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Population Structure and Spatial Delineation of Consumer
Communities in the Everglades National Park

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Joel Trexler¹ and William F. Loftus²

¹Department of Biological Sciences, Florida International University

Miami, FL 33199

²USGS, Florida Integrated Science Center, Water and Restoration Sciences

Everglades NP Field Station, Homestead, FL 33034

Dr. Tonya Howington, ENP – Cooperative Agreement Representative

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I. Executive Summary

Tracking the population dynamics of small fishes and aquatic invertebrates that serve as prey for wading birds is a key target for performance measures (PMs) of the Monitoring and Assessment Plan (MAP) of the Comprehensive Everglades Restoration Plan (CERP) (Anonymous 2004). PMs have been developed to track the wet-season population sizes and dry-season concentrations of these prey items based on a conceptual model linking these measures to foraging success and, ultimately, nesting success, of several species of wading birds inhabiting the Everglades (Gawlik 2002). A key research question that remains is the spatial ecology linking fish concentrated in dry season refuges and drying pools where birds feed and wet-season populations. Quantifying the spatial scale over which fish respond to water-level fluctuations is a key step in tracking the spatial domain of impacts from hydrological operations and creating models to evaluate alternative management scenarios (DeAngelis et al. 1997; Gaff et al. 2004). An additional pressing issue for planning in CERP is the impact of levees and canals on shaping ecological dynamics of the surrounding wetlands. There is a pressing need to better understand the role of these engineered landscape features in order to best plan for their use, retention, or removal in future restoration scenarios. This project attempted to begin the challenging process of gaining insight into fish movement at the landscape scale in Everglades wetlands.

The Everglades presents a major challenge to gathering information on the spatial origins and eventual disposition of fishes in alligator ponds or drying pools. Radio transmitters can be used to track fish weighing approximately 5 grams or more to obtain direct information about their movements. This seemingly arbitrary minimum size is based on trade-offs in transmitter technology (battery life) and the rule-of-thumb that transmitter mass should not exceed 5% of the mass of the fish carrying it. Unfortunately, the most abundant fishes in the Everglades and in diets of most wading birds fall below this size limit. The complexity, openness, and size of the Everglades renders more passive mark-recapture approaches relatively uninformative for these abundant small species of fish in that recapture is unlikely. In this project, we employed genetic markers as an indirect method to trace the spatial dynamics of fish to overcome the limitation of

direct tracking methods. We report population genetic surveys of two species of small fish (eastern mosquitofish and bluefin killifish) and one larger fish (spotted sunfish), as well as one crustacean (riverine grass shrimp). Part of this effort included identification of new microsatellite loci for analysis of genetic structure in bluefin killifish. Also, we report a two-year study using radio transmitters to track the movement of a large fish, Florida gar. In all studies, we focused on understanding the relationship of movement and hydrological fluctuation within water management areas, as well as the impact of canals and levees on movement and in shaping landscape patterns of population dynamics.

Both genetic and radio-tracking data indicated that large fish use the canals as dry-season refuges and as corridors for movement. Samples of spotted sunfish collected at several widely spaced areas in canals were more homogeneous genetically than samples from alligator ponds in marshes that were more closely positioned in the landscape. Typically for genetic data, movement that is restricted to local sites yields increasing genetic differences with increasing geographic distance; we did not observe this pattern in Everglades sunfish. The simplest explanation for our results is that canals are a destination of fishes from large areas of the Everglades and/or fish within the canals are moving and intermixing over longer distances than those resident in the wetlands. The former pattern is consistent with our findings for Florida gar. In the wet season, we found that radio-tagged gar that were distant from canals remained relatively stationary, making short distance movements primarily at night. However, as the local water depth dropped to approximately 20 cm, most tagged gar moved long distances, often toward canals; a number of our fish tagged in marsh locations in WCA-3A distant from canals moved into either the L-29 or L-28 canals as water levels dropped. With falling water levels, many gar traveled as far as 10 kilometers into deeper sites in a matter of weeks .

Patterns of genetic diversity in small fishes (eastern mosquitofish and bluefin killifish) were more clearly linked to whether the point at which they were collected had dried in the past year, rather than to the distance separating the collection sites. We examined population genetic patterns across the southern Everglades (WCA 3A, WCA

3B, Shark River Slough, and Taylor Slough) in two different years to test for repeatability of our results and contrast spatial and temporal patterns of genetic variance. In all cases for both species, we observed less heterogeneity among samples collected at sites that had not dried in the last year than among sites that had dried in the past year. This suggests that sites that are dried are re-colonized by fish from a variety of local sites. Regional drying events force mixing at several scales because we also observed evidence of population mixing at sites that had not dried in a drought year. We noted small amounts of variation among water management units (WCA-3A, Shark River Slough, Taylor Slough) in eastern mosquitofish and bluefin killifish, but not grass shrimp or spotted sunfish. The presence of small regional differentiation for these fish species is not surprising because the levees have been in place for 100 to 200 generations (depending on the levee), both have large population sizes, and mosquitofish are considered to be strong dispersers (little is known about dispersal ability of bluefin killifish). Conservatively, $1/m$ generations (where m is the migration rate) are required for population genetic differentiation to appear when a new spatial division is created. For example, if historically there were no genetic separation where the Tamiami Trail was built (complete mixing of individuals) and migration was reduced to 1% of the population exchanged each generation across the same boundary, 100 generations would be adequate to observe the new level separation with genetic markers. Thus, the presence of a genetic difference, albeit small, is indicative that the levees and restricted water flow across this boundary is a barrier to movement. Perhaps more important, the regionalization of water management has changed the frequency and scale of drying in each new compartment (Taylor Slough dries the most frequently and WCA 3A the least frequently of our study area), which affects the population structure of the fish differently in each region. Analysis of migration patterns reflected in patterns of genetic differentiation indicated some possible corridors of movement. For example, we estimated higher migration rates among sites along the margin of the main road in Everglade National Park from the Park Headquarters to Flamingo, than from Taylor Slough to the edge of the road or along the center of the slough.

Grass shrimp are very abundant invertebrates that serve as food for many fish species, some small wading birds, and even small alligators. They reach tremendous densities at times in the dry season. We found no evidence of spatial genetic structure for grass shrimp, unlike our data on mosquitofish. This may be the result of their very large population sizes.

This study determined that large predatory fishes are moving long distances across the Everglades to reach dry-season refuges. In WCA 3A, these refuges are often canals. In contrast, canals are barriers to small fishes, probably at least in part because of the accumulated predators found there. Small fish populations are heterogeneous at the scale of 10 kms or less, suggesting that they are converging on refuges over distances of this size or smaller. Spatially explicit models developed for assessment should incorporate different scales of movement by small and large fishes. An accumulation of predators in canals and long-hydroperiod marshes may reduce the abundance of fishes consumed by wading birds. It is possible that models will show that the presence of canals permit the persistence of larger populations of long-lived predatory fishes that would perish without them. The greater abundance of predators would probably reduce the abundance of small fish that they consume (and the density of small fish is lower in WCA 3A than in Shark River Slough at present), though no models have examined if this effect would yield reduced opportunities for foraging by wading birds.

Additional Studies that Received Partial Funding from this Agreement

Genetic markers are powerful tools for tracking species identity, in addition to population structure. During the course of this project, a new species was observed to have been introduced into the United States, the Asian swamp eel. Two populations were found in southeast Florida. This species presented a number of challenges to managers because of its extreme tolerance of low-oxygen conditions and its potential to tolerate relatively cool water temperatures. Unfortunately, the taxonomy of swamp eels is poorly resolved because of their greatly reduced anatomy and general paucity of traditional taxonomic characters. The natural range of the nominal species, *Monopterus albus*, extends from Indonesia to central China, from tropical to temperate climates. Thus, the

source of our introductions was important to determine in order to evaluate its potential to spread. We worked with Dr. Tim Collins, FIU, to use genetic markers to characterize the four known populations of swamp eels relative to a collection of tissues we assembled. This work demonstrated that the north Miami-Dade County introduction was from a different source than the one in the southern part of the county. This work also implied that the south Miami-Dade population may be relatively small, and led to further efforts at management actions targeting these fish (Robichaux 2000). Another dimension of this project was to explore the potential use of stable isotopes as spatial markers of aquatic animals. This contributed to the development of isotopic techniques in our laboratories in an exploratory project. We found no consistent spatial patterns in stable isotopes of carbon or nitrogen. Future directions will be to analyze sulfur isotopes as possible signatures of use of estuarine habitats. This work has also led us to investigating the use of elemental analysis of fish ear bones (otoliths) for future projects.

Reference Cited

- Anonymous. 2004. CERP Monitoring and Assessment Plan: Part 1. Monitoring and Supporting Research – January 2004. Comprehensive Everglades Restoration Plan, Restoration Coordination and Verification (RECOVER). SFWMD Planning Document.
- DeAngelis, D. L., W. F. Loftus, J. C. Trexler, and R. E. Ulanowicz. 1997. Modeling fish dynamics in a hydrologically pulsed ecosystem. *Journal of Aquatic Ecosystem Stress and Recovery* 6:1-13
- Gaff H, J. Chick J, J. Trexler, D. DeAngelis, L. Gross L, and R. Salinas. 2004. Evaluation of and insights from ALFISH: a spatially explicit, landscape-level simulation of fish populations in the Everglades. *Hydrobiologia* 520: 73-87
- Gawlik, D. E. 2002. The effects of prey availability on the numerical response of wading birds. *Ecol. Monogr.* 72: 329-346.
- Robichaux, M. 2000. Alien Invasion: Plague of Asian Eels Highlights Damage From Foreign Species --- Latest of Many Interlopers, These Creatures Could Threaten the Everglades --- Gender-Benders Won't Die. *The Wall Street Journal*. Sep 27, 2000, page A1

Personnel:

Joel Trexler, Principal Investigator
William F. Loftus, Project Coordinator

Douglas Creer, Post-doctoral Research Assistant
Thomas McElroy, Post-doctoral Research Assistant

Karen Kandl, Post-doctoral Research Assistant

Janette Garcia, Laboratory Assistant

Sharon Cross, Laboratory Assistant

Oral Presentations and Posters

- 2005 Extinction, recolonization, and spatial population genetic structure in ecological time. Trexler, McElroy, and Creer. Society for the Study Evolution, Fairbanks

Extinction, recolonization, and spatial population genetic structure in ecological time. Trexler, McElroy, and Creer. Ecological Society of America, Montreal.

- 2004 Extinction, recolonization, and spatial population genetic structure of mosquitofish. Trexler, McElroy and Ruetz. Society for the Study Evolution, Boulder.

Extinction, recolonization, and metacommunity structure in Everglades wetlands: spatial dynamics of aquatic communities driven by recurrent disturbance. Trexler, McElroy, Ruetz, and Loftus. First National Conference on Ecosystem Restoration (NCER)

- 2003 Patterns Of Movement Of Florida Gar (*Lepisosteus platyrhincus*) In The Everglades Revealed By Radio Telemetry. Lawrence F. Wolski, J. C. Trexler, Jason Knouft, Carl Ruetz III, William F. Loftus. Greater Everglades Ecosystem Research Symposium GEER

- 2002 Population structure of spotted sunfish (*Lepomis punctatus*) in the Florida Everglades as revealed by DNA microsatellite analysis T. McElroy and Trexler. American Society of Naturalists, Banff

- 2001 Gene flow patterns in *Gambusia holbrooki* from the Florida Everglades: A comparison of FST and coalescence-based maximum likelihood estimates. T. McElroy and Trexler. Society for the Study of Evolution

Population structure of spotted sunfish (*Lepomis punctatus*) in the Florida Everglades as revealed by DNA microsatellite analysis. J. Garcia, T. McElroy, Trexler. Society for the Study of Evolution (Poster)

- 2000 Genetics of introduced Asian swamp eels within the genus *Monopterus* (Synbranchidae). T. Collins, Trexler, L. Nico. American Society of Ichthyologists and Herpetologists

Population structure of the eastern mosquitofish, *Gambusia holbrooki*, during a severe dry-down in the Florida Everglades. (Poster) T. McElroy, Trexler. Society for the Study of Evolution

Genetic Analysis of Introduced Predatory Asian Swamp Eels (*Synbranchidae*) — Timothy Collins, Joel Trexler and Timothy Rawlings, Leo G. Nico. Greater Everglades Ecosystem Research Symposium GEER

- 1999 Use of microsatellites in examining effects of local extinction in populations of mosquitofish in the Florida Everglades. K. L. Kandl and J.C. Trexler. Society for the Study of Evolution.
- 1998 Population structure of mosquitofish and grass shrimp in the Florida Everglades. (Poster) K. Kandl and J. C. Trexler. Am Soc of Limnologists and Oceanographers/Ecological Society of America joint meeting

Publications that acknowledge this project

Collins, T., J. C. Trexler, L. G. Nico, and T. Rawlings. 2002. Genetic diversity in a morphologically conservative invasive taxon: Multiple swamp eel introductions in the southeastern United States. *Conservation Biology* 16:1024-1035.

Creer, D. A., and J. C. Trexler. 2005. New polymorphic microsatellite loci in two fish species: bluefin killifish (*Lucania goodei*) and yellow bullhead (*Ameiurus natalis*). *Molecular Ecology Notes*, in press.

McElroy, T. C., L. L. Kandl, J. Garcia and J. C. Trexler. 2003. Extinction-colonization dynamics structure genetic variation of spotted sunfish (*Lepomis punctatus*) in the Florida Everglades. *Molecular Ecology* 12:355-368

Trexler, J. C., W. F. Loftus, C. F. Jordan, J. Chick, K. L. Kandl, T. C. McElroy, and O. L. Bass. 2001. Ecological scale and its implications for freshwater fishes in the Florida Everglades. Pp. 153 – 181, in J. W. Porter and K. G. Porter (eds.) *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook*. CRC, Boca Raton.

**II. Temporal dynamics of population genetic structure reveal
colonization dynamics of eastern mosquitofish in a dynamic aquatic
landscape.**

Running Title: Temporal Genetic Structure in Eastern Mosquitofish

Temporal dynamics of population genetic structure reveal colonization
dynamics of eastern mosquitofish in a dynamic aquatic landscape

T. C. McElroy¹, J. C. Trexler², and K. L. Kandl³

¹Department of Biological and Physical Sciences, Kennesaw State University, Kennesaw,
GA 30144

²Department of Biological Sciences, Florida International University, University
Park, Miami, FL 33199

³Department of Biological Sciences, University of New Orleans, New Orleans, LA
70148

Correspondence: T.C. McElroy, Department of Biological and Physical Sciences,
Kennesaw State University, 1000 Chastain Rd, Kennesaw, GA 30144-5591. Fax:
(770) 423-6625. E-mail: tmcelro@kennesaw.edu

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Abstract

We hypothesized that the seasonal hydrology of the Florida Everglades shapes population genetic structure of aquatic animals there by driving local extinction and colonization dynamics. Also, we hypothesized that fish recolonizing recently dried sites come from a small number of local sources (migrant pool colonization), in contrast to the alternative of dispersal from disparate sources (propagule pool colonization). Migrant pool colonization is revealed by increased heterogeneity among disturbed sites because of local founder effects, while propagule pool colonization is characterized by homogenization of disturbed sites. We combined analyses of allozyme and microsatellite loci from eastern mosquitofish (Poeciliidae: *Gambusia holbrooki*) inhabiting the Everglades to test these hypotheses by documenting temporal stability of population structure following local drying. We also hypothesized that levees and canal create a regional level of genetic variation by forming barriers to gene flow. In 1996 and 1999, we sampled at least 24 eastern mosquitofish from each of 20 sites distributed in three water-management regions of the Everglades and characterized genetic variation by analysis of 12 polymorphic allozyme loci and 3 polymorphic microsatellite loci. In both years and for both marker types, most genetic variation was present among individuals within populations, with most spatial variation attributed to local populations within regions; less than 1% of total genetic variation was partitioned among water-management areas. We observed more variance among local sites when we partitioned it between those that had dried within the past year and those that had not. In these analyses, recently dried sites displayed no spatial genetic variation, while sites that had not dried were spatially heterogeneous in both years for both markers. All samples were in Hardy-

Weinberg genotypic frequencies except for sites that had not dried in 1999. At many of these sites we observed fewer heterozygous individuals than expected, suggesting a Wahlund effect (mixing of individuals from sources with different allele frequencies). We propose a conceptual model of mosquitofish dispersal driven by hydrology: within water-management unit genetic structure develops among local sites in wet periods, while individuals from multiple sources mix in short-hydroperiod refuge sites; a small amount of regional variation is present among water-management units, consistent with their relatively short history (the oldest have been in place for approximately 200 mosquitofish generations). Documentation of non-equilibrium population structure through temporal sampling provides insight into ecological processes of dispersal and colonization in dynamic landscapes.

Key Words: Allozymes, colonization, extinction, Florida Everglades, *Gambusia holbrooki*, microsatellites, population dynamics, genetic structure

Introduction

Survival in refuges, migration from refuges, and colonization dynamics may shape population and community dynamics in seasonally fluctuating environments (Pulliam 1996; Chesson and Huntly 1997). The spatial scale of sources of colonists following local extinction affects the general 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 1991). The biological significance of general genetic structure and pairwise genetic structure for metapopulations depends on environmental factors (*e.g.*, loss or gain of habitat, dispersal barriers, or corridors) and demographic patterns (Castric *et al* 2001; Hansen *et al.* 2002; Charbonnel *et al.* 2002). Directly tracking the origins and endpoints of dispersing organisms is difficult at best, and can often only be accomplished by indirect means such as analysis of genetic markers or population genetic characteristics (Slatkin 1985).

Interpretations of genetic structure often assume the observed spatial patterns are relatively stable over time and through environmental fluctuations (Tessier and Bernatchez 1999). However, temporally unstable or non-equilibrium genetic structure may be typical of species inhabiting environmentally fluctuating landscapes. Analysis of temporal change in population genetic structure can provide insight to the ecological processes and scale of migration that create it (Hedrick and Gilpin 1997). Two models are used to characterize extreme patterns of recolonization of emptied habitat patches, migrant pool and propagule pool (Slatkin 1977). Migrant pool colonization results from a founder effect of colonists filling local sites from a single or small number of sources. It is characterized by increased population differentiation of local populations at these re-

filled sites compared to the stable source populations, though the distinction may be quickly eroded by ambient patterns of gene flow (Wade and McCauley 1988). In contrast, a propagule pool colonization has little effect on population genetic structure and is characterized by colonists drawn from many sources, possibly even homogenizing newly re-colonized populations. For example, McCauley et al. (1995) determined that colonists arrive from a small number of sources to re-populate roadside populations of the herbaceous plant white campion (*Silene alba*), following periodic local extinctions (migrant pool colonization).

The Florida Everglades is a large, shallow (depth typically < 1 m) wetland ecosystem extending from the southern shores of Lake Okeechobee to Florida Bay in Florida, U.S.A. Historically, water flowed southward in sheet flow across the ecosystem. The central sloughs are bounded by wetlands that dry annually (short-hydroperiod). These wetlands experience seasonal drying events (typically November to May) when aquatic organisms become concentrated in deep-water refuges such as alligator ponds (Loftus and Kushlan 1987; Trexler et al. 2001). Seasonal drying causes the local extinction of populations, with re-colonization coming from deep-water refuges. Drying may force population mixing by long-range movement of fishes into refuge habitats. Populations of fish that inhabit the short-hydroperiod wetlands persist in a state of numerical flux, whereas populations of aquatic organisms that inhabit long-hydroperiod areas, such as the central sloughs, fluctuate over longer time periods (Trexler *et al.* 2001; Ruetz et al. 2005; Trexler et al. 2005). Thus, some fraction of the total population of fish 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 reduce the extent of movement through the habitat. Previous work with spotted sunfish (*Lepomis punctatus*) detected significant genetic structure among short, but not long-hydroperiod, marsh 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) could not address the temporal stability of genetic structure.

We examined the variability at allozyme loci and microsatellite loci at two 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 re-sampled the 1996 sites to assess the effect of the 1999 drought on population genetic structure. We combined these data with hydrologic data collected from long-

term study sites to describe population dynamics and test the temporal stability of spatial genetic structure in eastern mosquitofish. We tested the following hypotheses: (1) periodic cycles of local extinction and colonization by mosquitofish structures their population genetic variation causing variation among sites nested within water management units to exceed variation among water management units; (2) local hydrology strongly influences population dynamics and genetic structure causing the magnitude of genetic difference between pairs of sites to be correlated with hydrological patterns rather than the geographic distance separating them; and (3) local sites are recolonized by fish from local refuges sites, increasing the genetic variation among disturbed sites relative to stable ones and consistent with migrant pool predictions; (4) these local patterns are not stable through time.

Materials and Methods

We made two field collections (March 1996 and March 1999) of eastern mosquitofish from 20 sites throughout WCA-3A, SRS, and TS to document their population genetic structure (Fig. 1). Six or more generations of mosquitofish passed between our sampling events and local densities dropped to zero one or more months at sites that dried during the study (Fig. 2, see Trexler et al. 2001, Ruetz et al. 2005). Up to fifty adult eastern mosquitofish were collected with dip nets from each location, depending on availability of fish (Range = 24 – 50). The sites were distributed among three 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 dry-down event in the Everglades at the end of the 1999 dry season. Some sites were

completely dry in the TS, forcing us to sample fish at locations nearby the 1996 collection sites. We estimated the number of days that had passed before each collection site last dried and the minimum water depth during the sampling interval at each site (see Ruetz et al. 2005).

Laboratory methods

Allozyme Analysis-. We used starch gel electrophoresis to document patterns of allozyme variation in the study species. Upon collection, specimens were transferred to the laboratory and stored in a -80°C freezer prior to genetic analyses. 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 loci before selecting 10 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 four different buffer systems: TC8, Lithium-Borate/Tris-Citrate (LIOH; Selander *et al.* 1971), Tris-Citrate-EDTA (JRP; Ayala *et al.* 1972).

Microsatellite Analysis-. Whole genomic DNA was isolated from muscle tissue by standard phenol-chloroform DNA extraction methods (Hoelzel and Green 1992). Approximately 30 mg of tissue was frozen in liquid nitrogen and crushed to a fine powder. The powder was suspended in STE buffer, pH 7.5 (0.1 M NaCl, 50.0 mM Tris,

1.0 mM EDTA), 10% SDS, and proteinase K (Promega). After a short incubation period at room temperature, the DNA was extracted using a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1), followed by cleansing with a solution of chloroform and isoamyl alcohol (24:1). The DNA was precipitated in a cold solution of 95% ethanol and 5M sodium chloride. It was then spun into a pellet and washed twice with 70% ethanol, before being re-suspended in Tris EDTA buffer (TE, pH 8.0).

Three microsatellite loci (Table 1) were amplified using a multiplexed polymerase chain reaction (PCR). Amplifications were performed in 15:1 volumes, each one containing 10X buffer, 25 :M MgCl₂, 250 :M dNTP's, 5 U/:l Taq DNA polymerase (Promega), and three 5 :M 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 to a ROX 350 standard and the genotypes were assigned using the GENESCAN (ABI) and GENOTYPER software packages.

Statistical Analyses-. We examined goodness-of-fit to Hardy-Weinberg expectations at each locus, and in global tests across loci and across populations using GENEPOP (Raymond and Rousset 1995). When only two or three alleles were observed, we used

the complete enumeration method of Louis and Dempster (1987), while for loci exhibiting more than three alleles we used the Markov chain method to estimate exact P values (Guo and Thompson 1992). We tested for patterns of disequilibrium coefficient (F_{IS} ; Weir 1990, p84) as a function of hydrology using a non-parametric test based on ranks (Kruskal-Wallis One-Way Analysis of Variance).

We used Weir and Cockerham's (1984) coancestry method to partition the total observed genetic variation. These partitions were attributable to variation among individuals within subpopulations (individuals collected in areas $< 1 \text{ km}^2$) relative to total diversity within their subpopulation (Θ_{IS}), among subpopulations within water-management units relative to the total diversity in that unit (Θ_{SS}), among water-management units (populations) relative to the total genetic diversity with each sampling time (Θ_{SP}), and among sampling times (Θ_{PT}) relative to the total genetic diversity. This provided spatial and temporal estimates of Wright's hierarchical F statistic of population subdivision based on a random-effects sampling model (Weir 1990). The data was also analyzed without time as a factor in order to assess spatial consistency of local and regional effects between the 1996 and 1999 samples independent of time. The Genetical Data Analysis program (GDA; Lewis and Zaykin 1997) was used to estimate hierarchical F's following Weir and Cockerham (1984), with bootstrapping across loci to estimate confidence intervals.

We analyzed the correlation of estimated pairwise Θ_{ST} for the 1996 and 1999 allozyme and microsatellite data in order to examine the overall temporal consistency and the specific (pairwise) temporal consistency of Θ estimates. In order to further investigate these relationships sites were divided into regions corresponding to water

management units or into two general categories based on the hydrology within the time of this study (1996 - 1999). For analysis of hydrological disturbance, we grouped sites into those that had dried within the past year (surface water depth <5 cm), and those that remained inundated throughout the time of this study. We analyzed the correlation of estimated pairwise Θ_{ST} for the 1996 and 1999 allozyme and microsatellite data within each of these categories. Pairwise Θ estimates were also correlated with pairwise hydrology differences (days since dried (DSD) and minimum water depth) for each sampling time to further investigate the influence of hydrology on population genetic structure. Significance of matrix correlations was assessed by a Mantel test (Mantel 1967).

We analyzed the correlation of estimated pairwise Θ_{ST} for allozyme and microsatellite data and geographic distance in order to assess the presence or absence of isolation by distance (IBD) for both sampling times (Slatkin 1977, 1993). IBD was calculated for each data set and then for subsets of each data set that considered regions and sites that shared a common hydrology (as stated above). These data were used to assess gene flow –genetic drift equilibrium according to Wright’s ‘isolation by distance’ model. Significance of matrix correlations was assessed by a Mantel test (Mantel 1967).

Results

The tests for global conformity to Hardy-Weinberg equilibrium across allozyme loci revealed that in 1996 most of the loci conformed to Hardy-Weinberg expectation; however, only four of the loci conformed to Hardy-Weinberg expectations in 1999 (Table 1). In 1999, we observed a significant deficiency of heterozygous individuals, as

might be expected from a rapid diminution of population size and population mixing (Hartl and Clark 1997). We observed a similar pattern in the microsatellite data (Table 1). For both markers, deviation from Hardy-Weinberg expectations at loci with low polymorphism resulted primarily from the lack of rare-allele heterozygotes. No indication of null alleles, mis-scoring from protein degradation, or other sources of error could be identified as responsible for the observed deviations.

We noted a significant heterozygote deficiency detected across loci, populations and globally in the 1999 samples. For both marker types, mosquitofish sampled in 1999 displayed less heterozygosity overall than mosquitofish sampled in 1996 (Table 1). Heterozygote deficiency of allozymes (a positive value of the fixation index, F_{IS}) did not differ between years or as a function of drying history (Fig. 3). However, microsatellite loci displayed heterozygote deficiency in the 1999 samples from sites collected that did not dry in the previous year, but not at sites that dried (Mann-Whitney $U = 21.0$, $P=0.030$; Fig. 3); this difference in 1999 was not reflected at the same sites in 1996.

For both marker types and in both years, most spatial genetic variation was at the local scale. We observed little additional genetic variation among regions than was observed among sites within the regions ($<1\%$, Table 2). Though small, some genetic variation was attributed to differences among regions (Θ_{PT}) in both markers in 1996, and in microsatellites but not allozymes in 1999. Generally, about 1% of the total genetic variation was present among sites, though again this result was consistent in both marker types and both years of the study (Table 2). When time was included in an analysis of genetic variance, it explained a significant amount of variance for both allozymes and microsatellites, but less than spatial factors (Allozymes: between years $\Theta = 0.020$;

Microsatellites: between years $\Theta = 0.001$, among sites within years $\Theta = 0.004$; no confidence intervals overlap zero). When inter-site variation was documented separately for each region, the presence of genetic differentiation among study sites within regions was not always consistent between the marker types (Table 3). The spatial-temporal pattern was consistent for allozymes and microsatellites within Taylor Slough, but not within Shark River Slough and Water Conservation Area 3A.

Hydrology consistently affected the pattern of inter-site heterogeneity in both years and from both marker types. In both 1996 and 1999, undisturbed sites (no drying event during the year before sampling) had Θ_{ST} values significantly different from 0 for allozymes and microsatellites, while dry-down sites did not (Table 4). The number of recently dried sites was greater in the 1999 sampling event than in the 1996 event.

Overall, there was a significant correlation between pairwise Θ_{ST} estimates from the 1996 and 1999 allozyme ($r = 0.245$, $P = 0.035$, $N = 190$) and microsatellite data sets ($r = 0.229$; $P = 0.020$, $N = 190$) (Fig. 4). In both cases, the slope of the relationship was much less than one, indicating greater differentiation between the same pairs of sample sites in 1996 than in 1999. These correlations were greatest when only sites that have been recently disturbed by dry-down events were included in the data set (allozymes, $r = 0.276$ $P = 0.110$ $N = 36$; microsatellites, $r = 0.542$ $P = 0.107$ $N = 36$; Fig. 4). There was no apparent correlation for Θ_{ST} estimates between years when only sites that had not recently dried were evaluated (allozymes, $r = -0.0066$ $P = 0.441$ $N = 55$; microsatellites, $r = 0.072$ $P = 0.242$ $N = 55$). The inter-year correlation of pairwise Θ_{ST} estimates with one site that had recently dried and one that had not was intermediate. Pairwise estimates for allozymes and microsatellites were significantly correlated in 1996 but not in 1999.

We observed no evidence for isolation by distance for any set or subset of the allozyme or microsatellite data sets (Fig. 5). However, the variance among the pairwise values was clearly greater in 1999 for both marker types. In that year, both marker types indicated modest numbers of population pairs with F_{ST} values greater than 0.04. In both data sets, these resulted from pairwise comparisons with at least one of the pair having dried in the past year before 1999 samples were taken (Fig. 4).

Discussion

There is increasing appreciation of the importance on non-equilibrium 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 indicative of such recruitment patterns (Hedgecock 1994; Hedrick 2005), which have important consequences for management of marine fishes (Larson and Julian 1999). Our data indicate temporally dynamic and spatially weak population structure that is linked to hydrological patterns rather than distance or regional habitat boundaries. Following Harrison (1991), this pattern 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. 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.

Our data indicate three levels of population structure in the dynamic wetland landscape of the Everglades. We noted small but significant portions of genetic variability partitioned among the water management units separated by levees (regional partition) that have been in place for 40 to 80 years (80 to 160 mosquitofish generations). Given the large census population sizes of mosquitofish in these areas, it might be surprising to see any partitioning of variability at this level. The largest, though still small, partition of spatial variability is among sites within water management units (these are separated by approximately 10 kms on average). This is also the scale of largest variation in mosquitofish density at the study sites (Trexler et al. 2001; Ruetz et al. 2005). This inter-site genetic variation was best resolved by categorizing sites based on recent history of drying. Finally, we also noted structure among individuals within populations related to heterozygosity; we observed evidence of mixing of individuals within long-hydroperiod sites following a drought year in the form of a shortage of heterozygous individuals (a Wahlund effect).

Hydrologic disturbance (drying events) appeared to be the most important factor shaping genetic variation in mosquitofish in this dynamic wetland landscape. This result

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 typically 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 to the temporal spacing of their samples (see also Trexler et al. 2001). Environmental drivers have been implicated in shaping genetic variation in a variety of other species (reviewed in Manel et al. 2003).

Our data indicate eastern mosquitofish display propagule pool colonization because recently extirpated and re-colonized populations were more homogeneous than populations at continuously inhabited sites. This homogeneity is lost as time passes following a local drought event and structure emerges at the longer-hydroperiod sites. Population genetics of eastern mosquitofish have been studied in detail and often show 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; McClenaghan et al. 1985, Kennedy et al. 1985, 1986) 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 dispersal (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 to populations from more stable reservoirs. The Hawaiian populations are relatively closed and the reservoirs are homogeneous compared to the large heterogeneous environment of the Everglades. Our data indicating heterozygote deficiency are consistent with a Wahlund Effect resulting from mixing (either spatially or temporally) groups of fish with different allele frequencies (Hedrick 2000).

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. 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). While 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 point questionable, if not absurd for moderately long-lived taxa. As humans have altered and continue to alter ecosystems, it seems likely that few habitats in North America or other parts of the developed and developing world have remained stable in ways relevant to regional-scale patterns of gene flow over the past 50 or so 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 proof supporting it (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.

Acknowledgements

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Literature Cited

- 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. University Presses of Florida, Tallahassee.
- Bossart, J. L., and D. P. Prowell. 1998. Is population genetics mired in the past? Reply from J.L. Bossart and D. Pashley Prowell. *Trends in Ecology and Evolution* 13:360-360
- 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., M. Quesnoit, R. Razatavonjizay, P. Bremond, and P. Janrue. 2002. A spatial and temporal approach to microevolutionary forces affecting population biology in the freshwater snail *Biomphalaria pfeifferi*. *American Naturalist* 160:741-755.

- Chesson P., and N. Huntly. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *American Naturalist* 150:519-553
- Chick, J. H., C. R. Ruetz III, and J. C. Trexler. 2004. Spatial scale and abundance patterns of large fish communities in freshwater marshes of the Florida Everglades. *Wetlands* 24:652-664
- Davis, S.M., L.H. Gunderson, W.A. Park, J.R. Richardson, J.E. Mattson. 1994. Landscape dimension, composition, and function in a changing Everglades ecosystem. Pages 419-444 in S.M. Davis and J.C. Ogden, editors. *Everglades: the ecosystem and its restoration*. St. Lucie Press, Delray Beach, Florida.
- Freckleton, R. P., and A. R. Watkinson. 2002. Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. *Journal of Ecology* 90:419-434.
- Gawlik, D. E. 2002. The effects of prey availability on the numerical response of wading birds. *Ecological Monographs* 72:329-346
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361-372.
- Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg K-L D (2002) Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology*, **11**, 2523-2535.
- Harrison, S. (1991) Local extinction in a metapopulation context: an empirical evaluation. *Biological Journal of the Linnean Society*, **42**: 73-88.

- Harrison, S., and A. Hastings 1996. Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology and Evolution* 11: 180-183
- Hartl DL, Clark AG (1997) Random genetic drift. In: *Principles of population genetics 3rd edition*, pp. 267-313. Sinauer Associates, Inc., Sunderland, MA, USA.
- Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? Pp 122-134. In: A. Beaumont, ed. *Genetics and Evolution of Aquatic Organisms*. Chapman Hall, London.
- Hedrick, P. W. 2000. *Genetics of Populations*, 2nd Ed. Jones and Bartlett, Sudbury, MA
- Hedrick, P. 2005. Large variance in reproductive success and the N-e/N ratio. *Evolution* 59:1596-1599
- Hedrick, P. W., and M. E. Gilpin. 1997. Genetic effective size of a metapopulation, pp 166-182. In: I. A. Hanski and M. E. Gilpin, eds. *Metapopulation Biology*. Academic Press, San Diego.
- Hoelzel AR, Green A (1992) Analysis of population-level variation by sequencing PCR-amplified DNA. In: *Molecular Genetic Analysis of Populations: A Practical Approach* (ed Hoelzel AR), pp. 159-186. IRL Press, Oxford, England.
- Kennedy, P. K., M. L. Kennedy, and M. H. Smith. 1985. Microgeographic genetic organization of populations of largemouth bass and two other species in a reservoir. *Copeia* 1985:937-945
- Kennedy, P. K., M. L. Kennedy, E. G. Zimmerman, R. K. Chesser, and M. H. Smith. 1986. Biochemical genetics of mosquitofish. V. Perturbation effects on genetic organization of populations. *Copeia* 1986:937-945

- Larson, R. J., and R. M. Julian. 1999. Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. California Cooperative Oceanic Fisheries Investigations Reports 40:94-99.
- Lewis PO, Zaykin D (1997) Genetic Data Analysis: computer program for the analysis of allelic data. Free program distributed by the authors at the GDA homepage at <http://chee.unm.edu/gda/>.
- Light SS, Dineen JW (1994) Water control in the Everglades: a historical perspective. In: *Everglades, the ecosystem and its restoration* (eds Davis SM, Ogden JC), pp. 47-84. St. Lucie Press, Boca Raton, FL.
- Loftus WF, Kushlan JA (1987) Freshwater fishes of southern Florida. *Bull. Florida State Mus., Biol. Sci.* **31**, 147-344.
- Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends In Ecology and Evolution* 18:189-197
- Mantel N (1967) The detection of disease clustering and generalized regression approach. *Cancer Research*, **27**, 209-220.
- McCauley D. E., J. Raveill J, and J. Antonovics. 1995. Local founding events as determinants of genetic structure in a plant metapopulation. *Heredity* 75:630-636
- McClenaghan, L. R., Jr. M. H. Smith, and M. W. Smith. 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. *Molecular Ecology*, **12**, 355-368.
- Murphy RW, Sites JW Jr., Buth DG, Haufler CH (1996) Proteins: Isozyme electrophoresis. 120 In: *Molecular systematics*, 2nd edition (eds Hillis DM, Moritz C, Mable BK), pp. 51-120. Sinauer, Sunderland, MA.
- Pulliam, H. R. 1988. Sources, sinks, and population regulation. *Am. Nat.*, 132:652-661
- Raymond M, Rousset F (1995) GENEPOP (version 3.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- Ruetz, C. R., III, J. C. Trexler, F. Jordan, W. F. Loftus, and S. A. Perry. 2005. Population dynamics of wetland fishes: Spatiotemporal patterns shaped by hydrological disturbance? *Journal of Animal Ecology* 74:322-332
- Scribner K, T., M. C. Wooten, M. H. Smith, P. K. Kennedy, and O. E. Rhodes. 1992. Variation in life-history and genetic traits of Hawaiian mosquitofish populations. *Journal of Evolutionary Biology* 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. Gene. VI. Univ. Texas Pub.*, **7103**, 49-90.
- Slatkin, M. (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253-262.
- Slatkin, M. (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393-430.

- Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264-279.
- Smith, M. H., K. T. Scribner, J. D. Hernandez, and M. C. Wooten. 1989. Demographic, spatial, and temporal genetic variation in *Gambusia*, pp 235-257. In: G. K. Meffe and F. F. Snelson, Jr. Eds. *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ
- Smith, M. W., M. H. Smith, and R. K. Chesser. 1983. Biochemical genetics of mosquitofish. I. Environmental correlates, and temporal and spatial heterogeneity of allele frequencies within a river drainage. *Copeia* 1983:182-193
- Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edition. Freeman and Company, New York.
- 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.). *Molecular Ecology*, **8**, 169-179.
- Trexler, J. C. 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: *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys. An Ecological Sourcebook* (eds Porter JW, Porter KG), pp.153-181. CRC Press, Boca Raton, FL.
- Trexler, J. C., W. F. Loftus, and S. Perry. 2005. Disturbance frequency and community structure in a twenty-five year intervention study. *Oecologia* 145:140-152

- Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995-1005.
- Weir BS (1990) Genetic data analysis. Sinauer, Sunderland, MA
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.
- Whitlock, M. C. 2004. Selection and drift in metapopulations, pp 153-173. In: I. Hanski and O. E. Gaggiotti, eds. Ecology, Genetics, and Evolution of Metapopulations. Elsevier Academic Press, Amsterdam.
- Whitlock, M. C., and D. E. McCauley. 1999. Indirect measures of gene flow and migration: F_{ST} not equal $1/(4Nm+1)$. *Heredity* 82:117-125
- Wolski, L. F., J. C. Trexler, E. B. Nelson, T. Philippi, and S. A. Perry. 2004. Assessing visitor impacts from long-term sampling of wetland communities in the Everglades. *Freshwater Biology* 49:1381-1390.

Table 1. Allozyme and microsatellite loci surveyed, the observed heterozygosity at each locus and the probabilities associated with goodness-of-fit to Hardy Weinberg Expectations (HWE) for each locus and each sampling time.

		Observed		HWE	
Locus	E.C. No.	Heterozygosity			
		1996	1999	1996	1999
Allozymes					
Adenosine Deaminase (Ada)	3.5.4.4	0.014	0.023	1.000	0.004*
Aspartate Aminotransferase (Aat-1)	2.6.1.1	0.015	0.007	0.690	0.001*
Gluc-6-Phos Dehydrogenase (Gpi-1)	5.3.1.9	0.315	0.340	0.615	0.170
Gluc-6-Phos Dehydrogenase (Gpi-2)		0.441	0.498	0.681	0.657
Isocitrate Dehydrogenase (Idh-2)	1.1.1.42	0.013	0.010	0.278	0.001*
Lactate Dehydrogenase (Ldh-2)	1.1.1.27	0.018	0.015	0.858	0.000*
Malate Dehydrogenase(Mdh-2)	1.1.1.37	0.190	0.100	0.300	0.163
Man-6-Phosphate Isomerase (Mpi-1)	1.1.1.40	0.319	0.174	0.957	0.000*
Phosphoglucomutase (Pgm-1)	5.4.2.2	0.035	0.033	1.000	0.720
Phosphogluconate Dehydro (Pgd-1)	1.1.1.14	0.316	0.209	0.999	0.001*
Microsatellites					
GAF 2		0.866	0.807	0.158	0.000*
GAF 3		0.857	0.734	0.000*	0.000*
GAF 7		0.874	0.786	0.321	0.000*

* differs statistically from Hardy-Weinberg Expectations

Table 2. Estimates of genetic diversity in mosquitofish collected from the southern Everglades. The same locations were sampled in 1996 and 1999 whenever possible. Θ_{SP} indicates the genetic variance in samples relative to among samples and 1_{PT} indicates the genetic variance in among samples relative to the total study. Upper and lower bound estimates are 95% confidence interval estimates from bootstrap sampling. Time was not included in this model in order to assess spatial effects within each sampling time

	Allozymes 1996		Allozymes 1999		Microsatellites 1996		Microsatellites 1999	
	Θ_{SP}	Θ_{PT}	Θ_{SP}	Θ_{PT}	Θ_{SP}	Θ_{PT}	Θ_{SP}	Θ_{PT}
Estimate	0.0106*	0.0033*	0.0072*	0.0004	0.003*	0.0008*	0.004*	0.0008*
Upper	0.0199	0.0069	0.0167	0.0024	0.005	0.001	0.007	0.001
Lower	0.0034	0.0004	0.0033	-0.001	0.002	0.0005	0.002	0.0003

independently.

* Indicates that the 95% confidence intervals did not cross zero

Table 3. Estimates of genetic diversity in mosquitofish collected from the southern Everglades among regions (Water Conservation Area (WCA), Shark River Slough (SRS), and Taylor Slough (TS)). The same locations were sampled in 1996 and 1999 whenever possible. Θ_{ST} indicates the genetic variance among samples relative to the total variance detected within the region. Time was not included in this model in order to assess spatial effects within each sampling time and water management region independently.

	Allozymes 1996	Allozymes 1999	Microsat 1996	Microsat 1999
	θ_{ST}	θ_{ST}	θ_{ST}	θ_{ST}
WCA	0.020*	0.002	0.003	0.006*
SRS	0.010*	0.015*	0.004*	0.002
TS	0.005	0.011	0.002	-0.003

* Indicates that the 95% confidence intervals did not cross zero

Table 4. Estimates of genetic diversity in mosquitofish collected from the southern Everglades among sites recently affected by a dry-down event and undisturbed sites. The same locations were sampled in 1996 and 1999 whenever possible. Θ_{ST} indicates the genetic variance among samples relative to the total variance detected within each data set. Time was not included in this model in order to assess spatial effects within each sampling time and data set.

	Allozymes 1996	Allozymes 1999	Microsat 1996	Microsat 1999
	θ_{ST}	θ_{ST}	θ_{ST}	θ_{ST}
Undisturbed	0.019*	0.011*	0.005*	0.004*
Dry-Down	0.007	0.009	0.001	-0.001

* Indicates that the 95% confidence intervals did not cross zero.

Figure Legends

Figure 1. Map illustrating sampling sites. Sites symbols indicate sites that dried between 1996 and 1999 sampling events.

Figure 2. Illustration of mosquitofish population dynamics from three of the 20 study sites, one from each of the three 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 regions dried and mosquitofish populations were locally extirpated. Hydrographs for these sites are in Chick et al. (2004, fig. 2) and sampling methods are described in Wolski et al. (2005).

Figure 3. Fixation Index (F_{IS}) reported separately by the history of site drying in 1999. Positive values indicate an excess of homozygous individuals within a population. A. Allozymes. B. Microsatellites

Figure 4. Pairwise F_{ST} values from 1996 plotted against pairwise F_{ST} for the same pair of sites in 1999. Pairs where one or both of the two sites dried in 1999 are plotted as filled points. Dotted line indicates pattern if there was no change in pairwise F_{ST} and the solid lines indicate the observed relationship and solid line indicates observed linear relationship. A. Allozymes B. Microsatellites.

Figure 5. Pairwise F_{ST} plotted against the geographic distance (kms) separating the pairs of sample sites. Horizontal dashed lines indicate arbitrary points separating bulk of data from high values of F_{ST} . A. 1996 Allozyme data B. 1999 Allozyme data.

Figure 6. Conceptual model of extinction-recolonization dynamics in the Everglades. A. Map of vegetation mosaic in cross-section of northern Everglades National Park.

The black areas are dense sawgrass covered ridges and white are deeper sloughs. Fish must move around ridges to move from short to long-hydroperiod sites (reprinted from Trexler et al. 2001). B. Cartoon of metapopulation structure with a patchy core population and satellite populations sending colonists in times of drying. Regional core populations may exchange few migrants (dotted arrows) and inter-slough migration may be limited in times of stable water levels.

