimens were transferred to the laboratory and stored in a −80 °C freezer. Whole-tissue extracts were prepared for electrophoresis by homogenization of tissues in approximately 500 µl of grinding buffer (0.025 M tris, pH 7.0, 0.025 M sucrose, 0.005 M β-mercaptoethanol). Eye, liver, and soma clips were pooled; intestines were removed from all but a few small specimens. This approach was based on preliminary analyses that showed no tissue-specific expression of the proteins examined.

We screened 32 putative loci before selecting 10 polymorphic loci to score on all individuals (Table 1). We followed standard techniques described in Selander et al. (1971) and Murphy et al. (1996), with 11% (w/v) starch gels. Tissue extracts from the mosquitofish were run on 3 different buffer systems: Tris–citrate pH 8.0 buffer (TC8; Selander et al. 1971), Lithium-Borate/Tris–Citrate (LIOH; Selander et al. 1971), and Tris–Citrate–EDTA (JRP; Ayala et al. 1972).

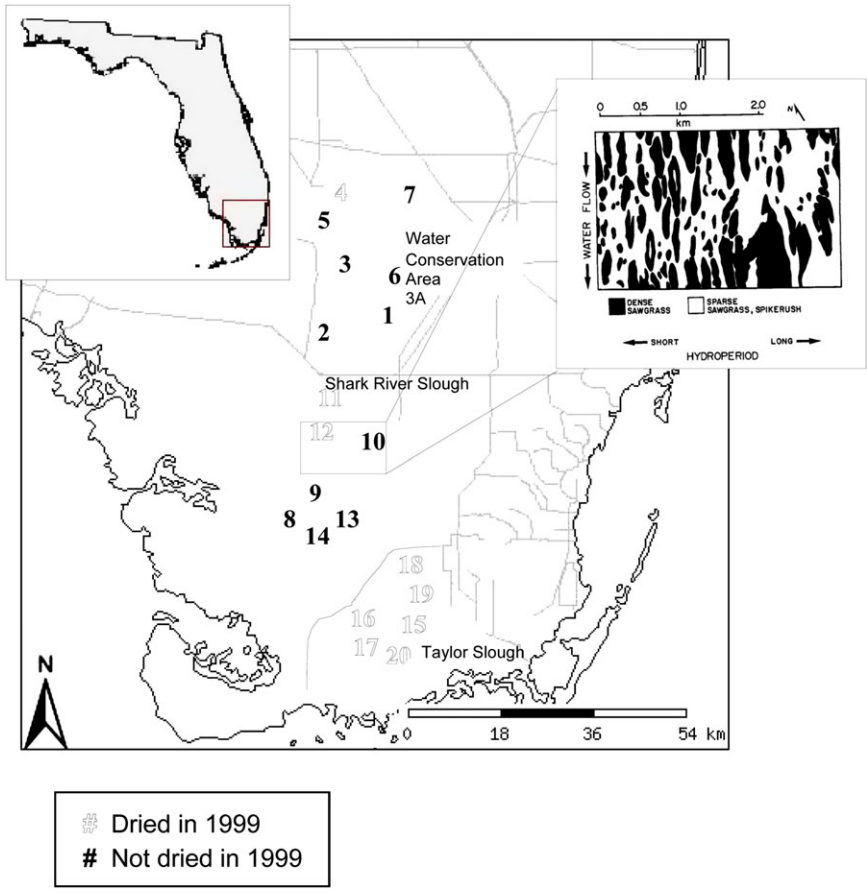

Figure 1. Map illustrating sampling sites. Site symbols indicate site number and which sites that dried between 1996 and 1999 sampling events (open numbers). The box inserted over SRS identifies where the habitat map insert was produced, illustrating the habitat mosaic of the Everglades. Black areas of the habitat map are dense sawgrass–covered ridges, and white areas are deeper sloughs. Fish must navigate around ridges to move from short- to long-hydroperiod sites (modified and reprinted from Trexler et al. 2001).

Microsatellite Analysis

Whole genomic DNA was isolated from muscle tissue by standard phenol–chloroform DNA extraction methods (Hoelzel and Green 1992). Three microsatellite loci (Table 1) were amplified using a multiplexed polymerase chain reaction. Amplifications were performed in 15 μl volumes, each one containing 10× buffer, 25 μM $MgCl_2$, 250 μM dNTP's, 5 U/μl Taq DNA polymerase (Promega), and three 5 μM primer sets, one of which was end labeled with a fluorescent dye (6-FAM, NED, or HEX; Applied Biosystems). The thermal cycling parameters were as follows: an initial 1 min denaturation at 94 °C, followed by 45 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C, and a final 10 min extension at 72 °C. The amplified samples were electrophoresed in 2.5% agarose gel to determine the presence or absence of a product. Following that, the amplified products were electrophoresed in 5% denaturing polyacrylamide gels on an ABI 377 automated DNA sequencer. The alleles were sized with respect to electrophoretic mobility compared with a ROX 350 standard, and the genotypes were assigned using the GENESCAN (ABI) and GENOTYPER software packages.

Statistical Analyses

Microsatellite and allozyme marker loci were combined for the analyses. We tested for deviations from Hardy–Weinberg expectations at each locus and in each population with analysis of molecular variance (AMOVA) (GENODIVE: Meirmans 2006). We tested for patterns of the disequilibrium coefficient Phi_{IS} as a function of hydrology using a nonparametric test based on ranks (Kruskal–Wallis one-way ANOVA). We tested the microsatellite loci for evidence of potential inbreeding and bottlenecks with BOTTLENECK (Cornuet and Luikart 1997). We also used MICROCHECKER (Van Oosterhout et al. 2004) to test for null alleles in the microsatellite data.

