Anurans as Biological Indicators of Restoration Success in the Greater Everglades Ecosystem

Alicia D. Dixon[1,*], William R. Cox[1], Edwin M. Everham III[2], and David W. Ceilley[2]

Abstract - The Picayune Strand Restoration Project is being conducted as part of the Comprehensive Everglades Restoration Plan to restore hydrology and habitat in Southwest Florida. This study evaluated the success of the restoration activities by examining anuran species richness and relative abundance in relation to various restoration treatments, which included restored areas, un-restored areas, and natural wetlands. Anuran observations were conducted using nocturnal audible call surveys and dip netting. Univariate results indicated that: the lowest species richness and relative abundance values occurred within the un-restored areas, richness significantly increased in all restored areas relative to un-restored areas, abundance increased in some restored areas but not others, and highest richness and abundance were documented in the natural wetlands. Multivariate analysis confirmed these patterns and also indicated that the anuran species assemblages were significantly different between restoration treatments. Furthermore, the presence or absence of *Lithobates sphenocephalus utricularius* (Southern Leopard Frog), *Gastrophryne carolinensis* (Eastern Narrow-mouthed Toad), and *Hyla femoralis* (Pine Woods Treefrog) may be used to document restoration success or hydrologic disturbance, respectively. These findings suggest that the restoration activities can be effective and that anurans could be used as performance measures of restoration success.

Introduction

Amphibian populations are influenced by numerous environmental factors including hydroperiod, food availability, access to suitable habitats, and presence/absence of predators (Mazzotti et al. 2008, Semlitsch 2000a). They are a significant indicator of ecosystem health because of their vulnerability to environmental stress caused by their specific biological needs, and because they exhibit the effects of stressors earlier than other organisms (Mazzotti et al. 2008, Welsh and Ollivier 1998). Amphibians are a major component of the indigenous biodiversity in almost every natural terrestrial and freshwater habitat in the Southeastern United States; therefore, their species diversity reflects habitat quality, as well as the consequences of environmental destruction or degradation (Knutson et al. 1999, Tuberville et al. 2005, Vitt et al. 1990). Urbanization primarily adversely affects anuran populations by loss of habitat and habitat fragmentation (McKinney 2002, Means 2008, Rubbo and Kiesecker 2005), but altered hydrology, ditching of isolated and ephemeral ponds, industrial silviculture, and fire

[1]Passarella and Associates, Inc., 13620 Metropolis Avenue, Suite 200 Fort Myers, FL. [2]College of Arts and Sciences, Department of Marine and Ecological Sciences, Florida Gulf Coast University, 10501 FGCU Boulevard South, Fort Myers, FL. [*]Corresponding author - AliciaD@Passarella.net.

suppression are also major threats (Means 2008). In addition, eutrophication and increased exposure to contaminants can cause a negative impact (Ehrenfeld 2000, McKinney 2002, Rubbo and Kiesecker 2005). Altered hydrology can reduce or even decimate potential breeding sites and can also increase exposure to contaminants that are detrimental to aquatic eggs and larvae (Duellman and Trueb 1986). However, hydroperiods affect amphibian species differently based on their larval periods, physiological tolerances, and predator avoidance (Mazzotti et al. 2008, Semlitsch 2000b).

The Picayune Strand Restoration Project (PSRP) is a major cooperative hydrologic restoration effort by the US Army Corps of Engineers (USACE) and the South Florida Water Management District (SFWMD) and is part of the Comprehensive Everglades Restoration Plan (CERP) (USACE and SFWMD 2004). Expected benefits from the PSRP include the restoration of historic natural wetland hydroperiods and floral and faunal communities, improved freshwater sheet-flow and storage, and the attenuation of surge flows, extreme forest fires, and prevalence of exotic species (Chuirazzi and Duever 2008, USACE and SFWMD 2004). In addition, anuran populations (along with other water-dependant fauna) are expected to show dramatic positive responses to the hydrological improvements, including both an increase in numbers and a return to their natural distribution patterns (USACE and SFWMD 1999).