Figure 1

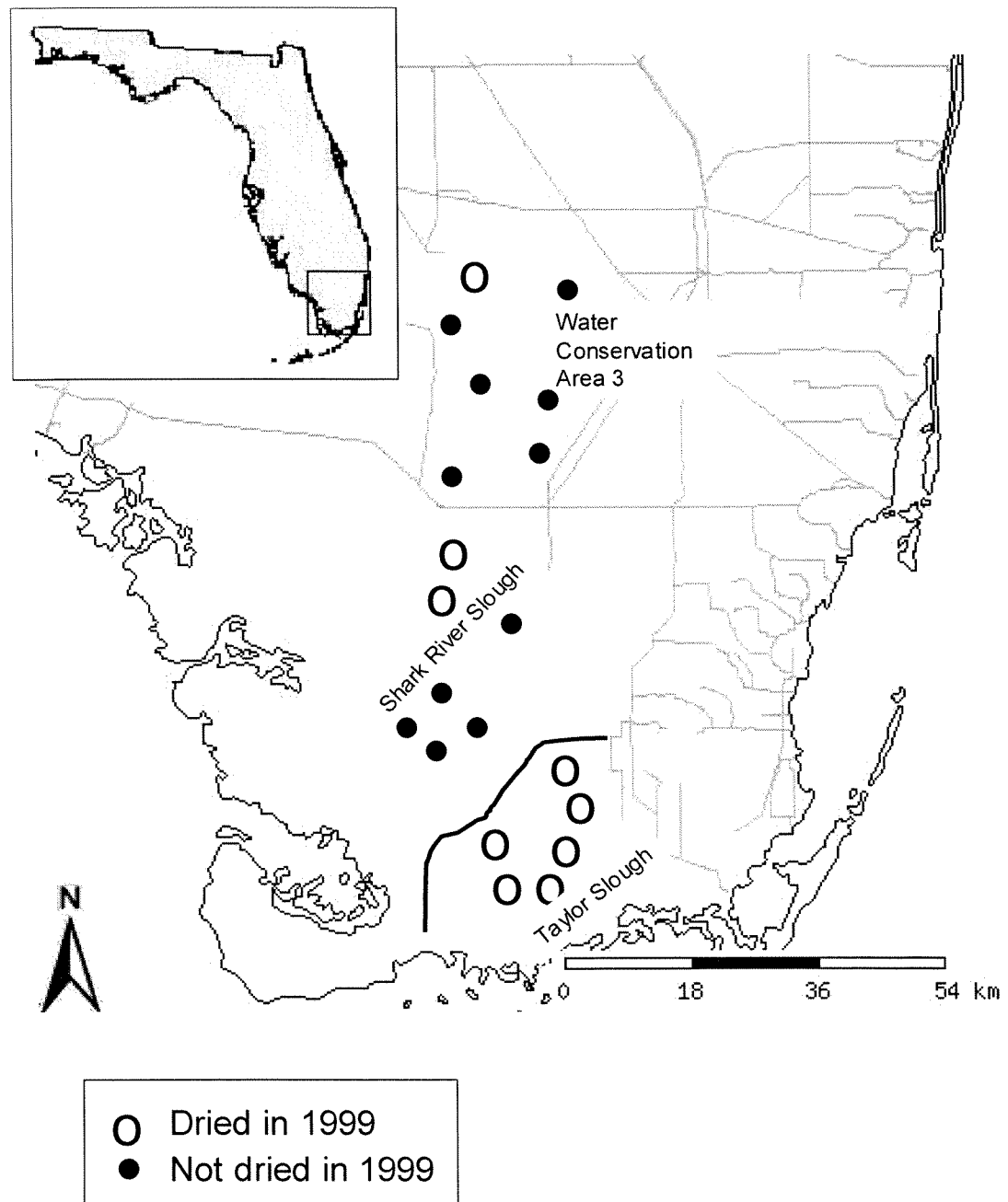


Figure 2

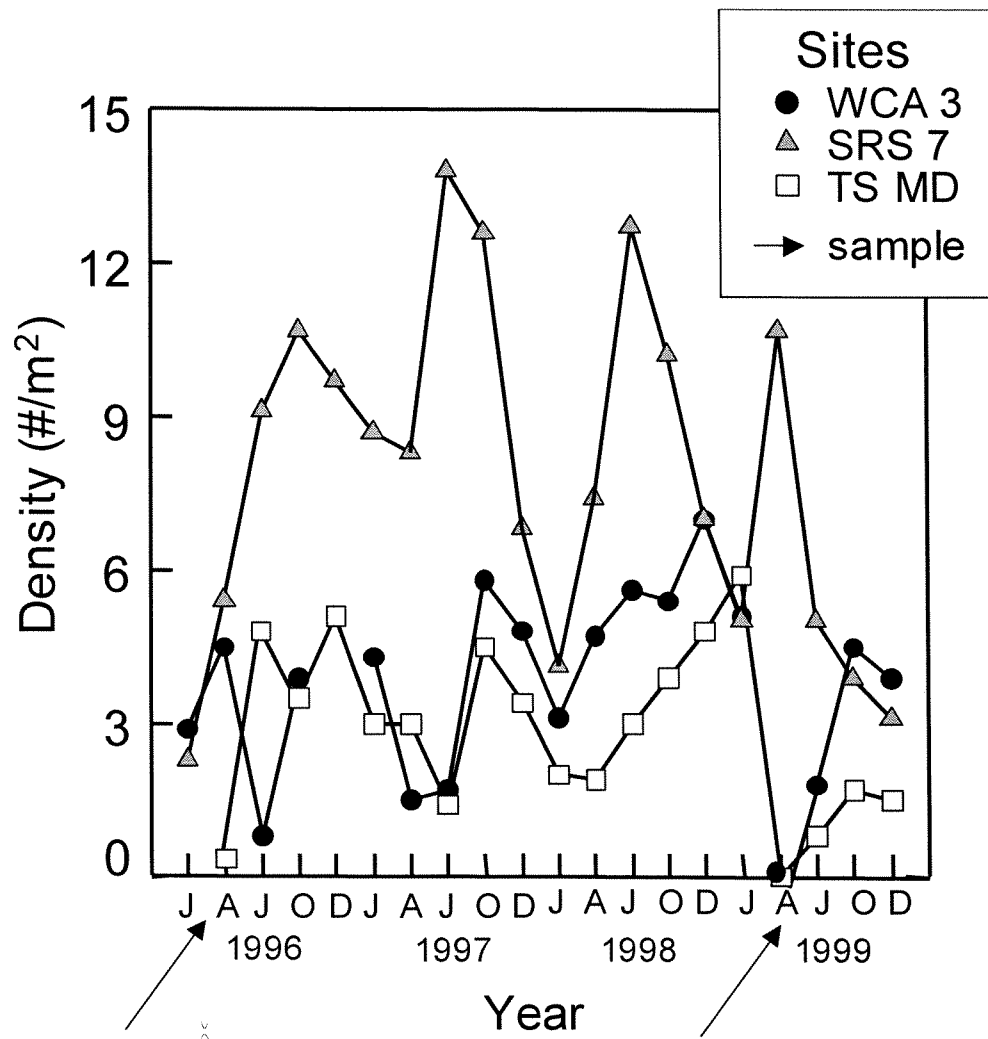


Figure 3

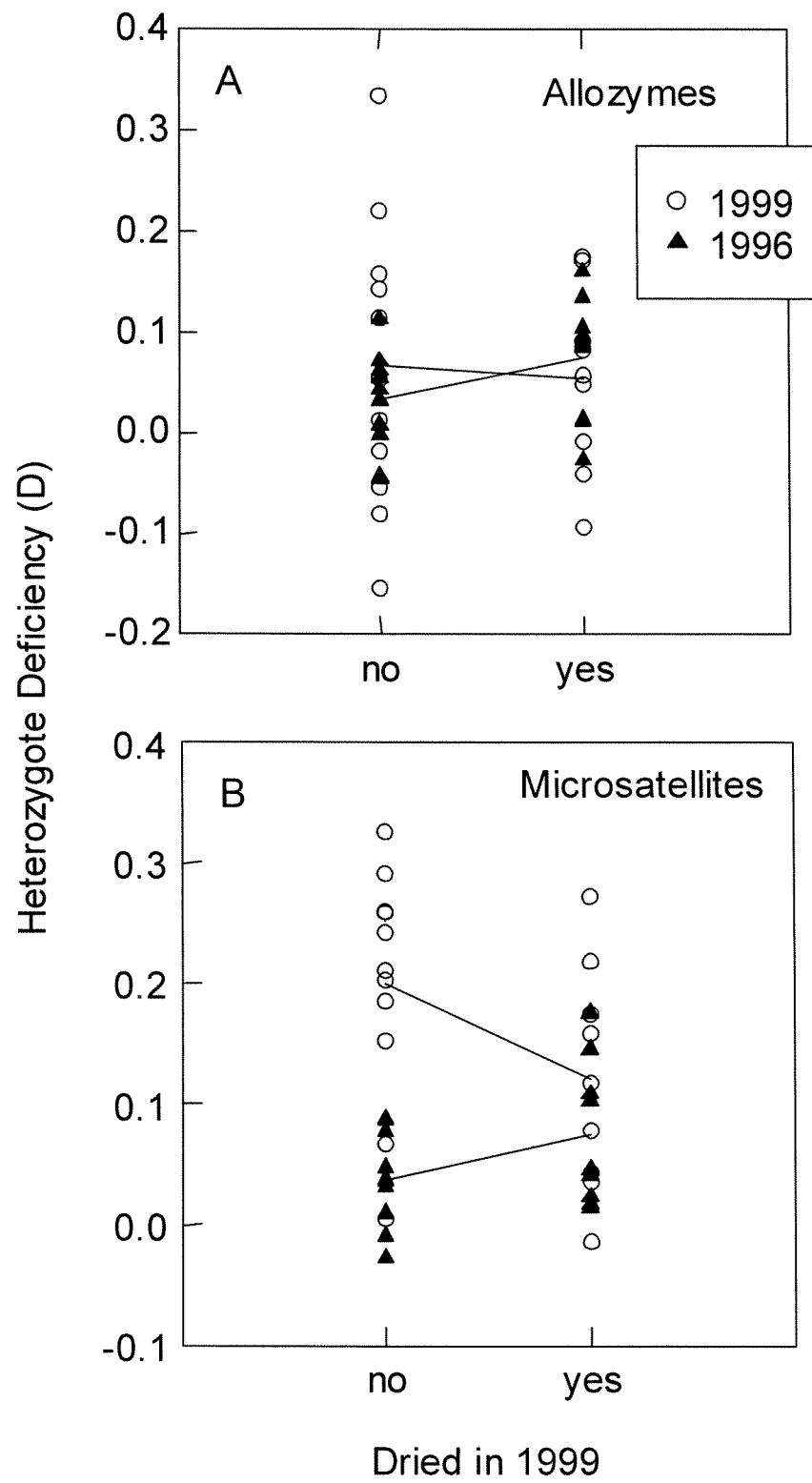


Figure 4

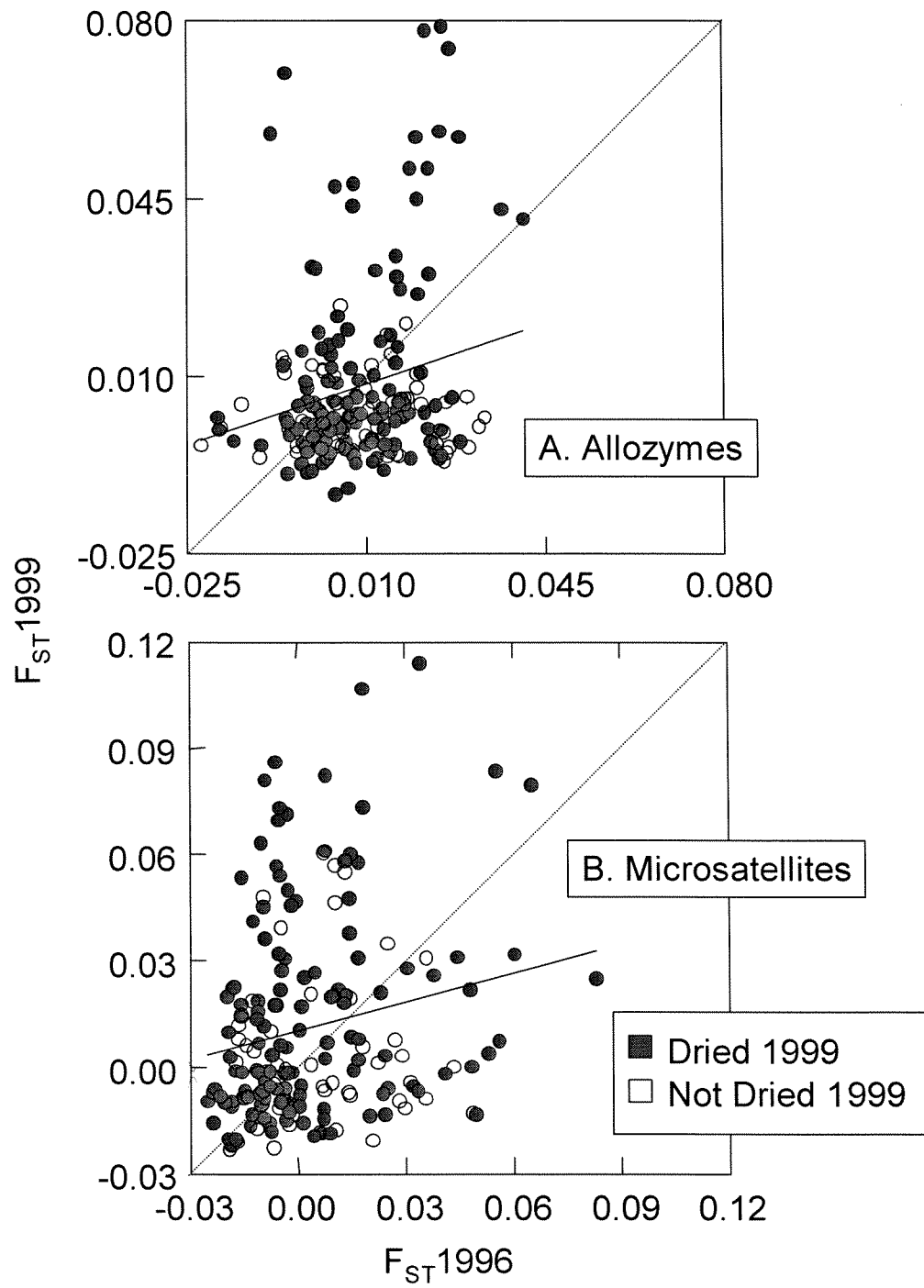


Figure 5

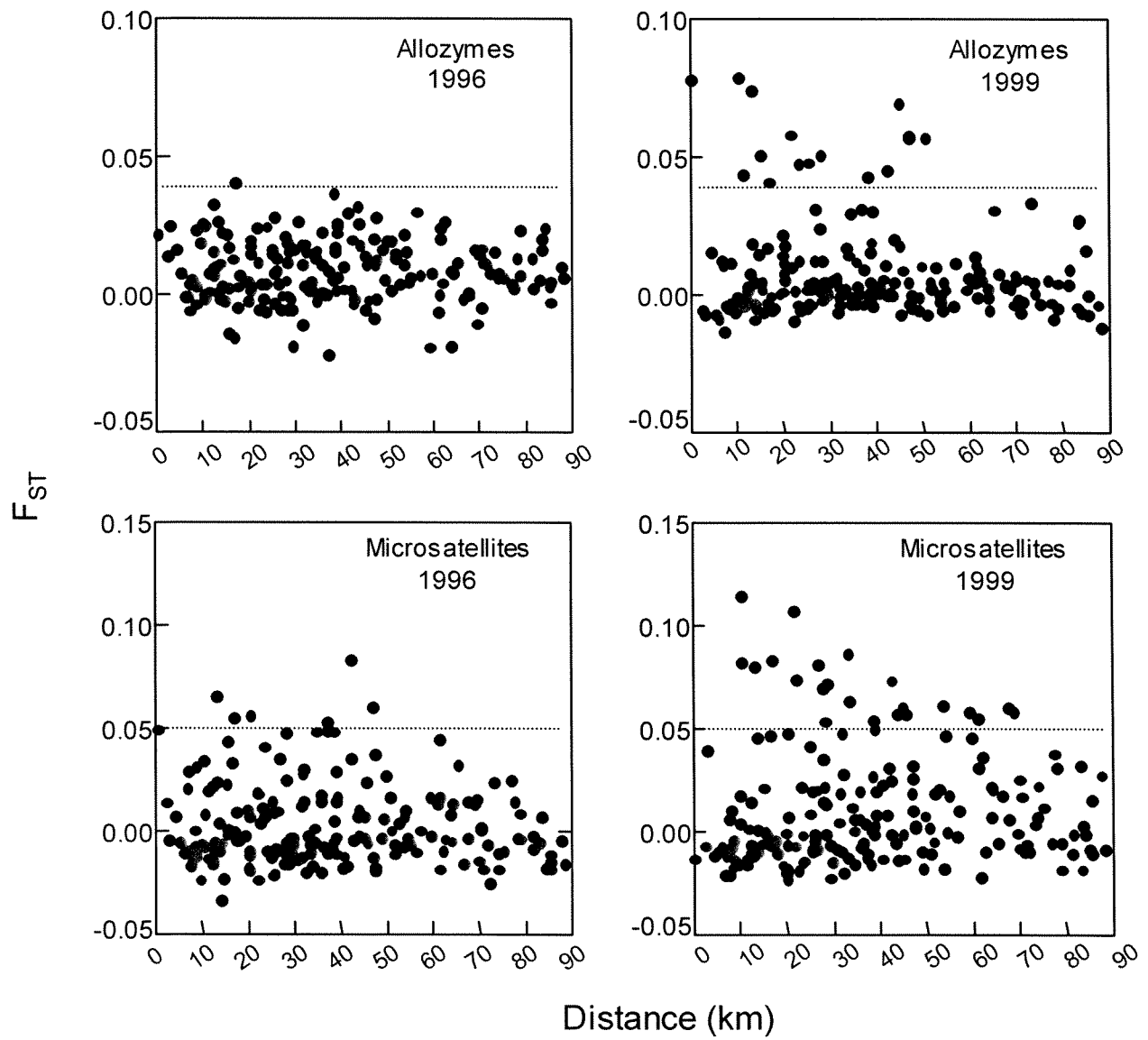
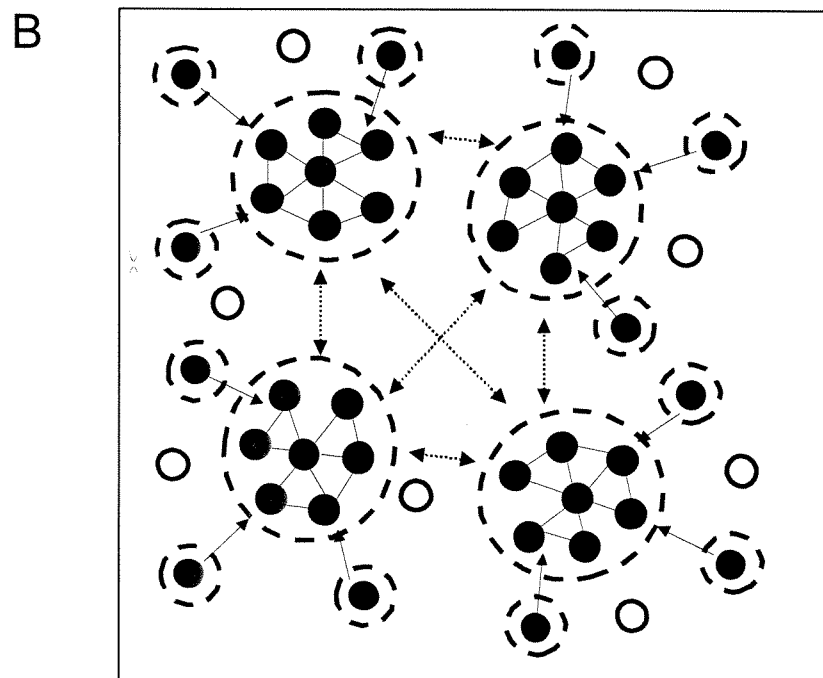
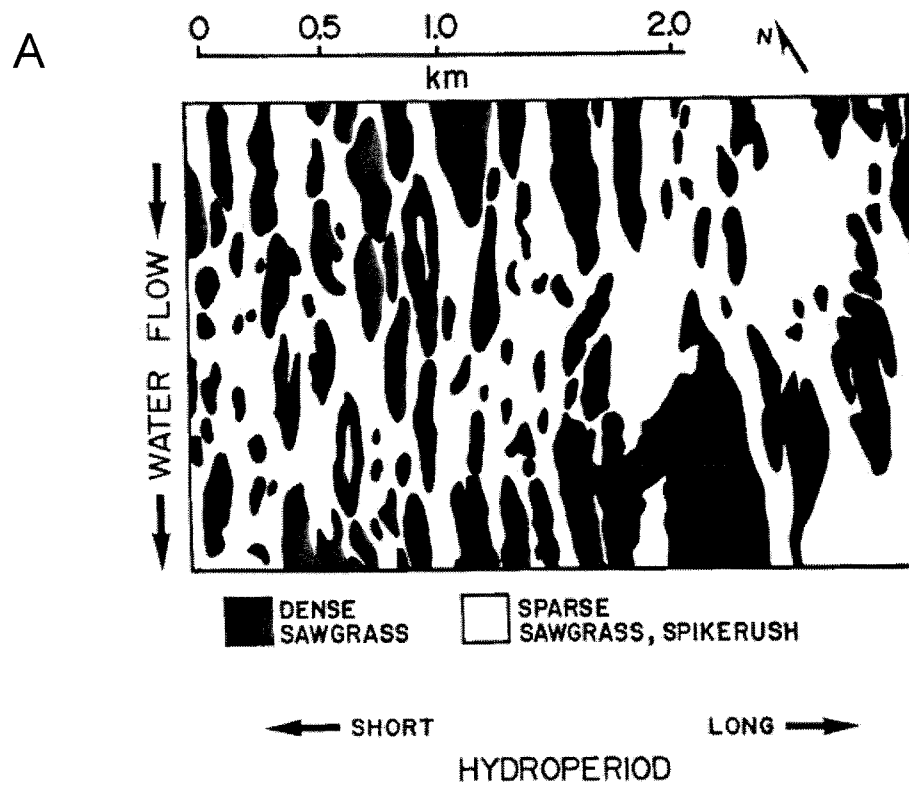


Figure 6



III. Population Genetics and Metapopulation Dynamics of Bluefin Killifish in a Seasonally Variable Wetland

Running Head: Killifish metapopulation dynamics in a wetland

Title: Population Genetics and Metapopulation Dynamics of Bluefin Killifish in a
Seasonally Variable Wetland

Douglas Creer and Joel Trexler

Department of Biological Sciences
Florida International University
Miami, FL 33199

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Abstract

Patterns of extinction and recolonization may affect local population genetic structure, depending on the mode of recolonization. This provides a potential tool for investigating metapopulation dynamics in localized populations. The bluefin killifish (*Lucania goodei*) is a common and widespread species of southeastern North America that prefers shallow-water habitat but quickly disappears from seasonally-flooded wetlands during drydown events. The short lifespan and generation time of this species allow its populations to show genetic differentiation in response to events on an annual time scale. In the Florida Everglades, in which this species is common, local sites commonly dry down during the annual dry season; however, the exact sites subject to drydown in a given year vary depending on weather-related and anthropogenic influences. We investigate the population dynamics of bluefin killifish in this variable environment using six microsatellite markers. We report data from fish collected in two successive years characterized by large differences in overall patterns of drying and rehydration. We found that small but significant genetic differentiation is detectable among populations, and that these differences strongly relate to hydrological patterns. These patterns of differentiation are not stable over time. In particular, widespread extinction and recolonization, driven by drydown events, leads to a loss of genetic differentiation between populations. We present a model for migration patterns of bluefin killifish in the Everglades, and discuss the significance of our findings with regard to the genetic consequences of extinction-recolonization dynamics. Our findings illustrate the importance of temporal genetic data

for analyzing metapopulation structure in dynamic environments, and the utility of correlating it with data from field sampling studies and environmental monitoring programs.

Introduction

Spatial structure involving local extinction and recolonization dynamics is characteristic of populations in nature, and much effort has been spent in characterizing this structure and its potential consequences. The metapopulation concept represents a major advance in this effort, and much attention has been devoted to creating models describing it and its consequences (Hanski & Gilpin 1991; REFS). However, there has also been debate over the applicability of such models in describing actual populations in the field (Harrison 1991; Hastings & Harrison 1994; Baguette 2004). Much of the application of metapopulation theory has been in assessing extinction risk for declining species occupying fragmented habitats (Baguette 2004). Current mathematical models appear to apply best to highly fragmented populations, in which migration between subpopulations is very limited (Hanski 2004).

Extinction-recolonization dynamics may play a major role in shaping populations that do not resemble the "classic" metapopulation first described by Levins (1969), and various conceptual models have been proposed to describe alternative forms of metapopulations (Harrison 1991). The rate of dispersal and recolonization of empty habitat patches is a critical factor. At high levels of dispersal, such that most individuals will migrate between patches during their lifespans, a panmictic "patchy population" results which does not exhibit the traits attributed to metapopulations (Harrison 1991). In nature, populations may exhibit a mix of patchy-population and metapopulation characteristics if migration rates vary among patches (Sutcliffe et al. 1997a).

Extinction-recolonization dynamics affect the genetic structure of populations in different ways, depending on the mode of recolonization and other factors (Slatkin 1977; Wade & McCauley 1988; Harrison & Hastings 1996). Slatkin (1977) noted that recolonization patterns fall between two extremes, a “propagule-pool” pattern in which new colonists come from a single source population, and a “migrant-pool” pattern in which new colonists represent a random mix of individuals from all potential source populations. The former pattern of recolonization leads to genetic structure being strongest in newly recolonized areas; under the latter pattern, genetic structure is stronger in undisturbed populations (Wade & McCauley 1988; McCauley et al. 1995). Extinction and recolonization may increase or decrease overall genetic structure of the metapopulation, depending on the specific pattern of recolonization, which in turn is linked to the overall likelihood of dispersal (Harrison & Hastings 1996).

While most metapopulation models regard habitat quality as constant, some effort has been made to address the effects of disturbance and habitat dynamism (e.g. Sutcliffe et al. 1997b; Amarasekare & Possingham 2001; Johst & Drechsler 2003). Many natural habitats are subject to recurring catastrophic events that may cause sudden extinction of local populations, and these events have the potential to drive metapopulation dynamics (Harrison 1991). Wetlands that experience periodic expansion and contraction due to variable precipitation and water flow may experience such disturbance events at a variety of time scales, and these disturbance patterns are known to have strong effects on the aquatic organisms found there (Wellborn et al. 1996; Corti et al. 1997).

The Everglades is a large wetland that covers much of south Florida. Its water is supplied by rainfall and by sheet flow from Lake Okeechobee at the north end of the system to Florida Bay at the south. At present, the Everglades region is subdivided by canals and levees that have been constructed during the last century. The system is divided into several water management units, with water flow between units subject to artificial manipulation. In addition, the southernmost part of the ecosystem, Everglades National Park, is divided into two natural drainages separated by a levee and a broad expanse of slightly higher ground (Loftus & Kushlan 1987). The westernmost of these, Shark River Slough (SRS), receives water from the Water Conservation Areas (WCA) to the north via artificial water-control stations that pump water across the levee that separates these regions. The smaller Taylor Slough (TS) at the southeast edge of the region receives water pumped from canals situated in developed areas on the border of the present-day wetlands (Trexler et al. 2002). Besides dividing the system, the canals also serve as permanent deepwater habitats, which influence population dynamics of fish species found there (Trexler et al 2002; Ruetz et al., 2005)).

Within the water management units, fish habitat in the Everglades is further fragmented by natural features of the environment related to small differences in elevation. Sloughs and flooded prairies dominated by spikerush (*Eleocharis cellulosa*) occupy the lower ground and provide habitat for most of the small fish in the ecosystem. This habitat is found in patches interrupted by more extensive areas of slightly higher ground dominated by dense sawgrass stands (*Cladium jamaicense*) and tree islands, which support relatively few fish and may hinder fish movement (Trexler et al. 2002). Average elevations tend to be lower and water depths greater along the central paths of

major drainages relative to the periphery of the system. Areas of deeper water also occur where creeks cut flow channels through the shallow prairies, in solution holes, and in small pondlike holes maintained by alligators (*Alligator mississippiensis*) that hold water during the dry season.

South Florida exhibits a strong wet-dry seasonality, with 75-85% of precipitation falling from May to October (Loftus & Kushlan 1987; Duever et al. 1994). The extent of flooded land varies seasonally, with maximal flooding occurring in the wet season and large portions of the landscape drying annually during the dry season. While the specific sites that dry down in any given year vary, the likelihood of drying events varies among sites. Sites located on relatively low ground, especially near the center of the major drainages, rarely dry completely, while sites nearer the periphery of the drainages are subject to frequent drydown events, leading to extirpation of aquatic animals (Trexler et al. 2002; Ruetz et al. 2005). Specific patterns of rainfall and anthropogenic factors related to water management may affect any particular site's chance of drying in a given year. In particular, the total amount of annual precipitation varies considerably, so that in some years much of the system dries and in others most sites remain flooded. In one sense, therefore, disturbance of aquatic habitats in this system is seasonal, but this variation in annual precipitation means that many local sites experience disturbance frequently but unpredictably (Trexler et al in press).

The source of new fish colonists in recently reflooded sites is a question that has not been fully answered in the Everglades. Trexler et al. (2002) proposed that large fishes migrate long distances to deepwater sloughs and canals during dry seasons, while small fishes, which presumably have less ability to move long distances, seek refuge

from drying in local alligator holes or creek channels. Radiotelemetry data on large fish has confirmed that these may migrate long distances (on a scale of tens of kilometers) in a matter of days. This technique cannot be used on smaller fishes because of their size, and the large population sizes of small fish species in the Everglades makes mark-recapture techniques impractical. Genetic markers may provide a better approach to study migration patterns in these species (Trexler et al. 2002).

The bluefin killifish (*Lucania goodei*) is a characteristic small (20-30mm) fish of the Everglades. It occurs throughout the system in suitable habitat, especially in shallow water with moderate vegetative cover, typically found in the flooded prairies and the edges of canals and deepwater sloughs (Loftus & Kushlan 1987). Regular surveys of aquatic life in the Everglades combined with measurement of hydrology show that *L. goodei* disappears quickly from marsh habitats during drydown events, and populations are slow to recover relative to sympatric fish species, taking several years to reach maximum density following a drying event (Ruetz et al. 2005, Trexler et al. in press).

Bluefin killifish may reach sexual maturity at three months post-hatching (Fuller & Travis 2004), and breeding occurs throughout most of the year (Foster 1967). Reproduction in this species is therefore not correlated with the disturbance regime, and multiple generations are likely to pass between disturbance events at most sites in the Everglades.

Genetic studies have previously been conducted on two other species of fish in the Everglades (Trexler et al. 2002; McElroy et al. 2003, in preparation). Both species differ greatly from bluefin killifish in natural history. One, the spotted sunfish (*Lepomis punctatus*) is a large-bodied fish which presumably has greater dispersal capability than

smaller-sized species; the other, the eastern mosquitofish (*Gambusia holbrooki*) is less subject to extinction in sites that dry down and recolonizes such sites much faster than bluefin killifish (Trexler et al. 2002; Ruetz et al. 2005). Population dynamics of bluefin killifish in the Everglades are strongly linked to drying history, while those of the other two species are not (Ruetz et al. 2005).

We report genetic data from collections of bluefin killifish made in two consecutive years that differed significantly in their hydrological dynamics. The first collections were made a few months following a severe dry period in which all short-hydroperiod collection sites (as defined by Ruetz et al. 2005) and several long-hydroperiod sites experienced drydown and consequent local extinction of fish. The dry season that intervened between these collections and those taken one year later was much less severe, and very few sites experienced drying in the interim period. We used microsatellite data to investigate patterns of genetic divergence among bluefin killifish populations sampled in both years. Our goals were to determine the pattern of recolonization following drying of sites, identify the scale at which genetic divergence occurs in this species, and characterize its population dynamics in relation to the physical properties of the landscape.

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Materials and Methods

We collected samples of bluefin killifish from 20 sites within the Everglades, including Water Conservation Area (WCA) 3A, Shark River Slough, Taylor Slough and the edges of canals bordering WCA 3A and WCA 3B (Fig. 1). Each site was located in

slough or wet prairie habitat, and the sites represent a sample of the many habitat patches available for this species in the study area. Collections were made in the early dry season (late November and December) in 2002 and 2003. We collected at least 20 fish at each site using minnow traps, hand nets, and small bar seines. Collected fish were placed on ice in the field and transferred to a -80°C freezer upon return to the laboratory. We excised caudal fins and peduncles from 20 fish from each site and extracted whole genomic DNA as described in Creer & Trexler (in review).

We developed four new microsatellite markers for bluefin killifish (designated Lg1, Lg4, Lg5, and Lg6) as described in Creer & Trexler (in review). In addition, we screened 30 loci developed in pupfishes (*Cyprinodon*) by Burg et al. (2002) to determine their suitability for work with this species. We amplified DNA by PCR from 14-20 individuals taken from Water Conservation Area 3A and Shark River Slough. Products were electrophoresed on 6% polyacrylamide slab gels and visualized by ethidium bromide staining. Two loci (AC17 and AC25) amplified reliably in bluefin killifish and proved to be polymorphic.

We amplified DNA from each sample in reactions in which either the forward or the reverse primer was labelled at the 5' end with fluorescent dye (6-FAM, HEX, or NED; Applied Biosystems and Integrated DNA Technologies). Reaction protocols are described in Creer & Trexler (in review). Each of the six loci were amplified in separate reactions. Reaction products (0.1-0.5µl) were mixed with 10µl high-density formamide along with a ROX-labelled size standard and analyzed on an ABI 3100 automated DNA sequencer (Applied Biosystems). In most cases, products from two PCRs using different fluorescent dyes were co-loaded into a single sample for analysis. Results of these

analyses were visualized using GeneScan 3.0 (Applied Biosystems), and allele identities were determined by eye. Where samples failed to amplify satisfactorily, this procedure was repeated up to two times, usually with annealing temperature lowered one degree and/or MgCl_2 concentration raised to 5mM, and with 1-2 μl of the products loaded to the automated sequencer.

We checked for linkage disequilibrium between loci and evaluated fit of the data to Hardy-Weinberg using GenePop v.3.2 (Raymond & Rousset 1995; see Creer & Trexler in review). We determined F_{st} values and assessed confidence intervals by bootstrapping using Genetic Data Analyzer (GDA; Lewis & Zaykin 2001). We chose F_{st} as the best estimator of differentiation because of the low level of genetic differentiation expected in this system (Balloux & Goudet 2002). In addition to assessing F_{st} for the entire region sampled in each year, we calculated values for the canal populations and for each major marshland subregion (WCA, SRS, TS) considered separately. We compared differentiation between and within subregions, calculating the global F_{st} in analyses in which all samples from within each subregion were treated as belonging to a single population, and comparing the resulting value to the mean of the F_{st} values from the three marshland subregions.

To determine whether the recolonization mode fits a migrant-pool or propagule-pool pattern, we compared genetic differentiation in disturbed versus undisturbed sites in both years. We assigned populations according to whether they had dried out in the year prior to sampling, and compared F_{st} values derived from dried-out versus undisturbed sites across the study area and (where applicable) within subregions. We expected to find greater divergence among newly recolonized populations than among undisturbed

populations under a propagule-pool model, and the opposite pattern if recolonization follows a migrant-pool pattern (Wade & McCauley 1988; McCauley et al. 1995). In a related analysis, we classified sites according to hydroperiod, using the classification of Ruetz et al. (2005). Two sites not considered in that paper (Pah-Hay-Okee and Invertebrate Site; see Fig. 1) were both classed as short-hydroperiod (unpubl. data). Canals were treated as long-hydroperiod sites. We calculated F_{st} among long-hydroperiod sites and among short-hydroperiod sites in both years. We also calculated F_{st} among short-hydroperiod sites that had not dried in the previous year using the 2003 data. In all cases, we examined populations both across the entire study area and within those subregions that had multiple populations in each category.

To examine stability of patterns of differentiation over time, we generated pairwise F_{st} values for all possible population pairs using GenePop v.3.2 (Raymond & Rousset 1995), and graphed the results, with the values from the 2002 collections on the x-axis and the corresponding values from the 2003 collections for each pair on the y-axis. Both of the populations that were not sampled in 2002 were excluded from this analysis. Increased average genetic differentiation in the 2003 samples was expected to yield a regression line with slope greater than one; conversely, a slope less than one would indicate less average differentiation in the 2003 samples.

Results

We collected complete samples of bluefin killifish from 18 sites in 2002 and from all 20 sites in 2003; a sufficient number of specimens could not be obtained from two of the short-hydroperiod sites in 2002 (Pah-Hay-Okee in SRS and the invertebrate site in

WCA; Figure 1). Drought during the year immediately preceeding the 2002 collections was severe, with 12 sites drying (defined as mean water depth <5cm). In contrast, in 2003 only two sites dried, and water levels throughout the system were generally higher than in the previous year.

Three of the six loci used exhibit strong heterozygote deficiency in all populations; the others do not deviate from Hardy-Weinberg expectations (see Creer & Trexler, in review, and Table 1 for details). Heterozygote deficiency in some, but not all, loci suggests the presence of null alleles. Redesigning primer sites had no effect on heterozygosity. While the effects of including null alleles in studies of broad patterns of population differentiation have not been explicitly characterized, current evidence suggests that they are unlikely to introduce significant bias in the results (Hare et al. 1996; Dakin & Avise 2004).

Overall genetic differentiation across the entire study region was weak in both years, and the bootstrap tests indicated these values are not significantly different from zero. Similarly weak differentiation was found within the major subregions (Table 2). The mean of F_{st} values calculated for populations within the marshland subregions was equal to the F_{st} value calculated between subregions in 2002; in 2003, the within-subregion mean was higher (Table 3).

In all cases, F_{st} values show greater differentiation among undisturbed populations relative to those that have experienced a recent drydown event (Table 4). This result strongly indicates that repopulation of dried sites most closely fits the migrant pool pattern of Slatkin (1997). The F_{st} value for all undisturbed populations in 2002, while relatively small, was significantly greater than zero (Table 4). The results of the

analysis of long- and short-hydroperiod sites are summarized in Table 5. In 2002, F_{st} values among long-hydroperiod sites were higher than among short-hydroperiod sites in all comparisons. In 2003, the opposite pattern was apparent, with sites within SRS providing the lone exception to the general pattern. Undisturbed short-hydroperiod sites in WCA and across the entire region showed the highest F_{st} values.

The slope of the regression line in the comparison of pairwise F_{st} values between years is negative and does not differ significantly from zero (Fig. 2). Patterns of pairwise population divergence in the two years are essentially uncorrelated. This indicates that patterns of genetic differentiation are not temporally stable, a result consistent with the shifts in patterns of divergence noted above.

Discussion

Overall, bluefin killifish in the Florida Everglades show little evidence of population subdivision, with most F_{st} values both overall and among different sets of populations being less than 0.01 (but see O'Reilly et al. 2004, regarding the magnitude of F_{st} values when highly variable microsatellite markers are used). This is consistent with hypotheses proposed by McCauley et al. (1995) and Harrison & Hastings (1996): that high turnover among populations and a migrant-pool pattern of recolonization should act to homogenize populations.

The F_{st} values observed among all populations were fairly similar in both years, albeit with a slight increase in 2003 (Table 2). The marshland subregions also seem to show an increase in genetic structure between years, though the canal sites do not. However, the results of the pairwise F_{st} comparison indicates that genetic structure at the

population level is highly unstable between years. Comparisons focusing on sites with similar hydroperiod characteristics (Table 5) shows that the slight increases observed in genetic structure is driven by a significant increase in differentiation among populations inhabiting short-hydroperiod sites, and that patterns of differentiation among sites grouped by hydroperiod changed greatly between years.

The pattern of differentiation summarized in Table 5, especially the shift in relative magnitude of F_{st} values among long- and short-hydroperiod sites between the dry and the wet years, suggests a model for population dynamics of bluefin killifish in the Everglades (Fig. 3). The model postulates that under typical flooded conditions, killifish in the core part of the system exist as a patchy population, with high levels of migration between areas of suitable habitat. Populations in peripheral short-hydroperiod habitat patches are more isolated from each other and the core populations (Fig. 3A). The system resembles a continent-island metapopulation under these conditions. During a severe drydown, however, the peripheral populations become extinct, and reduced water levels in the core region lead to fragmentation of the habitat, greatly curtailing migration among patches (Fig. 3B). Under drought conditions, the fish inhabiting the core region begin to resemble a classic metapopulation instead of a patchy population, if only briefly. After recovery from the drought, which might take one to two bluefin killifish generations, the populations in the core region begin again to exchange large numbers of migrants and revert to a patchy population, while each of the peripheral habitats is repopulated by migrants originating in multiple core-region patches (Fig. 3C). The greatest degree of genetic differentiation under this model occurs among short-hydroperiod sites following a long period without a severe drydown. The exception to

this in the present data is observed in SRS, where the lack of differentiation among the two short-hydroperiod sites that were undisturbed in 2003 (SRS 8, SRS 37) may be explained by their close geographic proximity, and therefore, their presumed greater capacity to exchange migrants (Fig. 1, Table 5).

Under this model, population structure of bluefin killifish in the Everglades is shaped primarily by hydrologic patterns and not by the more recently imposed anthropogenic subdivisions of this region. This also appears to be the case in both species from this region in which genetic structure has previously been investigated, though these species do not appear to show the pattern of extinction-recolonization dynamics seen here (McElroy et al. 2003; in preparation). The results summarized in Table 3 confirm the absence of strong genetic structure among subregions. This pattern may be because the levees that divide the subregions are not a strong barrier to fish migration, or because the subregions have been separated for too short a time for significant differentiation to occur (McElroy et al. 2003). Both bluefin killifish and eastern mosquitofish have shorter generation times than the spotted sunfish on which the latter hypothesis was based, and have undergone about 80-120 generations since the construction of the current water-control structures separating the WCA subregion from SRS and TS. This suggests that significant migration may occur through the pump stations that move water from WCA into SRS, and through the culverts that link SRS to TS. The open water that is found in the vicinity of these structures offers little or no preferred habitat for bluefin killifish and other small fishes, and harbors high concentrations of predatory large fish (JC Trexler, pers. obs.). However, no direct estimation of small-fish migration across these structures has been made.

The fine-scale refuge hypothesis proposed by Trexler et al. (2002) for small-bodied fish species in the Everglades does not appear to be consistent with the current data for this species. If the repopulation of the short-hydroperiod sites following drydown and rehydrations were accomplished primarily or solely by reproduction of remnant populations within the site, we would expect F_{st} values among these sites to be consistently high. The absence of temporal stability in population differentiation patterns (Fig. 2) also suggests turnover rather than a remnant-survival strategy (Østergaard et al. 2003). The low level of differentiation observed among recently dried sites strongly suggests that the primary source of fish in reconstituted populations is dispersal from multiple undried sites, which are likely to be located some kilometers distant. While this does not imply that bluefin killifish are capable of the long-range migrations observed in large-bodied fish in the Everglades, it does demonstrate a capacity for dispersal greater than previously suspected (Trexler et al. 2002). This result in bluefin killifish also suggests important differences between this species and the eastern mosquitofish, which appears to have a refuge-survival strategy in sites that do not dry completely (McElroy et al. in preparation).

The drying out of the marshland associated with local extinction in the Everglades represents a spatially correlated form of disturbance, which is considered a particular threat to the persistence a metapopulation (Harrison 1991; Johst & Drechsler 2003). The persistence of bluefin killifish under these conditions is due to the fact that while drying of the system affects all habitat patches, it does not affect them all equally. Drought events are likely to cause extinction at shallow, short-hydroperiod sites, but the deepest sites do not completely dry out even in severe droughts, and are essentially immune to

extinction over a moderately long time scale (Trexler et al. in press; OTHER REFS?). Schoener & Spiller (1987) demonstrated that metapopulations may be highly persistent even in the presence of consistently high levels of population turnover as long as a subset of the populations involved does not experience extinction. While the latter work considered turnover that was the apparent result of demographic stochasticity in small populations, the present study shows that frequent spatially correlated disturbances combined with variable site responses to disturbance may produce a similar pattern. Sutcliffe et al. (1997b) present an example of heterogeneous habitat preserving a metapopulation during a spatially-correlated disturbance event. It is worth noting that this study involved a system with mixed metapopulation and patchy-population attributes, though in this case the disturbance did not last long enough (<1 generation) to consider whether dispersal patterns changed during its course. Habitat gradients such as that from short- to long-hydroperiod are common in nature (e.g. Wellborn et al. 1996). This fact, coupled with the importance of disturbance as a cause of turnover (Harrison 1991), implies that the pattern seen in bluefin killifish in the Everglades may be common.

The apparent changes in migration patterns and dispersal ability discussed in the above model of bluefin killifish dynamics illustrates the necessity of using temporal data to investigate metapopulation structures in dynamic habitats. Most studies of genetic structure of populations involve samples from a single time period and assume stability of patterns of genetic differentiation over time (Tessier & Bernatchez 1999; Østergaard et al. 2003). Where studies have used samples of individuals collected over a time span greater than the species' generation time, stable patterns of differentiation have been observed in a number of instances. However, significantly, where unstable habitat

conditions exist, studies have shown that patterns of genetic structure are not stable over time, giving evidence that extinction-recolonization dynamics are important in these populations (Østergaard et al. 2003; and references therein). The present study also shows the importance of sampling at times when a dynamic habitat exhibits conditions representing different segments of its normal range. The distinct patterns showing the loss of genetic structure in recently dried-down sites, and the elevated structure among fish inhabiting long-hydroperiod sites, would not be apparent had we not sampled in both a relatively dry year and a much wetter one. A further element in the present study is the combination of field-sample and environmental data with population-genetic data. The former can demonstrate extinction-recolonization dynamics directly and show their environmental causes and correlates (e.g. Sutcliffe et al. 1997a,b). The latter allows inference of specific migration patterns influencing metapopulation dynamics, particularly in species with very large populations, and shows the genetic effects of those dynamics. Because of the extensive and varied data collection required, this approach has not been commonly used (but see McCauley et al. 1995). However, it shows considerable promise for understanding the complexities of metapopulation dynamics in nature.

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References

- Amarasekare P, Possingham M (2001) Patch dynamics and metapopulation theory: the case of successional species. *J. Theor. Biol.*, **209**, 333-344.
- Baguette M (2004) The classical metapopulation theory and the real, natural world: a critical appraisal. *Basic and Applied Ecology*, **5**, 213-224.
- Balloux F, Goudet J (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. *Mol. Ecol.*, **11**, 771-783.
- Burg TM, Wilcox JL, Martin A (2002) Isolation and characterization of polymorphic microsatellite loci in pupfish (genus *Cyprinodon*). *Conservation Genetics*, **3**, 197-204.
- Corti D, Kohler SL, Sparks RE (1997) Effects of hydroperiod and predation on a Mississippi River floodplain invertebrate community. *Oecologia*, **109**, 154-165.
- Creer DA, Trexler JC (in review) New polymorphic microsatellite loci in two fish species: bluefin killifish (*Lucania goodei*) and yellow bullhead (*Ameiurus natalis*). *Mol. Ecol. Notes*.
- Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. *Heredity*, **93**, 504-509.
- Duever MJ, Meeder JF, Meeder LC, McCollom JM (1994) The climate of south Florida and its role in shaping the Everglades ecosystem. In: *Everglades: The Ecosystem and its Restoration* (ed. Davis SM, Ogden JC) pp. 225-248. St. Lucie Press, Delray Beach, FL.
- Foster NR (1967) Comparative studies on the biology of killifishes (Pisces, Cyprinodontidae). Unpubl. Ph.D. dissertation, Cornell University, Ithaca, NY.

- Fuller RC, Travis J (2004) Genetics, lighting environment, and heritable responses to lighting environment affect male color morph expression in bluefin killifish, *Lucania goodei*. *Evolution*, **58**, 1086-1098.
- Hanski I (2004) Metapopulation theory, its use and misuse. *Basic and Applied Ecology*, **5**, 225-229.
- Hanski I, Gilpin M (1991) Metapopulation dynamics: brief history and conceptual domain. *Biol. J. Linn. Soc.*, **42**, 3-16.
- Hare MP, Karl SA, Avise JC (1996) Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. *Mol. Biol. Evol.*, **13**, 334-345.
- 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.
- Hastings A, Harrison S (1994) Metapopulation dynamics and genetics. *Ann. Rev. Ecol. Syst.*, **25**, 167-188.
- Johst K, Drechsler M (2003) Are spatially correlated or uncorrelated disturbance regimes better for the survival of species? *Oikos*, **103**, 449-456.
- Levins RA (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America*, **15**, 237-240.

- Lewis PO, Zaykin D (2001) Genetic Data Analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from <http://lewis.eeb.uconn.edu/lewishome/software.html>.
- Loftus WF, Kushlan JA (1987) Freshwater fishes of southern Florida. *Bulletin of the Florida State Museum, Biological Sciences*, **31**, 147-344.
- McCauley DE, Raveill J, Antonovics J (1995) Local founding events as determinants of genetic structure in a plant metapopulation. *Heredity*, **75**, 630-636.
- 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.
- McElroy TC, Trexler JC, Kandl KL, Ruetz CR III (in preparation) Population dynamics and the temporal stability of population genetic structure for the eastern mosquitofish (*Gambusia holbrooki*) in the Florida Everglades.
- O'Reilly PT, Canino MF, Bailey KM, Bentzen P (2004) Inverse relationship between F_{st} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Mol. Ecol.*, **13**, 1799-1814.
- Østergaard S, Hansen MM, Loeschke 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.
- Raymond M, Rousset F (1995) GENEPOP Version 3.2: population genetics software for exact tests and ecumenicism. *J. Heredity*, **86**, 248-249.