Following Hedrick (2005b), we used GENODIVE (Meirmans 2006) to partition the total observed genetic variance within each of our 2 sampling years. We used 2 different categorical models within each sample year to

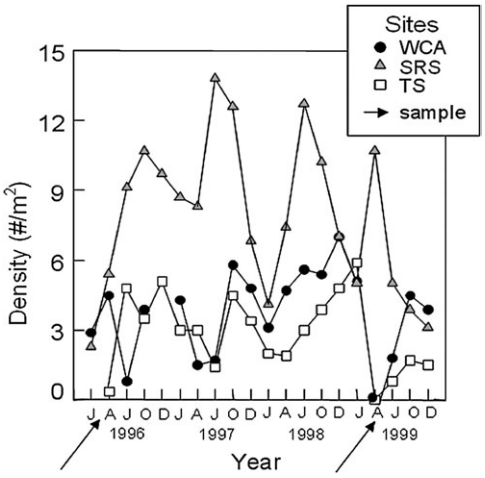

Figure 2. Illustration of mosquitofish population dynamics from 3 of the 20 study sites, one from each of the 3 regions. Density (#/m^2) is plotted by sampling event, and the times of genetic sampling for this study are indicated on the x axis by arrows. Note that sites in TS and WCA 3A regions dried and mosquitofish populations were locally extirpated. Hydrographs for these sites are in Chick et al. (2004, figure 2), and sampling methods are described in Wolski et al. (2004).

evaluate spatial genetic structure, water management units, and hydrological disturbance. In order to test the effects of water management units on population structuring among sites, we used partitions that were attributable to variation among individuals within subpopulations (individuals collected in areas < 1 km^2) relative to total diversity within their subpopulation (Phi_{IS}), among subpopulations within water management units relative to the total diversity in that unit (Phi_{CP}), among water management units (populations) relative to the total genetic diversity with each unit (Phi_{CT}). Statistical significance of these partitions was tested and interpreted in an AMOVA framework.

In order to investigate the effects of hydrologic disturbance on population structuring, sites were divided into 2 categories based on the hydrology within the time of this study (1996–1999). We grouped sites into those that had dried during the 1999 dry-down event (surface water depth < 5 cm) and those that remained inundated with water throughout the time frame of this study. We analyzed these groupings using AMOVA with Phi distance estimates calculated in GENODIVE (Meirmans 2006). Further, results were considered in the context of regional drying intensity. Water management units (Figure 1) differed with respect to regional drying intensity, where WCA 3A had the least amount of regional drying (1 of 7 sites dried), SRS was intermediate (2 of 7 sites dried), and TS (6 of 6 sites dried) had the greatest extent of regional drying.

We used GENODIVE to test for significant population structuring with time as an additional hierarchical factor within an AMOVA framework for the water management grouping scenario described above. This analysis assessed temporal stability of regional population structure that may have resulted from regional water management divisions. Statistical significance of these partitions was tested and interpreted in an AMOVA framework. We also analyzed the correlation of estimated pairwise Phi_{ST} for the 1996 and 1999 data in order to examine the temporal consistency and the specific (pairwise) temporal consistency of Phi_{ST} estimates.

We calculated the overall correlation of pairwise Phi_{ST} and distance separating sampling sites in order to assess the presence or absence of isolation by distance (IBD) for both sampling times (Slatkin 1977, 1993; Hutchison and Templeton 1999). Significance of matrix correlations was assessed by Mantel test (Mantel 1967). We also used GENODIVE to analyze IBD at different spatial scales to assess the geographic

Table 1 Allozyme and microsatellite loci surveyed

		Phi_{IS}		Alleles	
Locus	E.C. No.	1996	1999	1996	1999
Allozymes					
Adenosine deaminase (*ada*)	3.5.4.4	−0.005	0.254*	5	3
Aspartate aminotransferase (*aat*-1)	2.6.1.1	0.126	0.369*	3	2
Gluc-6-phos dehydrogenase (*gpi*-1)	5.3.1.9	0.054	0.014	2	2
Gluc-6-phos dehydrogenase (*gpi*-2)	5.3.1.9	0.055	−0.029	3	2
Isocitrate dehydrogenase (*idh*-2)	1.1.1.42	−0.146	0.237*	2	2
Lactate dehydrogenase (*ldh*-2)	1.1.1.27	−0.026	0.509*	2	2
Malate dehydrogenase (*mdh*-2)	1.1.1.37	0.106*	0.100*	2	2
Man-6-phosphate isomerase (*mpi*-1)	1.1.1.40	−0.054	0.132*	3	3
Phosphoglucomutase (*pgm*-1)	5.4.2.2	−0.026	0.004	3	3
Phosphogluconate dehydro (*pgd*-1)	1.1.1.14	−0.434	0.348*	3	3
Microsatellites					
gaf 2		0.056*	0.114*	27	25
gaf 3		0.050*	0.218*	36	33
gaf 7		−0.020	0.095*	22	20

The fixation index Phi_{IS} is indicated for each sampled year. The asterisk indicates a significant deviation from Hardy–Weinberg Expectations for that locus. A positive number indicates fewer heterozygotes observed than expected. The number of alleles detected for each sampling year is listed.

level of population structuring at the 2 sample times (1996 and 1999). Populations were grouped into geographic distance categories. Significance of matrix correlations between pairwise Phi_{ST} and distance separating the sites was assessed within each category by Mantel test (1000 permutations) (Mantel 1967; Sokal et al. 1986; Meirmans 2006).