Developing effective strategies for measuring and communicating restoration success/failure in large regional restoration projects is an extremely difficult, yet essential, task because ecological systems are so complex (Doren et al. 2009). Due to this complexity, it is important to select and monitor indicators that are representative of the system in question, integrate into responses to that system, clearly respond to changes in the system, can be effectively and efficiently monitored, and have results that can easily be communicated (Mazzotti et al. 2009). Anurans serve as excellent biological indicators of restoration within the PSRP because:

1. they are often locally abundant (Rocha et al. 2001, Waddle 2006, Watanabe et al. 2005) and can be found in all habitats and hydrological regimes in the Everglades (USGS 2004);
2. they integrate response to system processes (Mazzotti et al. 2008, Waddle 2006);
3. they respond to system changes via restoration (Mazzotti et al. 2008, Waddle 2006);
4. they can be effectively and efficiently monitored through audible-call surveys (Dodd 2003; Heyer et al. 1994; Pieterson et al. 2006; Rice et al. 2004, 2005, 2007) and dip netting (Bartoszek et al. 2007, Dodd 2003, Heyer et al. 1994, Means 2008); and
5. the results of the monitoring can be easily communicated (Addison et al. 2006; Bartoszek et al. 2007; Dodd 2003; Heyer et al. 1994; Pieterson et al. 2006; Rice et al. 2004, 2005, 2007; Waddle 2006).

An advantage of the PSRP as a study site is that it is currently in the process of being restored, allowing near immediate biological comparisons to be made within the various stages of restoration. This research investigated anuran use of the different restoration treatments including restored areas and un-restored areas in comparison to natural wetland areas. The main objective of this research was to use anuran species richness, relative abundance, and community structure as biological indications of the overall ecological condition of each restoration treatment. We expected that the species richness and relative abundance of amphibian populations would correspond with the quality of habitat that they were utilizing: highest in the natural wetlands, next highest in the restored sites, and lowest in the un-restored sites, effectively determining that anurans could be used as a performance measure of restoration success.

Study Site

The PSRP includes approximately 22,000 ha and is located in Collier County, FL. In the 1960s, the Gulf American Corporation attempted to develop this area into a large-scale residential subdivision by excavating a 77-km canal system and constructing 467 km of roads; however, the development failed before the vast majority of the homes were constructed. The main objective of the restoration project is to restore the ecology and hydrology to pre-drainage conditions. One of the key restoration components of the PSRP involves the plugging of the extensive canal system and removing the road network. Thus far, only the eastern-most canal (Prairie Canal) has been partially backfilled with non-asphalt material from the degraded roads; this restoration effort was completed in 2 phases, mid-2004 (north region) and mid-2007 (south region) (Fig. 1). Backfilling of canals has been used in the past as a useful habitat-restoration technique to return areas to a more natural hydrological regime and it also has potential to improve aquatic wildlife habitat (Turner et al. 1988, 1994).

The canal plugging resulted in numerous human-made pools that formed between the plugs. The dimensions of the pools were not precisely predetermined; therefore, they vary in shape, size, and depth, but all hold water throughout the year. Due to the native seed source in the soils and the restored hydrology, ephemeral wetlands have been created atop the canal plugs located between the permanent pools. Native herbaceous wetland ground cover dominated the restoration plugs within the northern region, while the newer restoration plugs in the southern region were dominated by bare ground. The 2 restored regions differed in ecological succession, which provided older restored (north region) and newer restored (south region) conditions.

The plugging of Prairie Canal has stopped the transport of water directly to the estuaries and has limited the draining of adjacent lands. The SFWMD is monitoring ground-water and surface-water elevations within the PSRP to document the hydrologic regime throughout the restoration process, and they have