- Ruetz CR III, Trexler JC, Jordan F, Loftus WF, Perry SA (2005) Population dynamics of wetland fishes: spatio-temporal patterns synchronized by hydrological disturbance? *J. Animal Ecology*, **2005**, 1-11.
- Schoener TW, Spiller DA (1987) High population persistence in a system with high turnover. *Nature*, **330**, 474-477.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theor. Pop. Biol.*, **12**, 253-262.
- Sutcliffe OL, Thomas CD, Peggie D (1997a) Area-dependent migration by ringlet butterflies generates a mixture of patchy population and metapopulation attributes. *Oecologia*, **109**, 229-234.
- Sutcliffe OL, Thomas CD, Yates TJ, Greatorex-Davies JN (1997b) Correlated extinctions, colonizations and population fluctuations in a highly connected ringlet butterfly metapopulation. *Oecologia*, **109**, 235-241.
- 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, Loftus WF, Jordan F, Chick JH, Kandl KL, McElroy TC, Bass OL (2002) Ecological scale and its implications for freshwater fishes in the Florida Everglades. In: *The Florida Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook* (ed. Porter JW, Porter KG) pp. 153-181. CRC Press, New York.
- Trexler JC, Loftus WF, Perry SA (in press) Disturbance frequency and community structure in a twenty-five year intervention study. *Oecologia*.

- Wade MJ, McCauley DE (1988) Extinction and colonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995-1005.
- Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Ann. Rev. Ecol. Syst.*, **27**, 337-363.

Table 1. Allele number, size range of fragments, sample size, and observed (Ho) and expected (He) heterozygosities for all loci, based on the 2002 collections. Data for the loci AC17 and AC25 are also given based on the 2003 collections; the remainder of the 2003 data are reported in Creer & Trexler (in review).

locus	# of alleles	size range	N	Ho	He
Lg1	32	130-254	360	0.936	0.945
Lg4	42	158-454	339	0.537	0.945
Lg5	30	94-234	343	0.478	0.909
Lg6	58	168-412	349	0.484	0.959
AC17	18	155-199	360	0.828	0.834
AC25	4	147-155	360	0.372	0.361
AC17-2003	17	165-223	400	0.835	0.849
AC25-2003	4	147-155	400	0.305	0.343

Table 2. Population structure within the total study region and within major subregions, expressed as F_{ST} values, and calculated for both years' collections. A * indicates that the 95% confidence interval determined by bootstrapping does not cross zero (a ^ is similar but indicates a 90% confidence interval). Values in parentheses indicate numbers of populations in each category.

	2002	2003
overall	0.0055 [^] (18)	0.0078 [^] (20)
WCA	0.0036 (5)	0.0061 (6)
SRS	0.0053 (6)	0.0069 [^] (7)
TS	0.0003 (3)	0.0104 (3)
canals	0.0080* (4)	0.0074 (4)

Table 3. Fst values calculated between marshland subregions, and the means of Fst values calculated among populations within subregions.

	2002 (dry year)	2003 (wet year)
between subregions	0.0030	0.0020
within subregions (mean)	0.0030	0.0078

Table 4. Differentiation among populations that experienced drydown and those that remained undisturbed, expressed as F_{st} values. All notations are as in Table 2.

	2002 (dry year)	2003 (wet year)
total dried down	0.0034 (10)	0.0007 (2)
total undisturbed	0.0086* (8)	0.0079^ (18)
WCA dried down	0.0030 (2)	NA (0)
WCA undisturbed	0.0044 (3)	0.0061 (6)
SRS dried down	0.0034 (5)	0.0007 (2)
SRS undisturbed	NA (1)	0.0072^ (5)

Table 5. Differentiation among populations in long- and short-hydroperiod sites, and among short-hydroperiod sites that did not experience drydown in 2003. All notations are as in Table 2.

	2002 (dry year)	2003 (wet year)
total long hydroperiod	0.0076 [^] (12)	0.0059 (12)
total short hydroperiod	0.0014 (6)	0.0092 [^] (8)
total undisturbed short hydroperiod	NA	0.0107* (6)
WCA long hydroperiod	0.0044 (3)	0.0010 (3)
WCA short hydroperiod	0.0030 (2)	0.0118* (3)
WCA undisturbed short hydroperiod	NA	0.0118* (3)
SRS long hydroperiod	0.0068 (3)	0.0089 (3)
SRS short hydroperiod	-0.0009 (3)	0.0054 (4)
SRS undisturbed short hydroperiod	NA	0.0012 (2)

Figure Captions

Figure 1. Map of the study area showing subdivisions and collection sites. Solid lines indicate canals and levees.

Figure 2. Graph of F_{st} values of all possible pairs of populations from the 2002 dataset plotted against values for corresponding pairs from the 2003 dataset.

Figure 3. Models of metapopulations dynamics for bluefin killifish in the Everglades.

A. Conditions in high-water year. Migration is common among core slough populations due to deeper water, leading to a patchy population structure.

Populations in short-hydroperiod sites are more isolated.

B. Drought event. Populations in short-hydroperiod regions on periphery of system suffer extinction. Migration among populations in central slough region restricted by low water, which accentuates isolation of habitat patches.

C. Rehydration following drought event. Short-hydroperiod sites receive migrants from multiple sites in the core slough region. High levels of migration restored in core region.

Figure 1

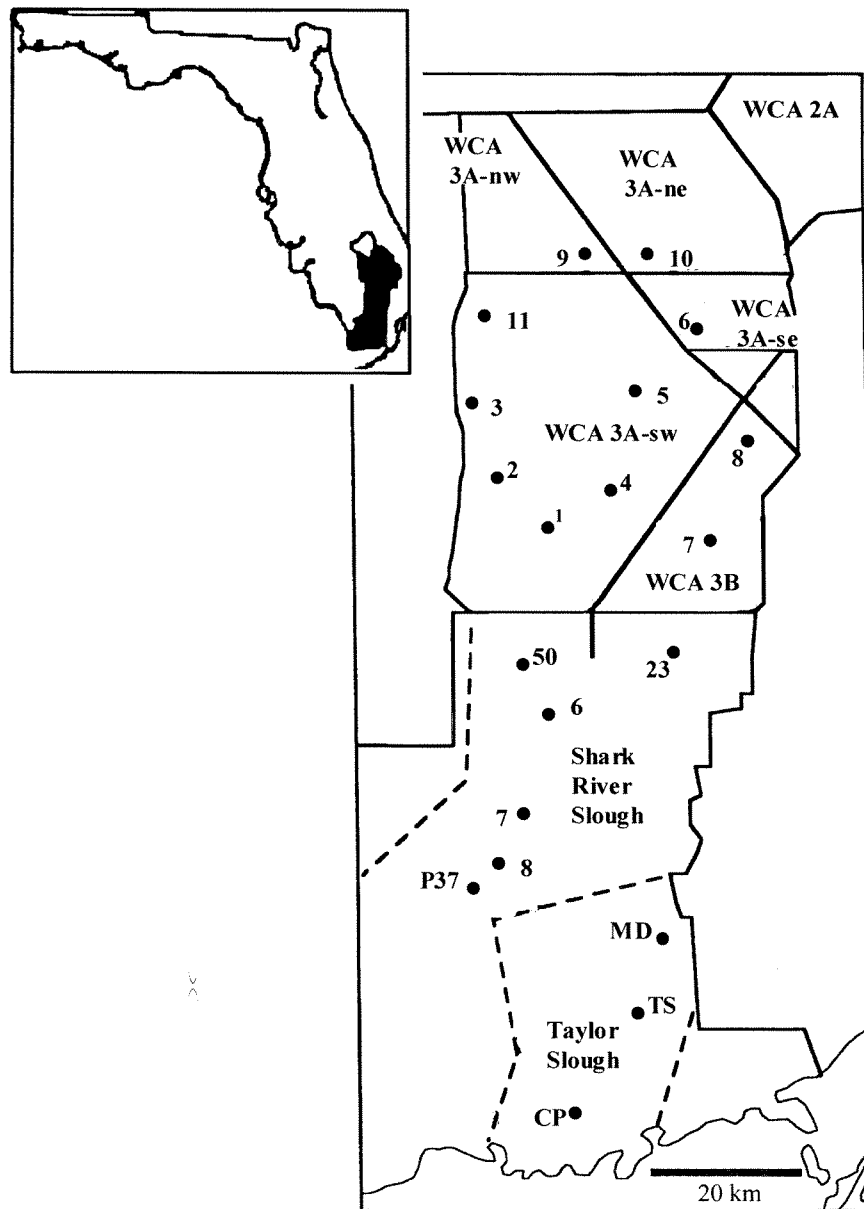


Figure 2

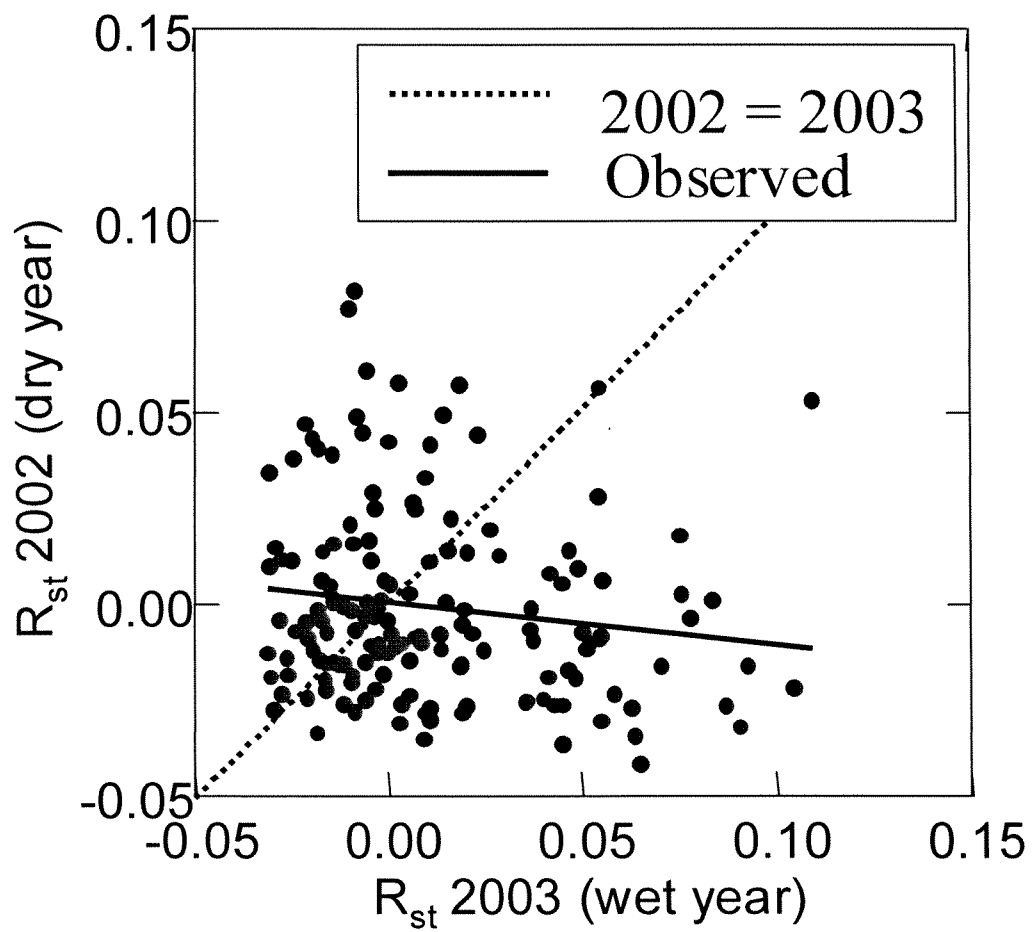
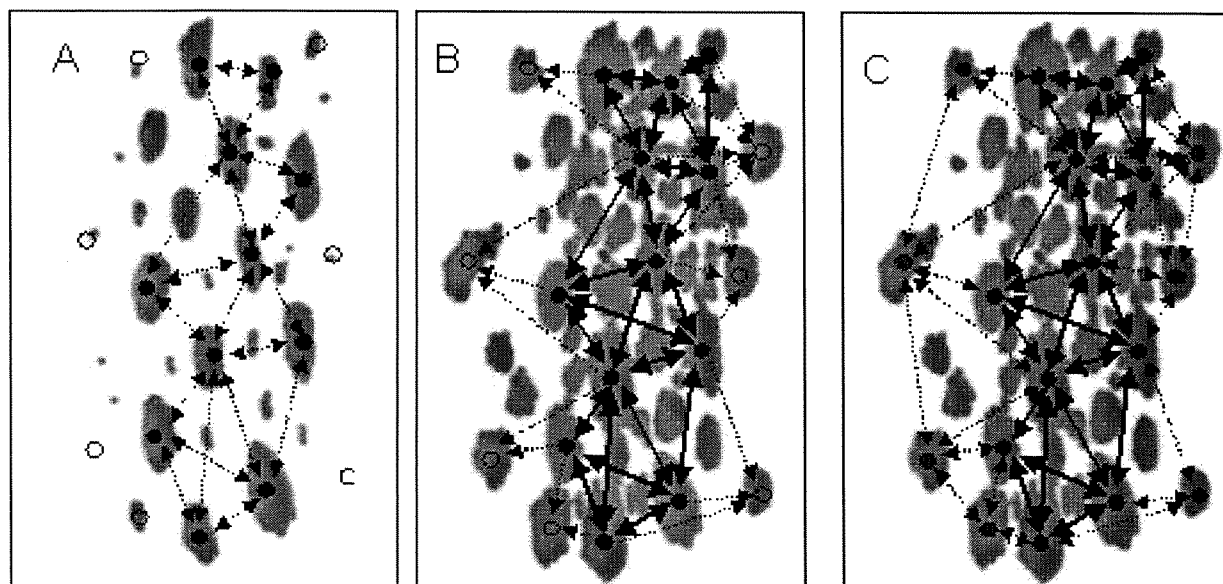


Figure 3



**IV. Extinction-colonization dynamics structure genetic variation of
spotted sunfish (*Lepomis punctatus*) in the Florida Everglades.**

Extinction-colonization dynamics structure genetic variation of spotted sunfish (*Lepomis punctatus*) in the Florida Everglades

T. C. MCELROY,* K. L. KANDL,† J. GARCIA* and J. C. TREXLER*

*Department of Biological Sciences, Florida International University, University Park, Miami, FL 33199, USA, †Present address: Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148, USA

Abstract

The population genetics of aquatic animals in the Florida Everglades may be strongly influenced by extinction and colonization dynamics. We combined analyses of allozyme and microsatellite loci to test the hypothesis that two levels of population structure are present for spotted sunfish (Pisces: Centrarchidae: *Lepomis punctatus*) inhabiting the Everglades. We hypothesized that annual cycles of marsh dry-down increase local-scale genetic variation through a process of local extinction and colonization; we hypothesized that barriers to gene flow by levee/canal systems create a second, regional level of genetic variation. In 1996 and 1997, we sampled spotted sunfish from 11 Everglades sites that were distributed in three regions separated by levees. We documented patterns of genetic variation at 7 polymorphic allozyme loci and 5 polymorphic microsatellite loci. Most genetic variation was present among local populations, according to both types of genetic markers. Furthermore, samples from marsh sites were heterogeneous, while those from canals were not. These data supported our hypothesis that dry-down events and local population dynamics in the marsh have a significant effect on population genetic structure of spotted sunfish. We found no support for our hypothesis that water-management structures superimpose a second level of genetic structure on this species, possibly because canals obscure historical structure by facilitating gene flow or because the complete canal system has been in place for fewer than 20 generations of this species. Our data suggests a continent-island (canal-marsh) structure of populations with high gene flow among regions and recurrent mixing in marshes from canal and creek habitats.

Keywords: allozymes, extinction, Florida Everglades, *Lepomis punctatus*, microsatellites, migration, population structure

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Introduction

The genetic structure of a metapopulation may be shaped by the geographical scale and pattern of dispersal leading to colonization following local extinction events (Wade & McCauley 1988; McCauley *et al.* 1995). For example, colonists from a small number of source sites elevate the level of genetic variation among populations within a metapopulation (propagule-pool colonization). In contrast, when colonists from many sources re-populate a site, local extinction leaves little impact on metapopulation genetic

structure (migrant-pool colonization). F -statistics (F_{ST}) can be used to distinguish stable entities of a population, identify newly colonized sites and indicate the dominant mode of colonization (Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990). The genetic structure of a metapopulation and the dominant mode of colonization for a species may depend on the spatial scale and physical structure of the environment being considered.

The effects of habitat fragmentation on population genetic structure can vary widely and may even be concealed or diminished by population-level genealogical relationships that persist when gene flow no longer occurs between two populations. Pannell & Charlesworth (1999) noted that F_{ST} is stable to fluctuations in the number of

Correspondence: J. C. Trexler. Fax: 305 348 1986. E-mail: trexlerj@fiu.edu

occupied sites, such as in a population that is expanding and contracting over time. They cautioned that F_{ST} is relatively insensitive to the mode of colonization if the rate of extinction is less than or equal to the rate of gene flow between populations. Further, F_{ST} may be a poor estimate of population divergence because it is sensitive to the total diversity (the denominator) unrelated to the difference between populations. Thus F -statistics may underestimate genetic divergence between populations, especially when allelic diversity is high (Nagylaki 1998; Pannell & Charlesworth 1999).

The Everglades can be characterized as a south flowing river with a braided structure of relatively deep sloughs separated by densely vegetated shallow ridges, and bound by relatively short-hydroperiod wetlands. The slough habitats are routinely fragmented by seasonal dry-down events that temporarily reduce and/or eliminate connections between them (separated by hundreds of metres to kilometres) and concentrate aquatic organisms into deep-water refuges (Loftus & Kushlan 1987; Trexler *et al.* 2001). Starting before 1900 and culminating in the late 1960s (reviewed in Blake 1980; Light & Dineen 1994), canals that may enhance fish dispersal and levees that may limit or preclude migration by fish have subdivided the ecosystem into regional management units covering hundreds of square kilometres. These two forms of habitat fragmentation, seasonal dry-down events and structural fragmentation, differ in their implications for population genetic structure. Seasonal dry-downs may cause the local extinction of populations, and colonists may re-populate local habitat from a variety of sources. Additionally, dry-downs may encourage mixing by relatively long-range movement of fishes into refuge habitats. Movement of individuals into deep-water refuges from a diversity of marsh sites during a dry-down event may result in population mixing revealed genetically by deviation from Hardy-Weinberg equilibria and deficiency of heterozygotes (Wahlund effect: Hartl & Clark 1997). Structural fragmentation by canals may create corridors that facilitate directional movement, while levee barriers may reduce the dimensionality of movement through the habitat. Thus, structural fragmentation may create either corridors that homogenize populations, or barriers to gene flow facilitating genetic differentiation of subpopulations on either side.

We have used F -statistics to identify the metapopulation genetic structure and distinguish between two modes of colonization (propagule and migrant pool) for a common centrarchid, the spotted sunfish (*Lepomis punctatus*), found throughout the Florida Everglades. We combined analyses of allozyme and microsatellite loci to test the hypothesis that two levels of population structure may be present for spotted sunfish in the Everglades: one of local variation resulting from extinction and colonization; and a second of regional variation resulting from barriers to gene flow by

levees and canals superimposed over the historical pattern of a large unimpeded river. If extinction and colonization are important, relatively greater F -statistics will be observed between sites that are subject to extinction-colonization dynamics compared to sites that are stable. Further, if colonization occurs from many different sources, including some distant from the receiving population (migrant-pool colonization), then local extinctions will have little effect on the population genetic structure within water management units. In the latter scenario, relatively large F -statistics among regions remain plausible. In this case an island model, perhaps at the scale of management units, would be appropriate to describe genetic diversity and the greatest F -statistic would be expected at the regional scale. The greatest F -statistic would be recorded at the local scale within water management units if colonization were from a small number of local sites (propagule-pool colonization) (Slatkin 1977; Wade & McCauley 1988).

Materials and methods

Sample collection

In the Everglades ecosystem, spotted sunfish are relatively large (85 mm adult mean standard length [SL = length from the tip of the snout to the base of the caudal peduncle]), long-lived fish (sexual maturity at approximately 1 year); most Everglades fish are cyprinodontiformes with approximately annual life cycles (Loftus & Kushlan 1987; Trexler *et al.* 2001). We collected 278 spotted sunfish between 1996 and 1997 from eight canal and three marsh-pond sites distributed throughout Shark River Slough in Everglades National Park (ENP) and Water Conservation Areas 3A (WCA-3A) and 3B (WCA-3B) (Fig. 1; Table 1). Canal and levee constructions separate these three regions of the Everglades, with WCA-3B being completely isolated hydrologically from the rest of the ecosystem by a system of levees completed in 1963. Gates permit a regulated flow of water southward from WCA-3A to ENP. The sample size from each site ranged from 12 to 52, and the fish were obtained by angling and electrofishing. Upon collection, animals were transferred to the laboratory, standard length was measured, and they were stored at -80°C prior to genetic analyses.

Sampling was conducted in the dry season of 1996 to obtain specimens from areas where they were concentrated because of declining water levels. However, an insufficient number of specimens was collected, so additional samples were obtained in the dry season of 1997 and pooled into a single sample with the fish from the previous year. We considered that pooling between the 2 years was appropriate because spotted sunfish live several years, permitting the adult fish from both years to be treated as members of the same cohort. Also, there was no evidence

Site	Habitat	Region	Latitude	Longitude	N	SL
L1	marsh	WCA-3A	25.51.67	80.43.49	23	84.5 (5.85)
L3	canal	WCA-3A	25.59.45	80.50.35	25	92.7 (2.51)
L11	canal	WCA-3A	25.45.70	80.49.40	25	100.0 (4.03)
L21	canal	WCA-3A	26.06.54	80.36.26	14	93.2 (2.98)
L23	canal	WCA-3A	26.03.80	80.26.30	25	65.3 (1.74)
L25	canal	WCA-3A	26.08.82	80.34.23	26	95.1 (1.77)
L32	canal	WCA-3A	26.08.83	80.40.69	25	85.3 (1.96)
L12	canal	WCA-3B	25.45.70	80.40.40	25	80.5 (1.88)
L33	canal	WCA-3B	25.56.52	80.26.37	12	88.2 (5.82)
L34	marsh	ENP	25.41.42	80.45.61	19	86.4 (3.50)
L35	marsh	ENP	25.41.32	80.45.70	12	86.0 (5.73)

Table 1 Collection information for spotted sunfish in WCA-3A and -3B, and ENP. N indicates number of specimens analysed and SL indicates mean standard length (SE). Sites are shown in Fig. 1

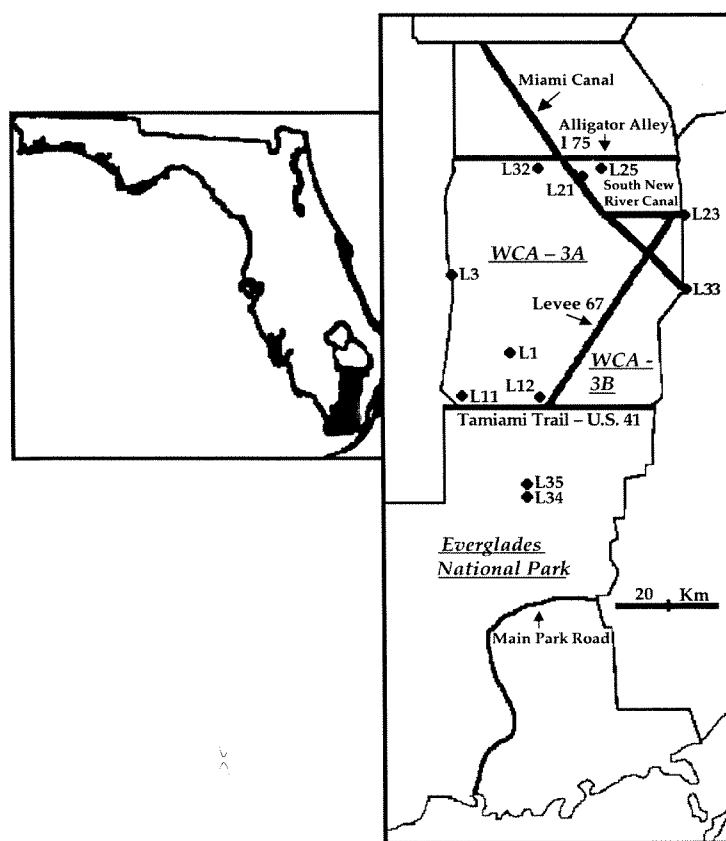


Fig. 1 Map of the regions sampled in the Florida Everglades showing the locations of the sample sites. The location of the sampling area is indicated in black on the Florida State map.

of significant interannual variation in allozyme or micro-satellite allele frequencies in these two samples (Trexler *et al.* 2001).

We estimated the density of spotted sunfish from the marsh habitat adjacent to one of our marsh sampling sites in ENP (L35) from a long-term fish-sampling programme

conducted by personnel of the park. These data were obtained by use of a 1-m² throw trap and approximately 105 samples were collected each year between 1978 and 1997. Fish were collected in February, April, July, October and December (for details of the sampling design see Loftus & Eklund 1994; Trexler *et al.* 2001). Juveniles of four

Enzyme	EC no.	N	P	H _O
Aconitase hydratase (AcoH)	4.2.1.3	3	1.0	0.52
Aspartate aminotransferase (Aat-1)	2.6.1.1	1	0.0	0.00
Aspartate aminotransferase (Aat-2)		2	1.0	0.27
Glucose dehydrogenase (Gcdh)	1.1.1.118	3	1.0	0.09
Isocitrate dehydrogenase (Idh-1)	1.1.1.42	2	1.0	0.17
L-Lactate dehydrogenase (Ldh-2)	1.1.1.27	2	1.0	0.00
Malate dehydrogenase (Mdh-2)	1.1.1.37	1	0.0	0.00
Malate dehydrogenase (Mdh-3)		1	0.0	0.00
Mannose-6-phosphate isomerase (Mpi-1)	5.3.1.8	1	0.0	0.00
Mannose-6-phosphate isomerase (Mpi-2)		1	0.0	0.00
Phosphoglucosmutase (Pgm-1)	5.4.2.2	4	1.0	0.39
Phosphoglucosmutase (Pgm-2)		1	0.0	0.00
Phosphogluconate dehydrogenase (Pgdh-1)	1.1.1.14	1	0.0	0.00
Phosphogluconate dehydrogenase (Pgdh-2)		1	0.0	0.00
Peptidase val-leu (Pep)	3.4.-.-	4	1.0	0.04
Totals			0.47	0.10

Table 2 Allozymes scored for allelic variation in spotted sunfish. EC. no. refers to Enzyme Commission number; Pep is a generic term. *N* indicates the number of alleles observed at each locus, *P* indicates the presence or absence of polymorphism in at least 1% of the individuals at each locus, and *H_O* is the observed heterozygosity for each locus

species of sunfish found in the Everglades are not easily distinguishable (these are *L. punctatus*, *L. marginatus*, *L. microlophus*, and *L. macrochirus*), though only spotted sunfish and *L. marginatus* are abundant. We summed the counts of spotted sunfish and unidentified juvenile sunfish to yield a generous estimate of the historical density of spotted sunfish at the sampling site prior to our collection for genetic analysis.

Allozyme analysis

We used starch-gel electrophoresis to document patterns of allozymic variation in spotted sunfish. 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 mercaptoethanol). We pooled eye, liver and soma clips; preliminary analyses indicated no tissue-specific expression of the proteins, justifying this approach. We screened 32 protein-encoding loci with horizontal starch-gel electrophoresis before selecting 15 resolvable loci to score on all individuals. Of these, seven loci were polymorphic (Table 2). We followed standard techniques described in Selander *et al.* (1971) and Murphy *et al.* (1996), with 11% (w/v) starch gels. All allozymes were resolved using a Tris-citrate EDTA buffer at pH 8.0 (Ayala *et al.* 1972).

Microsatellite DNA analysis

We surveyed nine potential microsatellite-locus primer pairs in spotted sunfish that had been developed in closely related species, *Lepomis auritus* (DeWoody *et al.* 1998) and *Lepomis macrochirus* (Colbourne *et al.* 1996). From these nine loci, we identified five polymorphic loci that amplified reliably (Table 3). Whole genomic DNA was isolated from

muscle tissue by standard phenol-chloroform DNA extraction methods (Hoelzel & Green 1992). The microsatellite loci were amplified using polymerase chain reaction (PCR) in 15 µL volumes, containing 1× buffer, 25 µM MgCl₂, 250 µM dNTP, 5 U/µL *Taq* DNA polymerase (Promega), and a 5 µM primer set. One primer of each pair was end-labelled with a fluorescent dye (6-FAM, NED or HEX; Applied Biosystems). The thermal cycling parameters, modified from Colbourne *et al.* (1996), were as follows: an initial 1 minute 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 minute extension at 72 °C. We electrophoresed the amplified samples in 2.5% agarose gel to determine the presence or absence of a product. 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 to a ROX 350 standard and the genotypes were assigned using the GENESCAN (ABI) and GENOTYPER software packages.

Statistical methods

We examined patterns of allelic and genotypic diversity in both allozymes and microsatellites. We calculated goodness-of-fit to Hardy-Weinberg expectations at each allozyme and microsatellite locus within each site, and in global tests across loci and across sites, using GENEPOP 3.2 (Raymond & Rousset 1995). When only two or three alleles were observed, we used the complete enumeration method of Louis & Dempster (1987), while we used the Markov chain method to calculate exact *P*-values for loci that exhibited more than three alleles (Guo & Thompson 1992). Linkage disequilibrium was tested for each pair of allozyme and microsatellite loci within each site using GENEPOP 3.2

Locus	Dye Marker	Locus Sequence Primer Sequences	N	H _O
RB7*	6FAM	not reported F: 5' - GTGCTAATTAAGGCTACTGTC R: 5' - TGTTCCTTAATTTGTTTGA	17	0.816
RB20*	HEX	not reported F: 5' - GGCTCTCTGTTAAATGAGGG R: 5' - GTTGGCTCTGTCAGAGTAAAAA	15	0.797
Lma 21**	HEX	(TC) ₁₉ (AC) ₁₁ F: 5' - CAGCTCAATAGTTCTGTGAGG R: 5' - ACTACTGCTGAAGATATTGTAG	14	0.683
Lma 29**	6FAM	(GT) ₃₀ F: 5' - CCTGTCTACTTGTGTATTC R: 5' - ATTGAGAGGCAAGCATATTC	17	0.731
Lma 87**	NED	(AC) ₁₅ (A) ₅ F: 5' - ATGACA CAGACTCACCATGTC R: 5' - CTCTGCCCCATAATCAGAC	14	0.566

*DeWoody *et al.* 1998.

**Colbourne *et al.* 1996.

Table 3 Primer sequences for spotted sunfish DNA microsatellites. Microsatellite fragments were sized based on migration relative to an ROX 350 standard. *N* indicates the number of alleles observed at each locus, and *H_O* is the observed heterozygosity for each locus. All samples were polymorphic for these loci

(Raymond & Rousset 1995). We used F-STAT (Goudet 2001) to estimate gene diversity and allelic richness separately for allozymes and microsatellites. We also tested for differences in gene diversity and allelic richness between allozymes and microsatellites using a Mann-Whitney's *U*-test with loci as replicates.

We hypothesized that sunfish from marsh sites that experienced seasonal dry-down events that varied in severity might experience significant reductions in their effective population size. The most recent severe drought occurred in 1989–90, when all of ENP and WCA-3B, and most of WCA-3A were dry. Thus, we examined the possible impact of demographic changes from hydrologic fluctuations or habitat fragmentation on allozyme and microsatellite diversity. We estimated deviations from expected heterozygosity and tested for evidence of recent population bottlenecks with BOTTLENECK (Cornuet & Luikart 1996; Piry *et al.* 1999). This program estimated heterozygosity excesses and deficiencies for each locus, and for each site, and tested significance using a one-tailed Wilcoxon test based on ranks. We used the infinite alleles model for analysis of the allozyme data, and both the stepwise and two-phase mutation models for analysis of microsatellites. In addition, an assignment test was performed according to the direct method (Rannala & Mountain 1997) with the leave-one-out option (GENECLASS software package; Cornuet *et al.* 1999). This permitted us to determine the likelihood that each individual's allozyme and microsatellite multilocus genotype belong to the site and/or region from which it was sampled (Paetkau *et al.* 1995). In order to increase the number of loci and thus the power of the population bottleneck and the assignment tests, allozyme

and microsatellite data were combined, and the above analyses were repeated. If local or regional sites are stable and genetically distinct, then recent migrants to a region or a local site might be detected and the source of these migrants could be identified.

To test for isolation by distance and equilibrium of gene flow and drift, we calculated pairwise estimates of *F_{ST}* and *R_{ST}* for allozyme and microsatellite loci, respectively (Slatkin 1993; Hutchison & Templeton 1999). We tested the significance of pairwise correlations between these values and the linear geographical distance separating sites using the Mantel permutation procedure (Mantel 1967) with the Spearman's rank correlation coefficient as the test statistic (GENEPOP 3.2; Raymond & Rousset 1995). Hydrographical distance between sites and linear geographical distance between sites were functionally equal because the Everglades is an expansive river-like wetland (i.e. with few exceptions, fish can move in all directions throughout much of the system). The patterns of the scatterplots were analysed according to Hutchison & Templeton (1999). Residual values from these analyses were classified as within or among regions, or within or among site types (canal or marsh). These values were analysed to test for regional division or site type patterns.

We used F-STAT (Goudet 2001) to compare gene diversity and allelic richness separately for allozymes and microsatellites among regions and between marsh and canal sites. We used Weir & Cockerham's (1984) co-ancestry method to hierarchically partition the total observed genetic variation for the allozyme and microsatellite data. These partitions were attributable to variation among individuals within sites (individuals collected in areas < 1 km²) relative

to total diversity within their site (θ_S), among sites within water-management units relative to the total diversity in that unit (θ_{SP}), and among water-management units (regions) relative to the total genetic diversity (θ_{ST}). This provided estimates of Wright's hierarchical F -statistic of population subdivision based on a random-effects sampling model (Weir 1990). The regions correspond to an area enclosed by levees and canals: WCA-3A, WCA-3B, and ENP (Fig. 1). F -statistic estimates were also calculated separately among all canal sites and among all marsh sites to compare genetic structure estimates between deep-water refuge sites and sites that may be subject to frequent extinction and colonization. The Genetical Data Analysis program (GDA 1.0; Lewis & Zaykin 1997) was used to estimate hierarchical F -statistics following Weir & Cockerham (1984), with bootstrapping across loci to estimate 95% confidence intervals.

To make statistical comparisons between the population-genetic patterns detected from allozymes and microsatellites, Weir & Cockerham's (1984) co-ancestry method was employed for both markers. Simulations performed by Ruzzante (1998) suggested that F_{ST} and R_{ST} perform similarly if mutation has not had enough time to affect allelic divergence of microsatellite loci. We tested for correlations of pairwise F_{ST} estimates between sites for allozymes and microsatellites with the Mantel permutation procedure performed as above.

Counts of sunfish collected in 1-m² samples deviated noticeably from a normal distribution, with many traps yielding zero specimens. Using SAS statistical software, we fit an overdispersed-Poisson linear model with a log linking function to provide estimates of annual density with asymmetrical confidence intervals lower bounded at zero (Littell *et al.* 1996).

Results

Hardy-Weinberg equilibrium and genetic diversity

Genotypic frequencies of allozymes were more consistent with Hardy-Weinberg predictions than genotypic frequencies of microsatellites. The genotypic frequencies of the allozyme loci were generally consistent with expectations of Hardy-Weinberg equilibrium; testing across loci within each site, we found only one case that did not conform to Hardy-Weinberg equilibrium expectations (L1). Additionally, we found evidence of heterozygote deficiency ($f < 0$) at 3 of the 7 polymorphic loci examined for that site (Table 4). Several of the microsatellite loci did not conform to Hardy-Weinberg predictions. Testing within each site indicated that genotypic frequencies within three of the sites were inconsistent with Hardy-Weinberg predictions (L1, L11, and L32). Additionally, there was significant heterozygote deficiency detected for some loci within those sites, though

different loci were deficient in heterozygotes among sites (Table 5). There was some evidence for linkage disequilibrium in allozymes (*Gadh* & *Pep*) and in microsatellites (Lma29 & Lma87).

We observed moderate variation at the 7 polymorphic allozymes surveyed (7 of the 15 loci examined were polymorphic = 46.7%, average heterozygosity of loci that were polymorphic = 0.25; Table 2). The number of alleles per polymorphic locus across all sites ranged from 2 (*Ldh*, *Idh-1*) to 4 (*Pgm-1* and *Pep*). We observed considerable variation in the five microsatellite loci studied (Table 5). The number of alleles per locus ranged from 14 (Lma21 and Lma87) to 17 (RB7 and Lma29; Table 5). The observed heterozygosity, averaged over all sites, ranged from 0.57 (Lma87) to 0.82 (RB7) (Table 3).

Compared to allozymes, microsatellites had a greater average gene diversity and allelic richness (Mann-Whitney's $U = 75.00$; $P < 0.001$). Matrices of allozyme and microsatellite pairwise F_{ST} estimates were significantly correlated (Mantel test, $r = 0.571$, $P = 0.009$), indicating concordance between the two types of molecular markers.

Recent bottleneck test

There were no indications that any of the studied sites experienced a recent, significant bottleneck. There was one site (L1) where the number of allozyme loci showing heterozygote excess was significantly greater than that expected from the allele diversity. Under the expectations of both models of mutation, there were no sites where the number of microsatellite loci showing heterozygote excesses was significantly greater than expected from the allele diversity. After applying a Bonferroni correction for multiple tests, none of these observations could be considered statistically significant ($\alpha = 0.05$).

Assignment test

The assignment test performed with the allozyme data did not reliably assign individuals to their site or region of sampling origin. Only 57 of 231 (24.7%) were correctly assigned to the site from which they were sampled, and 80 of 231 (34.6%) correctly assigned to WCA-3A, WCA-3B or ENP. Following King *et al.* (2001), we found that the inclusion of all allozyme loci produced the greatest assignment accuracy. The assignment test performed with the microsatellite data did not reliably assign individuals to their site or region of sampling origin. Only 48 of 283 (17.0%) were correctly assigned to the site from which they were sampled, and 163 of 283 (57.6%) were correctly assigned to WCA-3A, WCA-3B or ENP. The inclusion and exclusion of microsatellite loci did not alter the assignment accuracy. Similarly, the assignment test performed with a pooled data set of allozymes and microsatellites

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Table 4 Descriptive statistics for the 7 polymorphic allozyme loci from collections spotted sunfish at 11 sites in the southern Everglades. Number of samples genotyped (*N*), number of alleles per locus (*A*), gene diversity (H_E), allelic richness (*R*), inbreeding coefficient (*f*), and conformity to Hardy-Weinberg Equilibrium (HW)

Collection Site		Locus							Totals across loci
		Acon	Aat-2	Gcdh	Idh-1	Ldh-2	Pgm-1	Pep	
L1	<i>N</i>	23	23	23	13	23	16	23	
	<i>A</i>	2	2	3	2	2	3	3	
	H_E	0.476	0.231	0.565	0.269	0.474	0.283	0.482	
	<i>R</i>	2.000	1.992	2.992	2.000	2.000	2.932	2.777	
	<i>f</i>	-0.004	-0.128	0.692	-0.143	1.000	-0.103	0.730	0.402
	HW	1.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000
L3-	<i>N</i>	25	25	25	25	25	25	25	
	<i>A</i>	3	2	2	2	1	3	2	
	H_E	0.588	0.302	0.040	0.150	0.000	0.223	0.040	
	<i>R</i>	2.993	1.999	1.480	1.935	1.000	2.735	1.480	
	<i>f</i>	-0.089	0.072	0.000	-0.067	—	-0.079	0.000	-0.043
	HW	0.376	1.000	—	1.000	—	1.000	—	0.982
L11	<i>N</i>	25	25	25	25	25	25	25	
	<i>A</i>	3	2	1	2	1	3	1	
	H_E	0.538	0.327	0.000	0.150	0.000	0.279	0.000	
	<i>R</i>	2.969	1.999	1.000	1.935	1.000	2.473	1.000	
	<i>f</i>	0.034	0.020	NA	-0.067	—	-0.146	NA	-0.020
	HW	0.886	1.000	—	1.000	—	1.000	—	1.000
L12	<i>N</i>	25	25	25	25	25	25	25	
	<i>A</i>	3	2	2	2	1	3	1	
	H_E	0.587	0.245	0.078	0.078	0.000	0.681	0.000	
	<i>R</i>	2.986	1.993	1.735	1.735	1.000	3.000	1.000	
	<i>f</i>	-0.295	-0.143	-0.021	-0.021	—	0.119	NA	-0.078
	HW	0.051	1.000	1.000	1.000	—	0.326	—	0.612
L21	<i>N</i>	14	14	14	14	14	14	14	
	<i>A</i>	3	2	2	2	1	3	2	
	H_E	0.527	0.308	0.137	0.253	0.000	0.467	0.071	
	<i>R</i>	2.857	2.000	1.984	2.000	1.000	2.857	1.857	
	<i>f</i>	0.323	0.304	-0.040	-0.130	—	-0.376	0.000	0.028
	HW	0.238	0.348	1.000	1.000	—	0.468	—	0.772
L23	<i>N</i>	25	25	25	25	25	25	25	
	<i>A</i>	3	2	2	2	1	4	3	
	H_E	0.423	0.245	0.115	0.115	0.000	0.603	0.079	
	<i>R</i>	2.480	1.993	1.867	1.867	1.000	3.466	1.960	
	<i>f</i>	0.053	-0.143	-0.043	-0.043	—	0.006	-0.011	-0.013
	HW	1.000	1.000	1.000	1.000	—	0.647	1.000	1.000
L25	<i>N</i>	23	26	26	26	26	21	26	
	<i>A</i>	2	2	2	2	1	3	1	
	H_E	0.451	0.265	0.111	0.111	0.000	0.479	0.000	
	<i>R</i>	2.000	1.996	1.852	1.852	1.000	2.822	1.000	
	<i>f</i>	0.132	-0.163	-0.042	-0.042	—	-0.393	NA	-0.128
	HW	0.642	1.000	1.000	1.000	—	0.126	—	0.889
L32	<i>N</i>	23	25	25	25	25	25	24	
	<i>A</i>	3	2	1	2	1	2	3	
	H_E	0.611	0.245	0.000	0.150	0.000	0.040	0.082	
	<i>R</i>	2.992	1.993	1.000	1.935	1.000	1.480	2.000	
	<i>f</i>	0.074	-0.143	NA	0.067	—	0.000	-0.011	0.000
	HW	0.158	1.000	—	1.000	—	—	1.000	0.884

Table 4 Continued

Collection Site		Locus							Totals across loci
		Acon	Aat-2	Gcdh	ldh-1	Ldh-2	Pgm-1	Pep	
L33	N	12	12	12	12	12	12	12	0.078 0.865
	A	3	2	2	2	1	4	1	
	H _E	0.591	0.159	0.288	0.348	0.000	0.420	0.000	
	R	3.000	2.000	2.000	2.000	1.000	4.000	1.000	
	f	0.295	-0.048	-0.158	0.283	—	-0.189	NA	
	HW	0.169	1.000	1.000	0.403	—	1.000	—	
L34	N	17	19	19	17	19	18	19	0.128 0.086
	A	3	2	2	2	1	1	1	
	H _E	0.465	0.430	0.102	0.114	0.000	0.000	0.000	
	R	2.706	2.000	1.871	1.920	1.000	1.000	1.000	
	f	-0.265	0.633	-0.029	-0.032	—	NA	NA	
	HW	0.082	0.012	1.000	1.000	—	—	—	
L35	N	12	12	12	12	12	12	12	0.027 0.792
	A	2	2	2	2	1	3	1	
	H _E	0.462	0.288	0.167	0.288	0.000	0.595	0.000	
	R	2.000	2.000	2.000	2.000	1.000	3.000	1.000	
	f	-0.082	-0.158	1.000	-0.158	—	0.019	NA	
	HW	1.000	1.000	0.043	1.000	—	1.000	—	
Total all sites	N	179	186	186	174	184	173	185	0.126 0.000
	A	3	2	3	2	2	4	4	
	H _E	0.520	0.277	0.146	0.184	0.043	0.367	0.069	
	R	2.808	1.988	2.223	1.994	1.994	2.974	1.805	
	f	0.006	0.026	0.396	-0.034	1.000	0.078	0.556	
	HW	0.342	0.975	0.007	1.000	1.000	0.965	0.003	

Table 5 Descriptive statistics for five microsatellite loci in collections of spotted sunfish from 11 sites in the Everglades. Number of samples genotyped (N), number of alleles per locus (A), gene diversity (H_E), allelic richness (R), inbreeding coefficient (f), and conformity to Hardy-Weinberg equilibrium are reported

Collection Site		Locus					All loci
		RB7	Lma21	Lma29	RB20	Lma87	
L1	N	22	23	23	20	23	0.190 0.000
	A	13	7	11	11	5	
	H _E	0.918	0.791	0.848	0.850	0.610	
	R	9.025	5.561	7.472	7.152	4.004	
	f	0.257	0.120	0.282	0.176	0.073	
	HW	0.000	0.001	0.000	0.000	0.208	
L3	N	13	13	13	13	13	-0.106 0.432
	A	10	5	8	12	6	
	H _E	0.897	0.718	0.849	0.923	0.785	
	R	8.209	4.463	7.104	9.551	5.416	
	f	-0.029	-0.393	-0.177	0	0.020	
	HW	0.854	0.024	0.906	0.574	0.597	
L11	N	31	31	31	30	31	0.197 0.000
	A	13	8	12	12	7	
	H _E	0.887	0.711	0.891	0.923	0.751	
	R	8.111	4.659	8.344	9.059	4.967	
	f	-0.019	0.092	0.095	0.350	0.485	
	HW	0.184	0.114	0.271	0.000	0.000	

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Table 5 Continued

Collection Site		Locus					All loci
		RB7	Lma21	Lma29	RB20	Lma87	
L12	N	45	40	45	43	45	
	A	15	10	16	14	9	
	H _E	0.908	0.765	0.885	0.909	0.743	
	R	8.887	5.967	8.436	8.725	5.273	
	f	0.045	0.150	0.071	0.027	0.073	0.071
	HW	0.117	0.006	0.213	0.252	0.519	0.018
L21	N	23	22	23	22	21	
	A	12	9	11	15	8	
	H _E	0.914	0.766	0.813	0.935	0.785	
	R	8.888	6.079	6.998	10.173	5.976	
	f	0.144	-0.008	-0.016	0.076	0.150	0.072
	HW	0.428	0.441	0.938	0.250	0.231	0.517
L23	N	12	11	8	10	12	
	A	11	4	9	7	7	
	H _E	0.932	0.745	0.911	0.900	0.790	
	R	9.347	3.985	9.000	6.754	5.653	
	f	0.106	-0.098	0.039	0.444	0.194	0.143
	HW	0.131	0.026	0.735	0.001	0.331	0.002
L25	N	34	33	33	26	33	
	A	14	7	15	13	6	
	H _E	0.906	0.746	0.910	0.927	0.694	
	R	8.957	4.587	9.410	9.583	4.042	
	f	0.156	0.107	0.034	0.087	0.257	0.122
	HW	0.108	0.329	0.787	0.048	0.295	0.114
L32	N	50	43	51	30	44	
	A	14	9	12	14	10	
	H _E	0.905	0.744	0.900	0.918	0.754	
	R	8.719	4.792	8.329	9.387	5.134	
	f	0.116	0.000	0.390	0.129	0.187	0.169
	HW	0.009	0.009	0.000	0.094	0.006	0.000
L33	N	21	20	19	19	21	
	A	11	5	9	14	6	
	H _E	0.910	0.761	0.865	0.920	0.733	
	R	8.646	4.325	7.029	9.357	5.172	
	f	0.005	0.737	0.514	-0.087	0.545	0.317
	HW	0.157	0.000	0.000	0.888	0.000	0.000
L34	N	14	12	14	14	14	
	A	9	4	12	12	8	
	H _E	0.860	0.705	0.923	0.912	0.810	
	R	7.568	3.666	9.426	9.124	6.505	
	f	0.003	-0.301	0.149	0.139	0.207	0.053
	HW	0.491	0.354	0.361	0.195	0.253	0.317
L35	N	12	12	10	12	11	
	A	9	4	7	11	5	
	H _E	0.890	0.784	0.867	0.917	0.800	
	R	7.898	3.997	6.730	9.225	4.920	
	f	0.157	0.362	-0.154	0.000	-0.023	0.064
	HW	0.097	0.163	0.971	0.571	0.739	0.436
Total all Populations	N	305	279	294	257	289	
	A	17	14	17	15	14	
	H _E	0.902	0.749	0.878	0.913	0.750	
	R	8.78	5.20	8.51	9.11	5.30	
	f	0.131	0.091	0.161	0.152	0.226	0.143
	HW	0.000	0.000	0.000	0.000	0.000	0.000

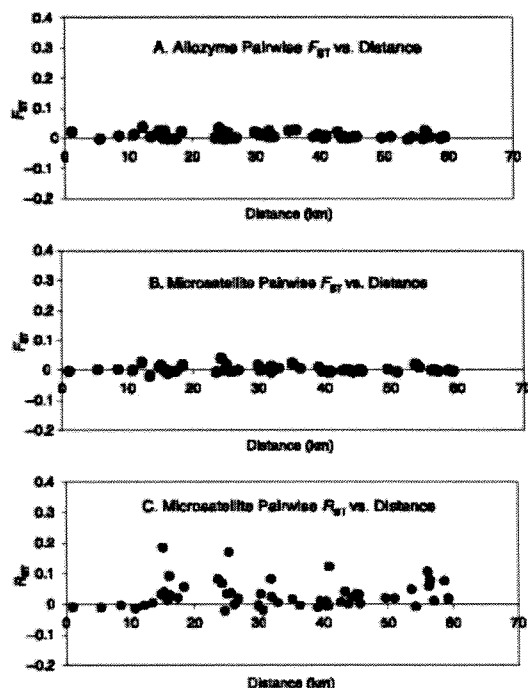


Fig. 2 Relationship between geographical distance and (A) θ_{ST} for the allozyme data (B) θ_{ST} for the microsatellite data, and (C) R_{ST} for the microsatellite data.

did not reliably assign individuals to their site or region of sampling origin. Only 58 of 231 (25.1%) were correctly assigned to the site from which they were sampled, and 114 of 231 (49.4%) were correctly assigned to WCA-3A, WCA-3B, or ENP. This suggests that there is not a unique multilocus genotypic distribution for the allozyme or microsatellite loci that described a site or region. This could result from routine mixing within and/or between the regions. The assignment test assumes Hardy-Weinberg equilibrium, which was partially violated by the microsatellite data.