Results

Hardy–Weinberg Equilibrium

The tests for conformity to Hardy–Weinberg equilibrium across allozyme and microsatellite loci (sampled sites were pooled) revealed that in 1996, most of the loci conformed to Hardy–Weinberg expectations (10 of 13); however, only 3 of the loci conformed to Hardy–Weinberg expectations in 1999 (Table 1). Deviations from Hardy–Weinberg equilibrium were consistently the result of fewer heterozygotes than expected in the pooled samples. The number of alleles detected in 1999 was slightly less than the number detected in 1996 (Table 1). There was no evidence for significant inbreeding or bottlenecking at microsatellite loci detected from BOTTLENECK (Cornuet and Luikart 1997) for both sampling times. Analyses run in MICROCHECKER (Van Oosterhout et al. 2004) indicated significant homozygote excess at the microsatellite loci for 2 of the 3 loci in 1996 (*gaf* 2 and *gaf* 3) and all 3 loci in 1999. There was no evidence of scoring errors or large allele dropout for the microsatellite loci at either sample time.

The tests for site-specific conformity to Hardy–Weinberg equilibrium across marker loci revealed that in 1996, 14 of 20 sites conformed to Hardy–Weinberg expectation; however, only 3 of the 20 sites conformed to Hardy–Weinberg expectations in 1999 (Table 2). Deviations from Hardy–Weinberg equilibrium detected by locus or collection site were consistently the result of fewer heterozygotes than expected. There was no relationship between region or hydrology and deviation from Hardy–Weinberg expectations. Overall heterozygote deficiency (a positive value of the fixation index, Phi_{IS}) increased between years (Tables 1 and 2, $P < 0.001$); however, the magnitude of change was not a function of specific drying history for each site. The increase in Phi_{IS} between years was greater in SRS than the other regions (Figure 3). There was no difference among regions in Phi_{IS} in 1996.

Spatial Genetic Structure

The spatial analysis revealed that in 1996, a significant amount of the total spatial genetic variation was explained at the among-sites within water management units scale (Phi_{SC} = 0.012; P = 0.001; mean distance between sites within regions = 13.5 km). This was not true for the 1999 samples (Phi_{SC} = 0.003; P = 0.054; Table 3). A smaller amount of the total genetic variation was attributed to differences among regions for both 1996 and 1999 samples (mean distance between sites among regions = 47.5 km; Table 3). Generally, 1% or less of the total genetic variation was attributable to differences among regions (Table 3).

Table 2 Sample sites surveyed (see Figure 1)

Site	Region	Dried in 1999	Phi_{IS} 1996	Phi_{IS} 1999
1	WCA 3A	No	0.018	0.178*
2	WCA 3A	No	0.067	0.106*
3	WCA3A	No	0.112*	0.121*
4	WCA 3A	Yes	0.058	0.182*
5	WCA 3A	No	0.068*	0.212*
6	WCA 3A	No	−0.006	0.122*
7	WCA 3A	No	−0.024	0.013
8	SRS	No	0.039	0.226*
9	SRS	No	0.06	0.086*
10	SRS	No	−0.052	0.196*
11	SRS	Yes	0.035	0.025
12	SRS	Yes	0	0.172*
13	SRS	No	0.144*	0.22*
14	SRS	No	0.119*	0.062*
15	TS	Yes	0.135*	0.33*
16	TS	Yes	0.08*	0.117*
17	TS	Yes	0.027	0.095*
18	TS	Yes	0.056	0.078*
19	TS	Yes	0.061	0.029
20	TS	Yes	0.032	0.098*

The fixation index Phi_{IS} is indicated for each sampled year. The asterisk indicates a significant deviation from Hardy–Weinberg Expectations. A positive number indicates fewer heterozygotes observed than expected.

Hydrologic Disturbance Analysis

When hydrology (short vs. long hydroperiod) was included as a hierarchical factor, it explained a small but significant amount of variance in 1996 and 1999 (Table 3). TS had and consistently has the greatest relative magnitude of drying

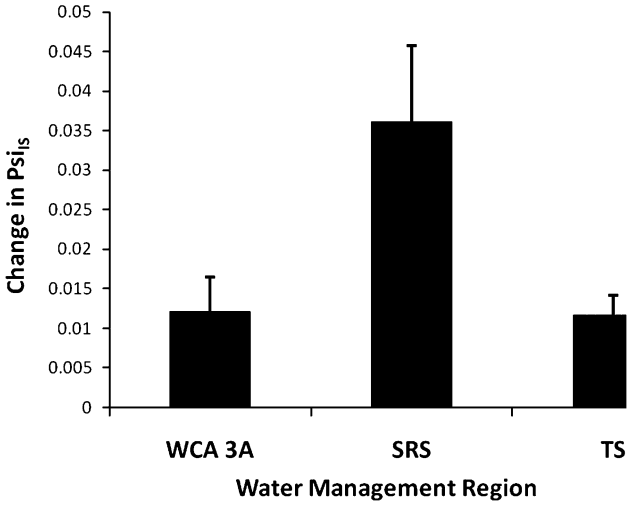

Figure 3. The average change plus standard error in the Fixation Index (Phi_{IS}) for each water management region (Water Conservation Area 3A [WCA 3A], Shark River Slough [SRS], and Taylor Slough [TS]). The change in Phi_{IS} for SRS was significantly greater that the other 2 regions ($P < 0.02$).