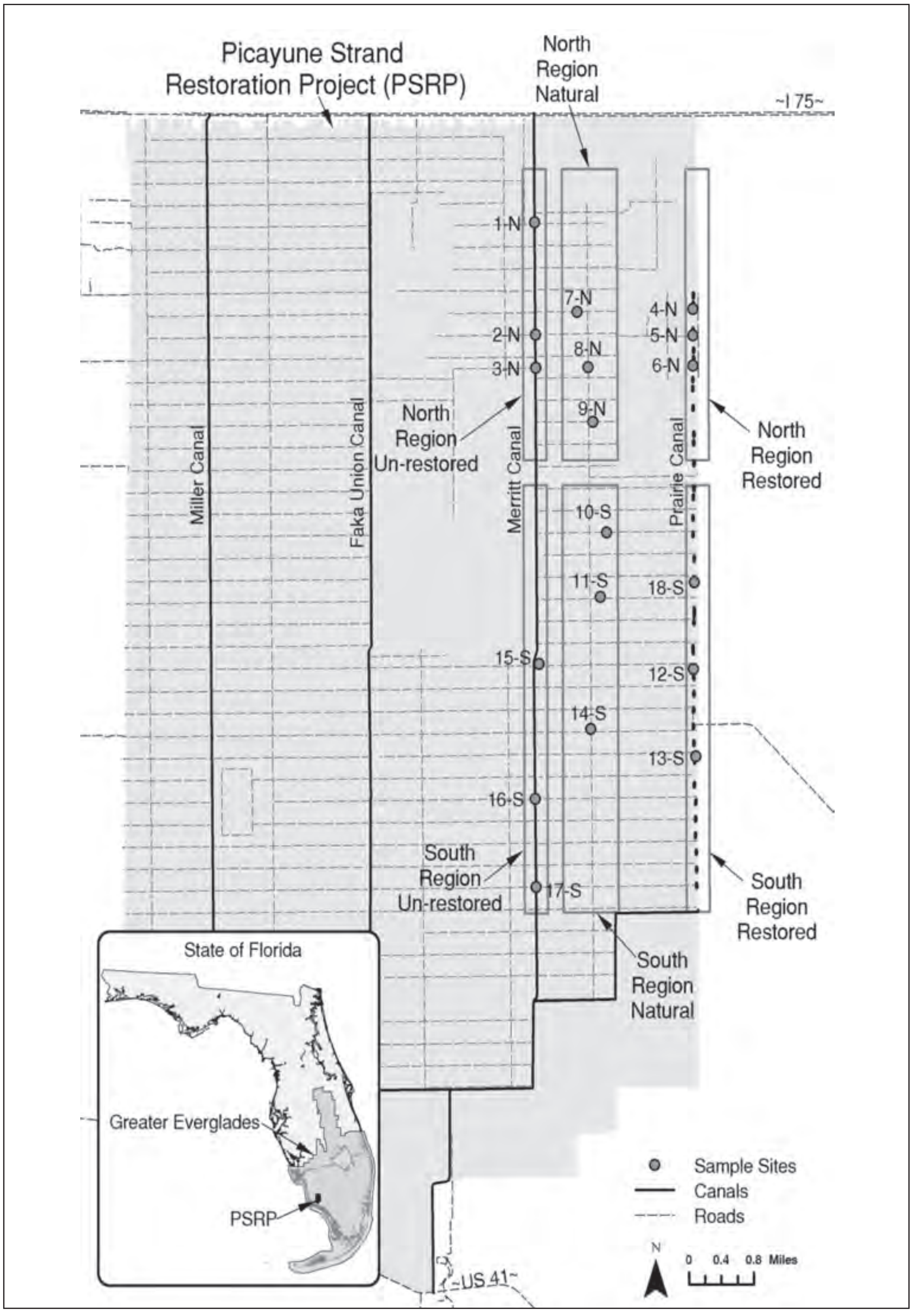


Figure 1. Project location map with anuran sample sites and north and south sample regions. The north region includes sites that have been restored since 2004, un-restored sites, and adjacent natural sites, with 3 replicates of each. The south region includes sites that have been restored since 2007, un-restored sites, and adjacent natural sites, with 3 replicates of each.

reported higher water levels near the restored Prairie Canal compared to near the un-restored Merritt Canal during the winter of 2006–2007 (Chuirazzi and Duever 2008). Plugging the canals, along with the other restoration mechanisms (installation of culverts under US 41, demolition clean-up, road removal, soil remediation, and the installation of pump stations and spreader channels) is anticipated to have significant positive effects both on-site and on adjacent lands, to help achieve the restoration goal for the greater Everglades ecosystem.