Isolation by distance

We found no correlations between pairwise estimates of geographical distance and F_{ST} from allozymes or F_{ST} and R_{ST} from microsatellite loci, further indicating a lack of spatially explicit genetic structure (Fig. 2). Furthermore, residual values calculated from these regressions did not reveal any significant patterns of deviation associated with region or site type. The overall pattern of the scatterplots was consistent between allozymes and microsatellites.

Table 6 Patterns of genetic diversity in spotted sunfish collected from the southern Everglades. Estimates reported for All Sites@ were hierarchically organized as sites within regions, and within each region or habitat type as listed. θ_{SP} indicates the genetic variance among sites within regions. θ_{PT} indicates the genetic variance in among samples relative to the total study

	Microsatellite data		Allozyme data	
	θ_{SP}	θ_{PT}	θ_{SP}	θ_{PT}
All Sites	0.0052*	-0.0001	0.0745*	-0.0068
Canal	NA	0.0021	NA	0.0485
Marsh	NA	0.0229*	NA	0.1446*

*Indicates 95% confidence interval does not cross zero.

Hierarchical genetic diversity

Gene diversity and allelic richness were not significantly different between WCA-3A, WCA-3B and ENP ($P > 0.05$) for allozymes and microsatellites. They were not significantly different between marsh and canal sites for allozymes ($P > 0.05$). However, gene diversity and allelic richness were significantly different between marsh and canal sites for microsatellites (gene diversity: $P = 0.022$; allelic richness: $P = 0.009$). Marsh sites had, on average, less gene diversity and allelic richness than canal sites.

Allozymes and microsatellites revealed consistent and significant genetic structure among sites within regions (Table 6). When considered separately, sites within WCA-3A displayed significant genetic structure, but sites in WCA-3B and ENP did not (i.e. bootstrap confidence intervals cross zero). The F -statistic estimates were an order of magnitude larger for allozymes than for microsatellites. This is consistent with expectations of F -statistic estimates calculated from low to moderately variable loci compared to highly variable loci (reviewed in Hedrick 1999). Canal sites were not genetically differentiated, whereas marsh sites were significantly heterogeneous (confidence interval of $\theta_{PT \text{ marsh}}$ did not cross zero). Based on a t -test re-sampling across loci with 1000 replicates, marsh sites were more heterogeneous than canal sites ($H_0: \theta_{PT \text{ canals}} = \theta_{PT \text{ marsh}}$; $P = 0.115$ for microsatellites and $P = 0.109$ for allozymes; Re-sampling test combining both data sets $F = 0.08$).

Spotted sunfish population dynamics

Spotted sunfish density in the marsh habitat near our ENP sampling sites fluctuated between approximately 0.5/m² to indistinguishable from zero over the 18 years prior to our study (Fig. 3a). No specimens of spotted sunfish (or of indistinguishable juveniles of the co-occurring congeners) were collected in 1988 or 1989, and very few specimens were collected in 1990. These latter low-density years overlapped with a relatively extreme 2-year drought event

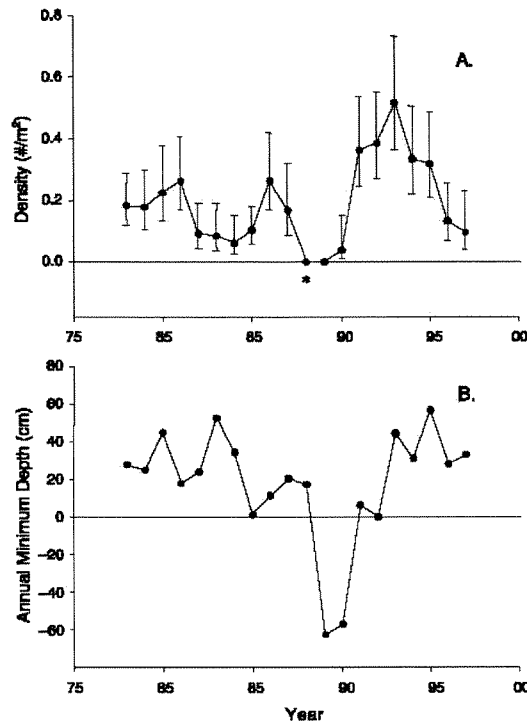


Fig. 3 Long-term record of density of spotted sunfish in marsh habitat adjacent to site L35 in ENP. (A) The average density of spotted sunfish (including all indistinguishable juvenile sunfish) in a marsh habitat with 95% confidence intervals estimated on an annual basis from 1978 to 1997. Note that no spotted sunfish were collected in 1988 or 1989, and very few in 1990. Asterisk indicates year when sampling was missed for 2 of the 5 months. (B) Annual minimum water depth at the collection site between 1978 and 1997.

in 1989 and 1990. A caveat is required for the low estimate in 1988; that is the only year of the 2-year record where sampling events were missed but water levels were above the ground surface; no samples were collected in April or May of that year.

Discussion

The spotted sunfish data were not consistent with use of regional-scale island models (at the spatial scale of water management units or greater) to describe the population structure for this species. The data suggested that spotted sunfish populations could be described as metapopulations that are not at equilibrium between gene flow and genetic drift. We propose that local-scale population dynamics have a significant effect on the population genetic structure of spotted sunfish in the Everglades. We

found no evidence of additional regional-scale variation. There are three possible sources of the lack of regional structure: historical population structure has been lost because of the homogenizing effects of canals as conduits for dispersal; the ecosystem historically had high gene flow and the canals and levees have not altered that situation; or, no genetic structure existed historically and, though the modern management system now limits gene flow, inadequate time has passed (approximately 20 generations of spotted sunfish) for the large regional populations to differentiate. Below, we elaborate on these conclusions.

Hardy-Weinberg equilibrium

Some sites were deficient in the frequency of heterozygotes, especially for microsatellite loci. Heterozygote deficiencies can arise from inbreeding, underdominant selection, mixing of genetically differentiated populations at a site (spatial Wahlund effect), or mixing of cohorts (temporal Wahlund effect) (Hartl & Clark 1997). The concordance we observed between allozyme and microsatellite markers (pairwise F_{ST} Mantel test) suggests that gene flow and genetic drift were the major causes of any observed patterns of population differentiation (cf., Estoup *et al.* 1998; Ross *et al.* 1999). The absence of widespread patterns of heterozygote deficiency suggests that Wahlund effects are not principal factors in the genetic structuring of local populations of spotted sunfish.

The coefficient of variation (CV) of body size of individuals collected from site L1 was much greater than that of any other site sampled ($CV_{L1} = 8.91$; $CV_{OTHER\ SITES} = 2.76$). The average size was not significantly different among sites with the exception of site L23 (Table 1). Although definitive discrimination between spatial and temporal Wahlund effects is beyond the power of our data, we pooled individuals into 1.0 cm size classes across all sites (6 size classes total, sample sizes within each class ranged from 14 to 39). We were unable to create size-based cohorts within sites because of low sample size. We re-tested for consistency with Hardy-Weinberg expectations within size classes for the microsatellites, and found only one locus (M87) that significantly deviated from expectations and only one of the cohorts was inconsistent with predicted frequencies (size class 6, 10.0 + cm, a mixture of larger/older classes). This suggests that temporal Wahlund effects should be considered in future studies and highlights the possibility that temporal variation and genetic differentiation between sites may be transient in time (Tessier & Bernatchez 1999). Genetic structure could be modified each generation by the addition of new cohorts and the mixing of old cohorts, depending on the connectivity of sites throughout the Everglades marsh. Thus, unique genetic signatures within sites may be masked by temporal variation (Whitlock & McCauley

1990; David *et al.* 1997); however, this does not preclude the possible effects of spatial mixing. It is interesting to note that pooling among sites within regions or among regions did not improve the fit of the data to Hardy-Weinberg expectations.

Patterns of genetic diversity

Allozyme and microsatellite loci differed in the amount of genetic diversity they showed, and in the patterns of gene diversity and allelic richness among sites. Highly variable microsatellite loci are likely more sensitive to these measures of genetic differentiation (Hedrick 1999). Both markers showed no evidence for recent population bottlenecks, and assignment tests did not reliably assign individuals to the site or region from which they were sampled. The assignment test failed to identify a unique genetic signal for regions and sites within regions. Thus, colonization of the marsh habitat after the dry season is not likely the result of a very small number of individuals that survive through the dry season in a deep-water refuge or colonize from canals to populate the site through *in situ* reproduction. Rather, the data indicate that a moderate to large immigrant population of spotted sunfish probably colonizes marsh sites. Also, a recent severe drought in 1989–90 did not cause a detectable bottleneck in those marsh sites, or subsequent colonization of the marsh sites from canal sites has masked the effects of the bottleneck.

We observed no evidence for isolation by distance in either allozymes or microsatellites, conforming to Hutchison & Templeton's (1999) case II. This pattern suggests that populations of spotted sunfish are not in equilibrium with respect to gene flow and genetic drift, possibly because of a recent colonization event from a relatively homogeneous source population. This conclusion is supported by the hierarchical population structure analyses, which did not reveal population structure for allozymes or microsatellites at the regional scale. Small but significant population structure and genetic differences were detected at the local level, which were likely the result of extinction and colonization effects at local spatial scales.

Local scale extinction-colonization dynamics of fish are tightly coupled with the natural hydrologic cycles of the Everglades (Trexler *et al.* 2001). We illustrated that the pattern with a 20-year record of spotted sunfish density in the marshes adjacent to one of our sites used for sampling genetic variation. Approximately 6 years prior to our study, the density of spotted sunfish dropped very low (indistinguishable from zero) for 1 to 2 years (1989 and 1990, we exclude the 1988 result because of low sample size). In 1990, alligator ponds in the vicinity of this marsh-sampling site dried, in addition to the marsh, because ground water depths dropped below 60 cm from the ground surface (Fig. 3b). We believe that this pattern of

population dynamics is linked to our finding of modest population structure among marsh sites, but not among canal sites for spotted sunfish. This pattern is present even though the average distance between the marsh sites (16.8 km) was less than ($P = 0.104$) the average distance between canal sites (30.2 km). Such a pattern of population genetic structure is expected when a group of recently colonized populations is compared to populations that are not subject to extinction and colonization turnover (Whitlock & McCauley 1990). Additionally, the low F -statistics and small difference between canal and marsh F -statistics suggests that spotted sunfish metapopulations are intermediate along a gradient between propagule pool and migrant pool patterns (McCauley *et al.* 1995). One might also expect this result if 'routine' migration has diluted the effects of a population crash on patterns of genetic variation, possibly as in our study where the crash was noted in 1990, six or more years prior to our collections.

Our data suggest that canal sites yield a homogeneous pool of colonists for marsh sites that dry routinely. We hypothesize that marsh sites are colonized by a modest number of individuals from such sources representing a subset of the total genetic diversity present there, but not diminishing genetic variation enough to be detected statistically as a 'bottleneck'. We propose that these colonization events create a modest genetic signature in the resulting marsh populations that may be short lived. Thus, a continent-island (canal-marsh) population structure best describes spotted sunfish genetics in the Everglades.

Genetic variation of Everglades spotted sunfish may not be at equilibrium at the regional level, in addition to the local dynamics already discussed. We do not know the historical population structure of sunfishes in the Everglades, but the regional habitat structure we sampled is a relatively recent addition to the Everglades ecosystem. The Tamiami Canal, separating WCA-3A and ENP, was originally constructed in 1926, and WCA-3A was enclosed by levees in 1963, thus the present regional habitat structure has been in place for 35–80 years. A minimum generation time for spotted sunfish is 2 years, so that 18–40 generations have passed since the regional structure was superimposed on the ecosystem. If the historical population was unstructured and the canals and levees form barriers to gene flow, the contemporary populations may not be at equilibrium with these changes. Genetic differentiation by drift, even with complete isolation (which is only possible for WCA-3B), is unlikely to have occurred in this short time without strong limits to dispersal and severe population bottlenecks (e.g. Arnaud *et al.* 2001).

The Everglades' natural hydropattern has been disrupted by recent water management practices. Regions of the Everglades that were historically flooded only a fraction of the year, are now flooded for prolonged periods of time, while other areas are dry much longer each year than

they were historically (Fennema *et al.* 1994). Also, historical patterns of sheet flow (flow of water across the wetland landscape driven by rainfall events) have largely been replaced with pulsed flow tied to management needs, resulting in lacustrine (southern WCA-3A) or stagnant conditions (WCA-3B) at some locations (Light & Dineen 1994; DeAngelis *et al.* 1997). Hydrologic patterns may affect the efficiency of colonization and exploitation of marsh habitats that become seasonally available to aquatic species in the Everglades. Our data suggest that patterns of periodic dry-down may create recurrent mixing events for spotted sunfish and, in this way, water management decisions may shape patterns of genetic diversity of this species in the contemporary Everglades ecosystem.

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References

- Arnaud JF, Madec L, Guiller A, Bellido A (2001) Spatial analysis of allozyme and microsatellite DNA polymorphisms in the land snail *Helix aspersa* (Gastropoda: Helicidae). *Molecular Ecology*, **10**, 1563–1576.
- 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*. University Presses of Florida, Tallahassee.
- Colbourne JK, Neff BD, Wright JM, Gross MR (1996) DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using (GT)_n microsatellites and its potential for assessment of mating success. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 342–349.
- Cornuet J-M, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.
- Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.
- David P, Perdieu M-A, Fernet A-F, Jarne P (1997) Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. *Evolution*, **51**, 1318–1322.
- DeAngelis DL, Loftus WL, Trexler JC, Ulanowicz RE (1997) Modeling fish dynamics and effects of stress in a hydrologically pulsed system. *Journal of Aquatic Ecosystem Stress and Recovery*, **6**, 1–13.
- DeWoody JA, Fletcher DE, Wilkins SD, Nelson WS, Avise JC (1998) Molecular genetic dissection of spawning, parentage, and reproductive tactics in a population of redbreast sunfish, *Lepomis auritus*. *Evolution*, **52**, 1802–1810.
- Estoup A, Rousset F, Michalakis Y, Cornuet J-M, Adriamanga M, Guyomard R (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology*, **7**, 339–353.
- Fennema RJ, Neidrauer CJ, Johnson RA, MacVicar TK, Perkins WA (1994) A computer model to simulate natural Everglades hydrology. In: *Everglades, the Ecosystem and its Restoration* (eds Davis SM, Ogden JC), pp. 249–289. St Lucie Press, Boca Raton, FL.
- Goudet J (2001) *FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices*. Version 2.9.3. <http://www.unil.ch/izea/software/fstat.html>. Updated from Goudet.
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.
- Hartl DL, Clark AG (1997) Random genetic drift. In: *Principles of Population Genetics*, 3rd edn, pp. 267–313. Sinauer Associates, Inc., Sunderland, MA.
- Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.
- Hoelzel AR, Green A (1992) Analysis of population-level variation by sequencing PCR-amplified DNA. In: *Molecular Genetic Analysis of Populations: a Practical Approach* (ed. Hoelzel AR), pp. 159–186. IRL Press, Oxford.
- 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**, 1898–1854.
- King TL, Kalinowski ST, Schill WB, Spidle AP, Lubinski BA (2001) Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. *Molecular Ecology*, **10**, 807–821.
- Lewis PO, Zaykin D (1997) Genetic Data Analysis: computer program for the analysis of allelic data. <http://chee.unm.edu/gda/>.
- Light SS, Dineen JW (1994) Water control in the Everglades: a historical perspective. In: *Everglades, the Ecosystem and its Restoration* (eds Davis SM, Ogden JC), pp. 47–84. St Lucie Press, Boca Raton, FL.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) *SAS System for Mixed Models*. SAS Institute Inc, Cary, NC.
- Loftus WF, Eklund AM (1994) Long-term dynamics of an Everglades small-fish assemblage. In: *Everglades, the Ecosystem and its Restoration* (eds Davis SM, Ogden JC), pp. 461–484. St Lucie Press, Boca Raton, FL.
- Loftus WF, Kushlan JA (1987) Freshwater fishes of southern Florida. *Bulletin of the Florida State Museum, Biological Science*, **31**, 147–344.
- Louis EJ, Dempster ER (1987) An exact test for Hardy-Weinberg and multiple alleles. *Biometrics*, **43**, 805–811.
- Mantel N (1967) The detection of disease clustering and generalized regression approach. *Cancer Research*, **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.
- Murphy RW, Sites JW Jr, Buth DG, Haufler CH (1996) Proteins:

- Isozyme electrophoresis. 120. In: *Molecular Systematics*, 2nd edn (eds Hillis DM, Moritz C, Mable BK), pp. 51–120. Sinauer, Sunderland, MA.
- Nagylaki T (1998) Fixation indices in subdivided populations. *Genetics*, **148**, 1325–1332.
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.
- Pannell JR, Charlesworth B (1999) Neutral genetic diversity in a metapopulation with recurrent local extinction and recolonization. *Evolution*, **53**, 664–676.
- Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.
- Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences USA*, **94**, 9197–9221.
- Raymond M, Rousset F (1995) GENEPop Version 32: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Ross KG, DeWayne Shoemaker D, Krieger MJB, Deheer CJ, Keller L (1999) Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Molecular Biology and Evolution*, **16**, 525–543.
- Ruzzante DE (1998) A comparison of several measures of genetic distance and population structure with microsatellite data: Bias and sampling variance. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1–14.
- 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*) studied gene VI. *University Texas Publications*, **7108**, 49–90.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology*, **12**, 253–262.
- Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.
- 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.). *Molecular Ecology*, **8**, 169–179.
- 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: *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys An Ecological Sourcebook* (eds Porter JW, Porter KG), pp. 153–181. CRC Press, Boca Raton, FL.
- Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.
- Weir BS (1990) *Genetic Data Analysis*. Sinauer, Sunderland, MA.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: Genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.

Thom McElroy, Karen Kandl, and Joel Trexler are interested in the role of population dynamics in shaping population genetic structure of aquatic organisms in fluctuating environments. This work is part of our larger project on the metacommunity dynamics of aquatic organisms in the Florida Everglades. Janette Garcia assisted with microsatellite analysis in developing her senior undergraduate thesis at Florida International University.

**V. Metapopulations or Patchy Populations? Population Structure of
Three Species of Aquatic Animals from the Florida Everglades**

Right Running Head: POPULATION TURNOVER AND GENETIC STRUCTURE

Left Running Head: J. C. TREXLER ET AL.

Metapopulations or Patchy Populations? Population Structure of Three Species of Aquatic Animals from the Florida Everglades

Joel C. Trexler¹, Karen L. Kandl^{1,2}, and C. Frank Jordan³

¹Department of Biological Science, Florida International University,

Miami, Florida 33199

Telephone: (305) 348-1966

E-mail: trexlerj@fiu.edu

²E-mail: kandlk@fiu.edu

³Department of Biological Science, Campus Box 25, Loyola University New Orleans,

New Orleans, Louisiana 70118

Telephone: (504) 865-3829

E-mail: jordan@loyno.edu

Abstract.-Dynamics in metapopulations are important for their effects on the partitioning of genetic diversity, but are the dynamics of “patchy populations” that are rapidly recolonized after local extinction of similar significance? We examined population structure in three species of aquatic animals from the Florida Everglades to test hypotheses about the effect of recurrent local extinction and recent regional sub-division on genetic diversity. The Everglades is a large wetland that experiences wet-dry season fluctuation in water level and was subdivided into water management units by canals and levees between the 1907 and 1963. Two species of fish, the eastern mosquitofish (*Gambusia holbrooki*) and the spotted sunfish (*Lepomis punctatus*), and one crustacean, the riverine grass shrimp (*Palaemonetes paludosus*), were chosen for study based on their population dynamics. All three species displayed levels of heterozygosity typical for their taxonomic group. Genetic differentiation, as indicated by Weir and Cockerham’s F_{ST} , was greatest at a local scale for all three species. The eastern mosquitofish displayed statistically significant, but weak, regional population structure related to the water management structures ($F_{ST} = 0.003$), with more heterogeneity among local subpopulations ($F_{SP} = 0.010$). Mosquitofish from a water management unit isolated from the surrounding Everglades for 60 to 90 generations had low heterozygosity ($\overline{H} = 0.127$, study $\overline{H} = 0.149$). Subpopulations from canals and short-hydroperiod marshes had relatively high heterozygosity, compared to those from long-hydroperiod marshes. Spatial autocorrelation of allele frequencies was weak at short distances, and absent between subpopulations separated by >10km. Averaged across loci, mosquitofish subpopulations from short-hydroperiod marshes had greater F_{SP} than those from nearby long-hydroperiod marshes, consistent with a model of migration into the short-hydroperiod marshes from local long-hydroperiod marshes. Grass shrimp displayed

no population structure among water management units, but weak differentiation among subpopulations ($2_{SP} = 0.006$). Heterozygosity did not differ among water management units or in relation to hydroperiod. There was no evidence of isolation by distance in this species, and population structure did not differ between short and long-hydroperiod marshes. Spotted sunfish displayed no statistically-significant structure at the regional or local level, though 2_{SP} ($= 0.02$) was high for this study. The confidence intervals around all genetic parameters for this species were large because of marked inter-locus variability. Heterozygosity did not differ among regions or in marsh versus canal sites. 2_{SP} from canal samples was less than from marsh samples for 3 of 4 polymorphic loci.

All three species studied could be described as inhabiting “patchy” populations that periodically go extinct, but are recolonized quickly. For mosquitofish, and possibly spotted sunfish, local population dynamics appear to have affected their population genetic structure. Grass shrimp may have been less affected by local population dynamics, possibly because they have large effective population sizes through drought events. These data indicate that the dynamics of patchy populations can affect population genetic structure, probably as a result of spatially-structured recolonization.

Key words.-Metapopulations, colonization, local extinction, population structure, Everglades, mosquitofish, sunfish, grass shrimp, isolation by distance, F-statistics

The spatial pattern of subpopulation numerical dynamics, including local recolonization following extinction and the origin of colonists, has important implications for population genetic structure (Slatkin 1977; Wade and McCauley 1988; Hastings and Harrison 1994). Harrison (1991) identified several patterns of population structure to illustrate the complexity of spatial population dynamics and migration, and to provide a basis for discussion of metapopulations (Fig. 1). Her examples range from Levins' metapopulation, a "population of populations," to "patchy populations" with short-lived local extinctions and complete mixing of colonists from many sources. In spite of the diversity of demographic dynamics in nature, techniques for estimating gene flow from population-genetic structure (Slatkin 1985, 1987) derive from the assumption of allele frequencies near equilibrium with contemporary demography. While these techniques are indeed robust to historical effects in the not-too-recent past (Crow and Aoki 1984; Slatkin 1993, but see Templeton et al. 1995), persistent effects of local, contemporary, population fluctuation remain poorly examined empirically (Boileau et al. 1992). To remedy this, recent theoretical advances provide a framework for testing hypotheses about population genetic structure in metapopulations not at equilibrium (Slatkin 1977; Wade and McCauley 1988; Whitlock and McCauley 1990).

For many species, some fraction of their populations exist in a state of numerical flux from periodic extinction and recolonization. Wade and McCauley (1988) describe such a metapopulation with stable subpopulations that provide colonists to less-stable subpopulations that periodically go extinct (Fig.1, B & D). Their model presents a dichotomy between the number of initial colonists following extinction, and routine migration among subpopulations. A key result is that some patterns of recolonization can

enhance genetic divergence of the fluctuating subpopulations, relative to the stable ones providing colonists. Two extreme forms of recolonization have been considered, the migrant pool and the propagule pool (Slatkin 1977; Wade 1978). In migrant-pool recolonization, colonists arrive as a random sample of individuals from the entire metapopulation, while in propagule-pool recolonization colonists are derived from a single subpopulation. Propagule-pool recolonization yields founder effects that persist a length of time determined by the rate of gene flow among subpopulations that resumes after recolonization (Wade and McCauley 1988). In reality, recolonization dynamics fall on a continuum from propagule pool to migrant pool (Whitlock and McCauley 1990). The geographic origin of colonists and the spatial scale of propagule mixing are key factors in both genetic and demographic models of metapopulations.

Population genetics of aquatic animal populations in the Florida Everglades, U.S.A., may be strongly influenced by local extinction and recolonization dynamics. The Everglades is a large, geologically young (.5,000 years old, Gleason and Stone 1994), wetland that is highly influenced by the subtropical wet-dry seasonal environment of south Florida (Fennema et al. 1994). In the annual dry season from November to May, large portions of the drainage basin may dry, forcing aquatic animals into deep-water refugia or stranding them in isolated pools where they die. Solution holes in the limestone basement rock, depressions maintained by alligators, and a core long-hydroperiod region that seldom dried served as refugia for aquatic animals in the historical Everglades (Loftus and Kushlan 1987). In infrequent drought years, even this core long-hydroperiod region may have dried, and only rare solution holes reaching into the ground water and permanent bodies of

water to the north (Lake Okeechobee) and south (Florida Bay) remained as refugia (Loftus et al. 1992). The most recent system-wide drought occurred from 1989-1990.

Starting in 1907, the Everglades were subdivided by canals and levees into water management units, called Water Conservation Areas (WCA). The last major levee was completed in 1963 (Light and Dineen 1994). Water continues to flow south through most of the water management units by way of water control structures, and under control of water management agencies. In the southern Everglades, one water management unit has been completely isolated by levees since 1963 (WCA-3B). Hydrological dynamics are now regulated independently in each management unit, though highly dependent upon rainfall, and canals serve as permanent deep-water refugia for aquatic animals.

We hypothesize that two levels of population structure may be present for aquatic animals living in the Everglades: one of regional variation resulting from barriers to gene flow by levees and canals superimposed over the historical pattern of a large unimpeded river; and a second resulting from hydroperiod and local extinction and recolonization. If migrant pool recolonization occurs, local extinctions in short hydroperiod areas will have little effect on population genetic structure within management units. In this case an island model, perhaps at the scale of water management units, may be appropriate to describe genetic diversity, and the greatest F-statistic would be seen at a regional scale. On the other hand, if recolonization is better described by a propagule pool, populations within short hydroperiod areas will be more heterogeneous (i.e., have higher F_{SP} values) than those within nearby long hydroperiod marshes. Also, the propagule pool model is indicated if the largest F-statistics are at the scale of local subpopulation samples.

Materials and Methods

Study species

We studied three common aquatic species from the Everglades: eastern mosquitofish (*Gambusia holbrooki*), spotted sunfish (*Lepomis punctatus*), and riverine grass shrimp (*Palaemonetes paludosus*). These species were chosen as typical of small and large fishes and aquatic invertebrates. Eastern mosquitofish are among the most abundant fishes in the Everglades (Loftus and Eklund 1994) and are known to be highly vagile (Brown 1985, 1987). Spotted sunfish is the only relatively long-lived and large fish species that could be caught in Everglades marshes in numbers necessary for this study (large fish are rare in the Everglades: Loftus and Kushlan 1987), but is much less abundant than mosquitofish. Grass shrimp are among the most abundant large aquatic animals in the Everglades (Turner and Trexler 1997). Mosquitofish and grass shrimp complete two to three generations per year in south Florida, while the spotted sunfish has a multi-year life cycle. Sampling studies indicate local extinctions over much of the Everglades in the drought years of 1989 and 1990, and recurrent extinction in short hydroperiod marshes for mosquitofish and grass shrimp (Fig. 2). Spotted sunfish probably displayed similar patterns but were not sampled in large numbers.

Field methods

We collected eastern mosquitofish, spotted sunfish, and grass shrimp throughout Water Conservation Areas 3A and 3B, and Everglades National Park to document their population-genetic structure (Fig. 3). Grass shrimp and mosquitofish were easily collected with dip nets, so we examined up to 50 specimens from each of 52 locations. These sites

were distributed among five water management areas to permit comparisons within and among areas separated by water control structures and canals (Fig. 3). Spotted sunfish were sampled in deep-water sites by angling and electrofishing. We were successful in collecting at least 20 specimens from each of 15 sites. Each site was sampled in the dry season of 1996. Sampling focused on the dry season to obtain specimens from areas where they were concentrated because of declining water levels. Too few spotted sunfish were collected from some sites in 1996, so additional specimens were collected in the dry season of 1997 and pooled into a single sample. We found no evidence of inter-annual variation in allele frequencies in these samples.

Study sites were categorized into four classes describing their hydroperiod (Table 1). These classes were short hydroperiod (average inundation with water between 1965 and 1995 <240 days per year, or marsh surface exposed for any period of time in 5 or more years between 1985 and 1995), intermediate hydroperiod (average inundation 240 to 330 days per year, or marsh exposed in 2 to 5 years between 1985 to 1995), long hydroperiod (average inundation more than 330 days per year, or marsh exposed in 2 or fewer years between 1985 and 1995), and permanently inundated canals. These determinations were made from simulations run by the South Florida Water Management Model (Fennema et al. 1994) and first-hand field experience at the study sites (W. Loftus, S. Coyne, and J. Trexler, pers. obs.).

Laboratory methods

We used starch gel electrophoresis to document patterns of allozyme variation in our 3 study species. Upon collection, animals were transferred to the laboratory and stored in a -80°C freezer prior genetic analysis. 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, 0.25 M sucrose, 0.005 M mercaptoethanol). For grass shrimp, the abdomens were removed and stored separately for later analysis; eye, liver, and soma clips were pooled for the two fish species, though intestines were not removed from small mosquitofish. Preliminary analyses indicated no tissue-specific expression of the proteins examined in the fish species, justifying this approach.

We surveyed 11 to 15 presumptive protein-encoding loci with horizontal starch-gel electrophoresis for the three species (Table 2). For grass shrimp and mosquitofish, we screened 32 loci before settling on 11 and 15 loci to score on all individuals; we screened 37 loci in spotted sunfish before choosing 15 to score on all specimens (Table 2). We followed standard techniques described in Selander et al. (1971) and Murphy et al. (1996), with 11% (w/v) starch gels. A Tris-Citrate pH 8.0 buffer (TC8; Selander et al. 1971) was used for all loci scored for grass shrimp and spotted sunfish. Mosquitofish were run on three different buffer systems: TC8, Lithium-Borate/Tris-Citrate (LIOH; Selander et al. 1971), and Tris-Citrate-EDTA (JRP; Ayala et al. 1972).

Statistical methods

Consistency with Hardy-Weinberg frequencies is a simple test of many of the assumptions of further genetic analyses of population structure. We examined goodness-of-fit to Hardy-Weinberg expectations at each locus, and in global tests across loci and across populations using GENEPOP (Raymond and Rousset 1995) and BIOSYS (Swofford and Selander 1981). When only two or three alleles were observed, we used the complete enumeration method of Louis and Dempster (1987), while for loci exhibiting more than three alleles, we used the Markov chain method to estimate exact P values (Guo and

Thompson 1992). No loci deviated significantly from Hardy-Weinberg frequencies in global tests, so these will not be reported further. After testing for consistency with distributional assumptions, patterns of heterozygosity were examined using analysis of variance (Archie 1985).

We used Weir and Cockerham's (1984) coancestry method to partition the total genetic variation observed for each species. These partitions were attributable to variation among individuals within subpopulations (individuals collected in areas $<1\text{km}^2$) relative to total diversity within their subpopulation (F_{IS}), among subpopulations within water-management units relative to the total diversity in that unit (F_{SP}), and among water-management units (populations) relative to the total genetic diversity (F_{PT}). This provided estimates of Wright's hierarchical F statistics of population subdivision based on a random-effects sampling model (Weir 1990). The populations correspond to individuals within areas enclosed by levees and canals, such as Water Conservation Area 3A south of I-75 and east of the Miami Canal, or Water Conservation Area 3B (see Fig. 3 for region names). The Genetical Data Analysis program (GDA; Lewis and Zaykin 1997) was used to estimate hierarchical F's following Weir and Cockerham (1984), with bootstrapping across loci to estimate confidence intervals.

F_{SP} can be used to estimate the spatial scale for isolation by distance (IBD) by examination of pairwise estimates of its value for all samples relative to the geographic distance separating them, on log-transformed scales (Slatkin 1993). In cases where many values are zero, or negative, Slatkin (1993) suggested that G_{SP} (Nei 1973) may be substituted for F_{SP} because G_{SP} does not take negative values. We estimated G_{SP} with GENEPOP (Raymond and Rousset 1995). The use of all possible pairwise comparisons of

populations raises a statistical problem of independence in the regression. This problem was resolved by adjusting the number of degrees of freedom downward to reflect the actual number of populations used in the comparison (Hellberg 1994). We used analysis of covariance (ANCOVA) to determine if the scale of genetic differentiation was affected by potential barriers to gene flow. We compared subpopulations separated by a particular geographic distance within a water management unit to those separated by the same distance but with a levee or canal between them (i.e., in different management units).

To supplement analyses of F-statistics, we performed a geostatistical analysis to document the spatial scale of autocorrelation in allele frequencies (Epperson and Li 1996). Both spatial autocorrelation and regression of pairwise F-statistics make three assumptions of the data: stationarity, isotropy, and sampling at the appropriate scale (Cressie 1991). Stationarity requires that relationships among all subsets of samples are maintained across the study area, i.e., the rate of change in the dependent variable across space does not change in different regions of the study area. Isotropy indicates that spatial processes occur similarly in all directions within the study area, e.g., unidirectional gene flow could yield anisotropy in allele frequencies. Finally, the spatial separation of sampling sites must be less than the scale of the ecological process under study, but the spatial extent of sampling sites must be extensive enough to capture the process. We employed an exploratory data analysis to check the validity of these assumptions prior to spatial autocorrelation and to describe patterns in our data that may not conform to the assumptions of geostatistical analyses (Statistical Sciences 1995; Mathsoft 1996). We used a nonparametric generalized additive model (GAM) with locally weighted regression (LOESS) to test for spatial pattern in the frequency of the most common allele of all variable loci (Cleveland 1993). The

model included LOESS regression in several north-south and east-west compass directions to test the isotropy assumption; no rotation gave a better fit than the north-south dimension, probably because this corresponds roughly to the flow of water so we will not discuss this further. We chose to group our data into five distance classes for spatial autocorrelation in order to assure a minimum of 25 population pairs in each bin. Other grouping schemes did not change our results. A correlation of at least 0.30 for mosquitofish and grass shrimp, and 0.44 for sunfish, was significant at the $\alpha = 0.05$ critical level.

During a drought in the dry seasons of 1989 and 1990, all short and intermediate hydroperiod sites were dry. A sparse scattering of aquatic refugia in ponds maintained by alligators may have persisted in the long-hydroperiod sites; however, only canal sites undoubtedly remained as aquatic refugia in those years. In many or most years since 1990, depending on local elevation, the short and intermediate hydroperiod sites dried and were re-colonized from long-hydroperiod sites (J. Trexler, pers. obs.). We tested the hypothesis that propagule-pool recolonization dynamics described the genetic diversity in our study area by comparing estimates of 2_{SP} estimated separately for sites grouped by hydroperiod following McCauley et al. (1995). We estimated 2_{SP} with a jackknife procedure (Hollander and Wolfe 1973), resampling across subpopulations to diminish the influence of individual samples. Resampling was desirable because our sample sizes in each hydroperiod category from each water management unit were small. We used non-parametric statistics (Friedman's test) to test hypotheses treating loci as independent replicates because the sampling distribution of 2 is unknown. We had small numbers of replicates (loci) and this test is less influenced by outliers than parametric test statistics.

The probability of rejecting the null hypothesis was combined across tests from separate water management units using Rice's (1990) sequential Bonferroni procedure.

Results

Mosquitofish

Genetic diversity.-Mosquitofish displayed extensive genetic variation at the allozyme loci examined. Though 15 loci were scored for all individuals, one (Fum) was monomorphic in all populations and 2 others proved unreliable, leaving 12 that provided data from all 52 study populations. Seven of the loci screen yielded two alleles, four yielded three, and two yielded four alleles (Table 2). In an average population, 61% of the loci screened were polymorphic in 1% or more of the specimens examined (Table 3). The average heterozygosity at the 12 loci retained was 0.15 (range 0.001-0.473).

No global pattern of deviation from Hardy-Weinberg expectations was observed. Only 17 out of 395 tests for consistency with the Hardy-Weinberg expected genotypic ratios were significant, less than the 20 expected by chance at the 5% testing level. No indication of null alleles, mis-scoring from protein degradation, or other sources of error could be identified as responsible for the deviations observed. For all loci but Gpi-2, non-compliance with Hardy-Weinberg expectations resulted from rare alleles (alleles with an expected value less than 5). Genotypic frequencies of Gpi-2 diverged significantly from expectations in two subpopulations.

The level of heterozygosity varied among water management areas, and among hydroperiods. Heterozygosity was highest in WCA-3Ase, and lowest in WCA-3B; canals had the highest heterozygosity and long-hydroperiod sites had the lowest (Table 4). There was a significant water-management-unit by hydroperiod interaction.

Population structure and isolation by distance.-Hierarchical F-statistics indicated that most of the genetic diversity was at the local level. Variation among subpopulations within populations was greater than variation among populations ($2_{SP} > 2_{PT}$; Table 5). Statistically significant within population subdivision was noted ($2_{SP} = 0.0106$); a small, though statistically significant, amount of additional variation was noted among populations ($2_{PT} = 0.0033$). There was no evidence of systematic deviation from Hardy-Weinberg allele frequencies within populations at this level of analysis (95% confidence interval for F_{IS} included 0.0; Table 5).

To further examine the source of the among-subpopulation variation, we compared estimates of 2_{sp} among populations using estimates from each locus as replicates. Populations (=water management units) differed in the amount of heterogeneity among subpopulations as measured by 2_{sp} (Friedman test statistic = 15.05, $P=0.020$), with the highest in Taylor Slough and lowest in WCA-3B (Table 6). The source of these results can be seen in the comparison of heterozygosity among water management units. Higher heterozygosity was observed in two of the management units in the northern part of the study area (WCA-3Anw and se), while significantly less heterozygosity was observed in WCA-3B (Table 4; Fig. 4). One possible source of the high variation among subpopulations within regions is differences in hydrology (canal sites, short, intermediate, and long hydroperiod). Canal and short hydroperiod sites displayed significantly higher heterozygosity on average, than long and intermediate hydroperiod sites (Table 4; Fig. 4). More canal sites occur in the northern portion of the study area. There is a significant hydroperiod by region interaction, mostly seen in TS

where the rank order of heterozygosity for short and long hydroperiod sites is the converse of the study-wide means (Fig. 4, Table 4 least-square means for hydroperiod).

We found no evidence of significant IBD in mosquitofish within water management units. However, comparisons of $\hat{M}$ estimated from subpopulations separated by canals or levees to estimates from more contiguous subpopulations separated by the same geographic distance provided evidence of reduced gene flow. $\hat{M}$ estimated from G_{ST} was unrelated to the Euclidean or water-flow distance separating subpopulations. However, $\hat{M}$ between subpopulations separated by a given distance and within the same water-management was higher than $\hat{M}$ between subpopulations from different units but separated by the same geographic distance (Fig. 5; ANCOVA:

$\hat{M} = \text{constant} + \ln[\text{distance}] + [\text{presence/absence of structure}]$, distance $F_{1,51}=2.285$, $P>0.2$, structure $F_{1,51}=12.26$, $P=0.001$, $R^2 = 0.012$; the interaction was not significant and was dropped from the model). Note that the effect of water control structures, while present, explained only a little over 1% of the total variance in pairwise comparisons. Analyses of G_{SP} and 2_{SP} gave the same qualitative results.

A complex pattern of spatial variation was revealed from three loci, with allele frequency changes over short geographic distances; autocorrelation, if present, was at a scale of 10-km or less. Allele frequencies of *Idh-1*, *Mdh-1*, and *Mpi* displayed spatial heterogeneity from north to south in the study area (Fig. 6; GAM regression: *Idh-1* $F_{2,4}=12.99$, $P<0.0001$, *Mdh-1* $F_{2,4}=2.73$, $P=0.068$, *Mpi* $F_{2,4}=2.80$, $P=0.063$). Two of the

five loci showed positive autocorrelation in the 3.1 and/or 10.8 km distances classes (Fig. 7; Idh-1, Mdh-1). Autocorrelation was not different from zero in comparisons over longer distances, except for one locus that indicated a significant negative correlation at the spatial scale of 40 kms. The non-linear spatial pattern of allele frequency change, especially at IDH-1, violates the stationarity assumption of spatial autocorrelation and decreases the correlations observed.

We found some evidence that nearby long-hydroperiod and canal subpopulations were more similar to each other than were those in short and intermediate hydroperiod marshes by combining non-significant results from the three largest management units. 2_{SP} from relatively short-hydroperiod sites was greater than that from longer hydroperiod sites in the same region in a majority of cases in Taylor Slough, Shark River Slough, and WCA-3Asw (Table 7). Though only one individual comparison was close to significance at the $\alpha = 0.05$ level (WCA-3Asw), the combined probability of the three tests was very close to that critical level ($z_p = 1.498$, $P = 0.067$). When all data were used to estimate 2_{SP} without regard to regional location, no statistically significant pattern emerged (Friedman statistic 0.091, $P = 0.763$). The rank order of the median 2_{SP} estimates was short > long > canal (Table 7).

Grass shrimp

Genetic diversity.-Grass shrimp displayed extensive genetic variation at the allozyme loci examined. Of the 12 loci scored for all individuals, 4 (Aat-1, Gapdh, Ldh-2, and Idh-1) proved to be monomorphic in all of the populations surveyed, and 3 others showed little variation (Mdh-2, Mdh-3, and Mdhp). Two of the loci screened yielded two alleles, 1 yielded three, 1 yielded 4, 1 yielded 7, and 2 yielded 9 alleles (Table 2). In

an average population, 44.0% of the loci screened were polymorphic at the 1% or greater level (Table 3). The average heterozygosity within a population across all loci was 0.161 (range 0.001-0.506).

Analysis of genotypic frequencies from shrimp indicated some deviation from the expectations of the Hardy-Weinberg law. Overall, more populations were out of Hardy-Weinberg frequencies than expected by chance (21 out of 262 tests, $B=2.24$, $P=0.0125$), though all of these resulted from the absence of heterozygotes of rare alleles. When rare allele classes were pooled, no significant deviations were noted. Twice as many populations as expected were not in Hardy-Weinberg frequencies at Mpi and Pgm (6 out of 51 tests, 3 expected at the 5% $\forall$ level), though none deviated from expectations when rare alleles were pooled.

There were no spatial patterns of heterozygosity in the shrimp samples. Heterozygosity did not differ among the study regions or by hydroperiod (Fig. 4; Model: region + hydroperiod + region*hydroperiod, $F_{17,33} = 1.144$, $P = 0.358$).

Population structure and isolation by distance. -Hierarchical F-statistics indicated no population structure related to water management units, though a small but significant amount of inter-subpopulation variation (2_{SP}) was present (Table 5). Although generally greater than zero (based on bootstrapped F-statistics), the regions were similar in the amount of variation among subpopulations (Table 6). At a local level, the level of subpopulation heterogeneity was similar in all the study regions.

We found no evidence of IBD in grass shrimp from the study area by regression of G_{SP} on geographic distance. The slope of $\ln(G_{SP})$ on $\ln(\text{distance})$ was not significantly different from zero (Fig. 5; ANCOVA:

$\hat{M} = \text{constant} + \ln[\text{distance}] + [\text{presence/absence of structure}]$, distance $F_{1,51}=0.355$, $P=0.5$, structure $F_{1,51}=2.031$, $P>0.2$, $R^2 = 0.002$; the interaction was not significant and was dropped from the model). This suggests that grass shrimp populations in our study area can be described by an island model. One population was uniquely differentiated compared to its neighbors (population number 13, Table 1), and the G_{SP} estimates for all pairwise comparisons with it were unusually small. However, there was no change in the outcome of the regression analysis when it was repeated excluding that population.

There was little evidence of spatial autocorrelation in allele frequencies of grass shrimp (Fig. 6). Only Pgm displayed spatial variation in allele frequencies, and only in the north-south dimension (Fig. 6; Nonparametric regression: Pgm $F_{2,3}=3.43$, $P=0.035$). The only statistically significant spatial autocorrelation was observed in the shortest distance class at a single locus (Fig. 7; Pep).

There was no evidence that hydroperiod affected population genetic structure in the grass shrimp. Unfortunately, our test of this hypothesis was not as strong as for the mosquitofish because inadequate data were available to test it in Taylor Slough; shrimp were not collected at enough long-hydroperiod sites to test the hypothesis there. Long hydroperiod marshes yielded estimates of 2_{SP} that exceeded those of short hydroperiod marshes (Table 7), the reverse of our predictions based on propagule pool recolonization of short hydroperiod marshes from nearby long hydroperiod ones. This lack of support for propagule pool migration was observed in both Shark River Slough and Water Conservation Area 3Asw.

Spotted Sunfish

Genetic diversity.- Spotted sunfish displayed the lowest genetic diversity of the three species examined by each of the measures we calculated (Table 3). Of the fifteen loci examined, four proved monomorphic in all populations surveyed. The average multilocus heterozygosity was 0.128. Acon and Pgm were particularly variable with heterozygosities exceeding 0.4, while Aat and Idh could be considered moderately variable ($0.05 < H < 0.4$). Thus, most of the information about population structure in this species came from these four loci.