Table 3 AMOVA and degrees of freedom (df) across allozyme and microsatellite markers using an infinite allele model

Source of variation	df	% Variation	Phi-value	SE	*P* value
(A) 1996 WMU					
Within individuals	322	93.4	Phi_{IT} = 0.066	0.017	—
Among individuals within sites	302	5.3	Phi_{IS} = 0.054	0.016	0.001
Among sites within WMU	17	1.2	Phi_{SC} =0.012	0.007	0.001
Among WMU	2	0.2	Phi_{CT} = 0.002	0.002	0.005
(B) 1999 WMU					
Within individuals	441	86.1	Phi_{IT} = 0.139	0.034	—
Among individuals within sites	422	13.4	Phi_{IS} = 0.135	0.035	0.001
Among sites within WMU	17	0.3	Phi_{SC} = 0.003	0.002	0.054
Among WMU	2	0.1	Phi_{CT} = 0.001	0.002	0.002
(C) 1996 Hydro					
Within individuals	322	93.4	Phi_{IT} = 0.066	0.017	—
Among individuals within sites	302	5.3	Phi_{IS} = 0.054	0.016	0.001
Among sites within hydro	17	1.3	Phi_{SC} = 0.013	0.007	0.001
Among hydro	2	0.1	Phi_{CT} = 0.001	0.002	0.041
(D) 1999 Hydro					
Within individuals	441	86.2	Phi_{IT} = 0.138	0.034	—
Among individuals within sites	422	13.4	Phi_{IS} = 0.135	0.035	0.001
Among sites within hydro	17	0.4	Phi_{SC} =0.004	0.002	0.017
Among hydro	2	0.1	Phi_{CT} = 0.001	0.001	0.044

Standard errors (SE) were obtained by jackknifing over loci, and significance was tested using 999 permutations. (A) Results from 1996 AMOVA with water management units (WMU) as hierarchical factor; (B) results from 1999 AMOVA with WMU as hierarchical factor; (C) results from 1996 AMOVA with hydrology (drying history as of 1999) as hierarchical factor; and (D) results from 1999 AMOVA with hydrology (drying history as of 1999) as hierarchical factor.

(6 of 6 sites). SRS had relatively moderate drying (2 of 7 sites), and WCA 3A had the least amount of drying (1 of 7 sites). There was no significant population structure detected among the collection sites located in TS for neither 1996 nor 1999. Significant population structure was detected among the collection sites in SRS in 1996 but not in 1999. Weak but significant population structure was detected among the collection sites in WCA 3A for both collection times (data reported below).

Temporal Genetic Structure

In a separate analysis with time included as a hierarchical factor, between year variation explained a significant amount of the total variance (Phi_{CT} = 0.066; % var = 6.6%; P = 0.001). The magnitude of temporal sample variation was high compared with the magnitude of among site and among water management unit sample variation within years. Correlations of pairwise Phi_{ST} estimates for all sites and between sampling years indicated no significant correlation between sampling years. There was a significant correlation for population genetic structure (pairwise Phi_{ST}) between years within WCA 3A (least amount of regional drying), but not for the other water management regions, although the significant relationship was driven by a single point (Figure 4).

Analysis of population structure within water management regions for each sampling time revealed significant population genetic structure in WCA 3A (least drying, 1 of 7 sites) for both sampling periods (1996: Phi_{ST} = 0.010, % var = 0.7, P = 0.034; 1999: Phi_{ST} = 0.011, % var = 0.8, P = 0.014). Significant population genetic structure was detected in SRS for the 1996 samples, but not the 1999 samples (moderate drying, 2 of 7 sites) (1996: Phi_{ST} = 0.035, % var = 2.3, P = 0.001; 1999: Phi_{ST} = 0.003, % var = 0.2, P = 0.21), and no significant population genetic structure was detected within TS for either sampling time (most extreme drying, 6 of 6 sites) (1996: Phi_{ST} = 0.008, % var = 0.5, P = 0.156; 1999: Phi_{ST} = −0.003, % var = 0.0, P = 0.771). SRS (moderate drying) had the greatest genetic equilibrium disturbance (change in Phi_{IS} within sites between sampling times compared with the other regions; Figure 3).

IBD and Spatial Autocorrelation

We observed no evidence for general IBD for the 1996 and 1999 data. However, IBD analysis of pairwise Phi_{ST} within distance categories for 1996 indicated significant correlation at small spatial scales between 1 and 25 km (Table 4). In 1999, IBD spatial autocorrelation of pairwise Phi_{ST} within distance categories indicated significant correlations at greater spatial scales ~100 and ~200 km (Table 4).