Methods

The study area was contained within the eastern portion of the PSRP because Prairie Canal was the only restored canal (to date), Merritt Canal was the closest major un-restored canal, and natural wetlands were located between the restored and un-restored areas. Also, road removal had been completed throughout this area. There were 3 restoration treatments (restored, un-restored, and natural). Natural wetlands were used as a control since these habitats represented the least disturbed areas within the PSRP, though their hydrology is assumed to also have been impacted by the canals. The natural wetlands included *Salix caroliniana* Michx. (Coastal Plain Willow) heads surrounded by *Taxodium distichum* (L.) Rich. (Bald Cypress) habitats. The un-restored treatment included disturbed/altered sites within and adjacent to Merritt Canal. The restored treatment included sites within and adjacent to the artificial canal plugs in the former Prairie Canal.

The study area was divided into north and south regions, which each included 3 replicate sampling sites of all 3 treatments (restored, un-restored, and natural; Fig. 1). The regions were separated in an attempt to differentiate between the older restored areas (north region), which were 4 years post-restoration, and newer restored areas (south region), which were 1 year post-restoration (Fig. 1). All 18 sites were sampled to inventory anuran species richness and relative abundance using nocturnal audible-call monitoring and dip netting. In an effort to randomize the sampling, both the nocturnal audible-call surveys and dip netting were conducted 1 site at a time, generally moving south to north during the first half of the study and north to south during the second half of the study, as we were aware that sampling at the same sites in the same order would have skewed the anuran data collected. The sampling was conducted during the wet season (late May through September) of 2008.

Nocturnal audible-call surveys

Anuran species that may be difficult to document throughout most of the year can be easily identified by nocturnal audible-call surveys during the breeding season (Dodd 2003). However, many amphibians are generalists, and hydrologic change may not affect the presence/absence of amphibians in an area as much as their relative abundance (Mazzotti et al. 2008, Meshaka et al. 2000). Therefore, nocturnal audible-call surveys were conducted to determine what anuran species were breeding within each sample site, and if breeding was taking place, to what intensity. These audible-call surveys were conducted on 31 May 2008, 14 and 28 June 2008, 2 August 2008, and 13 and 27 September 2008, beginning at sunset (7:30–8:30 p.m.) and continuing until all sample sites were monitored (1:00–2:00 a.m.).

At each sample site, anuran vocalizations were identified to individual species, and the intensities of their vocalizations were recorded over a period of 5 minutes. The intensity of calls were quantified using scaled values of 1 for small groups of individuals whose calls do not overlap, 2 for small groups where there is some overlap of calls between individuals of a species, and 3 for a chorus of overlapping calls as described in Pieterson et al. (2006) and USGS (2009). Since these surveys identified the largest number of species and also documented the relative abundance of each of those species, the majority of the analyses were focused on the audible-call data.

Dip netting

Dip-net surveys are an effective method for determining if breeding occurred, and if so, what species bred in that area (Means 2008). Therefore, dip netting was conducted to determine tadpole presence within the various restoration categories. Sampling of tadpoles was conducted using a standard D-frame aquatic dip net with mesh size of 1 mm. The net was swept 3 times within each sample site (Heyer et al. 1994). The net was worked vigorously within the vegetation, open water, and/or surficial bottom sediments within and atop the restored canal, natural wetlands, and un-restored canal when adequate standing water was present. Net contents were placed in a white pan and sorted with forceps. Samples were preserved in alcohol and identified in the laboratory utilizing the Altig (1970) tadpole key. If large numbers of indistinguishable tadpoles were concentrated in an area, then only a small, non-random representative sample was collected. The abundance of tadpoles was not quantified due to the time, staff, and budget constraints of collecting and identifying thousands of tadpoles. Anuran larvae species richness values were determined by the total number of species documented at least once at each site within each restoration treatment. The dip netting was conducted on 22 June 2008, 30 July 2008, and 3 August 2008.

Data analysis

The number of sampling events for each sample site and level of effort for each event were consistent. The call-intensity values were combined for each anuran species, in each sample site, for all audible-call sampling events, and overall abundance was calculated by combining all species and intensities for each site (Pieterson et al. 2006). To examine whether overall differences exist, mean richness and mean relative abundance among treatments and regions were compared using a general linear model (GLM), with region and restoration treatment as fixed factors and richness and total abundance as dependent variables. Tukey (HSD) test was used for post-hoc comparison between restoration treatments. Interpretation of any significant interaction effects were examined using the simple main effects approach suggested by Keppel (1991). Univariate tests were run with SPSS Version 16.0 for Windows.