No general pattern of deviation from Hardy-Weinberg expectations was observed in individual samples. Only 4 out of 61 tests indicated deviation from Hardy-Weinberg expectations (3 were expected at the $\forall = 0.05$ level), and all of these were from rare alleles (fewer than 5 observations expected).

There were no spatial patterns of heterozygosity in the spotted sunfish samples. Heterozygosity did not differ among the study regions or by hydroperiod (Fig. 4; Model: region + hydroperiod + region*hydroperiod, $F_{6,8} = 0.838$, $P = 0.574$, $R^2 = 0.386$).

Population structure and isolation by distance.- None of the partitions of genetic variation were significantly different from zero (Table 5, all confidence intervals include zero). However, the estimate for the among-regions partition was negative, while the partition estimate among subpopulations within regions was 0.02; the latter is the largest estimate in our study. The lack of statistical significance in our estimates arose from heterogeneity among loci (the estimates are bootstrapped across loci) because the confidence intervals are large for all of these estimates. For example, estimates of 2_{SP} were approximately zero for five loci (Acon, Aat-2, Idh, Mdh-2, and Pgd-1), 0.024 for Pep, 0.063 for Pgm-1, and 0.085 for Ldh.

Some evidence of isolation among water management units was observed for data from spotted sunfish. There was no evidence for IBD within water management units.

Geographic distance was not significant in any model we considered, however,

$\hat{\sigma}_M^2$ estimated between subpopulations from the same water management unit was higher than

$\hat{\sigma}_M^2$ estimated between subpopulations from different water management units (Fig. 5;

ANCOVA:

$\hat{\sigma}_M^2 = \text{constant} + \ln[\text{distance}] + [\text{presence/absence of structure}]$, distance $F_{1,13}=2.307$, $P>0.2$, structure $F_{1,13}=5.153$, $P=0.044$, $R^2 = 0.066$, the interaction was not significant and was dropped from the model). There was very little overlap in the geographic distance separating comparisons within and among management units (little overlap in two predicted lines in Fig. 5), so the within/between regions comparison was confounded with distance. These analyses do not reject the use of a simple island model to describe patterns of variation within water management units.

We examined spatial variation in allele frequencies and autocorrelation in this species, but samples sizes were too low to take full advantage of these techniques. We found no significant spatial variation in allele frequencies by fitting GAMs. However, two loci presented patterns that may have been significant with more samples (GAM: Ldh $F_{3,4}=2.79$, $P=0.138$, Pep $F_{3,4}=2.29$, $P=0.186$). Though none of the spatial autocorrelations were significant ($n=18$, minimum significant $r = 0.44$ at $\alpha = 0.05$), Acon, Ldh, and Pep displayed small negative correlations at the shortest distance comparisons.

Subpopulation variation among marsh sites was not significantly different from canal sites (Table 7). The direction of the comparison was consistent with the hypothesis that canals are home to more stable subpopulations (smaller 2) than marshes. However, marked inter-locus variation was indicated by the difference between the mean and median estimates of 2 , especially in marshes (Table 7). The most differentiated locus (Ldh) of the seven compared was inconsistent with the hypothesis, the other six were either not different or were consistent with the hypothesis.

Discussion

Everglades aquatic animal populations undergo marked inter-annual variation in density and are restricted to a limited number of refugia for portions of some years. Aquatic animals generally recolonize the marsh soon after water re-floods an area, but population sizes have been diminished and the source of colonists is generally not known. Harrison (1991) distinguished patchy populations from true metapopulations because, in a patchy population, recolonization is rapid and from spatially dispersed sources obscuring local from regional population dynamics. If recolonization is predominantly from a local source that has undergone a numerical bottleneck, local and regional dynamics can be uncoupled in both demography and genetics (Wade and McCauley 1988; Whitlock and McCauley 1990). Thus, the source of colonists following a local extinction can leave an imprint on genetic diversity for some time following local extinction and recolonization. This is manifest by the greatest partition of genetic diversity at the local scale in this and other studies (e.g., Ruckelshaus 1998) and supports the rejection of a pure migrant-pool model.

Different Processes at Different Spatial Scales

Our data indicate that different processes may be operating at different scales in the Florida Everglades. The spatial scale of recolonization following local extinction appears most important within water management units, while limitation of gene flow by some water management structures influences ecosystem-wide patterns. Recolonization from local sources is indicated by $2_{SP} > 2_{PT}$. Also, we observed little evidence of IBD within water management units in any of the three species. Spatial autocorrelation of allele frequencies indicated weak similarities among populations of mosquitofish at scales of 10km or less and no correlations at longer scales. This pattern is consistent with a stepping-stone migration model where exchange occurs only among adjacent subpopulations (Ibrahim et al. 1996), though the large inter-locus variation in our results clouds this interpretation. Such large interlocus variation is symptomatic of recently recolonized subpopulations. The distribution of F_{ST} following a perturbation may be highly non-normal and the rate of approach to equilibration depends on initial allele frequencies (Nei et al. 1977). The autocorrelation results are supported by the regressions of $\hat{M}$ on geographic distance within water management units. Regional variation is linked to habitat subdivision that has arisen in the past 60 to 200 generations of the organisms we studied (e.g., the low heterozygosity in WCA3B), while the local variation may have originated largely from the 1989-1990 drought and is routinely reconstituted each drought (e.g., the pattern of 2_{SP} in short and long hydroperiod sites in mosquitofish).

There was evidence of isolation among water management units in mosquitofish and spotted sunfish, but not grass shrimp. Distance-adjusted comparisons of $\hat{M}$ among management units were significantly different for both mosquitofish and spotted sunfish, but geographic distance and comparisons among units were confounded in spotted sunfish.

Regional variation in mosquitofish was also indicated by hierarchical analysis of genetic variation (F_{PT}), but was not significant in spotted sunfish (Table 5). The low heterozygosity of mosquitofish in WCA-3B may be our most striking indication of regional variation. WCA-3B has been isolated from the rest of the ecosystem since 1967. Fluctuating populations (Sjogren 1991), and those experiencing frequent turnover (Vrienhoek 1985; Waller et al. 1987), may display low heterozygosity (Hastings and Harrison 1994). Any differentiation at the regional scale corresponding to water-management units has arisen since the management system was installed over the past 30 to 80 years. The differences observed could have come from limitation in gene flow, or by marked differences in population dynamics among management units. Most of WCA3Ane and WCA3Anw regularly go dry and aquatic animal populations there may experience lower minima in population size than the other areas of the study.

Studies of population structure should explicitly identify the scale of processes that are intended for study (Hellberg 1994; Husband and Barrett 1994), and ideally should include information from scales above and below that of interest. In our study, mosquitofish loci *Idh-1*, and to a lesser extent *Mdh-1* and *Mpi*, displayed patchy changes in allele frequency (Fig. 6). Comparisons of samples from the southern end of the study show less spatial variation than those from the northern end. This is a violation of the stationarity assumption inherent in both spatial autocorrelation and regression-based IBD estimates of gene flow. Such patterns could arise from selection. However, regional differences in population fluctuation related to hydrological variation among water-management units may provide a more parsimonious explanation.

Origins of Population Structure

In mosquitofish, there was evidence that subpopulation differentiation was related to hydroperiod. We observed some evidence for greater differentiation among local subpopulations from short-hydroperiod marshes than among local subpopulations from long-hydroperiod areas. We hypothesize that this indicates long-hydroperiod marshes are source habitats for short-hydroperiod colonists. We have underestimated the difference in population structure between these two habitats following local extinction because at least 18 mosquitofish generations of gene flow have passed since the last system-wide dry down (Fig. 2). Exploring the pattern of migration further will require estimates of the number of initial colonists into short-hydroperiod regions after local extinction (Dybdahl 1994; McCauley et al 1995). Grass shrimp and sunfish did not display the pattern of genetic diversity predicted by our hypothesis of colonization of short hydroperiod marshes from nearby long hydroperiod areas. This may be because gene flow since the last dry-down has been common enough to eliminate any residual patterns from the recolonization process.

The population genetic structure of grass shrimp may be affected differently by population fluctuations than population structure of mosquitofish. The absence of structure and small estimates for 2_{SP} indicate that relatively large effective numbers of shrimp survive through droughts, at least compared to mosquitofish. Comparisons of F-statistics between species must be made with caution because they are potentially influenced by the absolute level of diversity in the genetic markers assayed. The heterozygosity was a little higher in grass shrimp than mosquitofish, and the number of alleles per locus was higher in grass shrimp. The number of polymorphic loci was higher in mosquitofish. The greater number of alleles per locus and higher heterozygosity in shrimp should have made it easier

to observe population structure in that species, if it were present. Thus, it appears that our results are in contradiction to biases that might be expected based on species differences in genetic diversity.

Genetic Diversity

Our estimates of heterozygosity were similar to those reported in other studies of these, or closely related, species. The eastern mosquitofish has been studied extensively in the southeastern United States and mean estimates of

$\overline{H}$ vary from 0.141 to 0.242 (Hernandez et al. 1995, Hernandez-Martich and Smith 1996); our estimates ranged from 0.127 to 0.184. Heterozygosity for the grass shrimp *P. pugio* ranged from 0.050 to 0.083 in Texas populations (Fuller and Lester 1980), while our estimates from the Everglades for *P. paludosus* were 0.108 to 0.235. Jennings and Philipp (1992) reported heterozygosities from 0.000 to 0.047 from *L. megalotis* sampled from throughout the United States, while we observed

$\overline{H}$ ranging from 0.072 to 0.167 from *L. punctatus* in the Everglades. Detailed comparisons among studies of genetic diversity estimated from allozymes is not warranted because the loci studied differ, the number of populations and specimens within populations was not the same, the spatial scale of sampling was not comparable, and laboratory techniques varied among studies. However, these estimates, and those of others (Baer 1998), indicate that Everglades aquatic animal populations display no lack of genetic diversity, as might be expected from the region's young geological age and isolation on the tip of a peninsula.

Caveat Emptor

We have followed the convention of estimating the gene flow parameter $N_e m$ (

$\hat{m}$) from our F-statistics using Wright's formula. However, this transformation assumes that spatial patterns of genetic diversity are approximately at equilibrium with temporal fluctuations in migration and effective population size. When populations are not near genetic equilibrium from a recent local extinction event, a simple interpretation of gene flow using F-statistics may be inappropriate (Slatkin 1985; Whitlock 1992a; Hellberg 1994; Husband and Barrett 1994; Ruckelshaus 1998). While all of the species we studied have high gene flow, it is not clear we can reliably estimate it from our F-statistics. Estimating gene flow rate from F-statistics can be biased by disequilibrium at the two temporal extremes: a "memory" of population-level genealogical relationships when gene flow no longer occurs, and disturbance from recent population extinction. Templeton et al. (1995) have provided a nested cladistic approach using parsimony assumptions to identify and circumvent the former bias. Several authors have addressed the latter form of bias through hypothesis testing based on predictions from theory (e.g., Whitlock 1992b; Dybdahl 1994; McCauley et al. 1995), as we have in this study. It is clear that analysis and interpretation of population genetic structure must be conscious of the natural history of the species under study and the temporal scale of processes shaping genetic variation. However, judicious use of these new methodologies can provide an insightful interpretation of genetic differentiation.

Conclusions

Our primary question was if population dynamics and migration of Everglades aquatic animals has affected their genetic differentiation. Based on population-genetic differentiation, the grass shrimp may be best described as a patchy population (Fig. 1,C) with homogenizing gene flow, while the mosquitofish may be more like a combination

metapopulation (Fig. 1, D) with local recolonization. Canal sites in WCA-3Asw appear to function as a core refuge for this species based on $\overline{H}$ and the pattern 2_{SP} ; long hydroperiod marshes may also be refugia for mosquitofish in the SRS and Taylor Slough. Though we have less statistical power to make a conclusion about spotted sunfish, they may be best described as a core-satellite metapopulation with canal and creek populations (in southern SRS) acting as the core. The spatial scale of population ecology of these three species differs, probably as a function of their population sizes, migratory patterns, and behavioral responses to droughts. Thus, the importance of local extinctions, and other local ecological phenomena, is not equal in these three species. Whether populations experiencing local extinctions and rapid recolonization are true metapopulations depends on the source of colonists. Local scale colonization dynamics can have consequences for the partitioning of genetic variation, and appears to for some species inhabiting the Everglades.

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Literature Cited

- Archie, J. W. 1985. Statistical analysis of heterozygosity data: independent sample comparisons. *Evolution* 39:623-637.
- Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourao, and S. Perez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics* 70:113-139.
- Baer, C. F. 1998. Species-wide population structure in a southeastern U. S. freshwater fish, *Heterandria formosa*: gene flow and biogeography. *Evolution* 52:183-193.
- Boileau, M. G., P. D. N. Hebert, and S. S. Schwartz. 1992. Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. *J. Evol. Biol.* 5:25-39.
- Brown, K. L. 1985. Demographic and genetic characteristics of dispersal in the mosquitofish, *Gambusia affinis* (Pisces:Poeciliidae). *Copeia* 1985:597-612.
- Brown, K. L. 1987. Colonization by mosquitofish (*Gambusia affinis*) of a Great Plains river basin. *Copeia* 1987:336-351.
- Cleveland, W. S. 1993. Visualizing data. Hobart Press, Summit, NJ.
- Cressie, N. A. C. 1991. Statistics for spatial data. John Wiley & Sons, New York.
- Crow, J. F., and K. Aoki. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *Proc. Natl. Acad. Sci. USA* 81:6073-6077.
- Dybdahl, M. F. 1994. Extinction, recolonization, and the genetic structure of tidepool copepod populations. *Evol. Ecol.* 8:113-124.

- Epperson, B. K., and T. Li. 1996. Measurement of genetic structure within populations using Moran's spatial autocorrelation statistics. *Proc. Natl. Acad. Sci, U.S.A.* 93:10528-10532.
- Fennema, R. J., C. J. Neidrauer, R. A. Johnson, T. K. MacVicar, and W. A. Perkins. 1994. A computer model to simulate natural Everglades hydrology. Pp.249-289 *in* S. M. Davis and J. C. Ogden, eds. *Everglades, the ecosystem and its restoration*. St. Lucie Press, Boca Raton, FL.
- Fuller, B., and L. J. Lester. 1980. Correlations of allozymic variation with habitat parameters using the grass shrimp, *Palaemonetes pugio*. *Evolution* 34:1099-1104.
- Gleason, P. J., and P. Stone. 1994. Age, origin, and landscape evolution of the Everglades peatland. Pp. 149-197 *in* S. M. Davis and J. C. Ogden, eds. *Everglades, the ecosystem and its restoration*. St. Lucie Press, Boca Raton, FL.
- Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:361-372.
- Harrison, S. 1991. Local extinction in a metapopulation context: an empirical evaluation. *Biol. J. Linn. Soc.* 42:73-88.
- Hastings, A., and S. Harrison. 1994. Metapopulation dynamics and genetics. *Ann. Rev. Ecol. Syst.* 25:167-188.
- Hellberg, M. E. 1994. Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. *Evolution* 48:1829-1854.
- Hernandez, J. D., J. M. Novak, M. H. Smith, and P. E. Johns. 1995. Genetic structure of mosquitofish populations in the Altamaha and Ogeechee drainages of Georgia:

- reporting an undescribed form in the Ocmulgee River. *Biochem. Sys. Ecol.* 23: 617-625.
- Hernandez-Martich, J. D., and M. H. Smith. 1996. Downstream gene flow and genetic structure of *Gambusia holbrooki* (eastern mosquitofish) populations. *Heredity* 79: 295-301.
- Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley & Sons, New York.
- Husband, B. C., and S. C. H. Barrett. 1994. Estimates of gene flow in *Eichornia paniculata* (Pontederiaceae): effects of range substructure. *Heredity* 75:549-560.
- Ibrahim, K. M., R. A. Nichols, and G. M. Hewitt. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* 77:282-291.
- Jennings, M. J., and D. P. Philipp. 1992. Genetic variation in the longear sunfish (*Lepomis megalotis*) Can. J. Fish. Aquat. Sci. 70:1673-1680.
- Lewis, P. O., and D. Zaykin. 1997. Genetic Data Analysis: computer program for the analysis of allelic data. Free program distributed by the authors at the GDA home page at <http://chee.unm.edu/gda/>.
- Light, S. S., and J. W. Dineen. 1994. Water control in the Everglades: a historical perspective. Pp. 47-84 in S. M. Davis and J. C. Ogden, eds. Everglades, the ecosystem and its restoration. St. Lucie Press, Boca Raton, FL.
- Loftus, W. F., and A.M. Eklund. 1994. Long-term dynamics of an Everglades small-fish assemblage. Pp 461-483 in S. M. Davis and J. C. Ogden, eds. Everglades, the ecosystem and its restoration. St. Lucie Press, Boca Raton, FL.

- Loftus, W. F., R. A. Johnson, and G. Anderson. 1992. Ecological impacts of the reduction of groundwater levels in the rocklands. Pp. 199-208 *in* J. A. Stafford and J. J. Simon, eds. Proc. 1st int. conf. of ground water ecology, American Water Res. Assoc., Bethesda, MD.
- Loftus, W. F., and J. A. Kushlan. 1987. Freshwater fishes of southern Florida. Bull. Florida State Mus., Biol. Sci. 31:147-344.
- Louis, E. J., and E. R. Dempster. 1987. An exact test for Hardy-Weinberg and multiple alleles. *Biometrics* 43:805-811.
- Mathsoft. 1996. S+ Spatialstats user's manual, version 1.0. MathSoft, Inc., Seattle, WA.
- McCauley, D. E., J. Raveill, and J. Antonovics. 1995. Local founding events as determinants of genetic structure in a plant metapopulation. *Heredity* 75:630-636.
- Murphy, R. W., J. W. Sites, Jr., D. G. Buth, and C. H. Haufler. 1996. Proteins: Isozyme electrophoresis. Pp. 51-120 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics, 2d edition. Sinauer, Sunderland, MA.
- Nei, M. 1973. Analysis of genetic diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA* 70:268-294.
- Nei, M., A. Chakravarti, and Y. Tatenno. 1977. Mean and variance of F_{ST} in a finite number of incompletely isolated populations. *Theoret. Popul. Biol.* 11:291-306.
- Rice, W. R. 1990. A consensus combined P-value test and family-wide significance of component tests. *Biometrics* 46:303-308.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 3.1): population genetics software for exact tests and ecumenicism. *J. Heredity* 86:248-249.

- Ruckelshaus, M. H. 1998. Spatial scale of genetic structure and an indirect estimate of gene flow in eelgrass, *Zostera marina*. *Evolution* 52:330-343.
- Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. R. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). *Stud. Gene. VI. Univ. of Texas Pub.* 7103:49-90.
- Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoret. Popul. Biol.* 12:253-262.
- Slatkin, M. 1985. Gene flow in natural populations. *Ann. Rev. Ecol. Syst.* 16:393-430.
- Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787-792.
- Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47:264-279.
- Sjogren, P. 1991. Genetic variation in relation to demography of peripheral pool frog populations (*Rana lessonae*). *Evol. Ecol.* 5:248-271.
- Statistical Sciences. 1995. S-plus guide to statistical and mathematical analyses, version 3.3. MathSoft, Inc., Seattle, WA.
- Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1. A computer program for analysis of allelic variation in genetics. Univ. Illinois Press, Urbana.
- Templeton, A. R., E. Routman, and C. A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographic distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140:767-782.

- Turner, A. M., and J. C. Trexler. 1997. Sampling aquatic invertebrates from marshes: evaluating the options. *J. N. Am. Benthol. Soc.* 16:694-709.
- Vrijenhoek, R. C. 1985. Animal population genetics and disturbance: the effects of local extinctions and recolonizations on heterozygosity and fitness. Pp. 265-285 *in* S. T. A. Pickett and P. S. White, eds. *The ecology of natural disturbance and patch dynamics*. Academic Press, New York.
- Wade, M. 1978. A critical review of the models of group selection. *Quart. Rev. Biol.* 53:101-114.
- Wade, M., and D. E. McCauley. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution* 42:995-1005.
- Waller, D. M., D. M. O'Malley, and S. C. Gawler. 1987. Genetic variation in the extreme endemic *Pedicularis furbishae*. *Cons. Biol.* 4:335-340.
- Weir, B. S. 1990. *Genetic data analysis*. Sinauer, Sunderland, MA.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
- Whitlock, M. C. 1992a. Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution* 46:608-615.
- Whitlock, M. C. 1992b. Nonequilibrium population structure in forked fungus beetles: extinction, colonization, and the genetic variance among populations. *Am. Nat.* 139:952-970.
- Whitlock, M. C., and D. E. McCauley. 1990. Some population genetic consequences of colony formation and extinction: Genetic correlations within founding groups. *Evolution* 44:1717-1724.

Table 1. Sample sites characterized by hydroperiod. See Fig. 3 for locations.

Mosquitofish and grass shrimp				
Region	Short	Intermediate	Long	Canal
Taylor Slough	37, 46, 32, 47, 50		48, 49, 38, 52	
Shark River Slough	31, 33, 34, 40, 41, 42, 43, 45, 54	39, 44, 53	36, 56, 57, 55	35, 51
WCA-3B		7, 8	13	15
WCA-3Asw	2, 14	3, 17	1, 4, 5, 16	11, 12, 19, 20
WCA-3Ase			22, 6	23, 21, 18
WCA-3Anw	9, 26			24, 27
WCA-3Ane	29, 10, 28			25, 30
Spotted Sunfish				
	Marsh		Canal, Creek	
	1, 2, 4, 7, 13, 14		3, 5, 6, 8, 9, 10, 11, 12, 15	

Table 2. Allozymes scored for allelic variation and the buffer systems used for each species. E.C. no. refers to Enzyme Commission identification number for each putative locus; PEP is used as a generic term because the specific enzyme scored is unknown (see Murphy et al. 1996:111). A blank space under number of alleles indicates that loci was not scored for that species. The number of alleles observed at each locus is reported. The number of polymorphic loci screened are indicated in parentheses next to the total number of loci screened. Superscripts after the enzyme name abbreviations indicate the buffer used for analysis of mosquitofish: 1) TC8.0, 2) JRP, 3) LIOH. The TC8.0 buffer was used for all loci in prawns and sunfish.

Enzyme	E.C. no.	prawn	No.of Alleles mosquitofish	sunfish
Aconitase Hydratase (Acoh)	4.2.1.3			3
Adenosine Deaminase (Ada) ¹	3.5.4.4		4	
Aspartate Aminotransferase (Aat-1) ¹	2.6.1.1	1	2	1
Aspartate Aminotransferase (Aat-2)		4		3
Fumerate Hydratase (Fumh) ³	4.2.1.2		1	
Glucose Dehydrogenase (Gcdh)	1.1.1.118			3
Glucose-6-Phosphate Dehydrogenase (G6pdh)	1.1.1.49			
Glucose-6-Phosphate isomerase (Gpi-1) ¹	5.3.1.9	9	2	
Glucose-6-Phosphate isomerase (Gpi-2) ¹			3	
Glyceraldehyde-3-Phosphate Dehydrogenase (Gapdh)	1.2.1.12	1		

Glycerol-3-Phosphate Dehydrogenase (G3pdh)	1.1.1.8		2	
Isocitrate Dehydrogenase (Idh-1) ²	1.1.1.42	1	3	3
Isocitrate Dehydrogenase (Idh-2) ²			2	
<i>L</i> -Lactate Dehydrogenase (Ldh-2) ²	1.1.1.27	1	2	2
<i>L</i> -Lactate Dehydrogenase (Ldh-3) ²			2	
Malate Dehydrogenase (Mdh-2) ¹	1.1.1.37	2	2	1
Malate Dehydrogenase (Mdh-3) ¹		3		2
Malate Dehydrogenase (Nadp ⁺) (Mdhp)	1.1.1.40	2		
Mannose-6-Phosphate Isomerase (Mpi-1) ¹	5.3.1.8	7	3	1
Mannose-6-Phosphate Isomerase (Mpi-2) ¹				1
Phosphoglucomutase (Pgm-1) ²	5.4.2.2	9	3	4
Phosphoglucomutase (Pgm-2)				1
Phosphoglucomutase (Pgm-3) ²			3	
Phosphogluconate Dehydrogenase (Pgdh-1) ¹	1.1.1.14		4	1
Phosphogluconate Dehydrogenase (Pgdh-2)				1
Peptidase val-leu (Pep)	3.4.-.-			4
Total number of loci screened		11 (7)	15 (14)	15 (8)

Table 3. Measures of genetic variation at loci examined averaged across study populations. P indicates the proportion of loci that were polymorphic in at least 5% of the individuals sampled, A is the average number of alleles at all loci examined, and H is the average heterozygosity.

Locus	Mosquitofish			Grass Shrimp			Spotted Sunfish		
	P	A	H	P	A	H	P	A	H
Aat-1	0.173	1.33	0.012	0	1.00	0	0	1.00	0
Aat-2				0.980	2.96	0.506	1.00 0	2.00	0.271
Acoh							1.00 0	2.80	0.557
Ada	0.327	1.69	0.020						
Gapdh				0	1.00	0			
Gpi-1	1.000	2.00	0.327	1.000	3.78	0.443			
Gpi-2	1.000	2.10	0.473						
Idh-1	1.000	2.02	0.277	0	1.00	0			
Idh-2	0.288	1.48	0.577				0.93 3	2.00	0.161
Ldh-2	0.365	1.65	0.019	0	1.00	0	0.20 0	1.27	0.023
Ldh-3	0.038	1.04	0.001						
Mdh-1	1.000	2.00	0.241						
Mdh-2				0.078	1.08	0.002	0	1.00	0
Mdh-3				0.196	1.22	0.006	0.06 7	1.07	0.003
G6pdh	0.596	2.04	0.049				0.33 3	1.40	0.021
Mpi	1.000	2.50	0.328	1.00	4.04	0.397	0	1.00	0
Pgm-1	0.500	2.00	0.036	1.00	4.73	0.288	0.93 3	3.20	0.461
Pgm-2							0	1.00	0
Pep				0.941	2.35	0.274	0.46 7	1.67	0.044
Mdhp				0.196	1.02	0.001			
All	0.607	1.82	0.150	0.440	2.12	0.161	0.41 1	1.62	0.128

Table 4. Analyses of heterozygosity from eastern mosquitofish by region and hydroperiod. The unbalanced design required use of type III sums of squares which do not sum to the model sums of squares because of confounded sources of variance. CD indicates the coefficient of determination for each source of variation. CDs indicate the minimum amount of variance explained by each factor because variables are confounded.

Source	SS	df	F	P	CD (%)
model	0.728	17	4.753	<0.001	70.4
region	0.184	5	4.080	0.005	17.8
hydroperiod	0.040	1	4.436	0.043	3.9
hydro*region	0.215	11	2.172	0.041	20.8
error	0.306	34			

Region	Least-squares means		N	Hydroperiod	Least-squares means		N
	trans-formed	untrans-formed			trans-formed	untrans-formed	
Taylor Slough	-1.911	0.148	8	Short	-1.911	0.148	18
Shark River Sl.	-1.918	0.147	16	Intermediate	-1.911	0.148	7
WCA-3B	-2.060	0.127	3	Long	-2.011	0.134	14
WCA-3Asw	-1.972	0.139	11	Canal	-1.811	0.164	13
WCA-3Ase	-1.695	0.184	5				
WCA-3Anw	-1.841	0.159	4				
WCA-3Ane	-1.977	0.138	5				

Table 5. Hierarchical analysis of genetic variation. Data for eastern mosquitofish and freshwater prawns are from samples collected in 1996, while those for spotted sunfish are from both 1996 and 1997. Upper and lower bounds for estimates are 95% confidence intervals obtained from bootstrapping over loci with 999 replicates. Results in parentheses were obtained after excluding a highly influential subpopulation (subpopulation 13 for freshwater prawns).

	f (F_{IS})	F (F_{IT})	2-Subpops (F_{SP})	2-Pops (F_{PT})
Eastern mosquitofish				
Estimate	0.0253	0.0354	0.0106	0.0033
Upper bound	0.0610	0.0764	0.0199	0.0069
Lower bound	-0.0018	0.0076	0.0034	0.0004
Grass shrimp				
Estimate	0.0124 (0.0127)	0.0179 (0.0171)	0.0055 (0.0044)	0.0005 (0.0002)
Upper bound	0.0397 (0.0406)	0.0487 (0.0466)	0.0117 (0.0076)	0.0017 (0.0010)
Lower bound	-0.0102 (-0.0107)	-0.0071 (-0.0073)	0.0023 (0.0023)	-0.0005 (-0.0007)
Spotted sunfish				
Estimate	0.0113	0.0314	0.0203	-0.0076
Upper bound	0.0997	0.1056	0.0579	0.0006
Lower bound	-0.0251	-0.0153	-0.0044	-0.0180

Table 6. Estimates of subpopulation variation within water management units. Median and mean estimates of 2_{SP} are reported. N indicates the number of variable loci in samples from each region that were used in the hypothesis tests.

Region	Mosquitofish			Grass shrimp			Spotted Sunfish		
	Median	Mean	N	Median	Mean	N	Median	Mean	N
Taylor Slough	0.0035	0.0078	11	-0.0015	0.0037	7			
Shark River Sl	0.0015	0.0024	11	0.0037	0.0060	7	0.020	0.058	6
WCA-3B	-0.0064	-0.0070	8	-0.0054	-0.0012	6	0.003	0.004	6
WCA-3Asw	0.0013	0.0060	11	0.0035	0.0028	6	0.030	0.048	6
WCA-3Ase	0.0033	0.0012	10	0.0014	0.0071	6			
WCA-3Anw	0.0009	0.0026	12	-0.0020	0.0001	6			
WCA-3Ane	0.0049	0.0109	11	-0.0012	-0.0001	6	-0.002	-0.001	7
Freidman test	15.054, P = 0.0198			4.371, P = 0.627			2.100, P=0.552		

Table 7. Tests of the effect of hydrology on metapopulation structure. P-values from Wilcoxon Sign Test are reported; the tests were repeated with all negative values set equal to zero and the results are reported in parentheses. N indicates the number of loci in each test. The long-hydroperiod category was omitted from WCA-3Asw test because of ambiguous separation of long and intermediate hydroperiod sites in this region.

Species\Region	Hydrolog y	Mean 2 _{SP}	Median 2 _{SP}	N	Wilcoxon Sign Test
Mosquitofish					
Taylor Slough	short	0.0083	0.0017	10	0.237 (0.343)
	long	0.0056	0.0008	10	
Shark River Sl	short	0.0038	0.0018	11	0.213 (0.228)
	long	0.0018	-0.0016	11	
WCA-3Asw	short	0.0107	0.0112	10	0.139 (0.058) - short vs canal
	long	0.0018	-0.0016	10	
	canal	0.0049	-0.0005	10	
Grass shrimp					
Shark River Sl	short	0.0048	0.0023	6	0.414
	long	0.0120	0.0090	6	
WCA-3Asw	short	-0.0004	0.0020	5	0.180
	canal	0.00251	-0.0004	5	
Spotted sunfish					
all regions	marsh	0.041	0.009	7	0.705 (0.770)
	canal	0.023	0.008	7	

Figure legends

Figure 1. Kinds of metapopulations re-drawn from Harrison (1991). In all cases arrows indicate the direction of dispersal, filled sites are occupied, unfilled sites are unoccupied, and dashed lines indicate habitat boundaries. We have excluded her “non-equilibrium metapopulation” (Harrison 1991:Fig 1,D) because it illustrates a case where no migrants are exchanged. A. Levins-type metapopulation, B. Core-satellite metapopulation, C. Patchy population, D. Intermediate case between B and C.

Figure 2. Density of mosquitofish and grass shrimp between 1985 and 1995 at typical short and long-hydroperiod marsh locations in the SRS. A system-wide drought occurred from 1989 to 1990. The marsh surface was exposed and many deep-water refugia dried out in the long-hydroperiod marsh. See Loftus and Eklund (1994) for details on data collection. Sampling months are indicated by letters: F = February, A = April, J = July, O = October, and D = December.

Figure 3. Map of the study area. Study sites are numbered. A. Sites where mosquitofish and grass shrimp were collected. B. Sites where spotted sunfish were collected.

Figure 4. Estimates of multilocus heterozygosity are plotted for each subpopulation grouped by the regions where they are found. A. Mosquitofish, B. Grass shrimp, C. Spotted sunfish. Symbols indicate the hydroperiod characterization of each site.

Figure 5. Pairwise estimates of G_{ST} versus distance separating each pair of subpopulations. Data are plotted on a natural log- natural log scale. The best-fit line regression lines are reported.

Figure 6. Frequency of the most common allele relative to position along a north-south axis in the Everglades. The zero kilometer point on the x-axis is the southern-most sample

site in the SRS. Lines indicate the best fit from LOESS regression. Data from mosquitofish are illustrated in panels A-C, and data from grass shrimp appear in panel D. Only statistically significant patterns are shown.

Figure 7. Correlograms plotted separately for each species. The correlation (ρ) of samples separated by the designated Euclidean distance is plotted against that distance.