Discussion

There is increasing appreciation of the importance on nonequilibrium dynamics of genetic variation in natural populations. For example, the Hedgecock effect describes ephemeral population structure formed by kin-structured recruitment of marine animals with planktonic larvae (Hedgecock 1994). Though controversial, temporally chaotic and spatially unstructured population genetics is

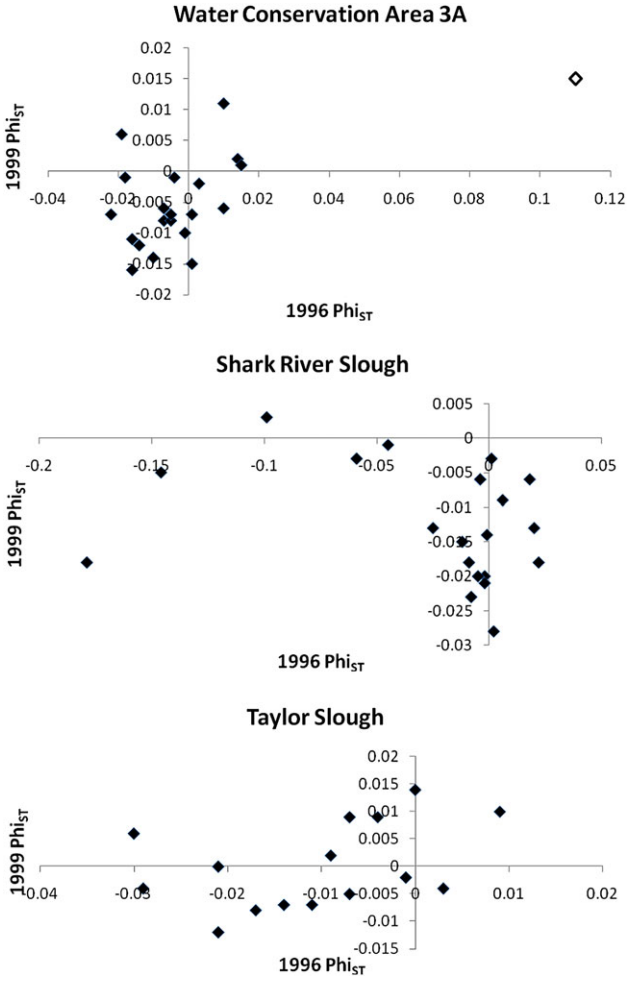

Figure 4. Correlations of pairwise Phi_{ST} between years for each water management region sampled. WCA 3A, $r = 0.625$, $N = 21$; SRS, $r = -0.270$, $N = 21$; and TS, $r = 0.404$, $N = 15$. Correlation value (r^*) was also calculated for WCA 3A without including the extreme data point (empty diamond) ($r^* = 0.361$).

indicative of such recruitment patterns (Hedgecock 1994; Hedrick 2005a), which have important consequences for management of marine fishes (Larson and Julian 1999).

Our data indicate temporally dynamic and spatially weak population structure driven by local hydrology. The analyses suggest that there has been a reorganization of spatial population structure between 1996 and 1999. It is important to note the shift from a population genetic structure that was consistent with Hardy–Weinberg expectations in 1996 to a general lack of equilibrium within populations in 1999. In 1999, there was consistently less heterozygosity observed than expected. These data are consistent with a Wahlund effect; however, the deficiencies of heterozygosity were not more pronounced for sites that had more severe drying. Although we expected greater genetic disequilibrium at disturbed sites than at undisturbed ones, this was not necessarily so. The Everglades is a large

Table 4 Spatial autocorrelations among sites within distance classes for each sampling time

Category	Distance (km)	Number of sites	1996, Phi	1996, *P* value	1999, Phi	1999, *P* value
1	1–25	10	0.096	0.037*	−0.030	0.350
2	26–50	20	−0.117	0.114	0.118	0.075
3	51–75	21	−0.065	0.182	−0.109	0.069
4	76–100	33	−0.025	0.360	0.172	0.010*
5	101–125	45	−0.048	0.242	−0.012	0.423
6	126–150	30	0.105	0.124	−0.169	0.083
7	151–175	21	0.036	0.324	−0.139	0.139
8	176–200	10	0.068	0.185	0.225	0.002*

Phi indicates the average pairwise Phi_{ST} for each distance category. The analysis used a Mantel test to assess statistical significance (1000 permutations for each distance category). Asterisk indicates statistical significance.

continuous ecosystem within modern water management units, similar to a braided stream. Drying events affect the entire region and may have "pushed" fishes from the disturbed regions far into the center of the system, creating region-wide mixing. In fact, we have tracked larger fish species with radio transmitters (Florida Gar *Lepisosteus platyrhincus*, Largemouth bass *Micropterus salmoides*, and Mayan cichlids *Cichlasoma uropthalmus*), and they display such patterns (Trexler JC, Parkos JJ, unpublished data). This study is novel in suggesting that even small fish such as eastern mosquitofish may be mixing over areas greater than 1 km.

Gambusia holbrooki is an early colonizing and persistent inhabitant of the Everglades aquatic ecosystem (Ruetz et al. 2005). A drying event in the Everglades aquatic system reduces the total available habitat space for aquatic organisms. As habitat area shrinks, aquatic organisms move into deep-water refugia (e.g., alligator holes or canals). TS has typically been an area of frequent drying disturbance. There was no significant population structure detected for either sampling period within TS. Lack of fine-scale population genetic structure has been associated with disturbance in a South Wales rare shrub, *Grevillea macleayana* (England et al. 2003) and Guanacaste Tree, *Enterolobium cyclocarpum* (Fabaceae) (Gonzales et al. 2010); whereas, spatial genetic structure was detected for both species in areas that were relatively undisturbed (England et al. 2003; Gonzales et al. 2010). The opposite pattern of disturbance effects on genetic structure was detected in a late-successional moss in subtropical cloud forests (Patiño et al. 2010). WCA 3A has typically been an area of relatively less annual dry-down disturbance. Before this study (1996), the area had not had a substantial dry-down event for more than 5 years. The data suggest that local structure is more temporally stable between these sites that were relatively less hydrologically disturbed. Significant population structure was detected for both years of the study among the sites located within WCA 3A. The magnitude of the detected structure and the amount of variation explained were consistent for both sample years because allele frequencies were probably not very different among the source sites.