Figure 1

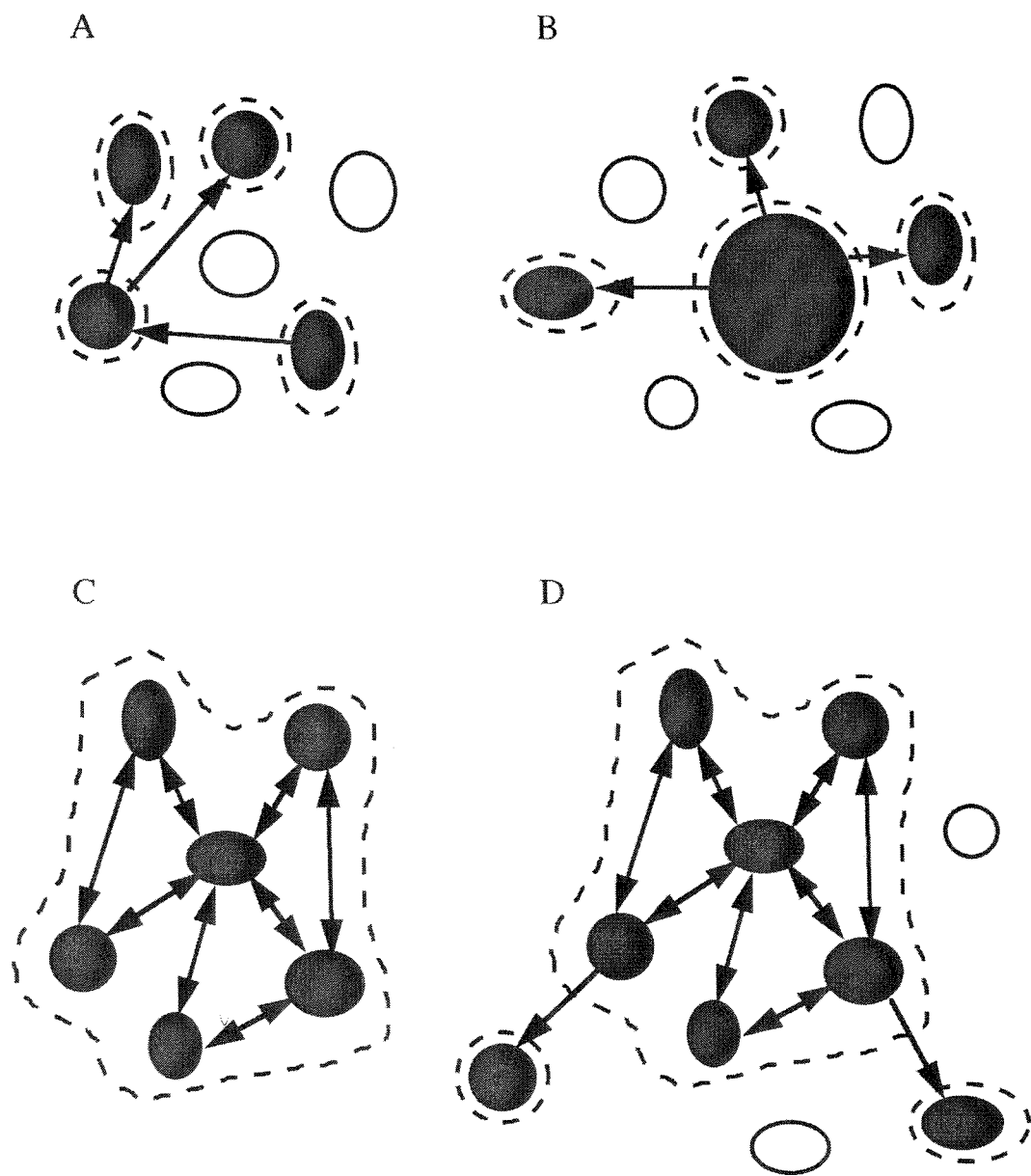


Figure 2

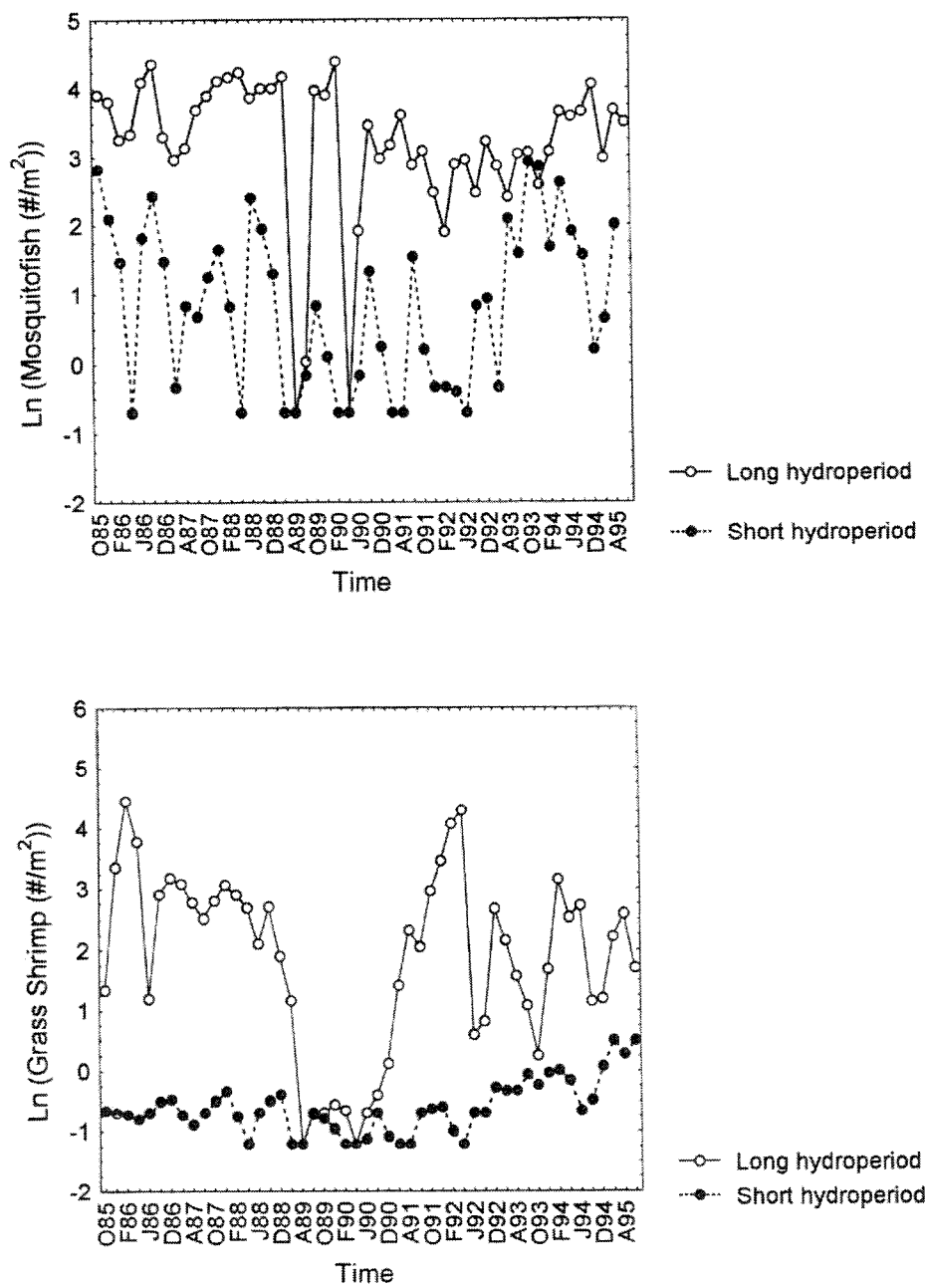


Figure 3

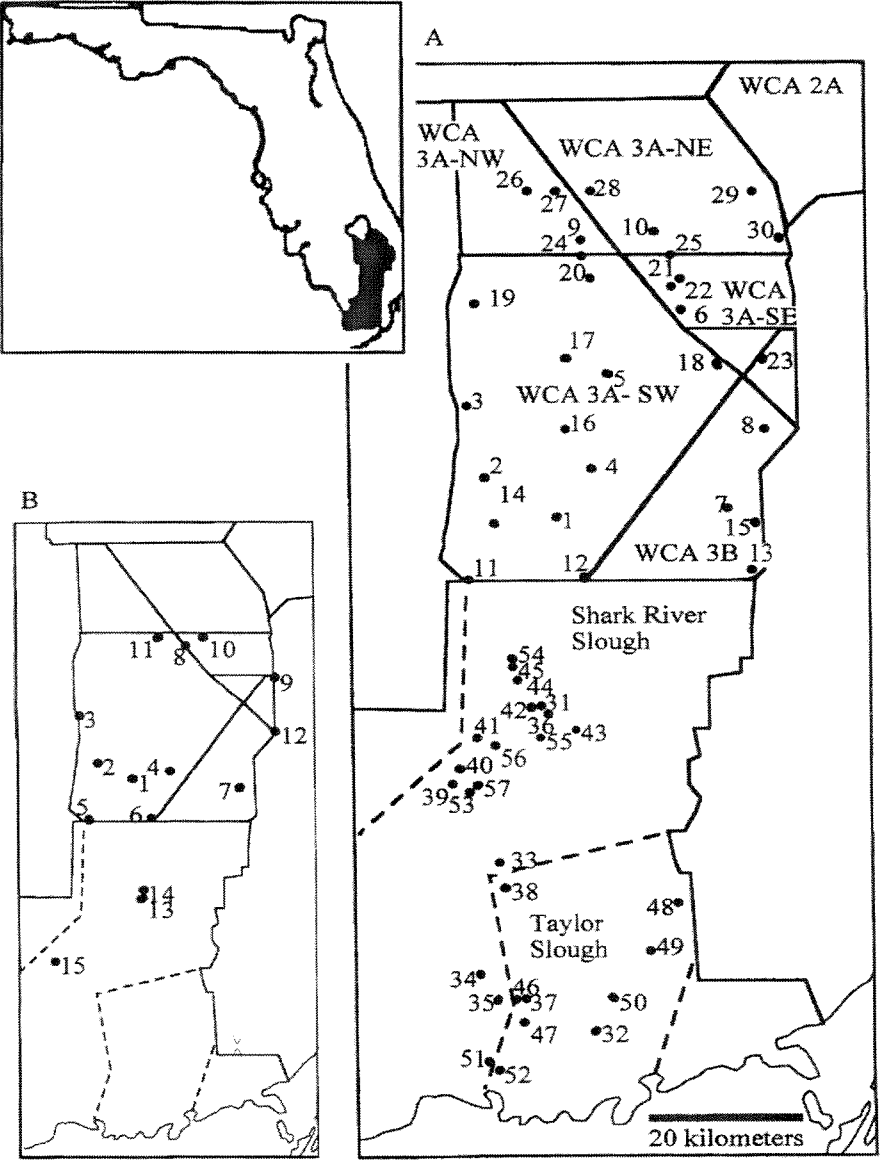


Figure 4

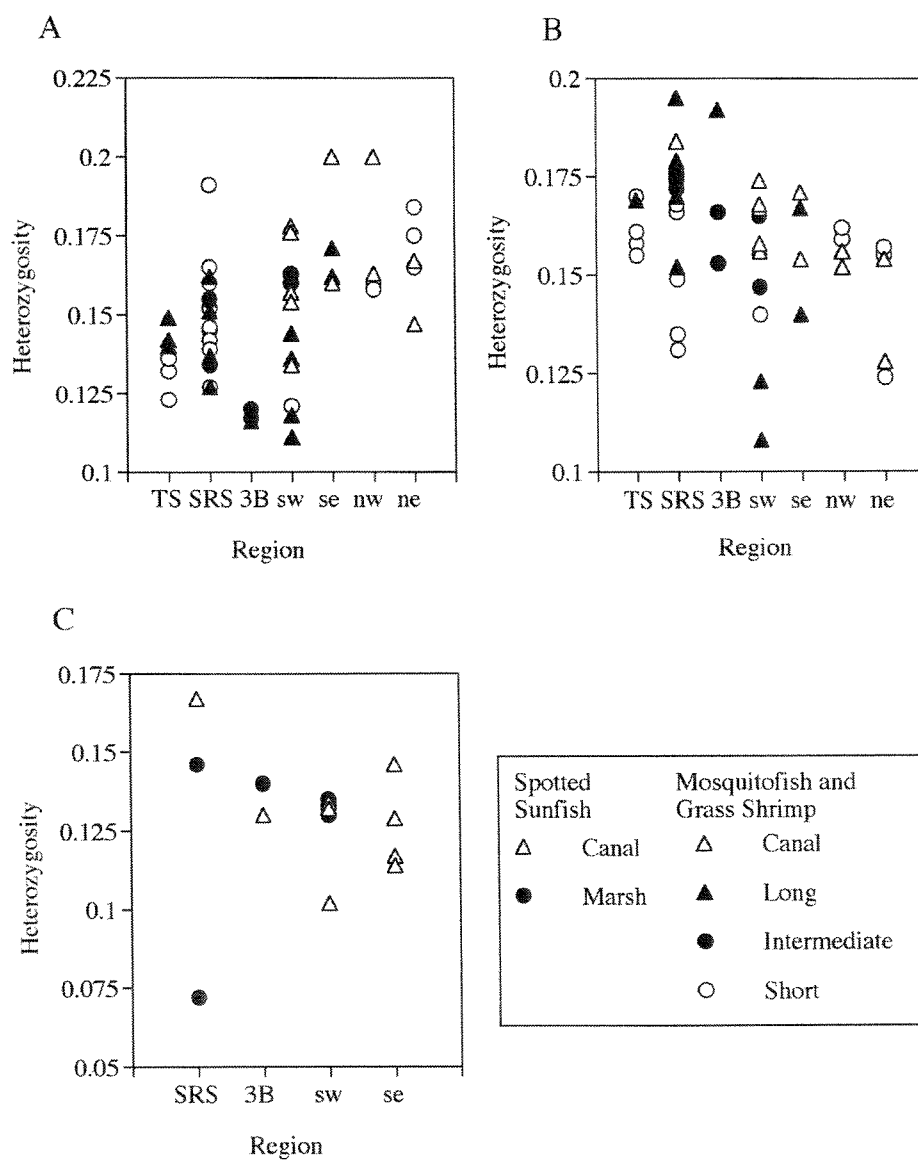


Figure 5

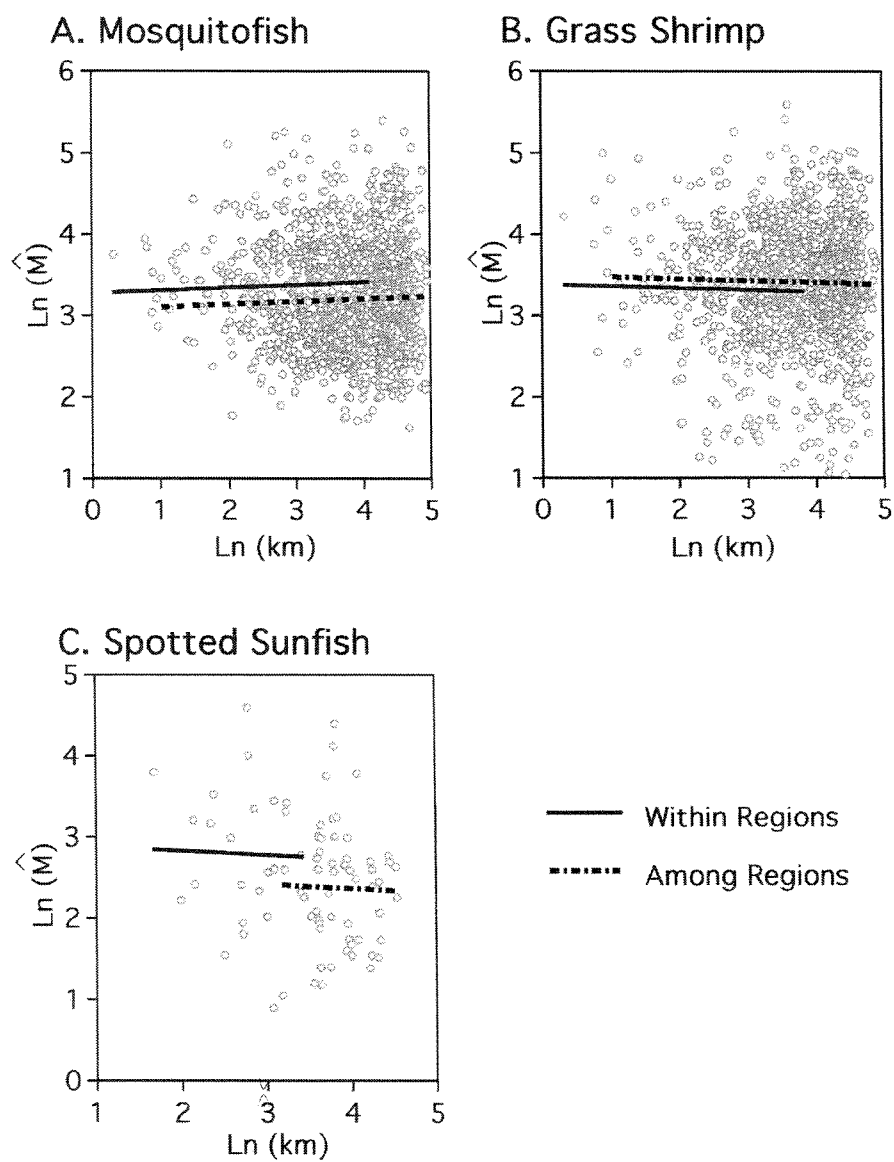


Figure 6

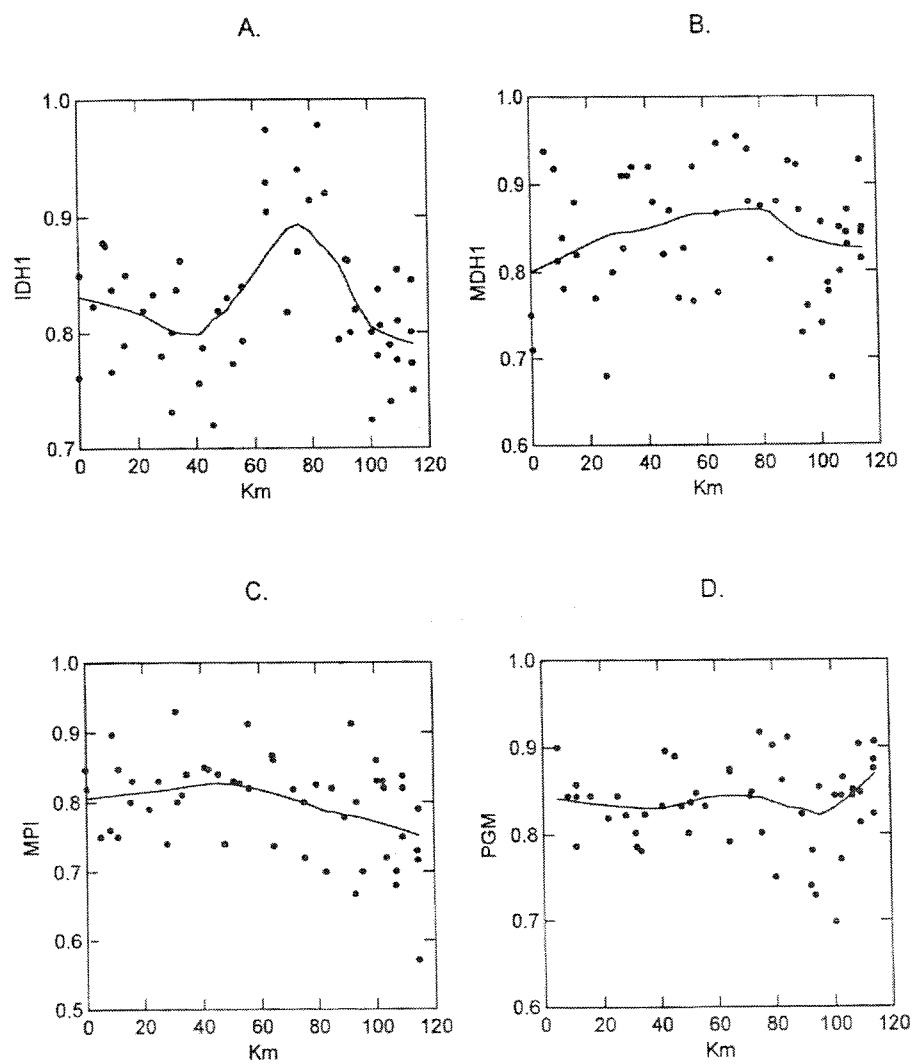
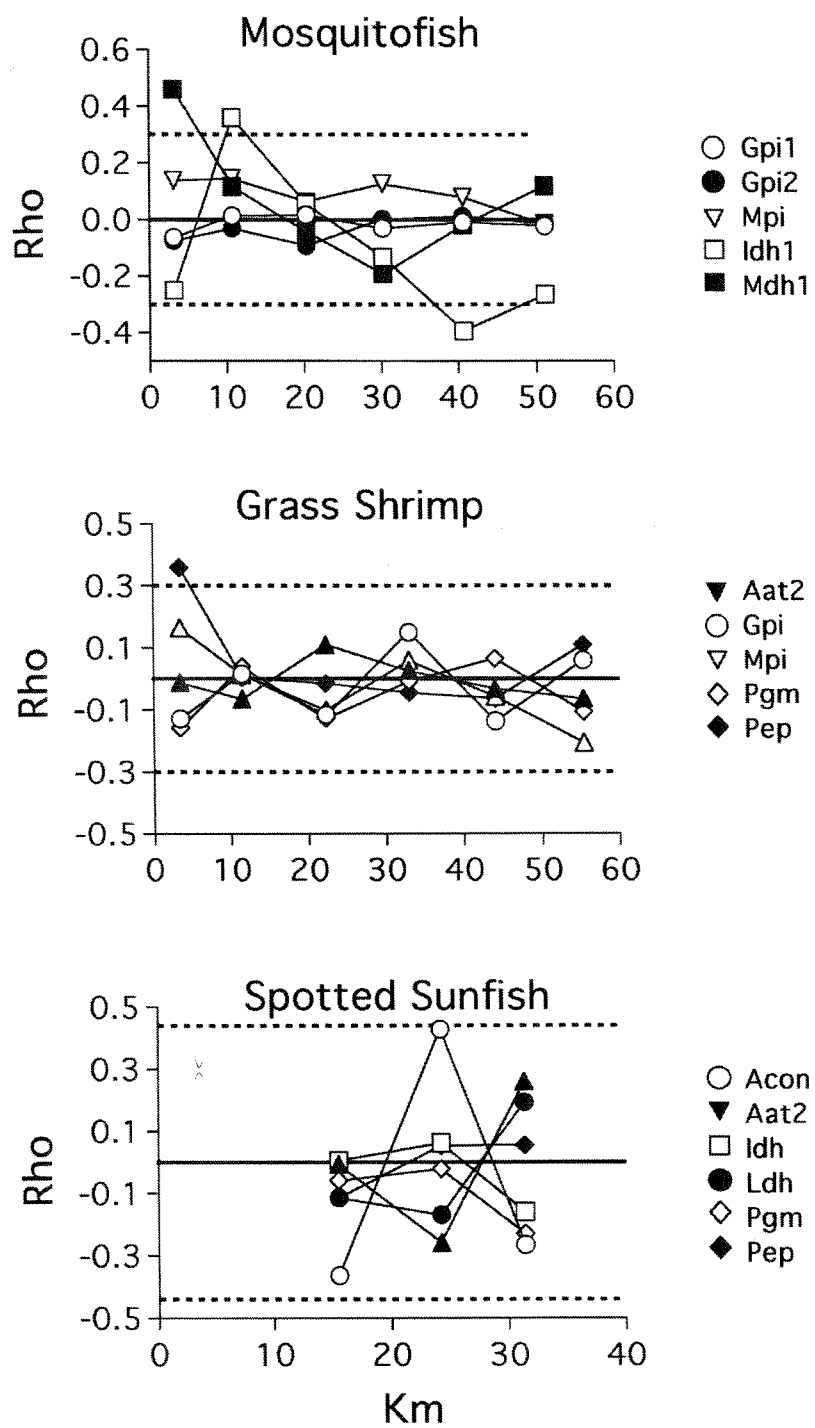


Figure 7



**VI. New polymorphic microsatellite loci in two fish species: bluefin
killifish (*Lucania goodei*) and yellow bullhead (*Ameiurus natalis*)**

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New polymorphic microsatellite loci in two fish species: bluefin killifish (*Lucania goodei*)
and yellow bullhead (*Ameiurus natalis*)

Douglas A. Creer and Joel Trexler

Department of Biological Sciences, Florida International University, Miami, FL 33199
USA.

Abstract

We identified four new polymorphic microsatellite loci in bluefin killifish (*Lucania goodei*) and five loci in yellow bullheads (*Ameiurus natalis*). We screened 400 killifish from 20 populations and 180 bullheads from nine populations, finding a high degree of polymorphism (nine to 54 alleles per locus; average expected heterozygosity 0.678 to 0.976). We found no evidence for linkage. Three of the loci found in bluefin killifish show heterozygote deficiency; the other loci do not deviate from Hardy-Weinberg expectations.

The yellow bullhead (*Ameiurus natalis*) is a common freshwater fish distributed throughout eastern North America. The bluefin killifish (*Lucania goodei*), while less widely distributed, is abundant in marshland habitats in Florida and the southern Atlantic coastal plain. This species has also figured prominently in studies of behavior, physiology, and selection (Fuller & Travis 2004, and references therein). Both species are abundant in the Florida Everglades. Cycles of drying and rehydration in this habitat lead to metapopulation dynamics in its fish populations that may be investigated using population-genetic data. We present the results of an effort to identify polymorphic microsatellite loci in both of these species.

We isolated whole genomic DNA from approximately 0.1g tissue from the region of the caudal peduncle (*Lucania*) or adipose fin (*Ameiurus*). Tissue samples were ground under liquid nitrogen and mixed with 250µl STE buffer, 37.5µl SDS solution (10%), and 6.3µl proteinase K solution (10mg/ml). Following digestion for 1-3 hrs at 55°C, we isolated nucleic acids by extracting twice with phenol-chloroform-isoamyl alcohol (25:24:1) and once with chloroform-isoamyl alcohol (24:1), using 1.5ml Phase Lock Gel Light tubes (Eppendorf) to separate the aqueous and organic layers. Final recovery was by ethanol precipitation, followed by resuspension in 50µl TE buffer.

We enriched the resulting whole genomic DNA for specific microsatellite markers following the protocol of Hamilton et al. (1999), incorporating modifications by P. Bentzen (pers. comm.). We digested whole genomic DNA with XmnI, DraI, and AluI (NEB), and simultaneously ligated cut fragments to double-stranded SNX linkers (T4 DNA Ligase, NEB) in the manner described in Hamilton et al. (1999). The thermal profile for the digestion/ligation reaction was: 22x(37°C 10min 16°C 30min) 1x(65°C

20min). We then amplified 2 μ l of the resulting product by PCR using the SNXf linker as primer (2.5mM MgCl₂). The thermal profile was 1x(95°C 5min) 40x(94°C 45s 62°C 1min 72°C 1min). The products of this reaction were hybridized to 5'-biotinylated oligonucleotide probes (GATA₅, GACA₄, CA_{9.5}), which had previously been complexed with streptavidin-coated magnetic beads (DynaL Biotech). Hybridization was carried out overnight at 38-45°C with rotation. After hybridization, the beads were washed with decreasing concentrations of standard saline citrate (SSC) buffer containing SNXf linker (0.5 ng/ μ l). We conducted four washes each with 2X SSC, 1X SSC, and 0.5X SSC; each wash was conducted at hybridization temperature for five minutes. A magnetic particle concentrator (DynaL Biotech) was used to stabilize the beads during washing. Following the washes, the beads were twice suspended in 50 μ l TE buffer and heated at 95°C for 15m, after which the beads were stabilized using the magnetic particle concentrator and the buffer containing the enriched DNA transferred to a clean tube.

We amplified the enriched DNA by PCR using the SNXf linker as primer with the following thermal profile: 40x(94°C 45s 62°C 30s 72°C 1min) 1x(72°C 20min). Products of this reaction were purified using Qiaquick spin columns (Qiagen), following the protocol supplied with that product. Products resulting from the enrichment with the CA probe were then further purified by electrophoresis on a 6% polyacrylamide vertical gel. Bands corresponding to a length of ca. 200-1000bp were excised from this gel, using a DNA ladder for size comparison. We extracted DNA from these bands by passive elution into a NaCl buffer at 37°C, followed by ethanol precipitation. We then repeated the PCR described at the beginning of this paragraph, using the gel-purified DNA as template, and repeated the spin-column purification.

We ligated the amplified DNA fragments into either TOPO TA vector (Invitrogen; used for GATA, GACA) or pDrive Cloning Vector (Qiagen; used for CA), and transformed the ligates into chemically competent *E. coli* cells (One Shot TOP10, Invitrogen). Transformants were plated and grown on Luria-Bertani (LB) agar plates containing 50-100mg/ml ampicillin. We screened the resulting colonies by direct amplification, using primers matching the regions adjacent to the vector insertion site (T3 and T7 for TOPO TA vector; T7 and Sp6 Upstream for pDrive Cloning Vector) combined with a third primer matching the desired repeat unit (GACA_{4.75}, TAGA_{5.75}, or CA_{9.5}). The presence of a double band when the product was electrophoresed was taken as evidence of the presence of a repeat unit. Such samples were re-amplified using only the primers matching the vector sequences. The resulting products were cleaned by treatment with ExoSAP IT (USB) and sequenced using Big Dye v2.0 or 3.1 (ABI), following the manufacturer's protocols and using T7, T3, or Sp6 Upstream as primers. Sequencing products were analyzed on an ABI 3100 automated DNA sequencer (ABI). Where sequences showed microsatellite repeats with usable flanking regions, we designed primers using Oligo Analyzer (IDT) and Amplify v1.2 (W. Engels, Madison, WI). Primer sets were tested on samples of about 10-20 fish of the appropriate species; sets that failed to amplify or showed no polymorphism were not screened further.

We recovered 17 new microsatellite loci for *L. goodei* and 23 for *A. natalis*. Of these, four and five, respectively, amplified and proved polymorphic in the initial screen (Table 1). We screened these loci further on samples of fish taken from the Everglades; *A. natalis* was sampled from nine sites and *L. goodei* from 20, with 20 individuals per site. PCR was carried out for all individuals using the thermal protocol 1x(95°C 3min)

35x(94°C 30s 50-57°C 30s 72°C 1min) 1x (72°C 30min), in a 10µl reaction with 0.5µM each primer and 2.5mM MgCl₂ (5mM for Lg1, Lg6). Annealing temperatures for each locus are shown in Table 1. One primer in each set was labelled at the 5' end with fluorescent dye (6FAM, HEX, or NED). We electrophoresed 0.1-0.5µl of the product together with a ROX-labelled size standard on an ABI 3100 sequencer (ABI) and visualized the results using GENESCAN v3.0 (ABI). We determined allele number and observed and expected heterozygosity and tested for linkage disequilibrium and departure from Hardy-Weinberg expectations using GENEPOP v3.4 (Raymond & Rousset 1995). We found no linkage disequilibrium between markers for a given species. Six of the loci showed no evidence of departure from Hardy-Weinberg expectations; however, Lg4, Lg5, and Lg6 are characterized by significant heterozygote deficiency ($p < 0.001$, Table 1), possibly a result of null alleles. This may cause problems with specific types of parentage analysis (Dakin & Avise 2004).

Acknowledgements

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References

- Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. *Heredity*, **93**, 504-509.
- Fuller RC, Travis J (2004) Genetics, lighting environment, and heritable responses to lighting environment affect male color morph expression in bluefin killifish, *Lucania goodei*. *Evolution*, **58**, 1086-1098.
- Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic libraries enriched for microsatellites. *BioTechniques*, **27**, 500-507.
- Raymond M, Rousset F (1995) GENEPOP (ver. 1.2): A population genetics software for exact tests and ecumenicism. *J. Heredity*, **86**, 248-249.

Table 1 Polymorphic microsatellite loci found for *Lucania goodei* and *Ameiurus natalis*. Primer names

beginning with Lg were found in the former species and those beginning with An in the latter. Where alternative primers are shown (designated f_b, r_b), the size range refers only to fragments amplified with the standard primer pair. P indicates the p-value in the test for departure from Hardy-Weinberg expectations.

Locus	Primer sequences	motif	°C	# of alleles	size range	N	H _o	H _e	P	GenBank Accession #
Lg1	f-CACATTAAACCATTCCTATATC r-AAATGGTGATGACCTTCT	(GATA) ₆ GA (GATA) ₁₁	51	33	134-266	400	0.923	0.944	0.907	DQ143903
Lg4	f-GAAATGCCTTATACCAAGCA r-CTCCAATTTCTATCGCTAGGTG	(TATC) ₃₈	50	45	142-418	398	0.460	0.939	<0.001	DQ143904
Lg5	f-CTCAGCATGCAGCATTTGGTG f _b -CATGCAGCATTTGGTCGTA r-TCTCCATCAGAGAATCACAGA	(TATC) ₂₀	57	29	94-226	393	0.476	0.892	<0.001	DQ143905
Lg6	r _b -GCAGCGTAAAGACGAAGACT f-CCTTCTCCAGGCTTTCAGTC r-GGGATAAACTCGTGTCTAAC	(TATC) ₆ (TGTC) ₃₃	52.5	54	168-400	398	0.427	0.960	<0.001	DQ143906
An7	r _b -ACTCGTGTCTAACTATAAG f-AGGATGCAGTCATCCATATC r-CTTATTTCAAAGTTATGATCTCG	(TATC) ₉ CATC (TATC) ₉	50	11	149-189	180	0.867	0.886	0.279	DQ143907
An11	f-TCAGTCTAATCTCTTCCACTTC r-TTCCTTAGAGGAAGAAGCTGC	(GATA) ₂ GA (GATA) ₆ GTTA (GATA) ₁₀ GAAA	56	33	171-307	180	0.956	0.954	0.386	DQ143908

An12	f-ACCATCTCAGTGGAGCCAA	TAAA(GAAA) ₄ (TATC) ₁₁	50	20	122-	180	0.872	0.907	0.696	DQ143909
	r-AAGAAACAGACTGCAACAT				202					
An13	f-TCATCTCAGGCATTTCAGA	(GATA) ₁₃	56	21	194-	180	0.900	0.906	0.978	DQ143910
	r-ATCTTCAGGATGTTGCACAT				282					
An17	f-CATGCCAAACCCACATCGT	(CA) ₈ (14bp)(CA) ₁₄	53	9	128-	180	0.678	0.646	0.962	DQ143911
	r-TCGGGACAAATCACGTGTAGT	TACA(TA) ₅			136					
	An17 alternate motif:	(CA) ₁₁ (TA) ₇			166-					
					176					

**VII. Effects of Season and Hydrology on Movement Patterns of
Florida Gar (*Lepisosteus platyrhincus*) in the Everglades**

Running Head: Florida gar activity patterns

Effects of Season and Hydrology on Movement Patterns of
Florida Gar (*Lepisosteus platyrhincus*) in the Everglades

Lawrence F. Wolski¹, Joel C. Trexler¹, Jason Knouft², Carl Ruetz III³, & William F. Loftus⁴

¹Department of Biological Sciences, Florida International University, Miami, FL 33199

²Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO

³Annis Water Resources Institute, Muskegon, MI

⁴U.S. Geological Survey, Homestead, FL

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The movement of predators across a landscape has important implications for their impact on prey. Since predators are often larger than their prey, it is probably common that the former move over larger areas than the latter. Predators that sample multiple prey patches across a landscape have the potential to stabilize prey population dynamics (refs). In a dynamic landscape, both predators and prey may be forced to continually move in order to remain in appropriate habitats. Aquatic ecosystems such as floodplains and wetlands are such dynamic landscapes for most fish that lack the ability for aerial respiration. While a variety of metapopulation and metacommunity structures have been described to evaluate the theoretical implications of local extinction and recolonization and patch-centered foraging (Harrison 1991; Leibold et al. 2004), the movement of animals like fish in dynamic ecosystems remain poorly characterized.

Radiotelemetry studies have been used to establish seasonal movement patterns (Knouft & Spotila, 2002, Ovidio et al, 2002, Burrell et al, 2000), home range size (Ferguson et al, 1999), population size estimation (Eberhardt, 1990), habitat usage (Masters et al, 2002, Huber & Kirchhofer, 1998), predator-prey interactions (Cronin et al, 2000), diel patterns (Snedden et al, 1999), and dispersal (Maehr et al, 2002, Strong & Bancroft, 1994) in numerous species. This information lends itself towards to quantification of parameters affecting foraging strategies and inter-habitat-patch movement (Byers, 2001, Railsback et al, 1999, Ruxton et al, 1999). Application of radiotelemetry is limited to relatively large fish because of transmitter size and to relatively shallow freshwater habitats where radio signals can be tracked. However, the type of information it yields is critical to analysis of spatial dynamics in ecological systems.

Florida gar (*Lepisosteus platyrhinchus*) is the most common piscivorous fish in the Florida Everglades, a large wetland the southeast United States, but impact on prey species there is poorly understood (Chick et al. 2004). The Everglades experience a wet and dry season hydrology, causing portion of the ecosystem to dry each year. Droughts, where the vast majority of wetland dry, occur every 10 to 20 years. Recurring disturbances on species whose generation time is relatively long (probably 7 to 10 years in the Everglades), requires that they move in and out of aquatic refuges within the life span of individual fish. The density of Florida gar in the Everglades is inversely related to the time since a local site has dried (Chick et al. 2004), and it is believed that this limits their impact on their prey (Trexler et al. 2005). However, the details of these interactions are heavily influenced by patterns of movement of gar. In this study, we assessed movement patterns Florida gar across the Everglades landscape to determine how they respond to hydrological fluctuation and mediate their impact on food-web dynamics.

Methods

Study Area

Beginning in March 2002, we monitored the movements of 93 Florida gar in the Everglades Wildlife Management Area, Conservation Area No. 3A (WCA-3A) and the Everglades National Park (ENP), Shark River Slough. An initial group of 29 fish were monitored from March to July 2002 in WCA-3A, a second group of 21 gar were monitored in WCA-3A between August, 2002 to April, 2003, and a final group of 4 gar were monitored from April 2003 to July 2003. We captured the fish in two widely separated areas of WCA-3A, one short-hydroperiod area to the

northwest (Site 3; 26.01 N, 80.82 W), and a second, longer hydroperiod area in south-central WCA3-A (Site 1; 25.86 N, 80.73 W), approximately 17 km apart. Similarly, in SRS, we collected fish in two areas, a long-hydroperiod area in the north (Site 6, 25.63 N, -80.73 W) and a short hydroperiod area in the southwest (Site 37, 25.47 N, -80.85 W), which is associated with the northeastern tributaries of the Shark River. Two groups of gar were monitored in SRS, the first group of 19 individuals were monitored from September 2003 through May 2004, and the second group of 20 individuals were monitored from April 2004 to September 2004. A final group of 24 gar were monitored in Taylor Slough of the Everglades National Park from May to December 2003.

WCA-3A, Taylor Slough and Shark River Slough are characterized by sedge-dominated (*Eleocharis* spp.) sloughs interrupted throughout with tree island complexes and thick stands of sawgrass (*Cladium jamacienses*). WCA-3A covers approximately 1200 km² and is bordered on all sides with deep-water canals. These canals were built to aid in water management between the WCAs and the Everglades National Park to the south. Shark River Slough covers approximately 1000 km² and Taylor encompasses approximately 100 km². These regions experience a seasonal wet and dry season, with water levels exceeding 1 m during the wet season. During the dry season, the marsh experiences a “dry-down”, which is most marked in the northwest regions of WCA-3A, Shark Slough and Taylor Slough where water levels may drop below 5 cm for an extended period of time. Canals and other deep water areas, such as alligator holes, remain as the only areas that contain standing water. On rare occasions, the severity of the dry down is such that

the alligator hole refugia also dry completely, resulting in a major die-off of larger fish species such as largemouth bass, bowfin, and gar (Kushlan, 1974).

Transmitter attachment

We collected gar using standard electrofishing techniques from an airboat (Chick et al, 1999), weighing and measuring each gar (TL, cm) as it was collected. Fish were anesthetized using MS-222 for surgical implantation of transmitters (SB-2, Holohil Systems, Ltd.). Transmitters were inserted into the body cavity of the gar through a ventral incision posterior to the pelvic girdle, which was closed with three to five sutures of braided nylon silk (3-0 Silk, Sherwood, Davis & Geck) and SuperGlue. We used transmitters of a size that maximized the range and lifespan of the transmitter but caused no harm or mechanical interference to the fish. The weight of the transmitter never exceeded 3% of the subject's mass. We released each fish only after it had regained equilibrium, and was swimming without difficulty in a holding tank. Each gar was released at the point it was obtained, and we monitored each animal for the lifetime of the transmitter.

Tracking Methods

In WCA-3, individual gar were located on a weekly basis from an airboat using a 1-MHz band radiotelemetry receiver (Wildlife Systems, Inc.) and a five-element Yagi antennae. Once fish were relocated, their latitude and longitude was noted with a hand-held global positioning system (Garmin 76; Garmin, Ltd) as well as the depth, temperature, and conductivity of the water at the

approximate location of the fish. Measurements were made at least 3m from the location of the fish to minimize disturbance to the fish. In addition, the top three types of vegetation in the immediate vicinity of the fish were also noted, as well as a general descriptor of the area (open marsh, canal, alligator hole, thick vegetation, tree island, etc.). If on two subsequent relocation attempts, the location of the transmitter did not change, technicians entered the water to identify if either the transmitter had been separated from the gar or the animal was actually present. If we were unable to locate a fish from the airboat on consecutive occasions, we used a helicopter or airplane to conduct an aerial survey of the area using a Communications Specialists R-1000 receiver and directional antennae. In Shark River Slough, relocations occurred on the same schedule, however, tracking was only performed from established airboat trails. If gar moved away from airboat trails, aerial surveys were conducted to establish contact. Relocations in Taylor Slough were performed either by foot or by aerial surveys.

We determined receiver accuracy by hiding several transmitters underwater throughout the marsh; technicians with no prior knowledge of the transmitters whereabouts attempted to relocate the transmitters from both airboats and airplanes. We were able to identify the location of the transmitter from the airboat within 3 m, with several cases less than 1 m. Relocations of the transmitter from the airplane were less than 100m from the actual position, with many relocations being within 50m of the transmitter.

Diel Movement Methods

Five 24-hour tracking sessions were completed over the course of this study, four of which were located in WCA-3A, and the last in Taylor Slough. Gar were relocated over a 24 hour period in 1 to 2 hour increments, often tracking up to 15 fish simultaneously. During the first sampling season, one diel study was conducted at Site 3. In the second sampling season, three diel studies were conducted, two at Site 1, and one at Site 3.

Data Analyses

Distance moved since previous location, direction moved from previous location, distance and direction from release point were calculated for each data relocation point. This data was grouped by gar, site, month, and slough. Directionality of movement was tested for all gar by using the Kolmogorov-Smirnov tests for uniformity. Distance moved by each fish was normalized by calculating the amount of time between successive relocations and the distance moved during this time. By this calculation, a metric of meters per day was used in all the following statistical tests. Nested ANOVA was used to investigate whether the distance traveled by gar were different at any specific site or within a particular slough. For all relocations, basic habitat notes were taken, and simplified at a later point to four main categories (canal/man-made, trails, alligator hole, marsh). This was done in order to incorporate data from all collection methods, airboat, airplane, and helicopter. Data was grouped by slough and site, and ANOVA was used to determine difference in basic habitat usage between the study areas. In addition to long-term data, data from the diel portion of the study was organized into 3-hour periods beginning at 0600 and continuing

through the data collection period. Analysis of variance was used to investigate rates of movements over the 24 hour period for all five diel recording periods.

Simulation methods

Over the course of a tracking period, the summation of the movements of an individual fish could be characterized by three general movement patterns: 1) random directional movements; 2) movements restricted to a particular area; or 3) non-random directional movements. To determine whether individual fish exhibited random, restricted, or directional movements, we generated simulations based on a Brownian motion model of dispersal. To parameterize the simulations, we calculated mean daily movements for each fish using the values determined from the telemetry data. Because animal movements generally exhibit a leptokurtotic curve, we also calculated the kurtosis of the distribution of movements for each fish. For each individual, 1000 movement simulations were generated with the same duration as the tracking of the actual fish. For example, if a fish was tracked for 60 days, each simulation was allowed to run for 60 iterations. At each iteration (day) of the simulation, the individual was allowed to move in any direction for a distance randomly chosen from a distribution of distances with a similar mean, variance, and kurtosis as the movements from the actual fish. On the last day/iteration of the simulation, the distance was calculated between the start and end point of the simulated movements. This process was repeated 1000 times for each individual to generate a null distribution of movements. The actual distance between the release point and the end point of tracking for the actual individual was then compared to the null distribution. If the distance between the start point and the end point for an actual fish

was less than the range of results from the simulations, then the fish was assumed to exhibit restricted movements. If the distance between the start point and the end point for an individual fish was greater than the range of results from the simulations, then the fish was assumed to exhibit non-random directional movements. If the distance between the start point and the end point for an individual fish was within the range of results from the simulations, then the fish was assumed to exhibit a random movement pattern.

Results

We tracked 12 sets of gar in WCA-3A and the Everglades National Park. Tracking periods for each set ranged from an average of 53 days post-release to 209 days post release. Similarly, average distances traveled during each tracking period differed as well, ranging from approximately 300meters from the release point to over 8 km. To investigate these differences, we normalized the distance traveled by each gar by dividing the length of movement by the amount of time in between relocations to yield estimates in meters moved per day. Nested ANOVA revealed no difference of move rate between sites nested within sloughs, but did indicate a difference of move rate between the sloughs themselves ($F_{2, 1728}=12.049$, $P<<0.001$).

Our long-term movement data indicated two patterns, foraging (or home-range establishment) and dispersal. The majority of animals that displaced less than 1 km while being tracked exhibited no apparent directional movement, consistent with foraging (Figure 1A). In contrast, gar that moved over 1 km generally displayed several relocations in a common direction within hundreds of meters of each other, followed by longer movements up to and exceeding 2 km

(Figure 1B). Kolmogorov-Smirnov tests for uniformity indicated no increase in directionality with increase in move length.

We grouped habitats used by gar into four categories: alligator hole; man-made canal; trail (natural or man-made); and marsh. These general categories were necessary because many of our observations made from fixed wing aircraft. We observed differences in habitat use between study areas, as well as sites within study areas ($F_{4, 2042}=112.89$, $P<<0.001$, Figure 2).

In addition to long-term monitoring of gar, we collected observations on diel movement and habitat-use at two sites in WCA-3A (Sites 1 & 3) by locating all fish at each site every two hours during a 24-hour period. Gar movements were minimal during the daylight hours and were increased at night ($F_{7, 302}=2.603$, $P=0.013$; Figures 3 and 4).

Simulation

We constrained the fish we selected for simulation analysis to individuals that had been tracked for at least 60 days with at least one contact per week. These constraints allowed us to assess movement patterns for 28 individuals. Of these 28 individuals, 5 exhibited restricted movements, 6 exhibited non-random directional movements, and 17 exhibited movement patterns that were within the range of random simulated movements.

Discussion

Long-term movements

The preliminary analysis of the long-term data indicates that there are two types of movement patterns, foraging (home range establishment) and dispersal (Maehr et al. 2002). The majority of animals that moved less than 1 km exhibited movement patterns that consisted of random movements across a fixed area, adopting a foraging strategy. Gar that moved over 1 km tended to display a pattern in which several relocations were within hundreds of meters of each other, followed by a longer movement sometimes in excess of 2 km. This type of movement can be considered a dispersal from one temporary home range to another. Kolmogorov-Smirnov tests for uniformity indicated no increase in directionality with increase in move length. Numerous parameters may play an integral role in determining the length of time a gar spends in any one home range. Local hydrology, prey availability and access to deep water refuges may all be factors in habitat usage.

Of the three factors listed, hydrology may be a controlling factor in long-term gar movement. During the first season, fish at Site 3 moved less as the water levels dropped. The majority of fish at Site 3 at this time were located in a single alligator hole, which had an average depth of ~50 cm during the dry-down event. Gar movements on a weekly basis were restricted to this alligator hole, probably because the surrounding marsh averaged between 10 and 20 cm. At the same time, fish at Site 1 were moving longer distances and with an apparent uniformity of direction, with water depths around Site 1 being much deeper than that of Site 3.

During the second season, both locales experienced high water levels and movement patterns were opposite those seen in the first season. Gar at Site 3 moved larger distances than those observed in the first season. Several of the fish travelled to the southeast, while others

moved shorter distances into the canal system near their release point. Researchers noted that at this time, the local concentrations of gar in the canals was extremely high, often observing several hundred gar at any one point along the canal. It is possible that the latter portion of the long-term movement study consisted of observations of gar moving towards deeper water areas in anticipation of a seasonal dry-down. The relocation to a deep water refuge may also be extremely important to females, which may be using the refuges as a place to produce their offspring. Loftus & Kushlin (1987) describe small juveniles caught in the spring among dense vegetation and deep water areas, and suggest that these areas may be used as nurseries. In addition, other species of gar have a reproductive cycle that peaks in the spring, and may be temperature dependent (Simon & Wallus, 1990). Female gar may move from an environment characterized by fluctuating water temperatures such as the open marsh to deep water refuges, such as canals and alligator holes, which may provide the animals with the environmental requirements and stability needed to produce offspring.

Habitat use

In addition to the long-term collected, habitat type and general descriptors of the immediate vicinity of the fish's location were collected. Gar were most often observed in the open marsh in WCA-3A, while in both SRS and TS frequented airboat trails (deeper than the adjacent marsh) and canals and creeks. Long-term movement patterns observed in SRS and TS imply that when the fish moved long distances, they used the airboat trails as corridors. While this also may be true of gar in the WCA, movements recorded there did not strictly follow these corridors.

In deep water areas, gar were often located in open marshes dominated by thick concentrations of bladderwort (*Utricularia* spp.) and lillies (*Nymphaea* spp.), habitats that are suited to an ambush predator (Snedden et al, 1999). In areas of low water depth, gar were most often found near the edges of deep water refuges and their associated vegetation. Other species of gar have been found to eat crayfish and centrarchids (Snedden et al, 1999), and our observation of gar in certain vegetation types may be in response to prey availability.

Diel Patterns

The analysis of diel movement indicated a lack of uniformity in the patterns of fish movement. However, at Site 3 in the first sampling season, gar were found in alligator holes and deeper areas of the marsh during the day, moving into shallower areas during the night. This may be in response to predation pressure in the shallow marsh during the daylight hours, as birds tend to aggregate to feed on fish in the shallow marshes. David & Closs (2001) also noted this movement strategy in kokpu (*Galaxius argenteus*), with fish remaining near shelter during the day and moving out into open regions of a pool during the night hours. At the long-hydroperiod site (Site 1), gar moved away from their starting locations and were displaced by the next morning. However, at the short-hydroperiod location (Site 3), gar moved away from their starting locations during the night, but returned near their starting positions before the next morning. Water levels at Site 3 were lower than at Site 1, and this “homing” movement pattern at Site 3 may be in response to predation risk in the shallow marsh during the daylight hours, as birds tend to aggregate to feed

on fish in the shallow marshes. At long-hydroperiod areas, the effects of predation risk may not be as large, as there are more areas of open marsh available to the fish to use as refuges.

We believe that our data reflect the ability to apply radiotelemetry data to numerous ecological questions. This study demonstrates the effectiveness of using radio transmitters to describe movement patterns of large, mobile fishes across a wetland landscape. We feel that data collected in his study will enable researchers to address questions regarding possible relationships between sex and body mass on habitat use and home range size. By collecting and analyzing movement data, we aim to develop a deeper understanding of survival, dispersal, and habitat use by these aquatic predators in the Everglades.

Acknowledgements

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Literature Cited

Burrell, K. H., Isely, J.J., Bunnell, D. B., Van Lear, D. H & Dollof, C. A., 2000. Seasonal movement of brown trout in a southern Appalachian River. *Transactions of the American Fisheries Society*, 129: 1373-1379.

- Byers, J.A., 2001. Correlated random walk equations of animal dispersal resolved by simulation. *Ecology*, 82: 1680-1690.
- Chick, J. H., Coyne, S. & Trexler, J. C., 1999. Effectiveness of airboat electrofishing for sampling fishes in shallow vegetated habitats. *North American Journal of Fisheries Management*, 19: 957-967.
- Cronin J. T., Reeve, J. D., Wilkens, R. & Turchin, P., 2000. The pattern and range of movement of a checkered beetle predator relative to its bark beetle prey. *Oikos*, 90: 127-138.
- David, B. O. & Closs, G. P., 2001. Continuous remote tracking of fish activity with restricted home ranges using radiotelemetry. *Journal of Fish Biology*, 59: 705-715.
- Eberhardt, L. L., 1990. Using radio-telemetry for mark-recapture studies with edge effects. *Journal of Applied Ecology*, 27: 259-271.
- Ferguson, S. H., Taylor, M. K., Born, E. W., Rosing-Asvid, A. & Messier, F., 1999. Determinants of home range size for polar bears (*Ursus maritimus*). *Ecology Letters*, 2:311-318.
- Huber, M. & Kirchhofer, A., 1998. Radio telemetry as a tool to study the habitat use of nase (*Chondrostoma nasus* L.) in medium-sized rivers. *Hydrobiologia*, 371/372: 309-319.
- Knouft, J. H. & Spotila, J. R., 2002. Assessment of movements of resident stream brown trout, *Salmo trutta* L., among contiguous sections of stream. *Ecology of Freshwater Fish*, 11: 85-92.
- Kushlan, J. A., 1974. Effects of a natural fish kill on the water quality, plankton, and fish population of a pond in the Big Cypress Swamp, Florida. *Transactions of the American Fisheries Society*, 103: 235-243.

- Leibold MA, M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601-613.
- Loftus, W. F. & Kushlan, J. A., 1987. Freshwater fishes of southern Florida. *Bulletin of the Florida State Museum , Biological Sciences*, 31(4): 147-344.
- Maehr, D. S., Land, E. D., Shindle, D. B., Bass, O. L. & Hootor, T.S., 2002. Florida panther dispersal and conservation. *Biological Conservation*, 106: 187-197.
- Masters, J. E. G., Welton, J. S., Beaumont, W. R. C., Hodder, K. H., Pinder, A.C., Gozlan, R. E. & Ladle, M., 2002. Habitat utilization by pike *Esox lucius* L. during winter floods in a southern English chalk river. *Hydrobiologia*, 483: 185-191.
- Ovidio, M., Baras, E., Goffaux, D., Giroux, F. & Philippart, J. C., 2002. Seasonal variations of activity pattern of brown trout (*Salmo trutta*) in a small stream, as determined by radio-telemetry. *Hydrobiologia*, 470: 195-202.
- Railsback, S. F., Lamberson, R. H., Harvey, B. C. & Duffy, W. E., 1999. Movement rules for individual-based models of stream fish. *Ecological monitoring*, 123: 73-89.
- Ruxton, G. D., Armstrong, J. D. & Humphries, S., 1999. Modelling territorial behaviour in animals in variable environments. *Animal Behavior*, 58: 113-120.
- Simon, T. P. & Wallus, R., 1990. Family Lepisosteidae. Pages 57-85 in Wallus, R., Simon, T. P. & Yeager, B. L., 1990. *Reproductive biology and early life history of fishes in the Ohio River drainage. Volume 1: Acispenseridae through Esocidae*. Tennessee Valley Authority, Chatanooga, Tennessee.

- Snedden, G. A., Kelso, W. E. & Rutherford, D. A., 1999. Diel and seasonal patterns of spotted gar movement and habitat use in the Lower Atchafalaya River Basin, Louisiana. *Transactions of the American Fisheries Society*, 128: 144-154.
- Young, M., 1994. Telemetry-determined diurnal positions of brown trout (*Salmo trutta*) in two south-central Wyoming streams. *The American Midland Naturalist*, 133: 264-273.

Table 1: Gar Movement Summary

SLOUGH	SITE	Number of Gar	Tracking Period	Average Number of Relocations	Days Post-Release	Average Distance From Release (m)
WCA	1	12	March, 2002 July, 2002	25.08 (2.93)	59.41 (20.29)	817.84 (1209.23)
WCA	2	2	March, 2002 July, 2002	8.50 (7.47)	52.94 (49.69)	2707.10 (2962.00)
WCA	3	15	March, 2002 July, 2002	9.67 (2.73)	53.70 (18.14)	2366.55 (1081.57)
WCA	1	11	August, 2003 April, 2003	22.64 (3.19)	161.90 (22.22)	1805.31 (1263.00)
WCA	3	10	August, 2003 April, 2003	15.30 (3.34)	138.71 (22.22)	8398.85 (1324.65)
WCA	1	4	May, 2003 July, 2003	8.60 (4.73)	55.01 (35.14)	314.47 (2094.45)
SRS	6	9	September, 2003 May, 2004	30.33 (3.52)	209.17 (23.42)	1192.08 (1396.30)
SRS	37	10	September, 2003 May, 2004	26.50 (3.34)	176.92 (22.22)	7594.32 (1324.65)
SRS	6	10	April, 2004 September, 2004	16.80 (3.34)	127.85 (22.22)	1036.75 (1324.65)
SRS	37	10	April, 2004 September, 2004	14.90 (3.34)	117.07 (22.22)	973.38 (1324.65)
TS	MD	24	May, 2003 December, 2003	10.88 (2.16)	98.24 (14.34)	420.48 (855.06)

Figure 1. A. Typical “Foraging” Track. Scale in meters. B. Typical “Dispersal” Track. Scale in Meters.

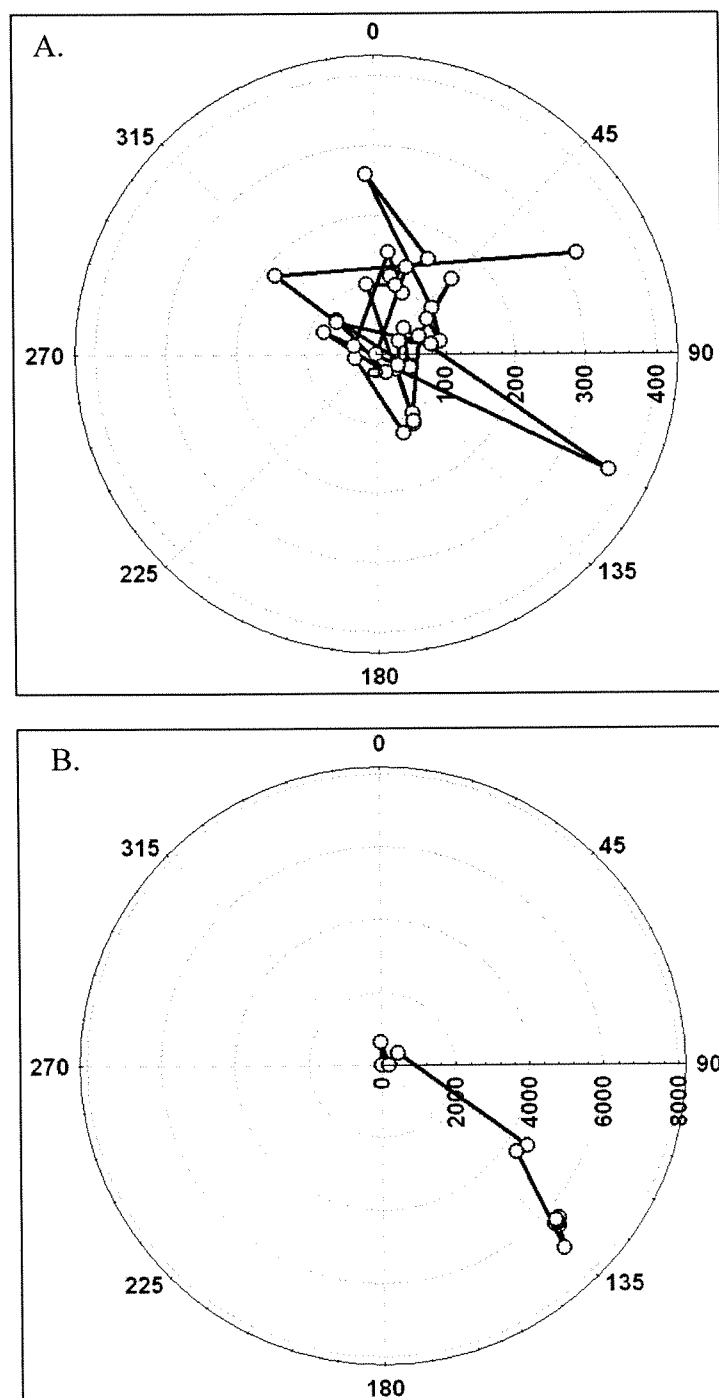


Figure 2. Habitat Use by Slough (needs to be as a percent of total with total number by region in the legend)

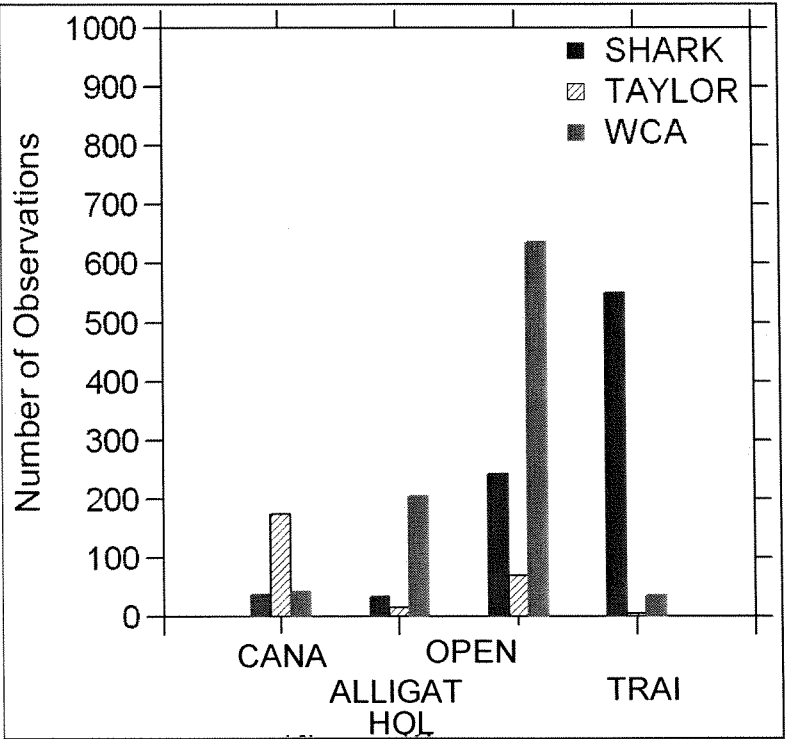


Figure 3. Diel Movement Patterns at Site 1, WCA. Periods run in 3-hour increments Starting at 0600 – 0900 (Period 1) through a 24-hour cycle, with the last time period (8) running from 0300 – 0600.

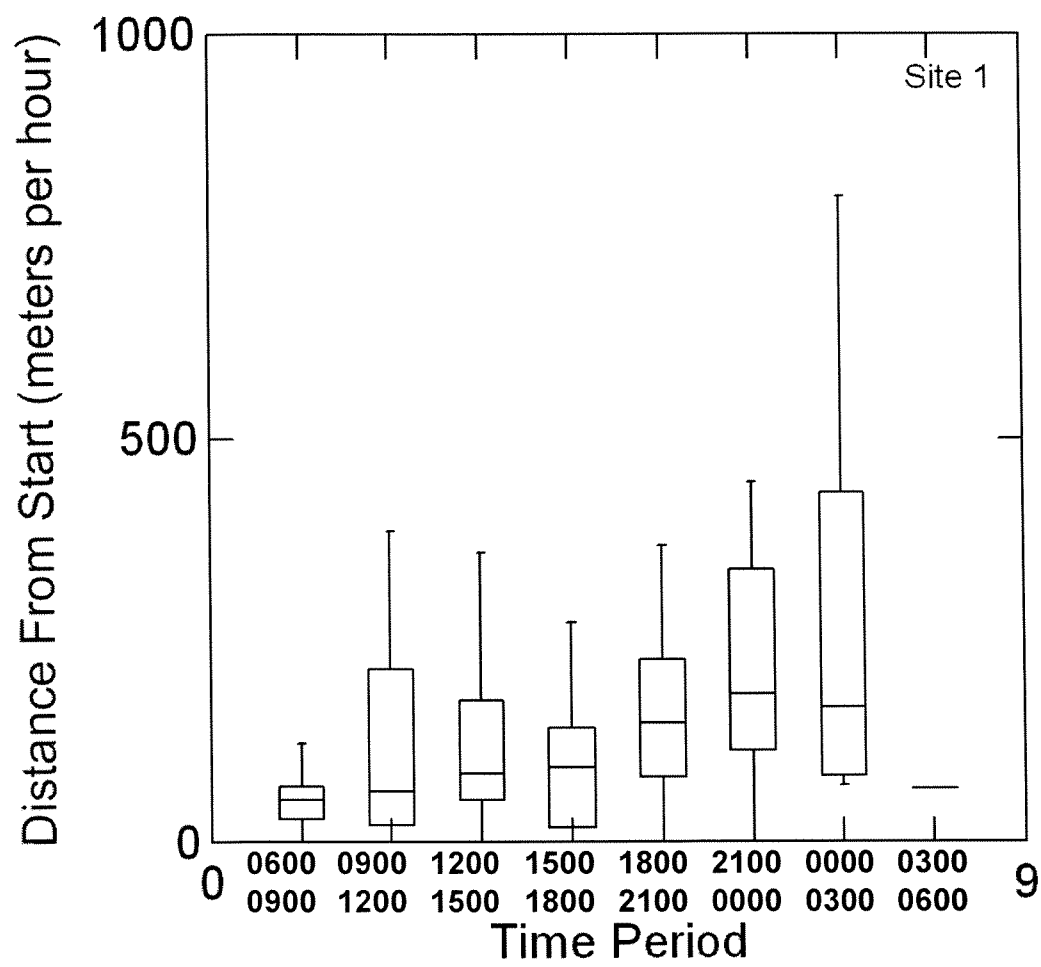
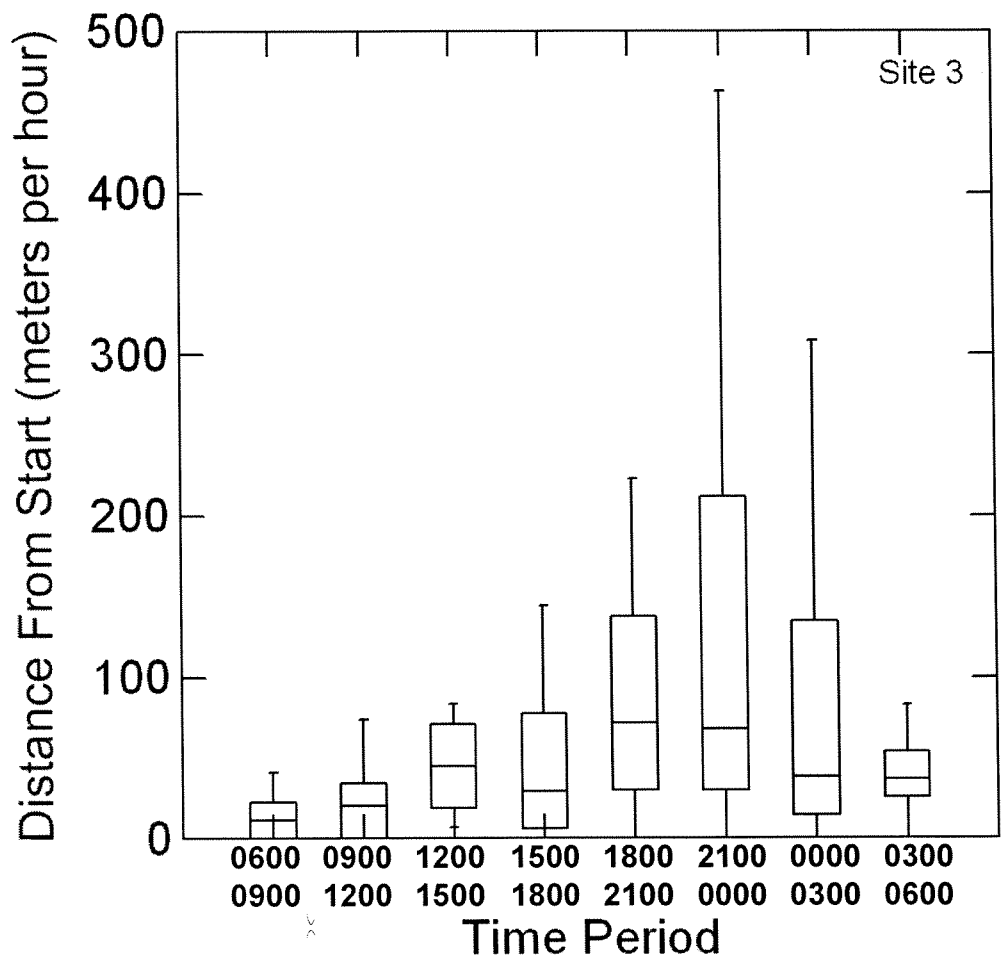


Figure 4. Diel Movement Patterns at Site 3, WCA. Periods run in 3-hour increments Starting at 0600 – 0900 (Period 1) through a 24-hour cycle, with the last time period (8) running from 0300 – 0600.



**VIII. Genetic diversity in a morphologically conservative invasive taxon:
Multiple swamp eel introductions in the southeastern United States**

Genetic Diversity in a Morphologically Conservative Invasive Taxon: Multiple Introductions of Swamp Eels to the Southeastern United States

TIMOTHY M. COLLINS,*† JOEL C. TREXLER,* LEO G. NICO,†
AND TIMOTHY A. RAWLINGS*

*Department of Biological Sciences, Florida International University, University Park, Miami, FL 33199, U.S.A.,

†United States Geological Survey, Florida Caribbean Science Center, 7920 NW 71st Street,
Gainesville, FL 32653, U.S.A.

Abstract: Genetic analysis of introduced populations, especially in morphologically conservative taxa, can clarify introduction histories, identify management units and source populations, provide a more realistic estimate of the frequency of successful invasion, and suggest strategies for preventing further introductions. In the last 7 years, populations of swamp eels, referred to the Asian genus *Monopterus* (Family Synbranchidae) on the basis of external morphology, have been discovered in aquatic habitats near Atlanta, Georgia; Tampa, Florida; North Miami, Florida; and most recently in close proximity to Everglades National Park in Homestead, Florida. Swamp eels are large predators capable of dispersal over land and have the potential to disrupt already threatened ecosystems. We analyzed mitochondrial DNA sequences from four known populations in the continental United States and samples from Malaysia, Indonesia, Vietnam, and two locations in China to determine introduction histories, source populations, genetic diversity, and relationships among populations. Our results indicate that there have been at least three independent introductions of genetically distinct forms. Introduced populations in close proximity (separated by <40 km) are genetically distinct. The level of sequence difference among introduced populations reaches levels seen among sister families of teleost fishes for the same region of the mitochondrial genome. These genetically distinct introduced populations in all likelihood represent at least two and possibly three species. Regardless of species status, these genetically distinct lineages may be expected to vary in ecological or life-history traits, representing different potential threats to the ecosystems where they have been introduced. Given the success of swamp eels in invading many habitats around the world, further study of these eels is warranted to elucidate the characteristics of successful invaders and invasions.

Diversidad Genética en un Taxón Invasivo Morfológicamente Conservativo: Introducciones Múltiples de Anguila de Pantano en el Sureste de Estados Unidos

Resumen: El análisis genético de poblaciones introducidas, especialmente en taxones morfológicamente conservativos, puede clarificar la historia de las introducciones, identificar unidades de manejo y poblaciones fuente, proporcionar una estimación más realista de la frecuencia de invasiones exitosas y sugerir estrategias para prevenir introducciones futuras. En los últimos 7 años, se han descubierto poblaciones de anguilas de pantano, referidas al género Asiático *Monopterus* (Familia Synbranchidae) con base en su morfología externa, en los hábitats acuáticos cerca de Atlanta, Georgia; Tampa, Florida; Miami Norte, Florida y más recientemente muy cerca del Parque Nacional Everglades en Homestead, Florida. Las anguilas de pantano son grandes depredadores capaces de dispersarse por tierra y tienen un potencial para alterar ecosistemas de por sí amenazados. Analizamos secuencias de ADN mitocondrial de cuatro poblaciones conocidas en Estados Unidos y muestras de Malasia, Indonesia, Vietnam y dos localidades en China para determinar la historia de las introducciones, poblaciones fuente, diversidad genética y relaciones entre las poblaciones. Nuestros resultados

*email collinst@fiu.edu

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indican que ha habido por lo menos tres introducciones independientes de formas genéticamente distintas. Las poblaciones introducidas en sitios cercanos (separados por <40 km) son genéticamente distintos. La diferencia de secuencias entre poblaciones introducidas alcanza niveles vistos entre familias hermanas de peces teleósteos para la misma región del genoma mitocondrial. Muy probablemente, estas poblaciones introducidas y genéticamente diferentes representan por lo menos dos y posiblemente tres especies. Independientemente del estado de la especie, se puede esperar que estos linajes genéticamente distintos varíen en cuanto a características ecológicas y de historia de vida, representando diferentes amenazas potenciales para los ecosistemas en donde han sido introducidos. Debido al éxito de las anguilas de pantano al invadir muchos hábitats en el mundo, se requiere mayor estudio de estas anguilas para dilucidar las características de invasores exitosos e invasiones.

Introduction

When biological invasions occur, ecosystems may be disrupted and the persistence of indigenous species threatened. To gauge the effect of an invasion on indigenous species and ecosystems and to plan prevention of future invasions, the most fundamental questions that can be asked are (1) How did these invasions occur? (2) How many species are involved? (3) What is the identity of the invader or invaders? (4) Where did these species come from? (5) What is known about the factors that control the abundance and distribution of these species over their native range? Answers to these questions may go a long way toward predicting the effects of biological invasions, as well as suggesting prevention, control, and management strategies (Courtenay & Stauffer 1984; Fuller et al. 1999).

Determination of the ecological and evolutionary diversity of introduced populations and identification of native source populations are therefore the necessary first steps in characterizing a recent invasion. Information on genetic variation clarifies the nature and extent of management units among populations of invasive species and can test competing hypotheses about the nature of introductions. Source-population data gives us information on factors controlling the distribution of invasive species over their native range and allows us to estimate the potential range of expansion of introduced populations as well as potential controls on this expansion. Examples of difficulties resulting from failure to properly account for genetic diversity and variation of superficially similar taxa are plentiful in the literature. Incorrect or misidentified biotypes have been introduced that are morphologically indistinguishable from the proper biotype but that differ in key traits from the correct biological control agent, leading to failure of biological control of pest insects in well-documented cases (e.g., Gonzalez & Gilstrap 1992). In several cases, proper matching of the climate requirements of different host races of parasitoids to the site of introduction has been critical to successful biological control (Gonzalez et al. 1979; Diehl & Bush 1984). In fishes, various misidenti-

fied species of tilapia have been introduced as food, game, or biological control agents, often with negative effects (e.g., Courtenay & Hensley 1979). Likewise, difficulties with species identification and formation of interspecies hybrids with different or unknown environmental tolerances and feeding habits have complicated the control and management of tilapia (Courtenay & Robins 1989; Fuller et al. 1999). An increasing appreciation of the importance of taxonomy for understanding and managing invasive species is reflected in a draft strategy by the European Platform for Biodiversity Research, which identified extending systematics and updating taxonomic knowledge of invasive species as research topics of high priority (Scott 2001).

In the last decade, swamp or rice eels, tentatively identified as belonging within the synbranchid genus *Monopterus*, have been found in aquatic habitats in several regions of the southeastern United States (Fuller et al. 1999). Swamp eels have many characteristics that make them potentially disruptive to already threatened native ecosystems. They are large predators, with adults reaching nearly 1 m in length, and they feed on a variety of animals, including fish. Piscivores are more likely to alter freshwater fish communities (Moyle & Light 1996), especially in cases such as the Everglades where species number is low and environmental variability and severity are high. Many swamp eels are air breathers, able to survive for long periods out of water, and capable of dispersal over land (Liem 1987; Graham 1997). Swamp eels produce mucus copiously, preventing dessication, and burrow as water levels drop (Liem 1967), making them extremely tolerant of drought. Hydrologic fluctuations, which appear to be an important factor controlling introduced fish populations in the Everglades (Trexler et al. 2001), are therefore unlikely to limit populations of swamp eels.

Given their amphibious abilities, synbranchids are capable of becoming established in habitats beyond the range of most other large fishes. Thus, invading eels are a potential threat to normally isolated populations of small fishes, frogs, crayfishes, and other small aquatic organisms. In addition, introduced swamp eels may also

compete for food with native fishes and wading birds. Swamp eels are relatively secretive and possibly nocturnal, often living in burrows or associated with dense mats of aquatic vegetation, complicating surveys and eradication of populations. There is evidence that synbranchids exhibit parental care, and some populations include individuals that undergo sex reversal, changing from female to male (Liu 1944; Liem 1963; Matsumoto & Iwata 1997).

Swamp eels were first discovered in North America near Atlanta, Georgia, in 1994 (Fig. 1:1), although population size structure suggests that the population may have been first introduced circa 1990 (Starnes et al. 1998). In 1997, two populations were discovered in Florida, one in Manatee County, near Tampa, and the other in Miami-Dade and Broward counties in North Miami (Fig. 1: 2, 3). More recently in late 1999, a population was discovered in the Homestead area of Miami-Dade County, near Everglades National Park (ENP) (Fig. 1:4). If the Homestead population had dispersed from North Miami, this would have entailed a rapid spread through 40 km of canals and water-control structures in a short period of time. Such rapid dispersal would suggest that there is little hope of containing or eradicating the population to prevent its spread to ENP. Field sampling, however, failed to recover eels from intervening canals.

Our initial question was, therefore, are the North Miami and Homestead populations genetically distinct? As sampling proceeded, we broadened the scope of our enquiry, using DNA sequence data to answer the following questions: Are introduced populations the result of a single event with subsequent "hopping" (sensu Kolar & Lodge 2001), or do they result from independent introductions? What is (are) the source(s) of introduced populations? What is the level of genetic diversity and genetic distinctness among these populations? What are the relationships among introduced and native populations?

Methods

Systematics and Distribution of Swamp Eels

The Synbranchidae is a family of eel-like percomorph fishes widely distributed throughout Central and South America, the Caribbean, tropical west Africa, and from the Indian subcontinent throughout Asia and the Indo-Australian archipelago to Australia. The native range of the genus *Monopterus* is restricted to the Old World and includes much of southern and eastern Asia, from the Indo-Malayan Archipelago, the East Indies, and Sulawesi, through Vietnam, Thailand, Laos, much of eastern China, and perhaps as far north as Vladivostok in far-eastern Russia. To the west it naturally occurs in Burma, India, Sri Lanka, Nepal, and Pakistan. One member of the genus

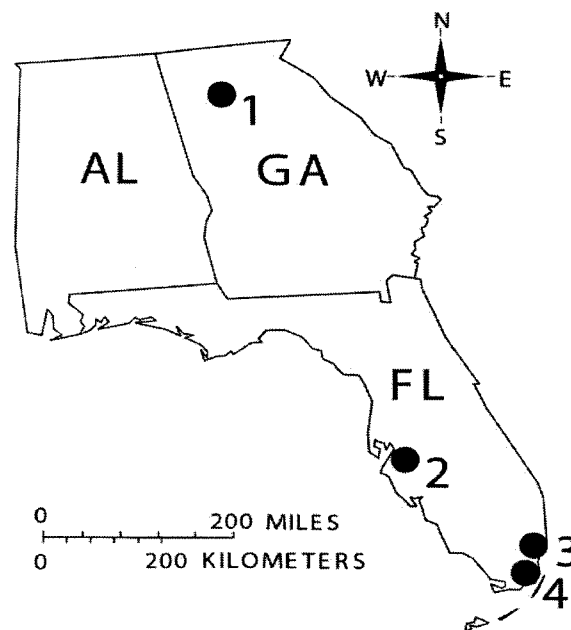


Figure 1. Locations of known swamp eel populations in the southeastern United States: Atlanta, Georgia (GA), 1; Tampa, Florida (FL), 2; North Miami, Florida, 3; Homestead, Florida, 4. See Table 1 for detailed locality information.

is also known from West Africa (Rosen & Greenwood 1976; Bailey & Gans 1998).

Based on the triangular shape and size of the external gill opening and other morphological characteristics, all populations introduced into the waters of the continental United States were initially assigned to *Monopterus albus*, commonly known as the Asian swamp eel, rice eel, or belut (Starnes et al. 1998; Fuller et al. 1999). The assignment is provisional, however, because of the lack of useful morphological characters to separate *M. albus* consistently from other members of the genus and the general confusion surrounding synbranchid taxonomy and systematics (e.g., Rosen & Greenwood 1976). In fact, because of its broad geographic distribution and reported regional morphological variation, *M. albus* is particularly problematic, and it is conceivable that what is referred to as *M. albus* actually is a species complex. *M. albus* naturally occurs in southern and eastern Asia.

Swamp eels have been introduced outside their native range in places other than the continental United States. It is unclear, however, whether populations along the periphery of synbranchid native ranges represent introductions. Swamp eels were introduced into the region

near Hami, Xinjiang Province, China, by the provincial army during the late nineteenth century Chin Dynasty (Wang et al. 1994). According to Matsumoto et al. (1998), *M. albus* was introduced to Nara Prefecture in Japan from Korea in the early 1900s. Some believe that the small population present in northeastern Australia is the result of an introduction (Merrick & Schmida 1984). Finally, a synbranchid identified as *M. albus* has become established on Oahu, Hawaii, from founding populations brought to the islands by Asian immigrants prior to 1900 (Brock 1960; Devick 1991).

Sampling, DNA Extraction, Amplification, and Sequencing

We sampled swamp eels from each of the four known localities in the continental United States, as well as China, Vietnam, the Malay Peninsula, and the East Indies (Table 1). Asian samples were either caught in the wild or obtained from local markets. *Synbranchus marmoratus* (family Synbranchidae) was sampled from Venezuela as an outgroup. Two of the swamp eels sampled from Vietnam were more closely related to *Synbranchus marmoratus* than to any other swamp eels sam-

pled and were therefore treated as additional outgroups (listed as unidentified synbranchid in Table 1). Specimens were either frozen or preserved in 70–95% ethanol. Voucher specimens for the United States populations are stored at the Florida Museum of Natural History, United States National Museum, and the North Carolina State Museum of Natural History.

We investigated relationships among populations of swamp eels by phylogenetic analysis of a portion of the 16S rRNA gene. This gene had been successfully employed for analyzing relationships from the level of populations to families and above (Palumbi 1996; Sullivan et al. 2000) and is therefore useful for investigating relationships of unknown phylogenetic depth. We extracted total genomic DNA from approximately 0.1 g of muscle tissue, following Collins et al. (1996). Polymerase chain reaction (PCR) amplifications were performed in 50 μ L of a solution containing 10–100 ng of genomic DNA, 200 μ M of each dNTP, 1 unit Taq Polymerase (Promega), 1.5 mM $MgCl_2$, each primer at 0.5 μ M, and buffer supplied by the manufacturer. The primers, designed by Kocher and Palumbi (Palumbi 1996), amplify an approximately 615-bp portion at the 3' end of the

Table 1. Localities sampled for swamp eels.

Locality*	Sample size	Taxonomic designation
Tampa, Florida		
Manatee County, Tampa Bay Basin, Frog Creek drainage, approximately 27°35'N, 82°29'W	11	<i>Monopterus</i>
North Miami, Florida		
Miami-Dade County, Turnpike Pond, Miramar (n = 2), Snake Creek Canal, C-9 (*n = 8)	10	<i>Monopterus</i>
Homestead, Florida		
Miami-Dade County, SW 296th Street & 205th Avenue, 25°29.47'N, 80°31.46'W (n = 1), C-113 Canal (n = 9)	10	<i>Monopterus</i>
Atlanta, Georgia		
Fulton County, pond(s) of Chattahoochee Nature Center, south-southwest of Roswell, 34°00.3'N, 84°22.8'W	10	<i>Monopterus</i>
Nanning, China		
Guanxi Province, Nanning and Nanhu markets, approximately 22°48'N, 108°20'E	9	<i>Monopterus</i>
Buobai, China		
Guanxi Province, Buobai (or Bobai) county market, approximately 22°12'N, 109°52'E	2	<i>Monopterus</i>
Ca Mau, Vietnam		
Southwestern Vietnam, approximately 8°46'N, 104°59'E	4	<i>Monopterus</i>
Kuala Lumpur, Malaysia		
Selangor Province, market in town of Petaling Jaya, approximately 3°7'N, 101°42'E	10	<i>Monopterus</i>
Jakarta, Indonesia		
Senen market, Jakarta-Pusat (central Jakarta), approximately 6°11'S, 106°50'E	2	<i>Monopterus</i>
Ca Mau, Vietnam		
Southwestern Vietnam, approximately 8°46'N, 104°59'E	2	unidentified synbranchid
Venezuela		
Apure State, Orinoco River Basin, Apure River drainage, Caño Caicara 07°33.51'N, 69°15.51'W	1	<i>Synbranchus marmoratus</i>

*Localities named for nearby major city, when possible, for ease of recognition.

16S (large subunit) mitochondrial ribosomal RNA gene. The PCR cycling parameters (MJ Research PTC 200, Woburn, Massachusetts) for the initial double-stranded amplification were 94° C for 30 seconds, 47°–49° C for 30 seconds, and 72° C for 45 seconds, repeated for 37 cycles. Free dNTPs and unincorporated dye-labeled nucleotides and primers were removed with the GeneClean kit (Bio 101 Company, Vista, California). The PCR products were quantified on a Dynaquant fluorometer (Pharmacia Biotech Inc., Piscataway, New Jersey). Cycle sequencing of 30–50 ng of the double-stranded PCR product was carried out with each primer and Big Dye terminators following the manufacturer's recommendation (PE ABI, Foster City, California). Sequences were determined on an ABI 377 automated DNA sequencer using 48-cm well-to-read plates.

To increase geographic and taxonomic sampling, we also attempted to determine sequences from formalin-fixed, ethanol-preserved museum specimens of swamp eels. We extracted DNA with a protocol developed for formalin-fixed tissue (Chase et al. 1998). Tissue samples were analyzed from *Monopterus albus* (University of Michigan Museum of Zoology numbers UMMZ 232761 and UMMZ 194392) collected in 1995 and 1969, respectively, and *Monopterus couchii* (UMMZ 208372) collected in 1977. These extractions yielded DNA fragments up to 250–500 bp in length, based on agarose electrophoresis and subsequent staining with ethidium bromide. We were, however, unable to amplify a product over a range of conditions with the 16S primers mentioned above. We therefore designed internal primers that amplified 250–375 base-pair regions of 16S. These primer pairs produced PCR products that unfortunately proved to be contaminants. Further optimization will be required to produce results for this gene region in formalin-fixed specimens of swamp eel.

Data Analysis

We aligned sequences with CLUSTAL W (Thompson et al. 1994) at the following settings: gap opening cost 10, gap extension 2.5, and transition-transversion bias. We subsequently examined alignments by eye to locate errors resulting from pairwise progressive alignment methods. We investigated relationships among mitochondrial haplotypes with maximum parsimony (MP) and minimum evolution (ME) in PAUP* 4.0 (Swofford 2000). We carried out a branch-and-bound search of all unique haplotype-locality combinations using parsimony informative characters, with all characters unordered and equally weighted. Gaps were treated as missing data. We based our parsimony bootstrap proportions on 100 replicates of branch-and-bound searches including all unique haplotype-locality combinations. We also carried out a heuristic search with all 71 sequences to illustrate levels of within-population haplotype variation.

We tested for base compositional equilibrium across taxa using a chi-square test implemented in PAUP* 4.0 (Swofford 2000). Patterns of nucleotide substitution mapped onto the set of most parsimonious trees (MacClade 4.0; Maddison & Maddison 2000) indicated an excess of transitions relative to transversions and different rates of change for the two types of transversion. Thus, Tamura-Nei distances based on all characters were used for distance analyses. Minimum-evolution bootstrap trees were based on 100 replicates, with starting trees obtained via random-sequence addition, 10 random-sequence additions per replicate, and tree bisection-reconnection branch swapping.

Results

The 71 synbranchid eels sequenced for a portion of the 16S mitochondrial rRNA gene yielded 18 unique haplotype-locality combinations. After removal of primer regions, these sequences varied in length from 556 to 578 base pairs (average 575). Sequences have been deposited in GenBank under the accession numbers AF512841–AF512858. Base composition did not vary significantly across taxa, with average base frequencies of 23% A, 21% C, 26% G, and 30% T. Using the parameters described above, we obtained an alignment of 586 positions. This alignment had an average of only 2.5 gaps per sequence, with the majority of these resulting from indels in *Synbranchus* and the unidentified Vietnamese synbranchid relative to the remaining taxa.

Introduced populations had low levels of within-population haplotype diversity and small haplotype-to-haplotype distances (Table 2; Fig. 2). The Atlanta and North Miami populations were represented by single haplotypes, whereas the Tampa population had two haplotypes that differed by a single substitution. The Homestead population also contained two haplotypes, these differing pairwise by four nucleotide substitutions (pairwise distance was six).

Nucleotide-sequence divergence varied widely among populations. One haplotype found in the Tampa population was identical to the North Miami haplotype, and the other Tampa haplotype differed from North Miami by a single nucleotide substitution. No haplotypes were shared among the remaining introduced populations. Uncorrected percent sequence difference among other introduced populations ranged from 3.5% to >10% (Table 2).

A branch-and-bound parsimony search of the 18 unique haplotype-locality combinations with informative characters yielded 24 most-parsimonious trees of 128 steps with ensemble retention indices of 0.870. This tree indicated strong bootstrap support for a clade of haplotypes from Tampa, North Miami, and Nanning, China (clade C; Fig. 3). A second well-supported clade was composed of Homestead, Vietnamese, Indonesian,

Table 2. Percent sequence difference matrix for pairwise comparisons among the 18 unique 16S rRNA haplotype-locality combinations of swamp eels.

Haplotype	Haplotype distance matrix number																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1Tampa	—																
3Nanning	0.17	—															
4Tampa	0.17	0.17	—														
1NorthMiami	0.17	0.17	0.00	—													
2Nanning	0.52	0.52	0.35	0.35	—												
8Nanning	0.87	0.87	0.70	0.70	0.35	—											
1Nanning	0.70	0.70	0.52	0.52	0.17	0.52	—										
11Buobai	2.96	2.96	2.78	2.78	2.44	2.78	2.26	—									
1Homestead	4.01	4.01	3.84	3.84	3.49	3.49	3.67	3.32	—								
1Jakarta	3.31	3.31	3.14	3.14	2.96	2.96	3.14	3.14	0.53	—							
1Vietnam	3.48	3.48	3.30	3.30	2.96	2.96	3.13	3.13	0.52	0.35	—						
8Homestead	3.65	3.65	3.48	3.48	3.13	3.13	3.30	3.30	0.70	0.87	0.52	—					
1KualaLumpur	4.17	4.17	4.00	4.00	3.65	3.65	3.83	3.48	1.22	0.70	1.04	1.57	—				
10KualaLumpur	4.00	4.00	3.83	3.83	3.48	3.48	3.65	3.30	1.05	0.52	0.87	1.39	0.17	—			
3KualaLumpur	4.01	4.01	3.84	3.84	3.49	3.49	3.66	2.96	0.70	0.52	0.52	1.05	0.52	0.35	—		
1Atlanta	10.17	10.17	9.99	9.99	9.81	9.81	9.99	10.35	10.18	10.34	10.69	10.52	10.51	10.34	10.35	—	
Unidentified synbranchid	17.01	17.01	16.83	16.83	16.83	16.83	17.00	17.18	16.69	16.50	16.83	17.00	16.29	16.47	16.86	14.66	—
<i>Synbranchus</i>	17.06	17.05	16.87	16.87	16.87	17.06	17.06	16.52	17.64	17.66	17.79	17.79	17.06	17.24	17.64	15.18	12.22

and Malaysian haplotypes (clade B). These two clades formed a third well-supported clade, along with the Buobai haplotype. This clade was in turn the sister group to the Atlanta haplotype (clade A). The unidentified synbranchid was basal to this clade, and, when additional percomorph fish sequences were added to the analysis (data not shown), was a sister to *Synbranchus marmoratus* (Fig. 3).

Discussion

Introduction Histories and Invasibility

Improving our understanding of the "epidemiology of invasions" (Mack et al. 2000) is critical if we are to control current invasions and, perhaps more significantly, prevent future invasions. Genetic data have much to offer in this regard, indicating, for example, the number of independent invasions and the geographic distribution of distinct invading populations (Bastrop et al. 1998; Kreiser et al. 2001). In addition, the nature of population expansion of invading species may be elucidated by population genetic studies (Wilson et al. 1999). When genetic sampling includes potential native populations and local source populations (e.g., pet stores, food markets), precise pathways of introduction may be determined with important lessons for preventing future introductions.

In the case of the swamp eels, the geographic distribution and phylogenetic relationships among haplotypes indicate that there have been at least three introductions

to North America from distinct source populations. The North Miami and Tampa populations possess closely related haplotypes and therefore could be derived from a common source. Additional data must be collected to determine whether these populations represent independent introductions or a single introduction and subsequent spread or transplant of individuals from the initial site. The Tampa and North Miami populations are genetically similar to haplotypes sampled from Nanning, indicating southern China as a potential source. The population from Homestead is nested within a clade of haplotypes sampled from Indochina, the Malay Peninsula, and the East Indies, identifying this broad region as a source. The Homestead population is clearly distinct from the North Miami population and is not part of a contiguous population extending from North Miami to Homestead. It is thus a recent introduction of limited distribution, making it a promising candidate, although by no means guaranteed, for successful eradication (Mack et al. 2000; Myers et al. 2000). Based in part on the results of our study, a program aimed at eradicating or controlling the Homestead swamp eel population is being pursued (J. Curnutt, personal communication). The Atlanta population is phylogenetically distinct from all sampled populations, and its source could not be determined from the available data.

Another important goal in studies of invasive species is the identification of probable invaders (Kolar & Lodge 2001) to prevent their introduction. The prevention of invasions is both more likely to succeed and more cost-effective than extirpation of established populations (Mack

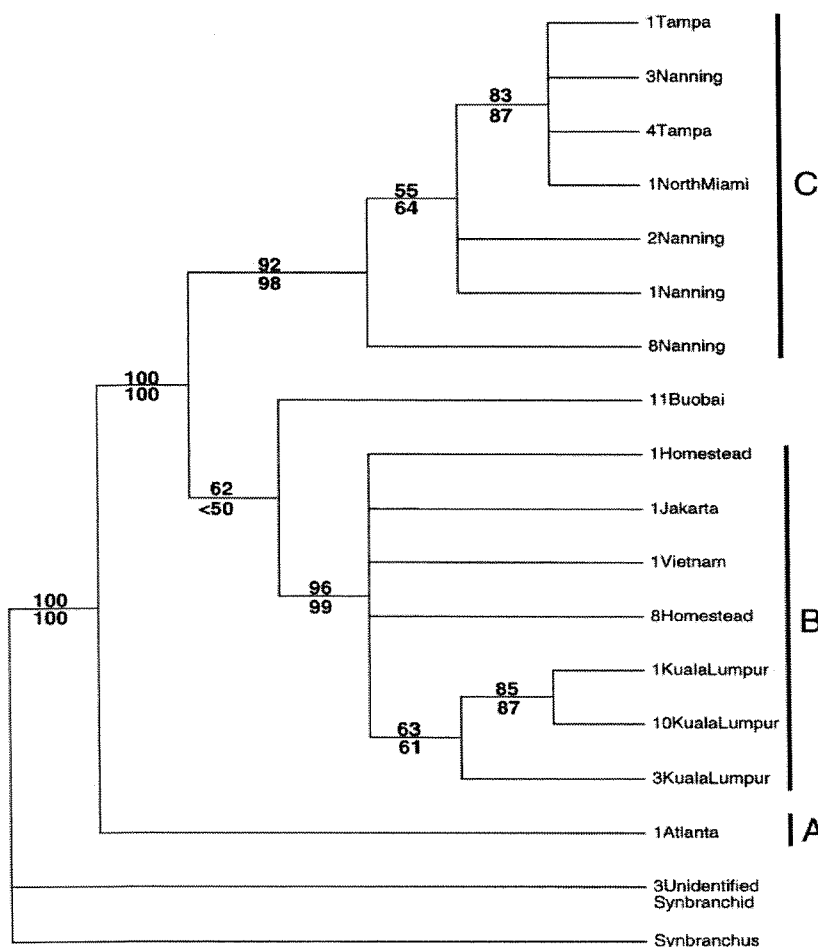


Figure 3. Strict consensus of the 24 most parsimonious trees resulting from a branch-and-bound search of parsimony-informative characters for the portion of the 16S gene sequenced from all unique swamp-eel haplotype-locality combinations. The ensemble retention index for characters on these 24 trees is 0.870. Bootstrap proportions are given above (parsimony) and below (minimum evolution) branches. Numerals preceding locality names are within-locality sample-identification numbers. Three clades with strong bootstrap support are labeled A, B, and C.

parameters, dispersal mechanisms, and the likelihood of invasion (Mack et al. 2000; Kolar & Lodge 2001). Whether the success of swamp eels is due to some innate properties of the clade, the fit between swamp eel biology and habitats in the southeastern United States, or the advantage of human-mediated dispersal remains to be determined. Molecular analyses may help sort out these alternatives because correct taxonomic apportionment and clarification of introduction histories may give a more accurate estimate of the numbers of invasions and the correspondence of traits to frequency of invasion.

It is not possible at this point to specify the agents of these independent introductions. Swamp eels have been sold at aquarium stores in Atlanta (Starnes et al. 1998), suggesting that Atlanta introduction may have resulted

from an aquarium release. At least one Florida introduction is in close proximity to a formerly active fish farm. Finally, swamp eel is a popular food item in many Asian cuisines, favored for its fine-textured meat and commonly sold in local markets.

Distinctness of Introduced Populations, Genetic Diversity, and Taxonomic Status: Implications for Management

Genetic diversity of introduced populations may give important clues to the size of the founding populations. In addition, populations with limited genetic diversity may be more susceptible to biological control than genetically diverse populations (Nissen et al. 1995). Genetic diversity within each of the four introduced populations

of swamp eel was generally low, as would be expected of populations that were likely founded from a small number of individuals. Although the Homestead population possessed only two haplotypes, these differed by four nucleotide substitutions, approaching the maximum within-population haplotype pairwise distance (5, Nanning) found among "native" samples. Genetic distance among introduced populations was remarkably high, with the Atlanta populations differing by >10% in uncorrected pairwise distance from the other North American populations. The difference between the A clade and the B and C clades exceeds that seen among intergeneric sister taxa in selected comparisons and is within the range seen between sister families of teleost fishes (Fig. 4). Moreover, it exceeds the amount of sequence difference seen among three pairs of snook species (family Centropomidae) inferred to have been isolated by the final emergence of the Isthmus of Panama approximately 3.5 million years ago (Donaldson & Wilson 1999; Tringali et al. 1999). The sequence difference between the B and C clades surpasses that seen among some of the snook geminates and ranges to the high end of the comparisons among intragenetic sister species analyzed (Fig. 4). The level of genetic difference among these populations suggests that they are unlikely to belong to a single species.

Species may, of course, vary in traits relevant to their ability to invade new habitats as well as in their effects on habitats and native species within those habitats. Unfortunately, the taxonomy and classification of swamp eels are problematic, primarily because of the simple external morphology of the family. The difficulties have been succinctly summarized by Rosen and Greenwood (1976): "Few groups of teleostean fishes have had so long and obscure a taxonomic history as the swamp eels . . . these fishes have a virtually featureless superficial anatomy and are rather variable in the very few external characters that can be measured or described precisely." The situation is particularly confusing within the genus *Monopterus*. Intraspecific variation and geographic ranges are not well characterized. Two named species, for example, are known only from holotypes, and Rosen and Greenwood (1976) suspect that several species may ultimately be found within the range of putatively broadly distributed taxa. It is therefore not surprising that it has proven difficult to assign the forms introduced into the southeastern United States with confidence to one or more species with proper binomials. Starnes et al. (1998) identified eels taken from near Atlanta as *Monopterus albus*. According to W. C. Starnes (personal communication), that determination was made according to the work of Rosen and Greenwood (1976) and was based on gill aperture, jaw morphology, and overall appearance. Those same characters were the basis for the tentative assignment of all Florida specimens to *M. albus* (Fuller et al. 1999).

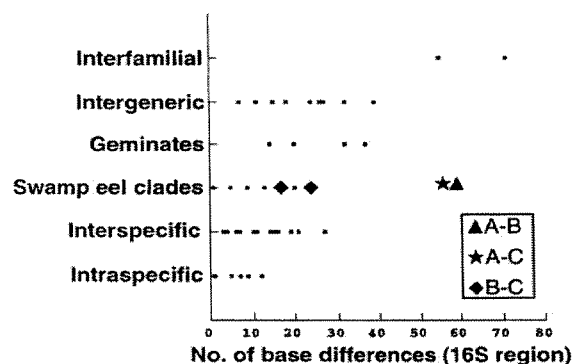


Figure 4. A comparison of the numbers of base differences between pairs of swamp-eel 16S haplotypes to numbers of differences over the same gene region in other pairs of fish taxa at a variety of taxonomic levels. Comparisons range from intraspecific to interfamilial. Interspecific comparisons are between terminal sister taxa (pairs of most closely related living species). The A, B, and C refer to clades of swamp eels from Fig. 3. Other swamp eel comparisons are within-clade and between clades B and C and the Buobai clade. Geminates refers to geminate species of snook thought to have been isolated by the emergence of the Isthmus of Panama 3.5 million years ago (Tringali et al. 1999). One of the geminates has speciated following the geminate split, so there are four comparisons for the three geminate pairs. Other data are from Duvernell and Aspinwall (1995), Simons and Mayden (1999), and Sullivan et al. (2000).

The proper generic and specific names that apply to the species being called *Monopterus albus* and the number of species that may ultimately prove to reside under this binomial are, however, in doubt. The binomials *Fluta alba* (Zuiew) (= *Monopterus albus*) and *Monopterus javanensis* Lacépède have also been used for what many authors consider a single species, although Rosen and Greenwood (1976) believe at least two species may be involved, one in China and another with a more southerly distribution, including Java. In his publication on the fishes of China, Nichols (1943) noted that "*Fluta alba*" specimens from south China were differentiable from other Chinese material he examined. The type specimen of *Monopterus javanensis* is from Java, whereas the type specimen of *Fluta alba* is presumed to be from Asiatic Russia. If these forms represent different species, it is possible that clade B, including specimens from Java, may include part or all of *Monopterus javanensis*. Clade C, including specimens from mainland China, might represent all of part of *Monopterus albus*. The type specimens on which these names are based are no

longer in existence, however, making a conclusive resolution unlikely. We have not sampled any individuals over the native range of *Monopterus* that are similar in mtDNA sequence to the Atlanta population.

In summary, the difficulties with synbranchid taxonomy and classification are profound and beyond the scope of this paper. For reasons we discuss below, however, it is imperative that the substantial variation recognized on the basis of mtDNA sequences not be obscured by grouping these forms under a single name, or simply as *Monopterus* spp. Until the taxonomy and classification of this group are satisfactorily resolved, we suggest that the introduced forms be known informally as *Monopterus* clade A, *Monopterus* clade B, and *Monopterus* clade C.

How many species are represented in these introductions? The answer depends on one's species concept. A phylogenetic species concept could certainly be applied to the distinct lineages shown in Fig. 3, but from the perspective of management of genetically distinct invasive populations, a biological species concept emphasizing the limits of gene exchange seems appropriate. We currently have no information on the potential for interbreeding among these populations. The admittedly approximate yardstick of degree of difference among introduced populations relative to recognized species (Fig. 4) suggests that, at a minimum, clade A is a distinct species. Clades B and C are more different over the sampled region of the 16S gene than many distinct species, including some thought to have been isolated for 3.5 million years. In addition, they are geographically separated over their native range based on current sampling. Of course, many more than three species may prove to be represented by the specimens sampled for this study once swamp eels are fully characterized over their native range.

The number of species diagnosed is ultimately less important than the recognition that these genetically distinct forms may vary in traits that will influence the effect of their introduction on native species and ecosystems. Just as biologists recognize evolutionarily significant units to conserve the genetic diversity of endangered or threatened indigenous species (Moritz 1994), those studying biotic invasions need to recognize distinct evolutionary lineages of invading taxa, evolutionarily significant invasion units (ESIUs). Swamp eels are conservative in external morphology but vary in other significant ways, including salinity tolerance from fresh to hypersaline waters, and habitat from clear flowing streams to sluggish or standing water with dense vegetation; they are cavernicolous and epigeal (Tyler & Feller 1996; Bailey & Gans 1998). They vary in the degree of development of the lung-like suprapharyngeal diverticula (Rosen & Greenwood 1976), which affects the efficiency of aerial respiration and hence their ability to disperse over land and to tolerate low-oxygen conditions. Swamp eels vary in sex ratio during development, with

some species or populations producing primary males and females and others producing only primary females that transform as adults to males (Liem 1968; Chan et al. 1972). Finally, swamp eels have a range from Japan and Russia south to Australia, and therefore will likely vary in temperature tolerance.

Variation in these traits has two important implications for conservation. First, these variations will affect the ability of introduced populations to persist in an area or undergo range expansion. For this reason, the ability to relate introduced populations to populations over the native range of *Monopterus* will be advantageous. Knowledge of the ecology and life history of these eels in their native habitat, and information on geographic range, temperature tolerance, predators, pathogens, and other checks on population size and growth may prove useful for management, control, and estimation of possible range expansion of introduced populations. Second, results of studies of the ecology, climatic tolerances, life-history traits, or susceptibility to biological controls of one introduced population should not be assumed to apply, in part or whole, to other introduced populations. For example, because numerous introduced species in Florida are from the tropics, temperature tolerance appears to be the most important factor controlling the demography of many invasive fish species in the state (Shafland & Pestrak 1982). Populations of the Mayan cichlid (*Cichlasoma urophthalmus*) exemplify this tendency, growing during typical years, only to crash precipitously during extreme cold events (Trexler et al. 2001). Based on the fact that the Atlanta populations of introduced swamp eels overwinter at latitude 34°N, one might conclude that cold events will have little effect on swamp eel populations in southern Florida. Because the Atlanta population represents a distinct lineage, however, its climatic tolerance may be irrelevant to the survival of introduced populations in peninsular Florida. The ecology of each genetically distinct population of swamp eels must be studied independently.

An important caveat to our study is that we have characterized these populations for a single maternally inherited marker. Because these introductions have likely resulted from the aquarium trade or the use of swamp eels as food items, we cannot rule out the possibility that there may have been some hybridization among populations of swamp eels characterized by distinct mtDNA lineages. This could occur by mixing of populations while in captivity, resulting in hybrid offspring. The absence of mixing of the major clades in any of the introduced or market-purchased populations argues against this possibility, as does the congruence of clades to broad-scale geographic distribution, with a southern clade B and a northern clade C. Nevertheless, these lineages should be characterized for one or more nuclear markers to determine definitively whether there has been any recent mixing of these lineages in captivity.

Our results suggest that genetic characterization of multiple populations of a putative invasive species, and identification of source populations, may provide data critical to the proper control, management, and determination of the frequency and nature of introductions, especially in the case of morphologically conservative taxa. The costs of these analyses have declined with technological advances and should no longer be considered extravagant or required only in exceptional circumstances. Analysis of genetic variation should be a routine part of an integrated approach to the prevention, control, and management of invasive species. Swamp eels have invaded habitats around the globe, suggesting that further investigation is merited to elucidate the characteristics of successful invaders and invasions.

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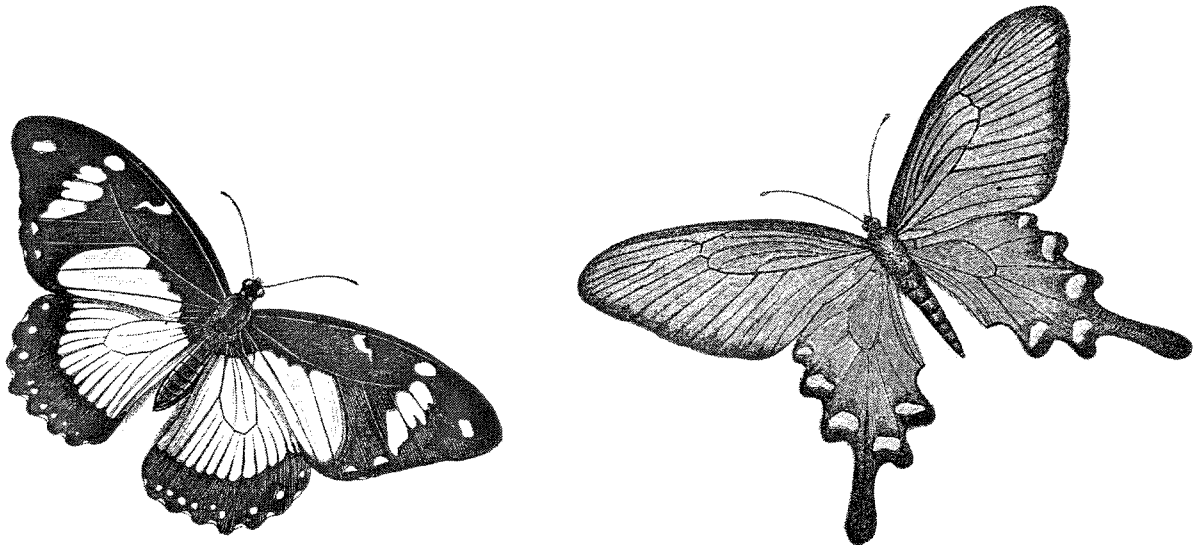
Note Added in Proof

Seiji Matsumoto kindly provided us with several samples of *Monopterus* from Nara Prefecture in Japan. These individuals are identical to the Atlanta haplotype for the 16S region we analyzed in this paper (unpublished data). This indicates Japan or Korea as the most likely source of the Atlanta population.