SRS has typically been an area of moderate dry-down disturbance. Before this study, the area had not had a substantial dry-down event for more the 3 years. Significant population structure was detected among 1996 but not among 1999 samples within this region. Furthermore, disturbance of genetic equilibrium detected as a general deficiency of observed heterozygosity was most pronounced SRS. The Wahlund effect should be detected in any mixing of samples in which the combined subpopulations had different allele frequencies. The magnitude of the departure from expectations is directly related to the magnitude of the variance in allele frequencies among sites and relative contribution to the admixture. In 1996, 2.3% of the variation was attributed among populations in SRS. This was associated with significant population structure and was the greatest amount for any of the regions or sample times. This dropped to 0.2% of the variation attributed among populations in 1999 with a concurrent deficiency of heterozygosity. This is consistent with a relatively even admixture of individuals from the sampled sites within this region.

Population genetic structure in *G. holbrooki* was dynamic in our study and driven by local hydrology. There was a reorganization of spatial population structure between 1996 and 1999. Fine-scale population genetic structure was detected in 1996, but not 1999. Further, only regional-level population structure was detected from the 1999 samples. The spatial restructuring is supported by the spatial autocorrelation analysis. That analysis indicated a significant correlation among sites in the 25 km distance class in 1996. In 1999, after a pronounced dry-down event, significant correlations were detected in the 100 and 200 km distance classes.

Following Harrison (1991), this pattern of population genetic structure may best be characterized as a "patchy population." Alternatively, a source–sink population structure (Pulliam 1988; Freckleton and Watkinson 2002) may apply, though we have not demonstrated that populations in short-hydroperiod regions fail to replace themselves by recolonization from local refuge sites as would be required by this model. This short-lived population structure has little impact on the response to local selection (Harrison and Hastings 1996) and the total population will probably evolve as a panmictic unit (though possibly with reduced effective size, reviewed in Whitlock 2004). Nevertheless, this dynamic population genetic structure reflects the pattern and scale of local movement and colonization of fish in response to water level fluctuation. These dynamics are linked to ecosystem function in the Everglades, such as the availability of small fish for consumption by wading birds (Gawlik 2002), and have important implications for ecosystem management. No field technique has permitted these patterns of movement to be studied directly because of the small size of the fish and large size and complexity of the habitat.

The scale of hydrological disturbance to spatial genetic variation revealed here is consistent with demographic analysis of synchronization of population dynamics of small fishes at these same study sites. Ruetz et al. (2005) found that hydrological synchrony among pairs of study sites was more strongly correlated with synchrony of population dynamics of several species than was distance separating the sites. Eastern mosquitofish stood out in that study by showing no significant synchronization by hydrology or distance; however, Ruetz et al. (2005) attributed this to the rapid colonization of mosquitofish to areas recently dried compared with the temporal spacing of their samples (see also Trexler et al. 2001). Similar to our interpretation, environmental drivers have been implicated in shaping genetic variation in a variety of other species (reviewed in Manel et al. 2003).

Population genetics of eastern mosquitofish have been studied in detail in other ecosystems, and they often display marked structure over relatively small spatial scales. For example, several studies have noted significant heterogeneity over distances of 6 or fewer kilometers (Smith et al. 1983; Kennedy et al. 1985, 1986; McClenaghan et al. 1985), and similar to our study, most spatial genetic variation is partitioned at the local (within site and among sites within regions) scale. A number of demographic explanations have been put forward for these patterns, including sex and age-specific demographic processes (Smith et al. 1989). Similar to our work, other studies employing temporal sampling have noted dynamic patterns of genetic variation for mosquitofish linked to environmental variation and eroded by intermittent gene flow (Smith et al. 1989). In contrast to our study, Scribner et al. (1992) noted increased heterozygosity in mosquitofish populations inhabiting fluctuating reservoirs in Hawaii compared with populations from more stable reservoirs. The Hawaiian populations are relatively closed and the environments within the reservoirs are homogeneous compared with the large and environmentally complex Everglades.

A key result of this study is that temporal sampling of spatial genetic structure provided a more compelling characterization of the underlying drivers of genetic variation in this system than could be revealed in a single sampling event. The relative magnitude of temporal sample variation was high compared with the magnitude of among-site spatial variation within years. It is unlikely that such a pattern would result as a statistical artifact caused by small sample sizes because the general pattern was consistent with regional hydrology (Kinnison et al. 2002). Small sample-size artifacts would only affect both spatial and temporal variation in this way if allele frequencies were changing at sites through time more than they differ among sites at the same time. We sampled sites scattered over a large spatial gradient (ca. 150 km by 25 km), leading us to conclude that temporal variation exceeding spatial variation was most unexpected.