Literature Cited

- Bailey, R. M., and C. Gans. 1998. Two new synbranchid fishes, *Monopterus roseni* from peninsular India and *M. desilvai* from Sri Lanka. Occasional papers 726. Museum of Zoology, University of Michigan, Ann Arbor.
- Bastrop, R., K. Jürss, and C. Sturmbauer. 1998. Cryptic species in a marine polychaete and their independent introduction from North America to Europe. *Molecular Biology and Evolution* 15:97-103.
- Brock, V. E. 1960. The introduction of aquatic animals into Hawaiian waters. *Internationale Revue der Gesamten Hydrobiologie* 45:463-480.
- Chan, S. T. H., O. Wai-Sum, F. Tang, and B. Lofts. 1972. Biopsy studies on the natural sex reversal in *Monopterus albus* (Pisces: Teleostei). *Journal of Zoology, London* 167:415-421.
- Chase, M. R., R. J. Etter, M. A. Rex, and J. M. Quattro. 1998. Extraction and amplification of mitochondrial DNA from formalin fixed deep-sea mollusks. *Biotechniques* 24:243-247.
- Collins, T. M., K. S. Frazer, A. R. Palmer, G. J. Vermeij, and W. M. Brown. 1996. Evolutionary history of Northern Hemisphere *Nuccella*: molecules, morphology, ecology, and fossils. *Evolution* 50:2287-2304.
- Courtenay, W. R., Jr., and D. A. Hensley. 1979. Survey of introduced non-native fishes. Phase I report. Introduced exotic fishes in North America: status 1979. Report. National Fisheries Research Lab, U.S. Fish & Wildlife Service, Gainesville, Florida.
- Courtenay, W. R., Jr., and J. R. Stauffer Jr. 1984. Distribution, biology and management of exotic species. Johns Hopkins University Press, Baltimore, Maryland.
- Courtenay, W. R., Jr., and C. R. Robins. 1989. Fish introductions: good management, mismanagement, or no management? *Reviews in Aquatic Sciences* 1:159-172.
- Devick, W. S. 1991. Patterns of introductions of aquatic organisms to Hawaiian freshwater habitats. Pages 189-213 in new directions in research, management, and conservation of Hawaiian freshwater stream ecosystems. Proceedings of the 1990 symposium on freshwater stream biology and fisheries management. Division of Aquatic Resources, Hawaiian Department of Land and Natural Resources, Honolulu.
- Diehl, S., and G. Bush. 1984. An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology* 29:471-504.
- Donaldson, K. A., and R. R. Wilson Jr. 1999. Amphiparic geminates of snook (Percoidae: Centropomidae) provide a calibration of the divergence rate in the mitochondrial DNA control region of fishes. *Molecular Phylogenetics and Evolution* 13:208-213.
- Duvernell, D. D., and N. Aspinwall. 1995. Introgression of *Luxilus cornutus* mtDNA into allopatric populations of *Luxilus chrysocephalus* (Teleostei: Cyprinidae) in Missouri and Arkansas. *Molecular Ecology* 4:173-181.
- Fuller, P. L., L. G. Nico, and J. D. Williams. 1999. Nonindigenous fishes introduced into inland waters of the United States. Special publication 27. American Fisheries Society, Bethesda, Maryland.
- Gonzalez, D., and F. E. Gilstrap. 1992. Foreign exploration: assessing and prioritizing natural enemies and consequences of preintroduction studies. Pages 53-70 in W. C. Kauffman and J. E. Nichols, editors. Selection criteria and ecological consequences of importing natural enemies. Entomological Society of America, Lanham, Maryland.
- Gonzalez, D., G. Gordh, S. N. Thompson, and J. Adler. 1979. Biotype discrimination and its importance to biological control. Pages 129-134 in M. Hoy and J. McKelvey Jr., editors. Genetics in relation to insect management. Working papers. Rockefeller Foundation, New York.
- Graham, J. B. 1997. Air-breathing fishes: evolution, diversity, and adaptation. Academic Press, San Diego.
- Kolar, C. S., and D. M. Lodge. 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16:199-204.
- Kreiser, B. R., J. B. Mitton, and J. D. Woodling. 2001. Single versus multiple sources of introduced populations identified with molecular markers: a case study of a freshwater fish. *Biological Invasions* 2:295-304.
- Liem, K. F. 1963. Sex reversal as a natural process in the synbranchiform fish *Monopterus albus*. *Copeia* 1963:303-312.
- Liem, K. F. 1967. Functional morphology of the integumentary, respiratory, and digestive systems of the synbranchoid fish *Monopterus albus*. *Copeia* 2:375-388.
- Liem, K. F. 1968. Geographic and taxonomic variation in the pattern of natural sex reversal in the teleost fish order Synbranchiformes. *Journal of Zoology, London* 156:225-238.
- Liem, K. F. 1987. Functional design of the air ventilation apparatus and overland excursions by Teleosts. *Fieldiana (Zoology)* 37:1-29.
- Liu, C. K. 1944. Rudimentary hermaphroditism in the synbranchoid eel, *Monopterus javanensis*. *Sinensia* 15:1-8.
- Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A.

- Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10:689-710.
- Maddison, D. R., and W. P. Maddison. 2000. MacClade: analysis of phylogeny and character evolution. Version 4.0. Sinauer Associates, Sunderland, Massachusetts.
- Matsumoto, S., and K. Iwata. 1997. Paternal egg guarding and mouth-brooding in the bubble nest in the swamp-eel, *Monopterus albus*. *Japanese Journal of Ichthyology* 44:35-41 (in Japanese with English abstract).
- Matsumoto, S., N. Nagai, S. Imanishi, H. Hasuike, and K. Masonori. 1998. An expanding distribution of a swamp-eel *Monopterus albus* (Teleostei: Synbranchidae) in Nara Prefecture. *Japanese Journal of Ecology* 48:107-116 (in Japanese with English abstract).
- Merrick, J. R., and G. E. Schmida. 1984. Australian freshwater fishes: biology and management. Griffin Press, Netley, Australia.
- Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* 3:401-411.
- Moyle, P. B., and T. Light. 1996. Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation* 78:149-161.
- Myers, J. H., D. Simberloff, A. M. Kuris, and J. R. Carey. 2000. Eradication revisited: dealing with exotic species. *Trends in Ecology & Evolution* 15:316-320.
- Nichols, J. T. 1943. The fresh-water fishes of China. American Museum of Natural History, New York.
- Nissen, S. J., R. A. Masters, D. J. Lee, and M. L. Rowe. 1995. DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. *Weed Science* 43:504-513.
- Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction. Pages 205-247 in D. M. Hillis, C. Moritz, and B. K. Mable, editors. *Molecular systematics*. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.
- Rosen, D. E., and P. H. Greenwood. 1976. A fourth Neotropical species of synbranchid eel and the phylogeny and systematics of synbranchiform fishes. Bulletin 157. American Museum of Natural History, New York.
- Scott, J. K. 2001. Europe gears-up to fight invasive species. *Trends in Ecology & Evolution* 16:171-172.
- Shafland, P. L., and J. M. Pestrak. 1982. Lower lethal temperatures for fourteen non-native fishes in Florida. *Environmental Biology of Fishes* 7:149-156.
- Simons, A. M., and R. L. Mayden. 1999. Phylogenetic relationships of North American cyprinids and assessment of homology of the open posterior myodome. *Copeia* 1:13-21.
- Starnes, W. C., R. T. Bryant, and G. C. Greer. 1998. Perilous experiment: the Asian rice eel in Georgia. *Georgia Wildlife: Urban Wildlife (Natural Georgia Series)* 7:60-70.
- Sullivan, J. P., S. Lavoué, and C. C. Hopkins. 2000. Molecular systematics of the African electric fishes (Mormyroidea: Teleostei) and a model for the evolution of their electric organs. *The Journal of Experimental Biology* 203:665-683.
- Swofford, D. L. 2000. PAUP*. Phylogenetic analysis using parsimony (and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673-4680.
- Trexler, J. T., W. F. Loftus, F. Jordan, J. Lorenz, J. H. Chick, and R. M. Kobza. 2001. Empirical assessment of fish introductions in a subtropical wetland: an evaluation of contrasting views. *Biological Invasions* 2:265-277.
- Tringali, M. D., T. M. Bert, S. Seyoum, E. Bermingham, and D. Bartolacci. 1999. Molecular phylogenetics and ecological diversification of the transisthmian fish genus *Centropomus* (Perciformes: Centropomidae). *Molecular Phylogenetics and Evolution* 13:193-207.
- Tyler, J. C., and I. C. Feller. 1996. Caribbean marine occurrence in mangroves of a typically fresh-water synbranchiform fish. *Gulf of Mexico Science* 14:26-30.
- Wang, X., M. Wu, Y. Ma, W. Liu, and W. Shi. 1994. Analysis of karyotype of *Monopterus albus* in Hami. *Journal of Fisheries of China* 18:157-159 (in Chinese).
- Wilson, A. B., K.-A. Naish, and E. G. Boulding. 1999. Multiple dispersal strategies of the invasive quagga mussel (*Dreissena bugensis*) as revealed by microsatellite analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2248-2261.



**IX. Interaction of hydrology and nutrients in determining trophic positions in a
detrital food web.**

Interaction of hydrology and nutrients in determining trophic positions in a detrital
food web

Alissa J. Williams and Joel C. Trexler*

Department of Biological Sciences

Florida International University, Miami, FL 33199

*Author for correspondence:

Joel C. Trexler

Florida International University

11200 SW8th Street

Miami, FL 33199

Phone: 305-348-1966

Fax: 305-348-1986

E-mail: trexlerj@fiu.edu

ABSTRACT

Theory predicts that trophic position is negatively correlated with disturbance and positively correlated with productivity, other things being equal. However, there is continuing debate about the importance of these factors in real food webs, partly because factors such as ecosystem size, multichannel energy flow, and biotic interactions also shape food webs. Furthermore, there has been little consideration of the effects of these abiotic gradients on detrital food webs. We estimated trophic position and carbon source for three secondary consumers (Florida gar, eastern mosquitofish, and riverine grass shrimp) from 20 sites representing gradients of productivity and hydrological disturbance in the Florida Everglades, U.S.A. In the Everglades, hydroperiod yields gradients of disturbance because drying events cause high mortality of fishes and macroinvertebrates. We characterized gross primary productivity at each site using light/dark bottle incubation and stem density of emergent vascular plants. We also documented nutrient status by analysis of total phosphorus (TP) in floc and periphyton, and the density of small fishes. Hydroperiod disturbance was characterized as the time since a site was last dried and the average number of days per year the sites were inundated for the previous 10 years. Food-web attributes were estimated in both the wet and dry seasons by analysis of $\delta^{15}\text{N}$ (trophic position) and $\delta^{13}\text{C}$ (food-web carbon source) from 702 samples of primary and secondary consumers. An index of carbon source was derived from a two-member mixing model with Seminole ramshorn snails used as a basal algal consumer and scuds (amphipods) as a basal detritivore. Detritus appeared to be the primary source of carbon at all but one study site, and though the relative contribution of detrital carbon was variable, there was no evidence that its contribution varied as a function of abiotic

factors or between seasons. Gar consistently displayed the highest estimated trophic position of the consumers studied (average trophic position = 4.5), with mosquitofish feeding at a slightly lower level (trophic position = 4.1), and grass shrimp fed at the lowest level (trophic position = 3.5). Trophic position was not correlated with any nutrient or productivity parameter, but did increase for grass shrimp and mosquitofish as the time following droughts increased. Trophic position of Florida gar was positively correlated with emergent plant stem density. Our study supports a multivariate approach to food-web analysis, both in the environmental drivers and in tracing the routes of energy.

Keywords: detritus, disturbance, food-chain length, food web, nutrients, stable isotopes, trophic position, wetlands

INTRODUCTION

Abiotic environmental factors affect food-web function both through the source of energy flow and by determining the trophic position of community members. The relative role of detrital and algal contributions to energy flow in aquatic food webs is an important, but unresolved, question (Moore et al. 2004; Sobczak et al. 2005). While photoautotrophic energy channels dominate in the literature, detritus-based “brown” food webs are well documented, notably in wetlands (Brinson et al. 1981; Mitsch and Gosselink 1993). The Florida Everglades is a large wetland characterized by unusually dense mats of periphyton that have been implicated as the primary energy source for aquatic food-webs (Browder 1996; Radar 1994), yielding some debate about the relative contribution of photoautotrophic and detrital energy channels. Gradients of phosphorus and hydroperiod are linked to periphyton and microbial production in the Everglades and other wetlands, affecting the rate of detrital production (Brinson et al. 1981; McCormick et al. 2002). Though dual detrital and photoautotrophic contributions to energy flow are widespread (Moore and Hunt 1988) and detrital contributions may equal or exceed photoautotrophic ones (Hairston and Hairston 1993; Wetzel 2001), details of the relative contribution of detritus in supporting higher trophic levels are poorly understood (Moore et al. 2004; Sobczak et al. 2005).

Food-chain length is determined both by addition and subtraction of top consumer species in a food web and changing diets of consumers (Post 2002a). Food-chain length may be affected by myriad abiotic and biotic factors in the environment, including resource availability, environmental stability, ecosystem size, colonization history, and predator-prey body size ratios (Pimm 1982; Briand and Cohen 1987; Post 2002a; Jennings and Warr 2003). Two expectations

derived from food-chain theory are that food-chain length will shorten along gradients of increasing disturbance and lengthen along gradients of increasing productivity (Pimm 1982; Briand and Cohen 1987). However, laboratory and field estimates often provide contradictory evidence to these predictions (Oksanen et al. 1981; Pimm 1982; Post et al. 2000), possibly because of a failure to account for detrital energy channels (Moore et al. 2004). Additionally, ecosystem size and connectivity effect spatial sorting of regional species pools, possibly affecting field results of studies of resource or disturbance effects on food webs (Post et al. 2000). Thus, a complex picture is emerging that emphasizes a hierarchy of historical, abiotic, and biotic factors interacting to constrain food-web characteristics (Post 2002a; Moore et al. 2004).

Significant empirical challenges confront description and analysis of food webs. Top consumers are commonly among the most mobile members of communities and their entry or departure from an area, and impact on local food-web dynamics, may be ephemeral and difficult to quantify. Thus, food-chain length *per se* may be difficult to define, particularly in spatially connected (open) systems. The trophic position of secondary consumers is determined directly by their own diet and indirectly by the diets of their food (Morin 1999). Recent work indicates that trophic omnivory (feeding on two or more trophic levels) is ubiquitous and has clarified empirical challenges for identification of the bases of trophic position; requisite averaging of consumption across trophic linkages, weighted by the relative assimilation of diet components, renders trophic position a less-precise measure than envisioned with simple food-chain models. Stable isotope technology provides estimates of trophic position, as well as insight into the origins of trophic variation within an ecosystem (Peterson and Fry 1987; Vanden Zander et al 1999; Post 2002b).

To accurately measure trophic relationships using isotopes, values must be compared relative to a baseline measurement that indicates spatial and temporal flux in isotope values (Post 2002b). Unfortunately, primary producers and bacteria frequently introduce complex temporal and spatial variability to the interpretation of isotopic signatures within a food web. In aquatic systems, algae, microbes, and detritus are typically found in mixed assemblages that cannot be easily separated into components that capture energy at the base of the food web and components recycling deceased consumers. Thus, periphyton mats, for example, function at multiple consumer levels. Furthermore, stoichiometric differences between algae and bacteria and consumers affect fractionation of carbon and nitrogen, complicating interpretation of isotopic data (Sterner and Elser 2002; Post 2002b). For these reasons, primary consumers have proven a logical choice as baseline proxies for primary producers in food-web analyses (Post et al. 2000; Post 2002b).

We report an analysis of trophic position and carbon source for selected components of food webs at 20 sites in the Florida Everglades, representing gradients of nutrient availability and hydrological disturbance. To characterize local food webs, we selected two consumers that are ubiquitous and representative of the top trophic level of relatively stationary animals. Also at each site, we sampled tissue for isotope analysis of a highly mobile predatory species that is among the top consumers of the aquatic Everglades food web. Our goal was to use stable isotopes to assess the impact of nutrient status, disturbance, and their interaction, as driving factors shaping this wetland food web. We hypothesized a synergistic effect of nutrient status and disturbance on this food web: trophic positions are positively correlated with nutrients (TP) and hydroperiod, and highest (indicative of more complex food webs) when these factors are both at maximum. We also

examined the relative contribution of algal and detrital sources to the food web to determine if these varied along nutrient and disturbance gradients.

MATERIALS AND METHODS

Study area and study species

We collected samples in September 2002 (rainy season) and February 2003 (dry season) to maximize extremes of water level at our study sites. Sixty-five percent of the annual rainfall in South Florida falls between the months of June and November (Davis 1994; Ali and Abtew 1999). We examined food-web characteristics at 20 sites within the freshwater Everglades ecosystem: 6 sites located within Shark River Slough (SRS) and 3 sites within Taylor Slough (TS) in Everglades National Park (ENP); and 11 sites in Water Conservation Areas 3A and 3B (WCA) (see Trexler et al. 2002 for map). Sites were selected to encompass a gradient of hydrology and productivity typical of the landscape in areas not receiving anthropogenic nutrient enrichment. With only two exceptions, sites were located at least 5 km apart. Fish community structure and biomass is maximally variable at this spatial scale (Trexler et al. 2002; Ruetz et al. 2004). Population genetic structure is also maximized at this scale and dispersal of small fishes among these sites appears to be limited (Trexler et al. 2002; McElroy et al. 2003).

Samples of representative animal groups from primary, secondary, and tertiary trophic levels were collected at each site. We selected Seminole ramshorn snails (*Planorbella duryi*) and scuds (*Hyallela azteca*) as the primary consumer species because they primarily feed on algae and detritus, respectively (Brown 1991; Covich and Thorpe 1991). These primary or basal consumers

were chosen to represent the two dominant energetic pathways found in Everglades food webs (Gunderson and Loftus 1993; Browder et al. 1994; Loftus 2000). These choices are supported by $\delta^{13}\text{C}$ data; green algae and snails are relatively depleted in ^{13}C compared to amphipods, floc, bulk periphyton, emergent vascular plants, and cyanobacteria (Table 1). Cyanobacteria are generally considered to be of low palatability because of both chemical and physical defenses from grazers (Steiman 1996; Geddes and Trexler 2002), though decomposing bacteria growing on dead cyanobacteria or sloughed polysaccharides may be consumed. Similarly, vascular plant detritus supports bacterial communities that are consumed. Use of basal consumers is supported by stoichiometric data indicating a marked departure in C:N for floc and periphyton samples from potential basal consumers and selected secondary consumers (Table 2). Riverine grass shrimp (*Palaemonetes paludosus*) and eastern mosquitofish (*Gambusia holbrooki*) were selected as secondary consumers, and top consumers of the aquatic food web were represented by Florida gar (*Lepisosteus platyrhinchus*), a piscivorous fish found throughout the freshwater marsh. Mosquitofish and grass shrimp feed differently, possibly in separate (but overlapping) compartments of the food web; grass shrimp are benthic consumers, while mosquitofish feed throughout the water column, though often near the water surface (Geddes and Trexler 2003). Florida gar were not present at all short-hydroperiod sites, suggesting variation in maximal food chain length by changes in local species composition as a function of time since dry (Trexler et al. 2005) not addressed in this study. All animals were collected using standard throw trapping, sweep netting, and electrofishing techniques (Trexler et al. 2002). All samples were frozen upon collection.

Gross primary productivity, nutrient status, and hydrological data were collected concurrently with food-web samples. At each site, we collected three samples of flocculent material (floc) using a 3-cm core (Childers et al. 2001), as well as three samples of surface periphyton mat, and combined the three of each into separate composites for isotope and total-phosphorus analysis (TP). TP has been shown to be a direct indicator of primary productivity in the freshwater Everglades (McCormick et al. 2001). Gross primary production (GPP) of algal mats was also measured at all sites each season using light/dark bottle incubation (Franson 1998). Light, water depth, and temperature were measured as covariates during the incubation experiments. Total vascular-plant stem density was also measured as an additional index of local production by these plants. Hydroperiod data were estimated two ways: (1) days since dry (DSD), the number of days since a site last dried, and (2) hydroperiod, the average number of days a site was wet per year for the preceding 10 years.

Stable Isotope Analysis

We analyzed muscle tissue for fish and grass shrimp, whole body without shell for snails, and whole body for scuds (Pinnegar and Polunin 1999; McCutchan et al. 2003). It was necessary to pool baseline consumers to yield adequate mass for mass-spectrometric analysis (0.4 mg needed per sample = 20 amphipods or 3 snails); all other animals were analyzed individually. Consistent with Sotiropoulos et al. (2004), our analyses indicated no effect of lipid extraction on soma isotope values; so all samples were run without extraction. Tissues were dried at 55° C for at least 24 hours prior to processing. Periphyton and floc samples were decarbonated by standard fuming

techniques (Chang et al. 1991). All samples were analyzed for $\delta^{15}\text{N}$ and decarbonated samples were analyzed for $\delta^{13}\text{C}$. A total of 702 samples were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a Finnigan Isotope ratio mass spectrometer (IRMS) at the SERC Stable Isotope Laboratory at Florida International University, Miami, FL. All isotopic values are reported using the standard delta (δ) notation. Delta values were calculated using the following standard equation for comparison to reference materials (DeNiro and Epstein 1978; Minagawa and Wada 1984). The isotopic standards used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were Pee Dee Belemnite (PDB) and AIR, respectively. The average isotopic lab error of replicate standards was $\delta^{15}\text{N} \leq 0.2\text{‰}$ and 0.1‰ for $\delta^{13}\text{C}$.

We used simple two-end-member-mixing models (reviewed in Post 2002b) to estimate indices of detritivory (I_D) and trophic position for each study site at both sampling seasons. $\delta^{13}\text{C}$ enriches minimally ($\sim 0\text{--}1\text{‰}$) as it moves through food webs (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001; McCutchan Jr. et al. 2003), providing an index of the relative contribution of carbon from our algae and detritus proxies (snails and amphipods). An index of detritory ($I_{D2}^{\text{nd}}_{\text{cons}}$) was calculated using the $\delta^{13}\text{C}$ values for the secondary and basal consumers ($C_2^{\text{nd}}_{\text{cons}}$, C_{snail} , and C_{scud}) and all predators sampled Post (2002b):

$$I_{D2}^{\text{nd}}_{\text{cons}} = (C_2^{\text{nd}}_{\text{cons}} - C_{\text{snail}}) / (C_{\text{scud}} - C_{\text{snail}}).$$

This index took values from 0 to 1, with 0 indicating a detritus-based food web and 1 a primarily algal-based one. If the $\delta^{13}\text{C}$ value for a secondary consumer exceeded a primary consumer, the

difference was treated as sampling error and set to 0 or 1, indicating 100% algal or detrital energy flow. Trophic position ($\text{TRPO}_{2^{\text{nd}} \text{ cons}}$) was calculated as the difference in $\delta^{15}\text{N}$ between the basal and higher-level consumers, weighted by the relative contribution of our energy-flow proxies (Post 2002b):

$$\text{TRPO}_{2^{\text{nd}} \text{ cons}} = 2 + (\text{N}_{2^{\text{nd}} \text{ cons}} - ((\text{N}_{\text{scud}} * \text{I}_{\text{D}2^{\text{nd}} \text{ cons}}) + (\text{N}_{\text{scud}} * (1 - \text{I}_{\text{D}2^{\text{nd}} \text{ cons}})))) / 3.4.$$

Two was added to the calculated TRPO value reflect the assumed trophic position of our baseline consumers and the ratio of $\delta^{15}\text{N}$ values was divided by 3.4 to reflect standard estimates of nitrogen fractionation in a trophic step. Though an abstraction, use of these constants has no impact on relationships calculated and facilitates discussion in the food-web context.

We used a backwards stepping multiple regression and analysis of covariance (for effects of season) to select models best describing the relationships between I_{D} and TRPO and our measures of hydroperiod, nutrient status, plant and fish density. This approach starts with a full model, including all possible independent variables, and then uses standard criteria to systematically eliminate independent parameters failing to contribute to explaining the dependent variable, in order to settle on a parsimonious final model (Younger 1979). The angular transformation (Zar 1999) was used on I_{D} to meet the assumptions of regression. Floc was absent at several of our study sites in the dry season and limited these analyses to wet season for some dependent variable. Throughout, we report the coefficient of variation (CV) for comparison of variability among groups.

RESULTS

Environmental gradients

Our study sites encompassed a range of hydrological and nutrient conditions (Table 1A). Hydroperiod (HydPd) ranged from 306 to 365 days (average for a 10-year period from 1992 to 2002) and DSD ranged from 27 to 3,304 at the time of sampling. Soil TP ranged from 93 to 999 ug/g; 450 ug/g is considered a maximum for natural variation in this system (Grunwald et al. 2004). Five of our 20 study sites exceeded the 450 ug/g benchmark for soil TP value, though only 2 exceeded 500 ug/g soil TP. Average density of emergent plant stems ranged from 9 to 497 stems/m². Gross primary production and stem density were more variable in the dry than the wet season, while periphyton TP was more variable in the wet season (Table 1A). Our estimates of fish density were similar between the wet and dry season, though the densities tended to be greater in the dry season (range 11-60 fish/m² in dry, 5 – 42 fish/m² in the wet).

Our independent variables were not correlated, with a few exceptions (Table 1B). Hydroperiod and DSD were strongly positively correlated, as were emergent stem density and periphyton TP; fish density and stem density were also correlated, though not strongly (Table 1B). Gross primary production varied independently from periphyton TP and emergent stem density, as well as from fish density. Overall, four groups of variables were identified: hydrology (DSD and HydPd); nutrient status and habitat structure (periphyton TP and emergent stem density); periphyton mat composition and/or function (GPP); competition and/or predation (fish density). We confirmed these patterns with a Principal Components Analysis (not reported).

Isotope Signatures of Periphyton and Floc

We observed marked variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of periphyton and floc samples (Table 2). Periphyton and floc isotope values spanned a very wide range, surpassing expected values based on data from primary consumers (Periphyton wet: average $\delta^{15}\text{N} = 2.637 \pm 0.462$, average $\delta^{13}\text{C} = -28.297 \pm 0.968$; Periphyton dry: average $\delta^{15}\text{N} = 3.481 \pm 0.839$, average $\delta^{13}\text{C} = -27.459 \pm 0.1507$; Floc wet: average $\delta^{15}\text{N} = 2.412 \pm 0.454$, average $\delta^{13}\text{C} = -29.024 \pm 0.901$). Periphyton and floc displayed differing patterns of variance in $\delta^{13}\text{C}$ (homogeneity of variance test: $F_{2, 50} = 3.486$, $P = 0.038$) and floc was much more variable in both isotopes than any other sample type (CV, Table 2). For some sites, the $\delta^{15}\text{N}$ values of periphyton were equal to or greater than secondary consumers, most likely from the high frequency of cyanobacteria into these samples (Table 2). $\delta^{13}\text{C}$ in Seminole ramshorn snails was only slightly less depleted than estimates of green algae, consistent with a selective algal grazing diet (other Everglades snail species display $\delta^{13}\text{C}$ intermediate between green algae and bulk periphyton and floc, suggesting a greater role for detritus in their diets, personal observation). In contrast, $\delta^{13}\text{C}$ of scuds was only a little less depleted than periphyton or floc, consistent with a detrital or cyanobacterial diet (Table 2).

As expected, floc and periphyton samples had much lower nitrogen content than animal tissues as indicated by C:N (Table 3). These samples were also much more variable in stoichiometry than the animals tissues (CV > 25% for floc and periphyton versus <20% for all animal tissues), probably indicative of the heterogeneous nature of these substrates. Scud samples were relatively nitrogen poor compared to the other animals (Table 3). This probably resulted

from sample preparation, which included carapaces in these small animals, but not which excluded similar materials from the larger animals.

Index of detritivory

The majority of our $\delta^{13}\text{C}$ data indicated our three consumers were more similar to scuds in their energy source than to ramshorn snails. Our I_D ranged from 0.69 to 0.83, indicating mixed but predominately detrital carbon flow in this food web (70 to 85% detrital carbon source; Table 4). We observed no seasonal or regional variation in I_D , though values did vary among sites within the seasons (CV ranged from 32 to 48%, Table 4). In the dry season, 9 out of 19 yielded $I_D > 0.9$ for mosquitofish and 7 out 19 sites for grass shrimp. In the wet season, $I_D > 0.9$ for 12 out 20 sites for mosquitofish, but only 5 out of 20 for grass shrimp. While this suggests a broader diet for grass shrimp in the wet season than for mosquitofish, I_D generally exceeded 0.7 for both species. Only a constant remained in our final model following backwards-stepwise regression with region, season, and six environmental parameters (DSD, HydPd, GPP, PeriphytonTP, stem density, and fish density) for transformed values of I_D of grass shrimp, mosquitofish, and gar.

Trophic Position

Mosquitofish and grass shrimp displayed increasing trophic level with increasing time following a drought (Table 5A, Fig. 1A, B). The effect was strongest for mosquitofish, whose trophic position ranged from 4 to 4.5 in the wet season in areas ranging from recently dried to those remaining inundated for over 8 years. In contrast, trophic position of gar increased with

emergent stem density in the area where they were captured in the wet season (Table 5A, Fig. 1C). Estimated trophic position for gar varied from 4.3 to 4.9 in the wet season, comparing sites with stem density near 0 to sites with over 350 stems m⁻².

Only mosquitofish displayed seasonal variation in trophic position, and this varied among regions (Table 5A). Their estimated trophic position was greatest in Taylor Slough compared to the other two regions in both seasons (typically by 0.2 – 0.3 trophic positions, Table 5B). Trophic position in Taylor Slough was greater in the dry season than in the wet.

DISCUSSION

Though many have argued that food-chain length should increase as availability of limiting nutrients increase, field studies have often failed to find such patterns (Power et al. 1996; Post 2002a). Our data also fail to support this concept. Neither our resident taxa (mosquitofish and grass shrimp) nor our mobile predator (gar) displayed any relationship between trophic position and measures of nutrient status or indicators of productivity. In contrast, we found evidence that time since a drought was positively correlated with trophic position of two secondary consumers in this ecosystem. Other work has shown that large predatory fishes are excluded by drought and require months to return from refuges, and even longer to gain robust population sizes (Chick et al. 2004; Trexler et al. 2005). Thus, droughts act as an environmental filter, restricting food-chain length by elimination of large predatory fish species. Our findings suggest that diets throughout the food web are also shifting in ways that complement changing species composition of top consumers. However, we found no evidence that the relative contribution of detritus contributed

to these changes. It is unclear if the shifting trophic position of intermediate consumers resulted from adding species of consumers feeding lower in the web, or from diet shifts to consume more high-trophic-level prey, or both. Also, our data provided no evidence of humped relationships of trophic position and time since drought as described by Power et al. (1996), even though our study sites bracketed very short and long time periods.

Our study provides empirical support for a dominant role of detritus for carbon flow in the Everglades. Past work by Browder et al. (1994) suggested an important algal route of carbon and energy in this ecosystem. While our data do not undermine her conclusion that periphyton mats are critical elements to energy flow in the ecosystem and may be considered the ‘base’ of the food web, their contribution appears to come after death or from materials sloughed off as the mat constituents grow. Recent work has supported the hypothesis that much of Everglades periphyton mats are not consumed directly by grazers (Geddes and Trexler 2003).

Choice of basal consumers for an isotopic analysis such as this is critical to its success. It seems unlikely that any taxa in this ecosystem feed solely on green algae embedded in periphyton mats. Indeed, $\delta^{13}\text{C}$ of Seminole ramshorn snails is less depleted than our estimate of green algae from Shark River Slough. More data are needed on isotopic values of the mat components, though the complexity of the mats has resisted routine separation. However, periphyton mats are home to a diverse infauna dominated by midge larvae and scuds (McCormick et al. 2004; Liston and Trexler 2005) that probably serve as major food sources for higher consumers.

Trophic position of Florida gar displayed a different pattern than our intermediate level consumers. Florida gar in the Everglades have a narrow diet breadth (Loftus 2000) feeding on

other fish, amphibians, grass shrimp, and crayfish. Gar can move long distances (Snedden et al. 1999). Tracking studies have shown Florida gar in the Everglades to travel 5-20 km in one day when water levels drop locally in the dry season (Wolski and Trexler, unpublished data). However, in the wet season when our isotopic data were gathered, most radiotagged gar moved relatively little, displaying nighttime foraging movements from depressions and ponds and returning to the same area each day. The density of small fish (intermediate level consumers) was positively correlated with emergent stem density in this study and such correlations are commonplace in the literature (Savino and Stein 1982; Rozas and Odum) . However, interpreting such a correlation literally may be unwise, because many environmental gradients covary with stem density, notably nutrient levels, and may be more directly tied to the observed pattern in trophic position.

Analysis of stable isotopes to characterize food webs has become commonplace, but these data do not address functional food-web relationships. Changing biotic interactions can alter food webs and shape community structure in ways that are not readily elucidated by a descriptive tool such as this. Additionally, key questions remain about the nature of ‘detrital’ carbon flow in this ecosystem. For example, it is unclear if a microbial loop (Fenchel 1988) provides a major route of energy flow in the Everglades, though this seems to be the likely linkage between the abundant but defended and unpalatable cyanobacterial primary producers and higher consumers. Such a link could explain the lack of a trophic position-productivity relationship in our data (Moore and Hunt 1988; Moore et al. 2004). Much work remains tracing routes of energy flow and biotic interactions to fully understand the functioning of this detrital food web.

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REFERENCES

- Ali and Abtew (1999) Regional Rainfall Frequency Analysis for Central and South Florida. In. South Florida Water Management District, West Palm Beach, FL
- Briand F, Cohen JE (1987) Environmental correlates of food chain length. *Science* 238:956-959
- Brinson, M. M., A. E. Lugo, and S. Brown (1981) Primary productivity, decomposition and consumer activity in freshwater wetlands. *Annual Review of Ecology and Systematics* 12:123-161
- Browder JA, Gleason PJ, Swift DR (1994) Periphyton in the Everglades: Spatial variation, Environmental correlates, and Ecological implications. In: Ogden JC (ed) *Everglades: The Ecosystem and its Restoration*. St. Lucie Press, Delray Beach, FL, pp 379-418

- Brown KM (1991) Mollusca and Gastropoda. In: Covich AP (ed) Ecology and classification of North American freshwater invertebrates. Academic Press Inc., San Diego, CA, pp 285-309
- Chang F-Y, Kao S-J, Liu K-K (1991) Analysis of organic and carbonate carbon in sediments. *Acta Oceanogr. Taiwanica* 27:140-150
- Chick, J. H., C. R. Ruetz III, and J. C. Trexler (2004) Spatial scale and abundance patterns of large fish communities in freshwater marshes of the Florida Everglades. *Wetlands* 24:652-664
- Childers DL et al. (2001) Quantifying the effects of low-level phosphorus enrichment on unimpacted Everglades wetlands with in situ flumes and phosphorus dosing. In: Porter KG (ed) The Everglades, Florida Bay, and Coral Reefs of the Florida Key: An ecosystem sourcebook. CRC Press, Boca Raton, FL, p 127 – 152
- Covich AP, Thorpe JH (1991) Crustacea. In: Covich AP (ed) Ecology and classification of North American freshwater invertebrates. Academic Press, Inc., San Diego, CA, pp 665-683
- Davis SM (1994) Phosphorus inputs and vegetation sensitivity in the Everglades. In: Odgen J (ed) Everglades: the ecosystem and its restoration. St. Lucie Press, Delray Beach, FL, pp 357-375
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506
- Fenchel, T. 1988. Marine plankton food chains. *Annual Review of Ecology and Systematics* 19:19-38

- Franson MH (1998) Biological Examination. In: Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC
- Gaye-Siessegger J, Focken U, Muetzel S, Abel H, Becker K (2004) Feeding level and individual metabolic rate affect $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in carp: implications for food web studies. *Oecologia* 138:175-183.
- Geddes P, Trexler JC (2003) Uncoupling of omnivore-mediated positive and negative effects on periphyton mats. *Oecologia* 136:585-595
- Grunwald S., K. R. Reddy, S. Newman, and W. F. DeBusk. 2004. Spatial variability, distribution and uncertainty assessment of soil phosphorus in a south Florida wetland. *Environmetrics* 15:811-825.
- Gunderson LH, Loftus WF (1993) The Everglades. In: Echternacht AC (ed) Biodiversity of the Southeastern United States. John Wiley & Sons, Inc., New York, pp 199-255
- Hairston, N. G., Jr., and N. G. Hairston, Sr. (1993) Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. *American Naturalist* 142:379-411
- Jennings. S., and K. J. Warr. 2003. Smaller predator-prey body size ratios in longer food chains. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270:1413-1417
- Liston, S. E., and J. C. Trexler. 2005. Spatial and temporal scaling of macroinvertebrate communities inhabiting floating periphyton mats in the Florida Everglades. *Journal of the North American Benthological Society*, in press.
- Loftus WF (2000) Accumulation and fate of mercury in an Everglades aquatic food web. In: Department of Biological Sciences. Florida International University, Miami, FL, p 295

- McCormick PV, Newman S, Miao S, Gawlik EE, Marley D, Reddy KR, and Fontaine TD . (2001) Effects of anthropogenic phosphorus inputs on the Everglades. In: Porter KG (ed) The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press, Boca Raton, FL, p 83 – 126
- McCutchan Jr. JH, Lewis Jr. WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *OIKOS* 102:378-390
- McElroy TC, Kandl KL, Garcia J, Trexler JC (2003) Extinction-colonization dynamics structure genetic variation of spotted sunfish (*Lepomis punctatus*) in the Florida Everglades. *Molecular Ecology* 12:355-368
- Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48:1135-1140
- Mitsch WJ, Gosselink JG. 1993. Wetlands, second edition. Van Nostrand Reinhold, NY
- Moore, J. C., E. L Berlow, D. C. Coleman, P. C. deRuiter, Q. Dong, A. Hastings, N C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. *Ecology Letters* 7:584-600.
- Moore, J. C., and H. W. Hunt. 1988. Resource compartmentation and the stability of real ecosystems. *Nature* 333:261-263.
- Morin PJ (1999) Community Ecology. Blackwell Science, Inc., Malden, MA

- Oksanen, L., S. D. Fretwell, J. A. P. Niemela. (1981) Exploitation ecosystems in gradients of primary productivity. *American Naturalist* 118:240-261
- Peterson, B. J., and B. Fry (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293-320
- Pimm, S. L. (1982) *Food Webs*. Chapman and Hall, NY
- Pinnegar JK, Polunin NVC (1999) Differential fractionation of d13C and d15N among fish tissues: implications for the study of trophic interactions. *Functional Ecology* 13:225-231
- Post DM (2002a) The long and short of food-chain length. *Trends in Ecology and Evolution* 17:269-277
- Post DM (2002b) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703-718
- Post DM, Pace ML, Hairston NGJ (2000) Ecosystem size determines food-chain length in lakes. *Nature* 405:1047-1049
- Power, M. E., M. S. Parker, and J. T. Wootton. 1996. Disturbance and food chain length in rivers, pp 286-297. In G. A. Polis and K. O. Winemiller. *Food Webs. Integration of Patterns and Dynamics*. Chapman & Hall, NY.
- Radar, R. B., and C. J. Richardson (1994) Response of macroinvertebrates and small fish to nutrient enrichment in the northern Everglades. *Wetlands* 14:134-146
- Rozas, L. P., and W. E. Odum. 1998. Occupation of submerged aquatic vegetation by fishes: testing the roles of food and refuge. *Oecologia* 77:101-106

- Ruetz, C. R., III, J. C. Trexler, F. Jordan, W. F. Loftus, and S. A. Perry (2005) Population dynamics of wetland fishes: Spatiotemporal patterns shaped by hydrological disturbance? *Journal of Animal Ecology* 74:322-332
- Savino, J. F., and R. A. Stein. 1982. Predator-prey interactions between largemouth bass and bluegills as influenced by simulated, submerged vegetation. *Transactions of the American Fisheries Society* 111:255-266.
- Snedden GA, Kelso WE, Rutherford DA (1999) Diel and seasonal patterns of spotted gar movement and habitat use in the lower Atchafalaya River Basin, Louisiana. *Transactions of the American Fisheries Society* 128:144-154
- Sobczak, W. V., J. E. Cloern, A. D. Jassby, B. E. Cole, T. S. Schraga, and A. Arnsberg. 2005. Detritus fuels ecosystem metabolism but not metazoan food webs in San Francisco estuary's freshwater Delta. *Estuaries* 28:124-137
- Sotiropoulos MA, Tonn WM, Wassenaar LI (2004) Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecology of Freshwater Fish* 13: 155-160
- Steiman, A. D. (1996) Effects of grazers on freshwater benthic algae, pp 341-373. In Stevenson, R. J., M. L. Bothwell, and R. L. Lowe, eds. *Algal Ecology*. Academic Press, San Diego.
- Sterner, R. W., and J. J. Elser (2002) *Ecological Stoichiometry*. Princeton University Press, Princeton.
- Trexler, J. C., W. F. Loftus, and S. Perry (2005) Frequency of disturbance limits Everglades fishes demonstrated with a twenty-five year intervention study. *Oecologia*, in press

- Trexler, J. C., W. F. Loftus, C. F. Jordan, J. Chick, K. L. Kandl, T. C. McElroy, and O. L. Bass
(2002) Ecological scale and its implications for freshwater fishes in the Florida Everglades.
Pp. 153 – 181, in J. W. Porter and K. G. Porter (eds.) *The Everglades, Florida Bay, and
Coral Reefs of the Florida Keys: An Ecosystem Sourcebook*. CRC, Boca Raton.
- Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation:
implications for aquatic food web studies. *Limnology and Oceanography* 46:2061-2066
- Vander Zanden MJ, Shuter BJ, Lester N, Rasmussen JB (1999) Patterns of food chain length in
lakes: a stable isotope study. *The American Naturalist* 154:406-416
- Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a
freshwater habitat gradient. *Annual Review of Ecology & Systematics* 27:337-363
- Wetzel, R. G. (2001) *Limnology*, 3rd ed. Academic Press, San Diego
- Younger, M. S. (1979) *A Handbook for Linear Regression*. Duxbury Press, North Scituate, Mass.
- Zar, J. H. 1999. *Biostatistical Analysis*, fourth edition. Prentice Hall, Upper Saddle River, NJ

Table 1. A. Description of study sites. Abbreviations are explained in the text. N is 19 for dry season and 20 for wet season. StdDev is the standard deviation of the mean based on inter-site variance and CV is the coefficient of variation. B. Matrix of pairwise comparisons of environmental parameters. * indicates $P < 0.05$ with Dunn-Sidak correction, $N = 39$ except for floc TP[@], which has $N = 19$.

A. Summary parameters by season

Parameters	Season					
	Dry			Wet		
	Mean	StdDev	CV	Mean	StdDev	CV
DSD (days)	1518.79	842.20	55.5	1280.01	1052.35	82.2
HydPd (days)	349.86	14.39	4.1	347.74	17.53	5.0
GPP (mg/C/year)	715.77	796.54	111.3	1219.01	730.61	59.9
Floc TP (ug/g)				335.29	207.84	62.0
Periphyton TP (ug/g)	116.92	60.18	51.5	143.21	145.71	101.7
Stem Density (#/m ²)	158.09	158.79	100.4	135.81	88.58	65.2
Fish Density (#/m ²)	23.12	12.82	55.4	21.56	12.00	55.6

B. Correlation Matrix

	DSD	HydPd	GPP	FlocTP [@]	PeriTP	StDen
HydPd	0.768*					
GPP	-0.173	-0.141				
FlocTP [@]	-0.043	-0.088	-0.401			
PeriTP	-0.122	-0.2	0.056	0.303		
StDen	-0.173	-0.291	0.396	0.099	0.554*	
FhDen	-0.024	-0.011	0.085	0.334	0.353	0.552*

Table 2. Mean $\delta^{13}\text{N}$ and $\delta^{15}\text{C}$ isotopic values for each material analyzed for this study. Average values for each study site are reported. N is 20 in all cases and is based on the mean of three replicate samples for each sample type from each site. StdDev is the standard deviation of the mean based on inter-site variance and CV is coefficient of variation. * Data from Loftus (2000).

	$\delta^{13}\text{N}$	stddev	CV	$\delta^{15}\text{C}$	stddev	CV
Floc	2.4	1.065	44.2	-29.0	9.097	31.3
Periphyton	2.6	0.986	37.4	-28.3	2.068	7.3
Green algae*	3.8			-32.0		
Blue-green algae*	2.2			-23.0		
Emergent vascular plants*	-5 to -1			-27 to -8		
Scuds	2.7	0.981	36.1	-27.7	1.958	7.1
Seminole ramshorn snail	5.0	1.629	32.3	-30.2	7.181	23.8
Riverine grass shrimp	8.2	0.833	10.2	-28.2	1.924	6.8
Eastern mosquitofish	10.0	1.039	10.4	-27.6	1.935	7.0
Florida gar	11.3	3.638	32.2	-27.6	8.643	31.3

Table 3. Carbon and nitrogen stoichiometry of samples analyzed for this study. C:N ratio is the ratio of Carbon to Nitrogen for all samples analyzed, N indicates sample size, StdDev is the standard deviation of the mean, and CV is the coefficient of variation.

Material	C:N ratio	N	StdDev	CV
Periphyton	13.6	41	3.751	27.6
Floc	11.3	25	3.201	28.2
Scuds	6.7	126	0.725	10.9
Seminole ramshorn snail	4.7	132	0.828	17.7
Eastern mosquitofish	4.0	123	0.150	3.8
Riverine grass shrimp	4.0	143	0.163	4.0
Florida gar	3.9	98	0.188	4.8

Table 4. Summary of Index of Detritivory (I_D) and trophic position (TRPO) for each species studied, reported by season. SD is the standard deviation of each parameter; CV is the coefficient of variation.

Parameter	Taxon	Season					
		Dry			Wet		
		mean	StdDev	CV	mean	StdDev	CV
I_D	Riverine Grass Shrimp	0.71	0.345	48.8	0.69	0.291	42.2
	Eastern Mosquitofish	0.72	0.343	47.7	0.83	0.263	31.8
					0.75	0.363	48.5
TRPO	Riverine Grass Shrimp	3.5	0.194	5.5	3.6	0.225	6.2
	Eastern Mosquitofish	4.0	0.182	4.5	4.1	0.237	5.7
					4.5	0.223	4.9

Table 5. A. Results from analysis of trophic position for each consumer species using backwards-stepping ANCOVA model (see text for factors appearing in initial model). Season indicates season used in analysis (both = wet and dry seasons). Only factors appearing in final model are shown (DSD = days since site dried). B. Effect sizes from significant pairwise comparisons of trophic position for mosquitofish (TD = TS dry season, SD = SRS dry season, TW=TS wet season, WD = WCA dryseason, etc).

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A. Final models

Species	Season	Stem density		Region x Season		DSD	
		F	P	F	P	F	R ²
Florida gar	Wet	$F_{1,16} = 9.759$	0.007				0.379
Eastern mosquitofish*	Both			$F_{2,33} = 5.452$	0.009	$F_{1,33} = 14.189$	0.434
Grass shrimp	Both					$F_{1,35} = 4.140$	0.105

* One case deleted (overly influential)

B. Effects sizes

Pairwise
Comparisons
TD-SD = 0.251
TD-TW= 0.320
TD-WD=0.230
TW-SW=0.251
TW-WW=0.230

Figure legends

Figure 1. A. Trophic position versus days since dried (DSD) prior to collection for the wet season sample. B. Trophic position versus DSD prior to collection for the dry season sample. An insufficient number of gar could be collected at this season for statistical analysis. C. Trophic position of Florida gar versus density of emergent vascular plant stems at the collection site. Only wet season data are plotted.

Figure 1.