By applying McCauley et al.'s (1995) hypothesis-testing approach to our data analysis, we found evidence for ecological drivers in a seemingly chaotic population (see also Ostergaard et al. 2003). In his 1985 paper, Slatkin noted that F_{ST} and the frequency of private alleles can be useful in estimating gene flow because conditional allele frequencies

reflect ongoing gene flow patterns after approximately $1/m$ generations, much less than $1/\mu$, which is the time required to reach overall genetic equilibrium (where m is the migration rate and μ is the mutation rate). Although this has given succor to many researchers employing indirect techniques to estimate gene flow (e.g., Trexler 1988), ecological realities of modern habitats render this solace questionable, if not unfounded for long-lived organisms in many places. As humans have altered and continue to alter ecosystems, it seems likely that few habitats in much of the developed and developing world have remained stable in ways relevant to regional-scale patterns of gene flow over the past 50 or more years ($=1/m$ for $m = 0.02$ and an annual species). Greater ecological realism is needed in analysis of gene flow, starting with discarding the pretense of population genetic structure equilibrium in the absence of supporting evidence (Bossart and Powell 1998; Whitlock and McCauley 1999; Charbonnel et al. 2002). On the other hand, tools such as microsatellites, and even allozymes, can be powerful to unearth spatial population structure with important applications when appropriate sampling designs are applied in a hypothesis-testing framework.

Funding

Cooperative agreement from the U. S. Geological Survey (USGS Cooperative No. 1445-CA09-95-0112, Sub-agreement No. 1). The Florida Coastal Everglades LTER project (NSF 99-10514) also provided financial support during the analysis and writing phase of the project.

Acknowledgments

Bill Loftus made the study possible and provided invaluable advice on all aspects of the work. We thank Joe Travis and Charlie Baer for helpful suggestions on analyzing these data.

References

Ayala JW, Powell JR, Tracey ML, Mourao CA, Perez-Salas S. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics. 70:113–139.

Blake NM. 1980. Land into water—water into land. A history of water management in Florida. Tallahassee (FL): University Presses of Florida.

Bossart JL, Powell DP. 1998. Is population genetics mired in the past? Reply from J.L. Bossart and D. Pashley Prowell. Trends Ecol Evol. 13:360.

Castric V, Bonney F, Bernatchez L. 2001. Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. Evolution. 55:1016–1028.

Charbonnel N, Quesnoit M, Razatavonjizay R, Bremond P, Janrne P. 2002. A spatial and temporal approach to microevolutionary forces affecting population biology in the freshwater snail *Biomphalaria pfeifferi*. Am Nat. 160:741–755.

Chesson P, Huntly N. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. Am Nat. 150:519–553.

Chick JH, Ruetz CR 3rd, Trexler JC. 2004. Spatial scale and abundance patterns of large fish communities in freshwater marshes of the Florida Everglades. Wetlands. 24:652–664.

Cornuet JM, Luikart G. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics. 144:2001–2014.

Davis SM, Gunderson LH, Park WA, Richardson JR, Mattson JE. 1994. Landscape dimension, composition, and function in a changing Everglades ecosystem. In: Davis SM, Ogden JC, editors. Everglades, the ecosystem and its restoration. Delray Beach (FL): St. Lucie Press. p. 419–444.

England PR, Whelan RJ, Ayre DJ. 2003. Effect of seed bank disturbance on the fine-scale genetic structure of populations of the rare shrub *Grevillea macleayana*. Heredity. 91:475–480.

Freckleton RP, Watkinson AR. 2002. Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. J Ecol. 90:419–434.

Gawlik DE. 2002. The effects of prey availability on the numerical response of wading birds. Ecol Monogr. 72:329–346.

Gonzales E, Hamrick JL, Smouse PE, Trapnell DW, Peakall R. 2010. The impact of landscape disturbance on spatial genetic structure in the Guanacaste tree, *Enterolobium cyclocarpum* (Fabaceae). J Hered. 101:133–143.

Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg K-LD. 2002. Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. Mol Ecol. 11:2523–2535.

Harrison S. 1991. Local extinction in a metapopulation context: an empirical evaluation. Biol J Linn Soc. 42:73–88.

Harrison S, Hastings A. 1996. Genetic and evolutionary consequences of metapopulation structure. Trends Ecol Evol. 11:180–183.

Hedgecock D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont AR, editor. Genetics and evolution of aquatic organisms. London: Chapman & Hall. p. 122–134.

Hedrick PW. 2005a. Large variance in reproductive success and the N-e/N ratio. Evolution. 59:1596–1599.

Hedrick PW. 2005b. A standardized genetic differentiation measure. Evolution. 59:1633–1638.

Hedrick PW, Gilpin ME. 1997. Genetic effective size of a metapopulation. In: Hanski IA, Gilpin ME, editors. Metapopulation biology. San Diego (CA): Academic Press. p. 166–182.

Hoelzel AR, Green A. 1992. Analysis of population-level variation by sequencing PCR-amplified DNA. In: Hoelzel AR, editor. Molecular genetic analysis of populations: a practical approach. Oxford: IRL Press. p. 159–186.

Hutchison DW, Templeton AR. 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.

Kennedy PK, Kennedy ML, Smith MH. 1985. Microgeographic genetic organization of populations of largemouth bass and two other species in a reservoir. Copeia. 1985:937–945.

Kennedy PK, Kennedy ML, Zimmerman EG, Chesser RK, Smith MH. 1986. Biochemical genetics of mosquitofish. V. Perturbation effects on genetic organization of populations. Copeia. 1986:937–945.

Kinnison MT, Bentzen P, Unwin MJ, Quinn TP. 2002. Restructuring recent divergence: evaluating nonequilibrium population structure in New Zealand chinook salmon. Mol Ecol. 11:739–754.

Larson RJ, Julian RM. 1999. Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. Calif Coop Ocean Fish Investig Rep. 40:94–99.

Light SS, Dineen JW. 1994. Water control in the Everglades: a historical perspective. In: Davis SM, Ogden JC, editors. Everglades, the ecosystem and its restoration. Boca Raton (FL): St. Lucie Press. p. 47–84.

Loftus WF, Kushlan JA. 1987. Freshwater fishes of southern Florida. Bull Fla State Mus Biol Sci. 31:147–344.

Manel S, Schwartz MK, Luikart G, Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol. 18:189–197.

Mantel N. 1967. The detection of disease clustering and generalized regression approach. Cancer Res. 27:209–220.

McCauley DE, Raveill J, Antonovics J. 1995. Local founding events as determinants of genetic structure in a plant metapopulation. Heredity. 75:630–636.

McClenaghan LR Jr, Smith MH, Smith MW. 1985. Biochemical genetics of mosquitofish. IV. Changes in allele frequencies through time and space. Evolution. 39:477–482.

McElroy TC, Kandl KL, Garcia J, Trexler JC. 2003. Extinction-colonization dynamics structure genetic variation of spotted sunfish (*Lepomis punctatus*) in the Florida Everglades. Mol Ecol. 12:355–368.

Meirmans PG. 2006. Using the AMOVA framework to estimate a standard differentiation measure. Evolution. 60:2399–2402.

Murphy RW, Sites JW Jr, Buth DG, Haufler CH. 1996. Proteins: isozyme electrophoresis. In: Hillis DM, Moritz C, Mable BK, editors. Molecular systematics. 2nd ed. Sunderland (MA): Sinauer. p. 51–120.

Ostergaard S, Hansen M, Loeschcke V, Nielsen EE. 2003. Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. Mol Ecol. 12: 3123–3135.

Patiño J, Werner O, González-Mancebo JM. 2010. The impact of forest disturbance on the genetic diversity and population structure of a late-successional moss. J Bryol. 32:220–231.

Pulliam HR. 1988. Sources, sinks, and population regulation. Am Nat. 132:652–661.

Pulliam HR. 1996. Sources and sinks: empirical evidence and population consequences. In: Rhodes OE Jr, Chesser RK, Smith MH, editors. Population dynamics in ecological space and time. Chicago (IL): The University of Chicago Press. p. 45–69.

Ruetz CR 3rd, Trexler JC, Jordan F, Loftus WF, Perry SA. 2005. Population dynamics of wetland fishes: spatiotemporal patterns shaped by hydrological disturbance? J Anim Ecol. 74:322–332.

Scribner KT, Wooten MC, Smith MH, Kennedy PK, Rhodes OE. 1992. Variation in life-history and genetic traits of Hawaiian mosquitofish populations. J Evol Biol. 5:267–288.

Selander RK, Smith MH, Yang SY, Johnson WE, Gentry JR. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud Genet VI Univ Texas Publ. 7103:49–90.

Slatkin M. 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theor Popul Biol. 12:253–262.

Slatkin M. 1985. Gene flow in natural populations. Annu Rev Ecol Syst. 16:393–430.

Slatkin M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution. 47:264–279.

Sokal RR, Smouse PE, Neel JV. 1986. The genetic structure of a tribal population, the Yanomama Indians. XV. Patterns inferred by autocorrelation analysis. Genetics. 114:259–287.

Smith MH, Scribner KT, Hernandez JD, Wooten MC. 1989. Demographic, spatial, and temporal genetic variation in *Gambusia*. In: Meffe GK, Snelson FF Jr, editors. Ecology and evolution of livebearing fishes (Poeciliidae). Englewood Cliffs (NJ): Prentice Hall. p. 235–257.

Smith MW, Smith MH, Chesser RK. 1983. Biochemical genetics of mosquitofish. I. Environmental correlates, and temporal and spatial heterogeneity of allele frequencies within a river drainage. Copeia. 1983:182–193.

Tessier N, Bernatchez L. 1999. Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). Mol Ecol. 8:169–179.

Trexler JC. 1988. Hierarchical organization of genetic variation in the sailfin molly, *Poecilia latipinna* (Pisces, Poeciliidae). Evolution. 42:1006–1017.

Trexler JC, Loftus WF, Jordan F, Chick JH, Kandl KL, McElroy TC, Bass OL. 2001. Ecological scale and its implications for freshwater fishes in the Florida Everglades. In: Porter JW, Porter KG, editors. The Everglades, Florida Bay, and Coral Reefs of the Florida Keys. An ecological sourcebook. Boca Raton (FL): CRC Press. p. 153–181.

Trexler JC, Loftus WF, Perry S. 2005. Disturbance frequency and community structure in a twenty-five year intervention study. Oecologia. 145:140–152.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol. 4:535–538.

Wade MJ, McCauley DE. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. Evolution. 42:995–1005.

Whitlock MC. 2004. Selection and drift in metapopulations. In: Hanski I, Gaggiotti OE, editors. Ecology, genetics, and evolution of metapopulations. Amsterdam (The Netherlands): Elsevier Academic Press. p. 153–173.

Whitlock MC, McCauley DE. 1999. Indirect measures of gene flow and migration: F-ST not equal 1/(4Nm+1). Heredity. 82:117–125.

Wolski LF, Trexler JC, Nelson EB, Philippi T, Perry SA. 2004. Assessing visitor impacts from long-term sampling of wetland communities in the Everglades. Freshw Biol. 49:1381–1390.

Received January 3, 2011; Revised June 27, 2011;
Accepted July 22, 2011

Corresponding Editor: Stephen Karl