Plymouth routines in multivariate ecological research, (PRIMER v6) was used to evaluate the nocturnal audible-call data and the dip-netting data separately (Clarke and Gorley 2006). Bray-Curtis similarity (Bray and Curtis 1957) was used to compare the percent similarity of anuran communities (species presence/absence and abundance) between all sites, with a resulting matrix used for additional analysis. A hierarchical agglomerative cluster analysis was used along with similarity profile significance test (SIMPROF) to search for significance in resulting clusters of sites using group-average similarity to construct the dendrogram. Non-metric multi-dimensional scaling (MDS) ordination (based on the Bray-Curtis similarity matrix) was created to illustrate in 2 dimensions the relative distances apart of all points in the same rank order as the relative dissimilarities. Points that are close together in the ordination represent high similarity, while points far apart represent very different values or dissimilarity.

To validate the MDS ordination, a non-parametric analog of analysis of variance, analysis of similarity (ANOSIM), was utilized to test for significance between a priori treatments. The ANOSIM analysis produces up to 999 random permutations of the data set to create a frequency distribution of the test statistic, R. ANOSIM produces a global R statistic for all observed values along with pair-wise tests of treatments to determine if there are significant site differences somewhere that are worth examining further and if there are specific pair-wise differences (Clarke and Gorley 2006). The similarity percentage test (SIMPER) was used to identify the contributions of individual anuran species to forming the Bray-Curtis similarity matrix as well as the similarity and dissimilarity within and between treatments, respectively. The SIMPER output lists the average abundance and (in order of importance) the contribution of each species to the total similarity within and dissimilarity between a prior treatments (un-restored, restored, and natural). The MDS ordination was overlaid with the abundance data of individual anuran species to visually examine the relative distribution of those species.

Results

Nocturnal audible-call surveys

Of the 13 anuran species documented, 12 were identified via nocturnal audible surveys (Table 1). The GLM indicated significant differences in species richness among treatments ($F = 7.8$; df = 2, 12; $P = 0.007$), but not between regions ($F = 0.714$; df = 2, 12; $P = 0.509$) (Fig. 2). Species richness was significantly lower in the un-restored sites than in the natural and restored sites (HSD Test: $P = 0.016$ and $P = 0.011$, respectively), but was not significantly different between natural and restored sites (HSD Test: $P = 0.977$). No significant interaction between the effects of region and treatment on species richness was detected ($F = 0.714$, df = 2, 12, $P = 0.509$).

The GLM also indicated that total abundance differed significantly among treatments ($F = 31.9$; df = 2,12; $P < 0.001$), but not among regions ($F = 0.526$; df = 1,12; $P = 0.482$), though an interaction between region and treatment

Table 1. Anuran species list by common name, scientific name, and authority documented by each sample method (scientific names per ITIS [2010]). A = audible-call survey, D = dip netting.

Common name	Scientific name	Authority	A	D
Cuban Treefrog	*Osteopilus septentrionalis*	Duméril and Bibron	X	X
Eastern Narrow-mouthed Toad	*Gastrophryne carolinensis*	Holbrook	X	X
Green Treefrog	*Hyla cinerea*	Schneider	X	X
Greenhouse Frog	*Eleutherodactylus planirostris*	Cope	X	
Little Grass Frog	*Pseudacris ocularis*	Bosc and Daudin in Sonnini de Manoncourt and Latreille	X	
Oak Toad	*Anaxyrus quercicus*	Holbrook		X
Pig Frog	*Lithobates grylio*	Stejneger	X	
Pinewoods Treefrog	*Hyla femoralis*	Bosc in Daudin	X	
Southern Chorus Frog	*Pseudacris nigrita*	LeConte	X	
Southern Cricket Frog	*Acris gryllus*	LeConte	X	
Southern Leopard Frog	*Lithobates sphenocephalus utricularius*	Harlan	X	X
Southern Toad	*Anaxyrus terrestris*	Bonnaterre	X	X
Squirrel Treefrog	*Hyla squirella*	Bosc in Daudin	X	X

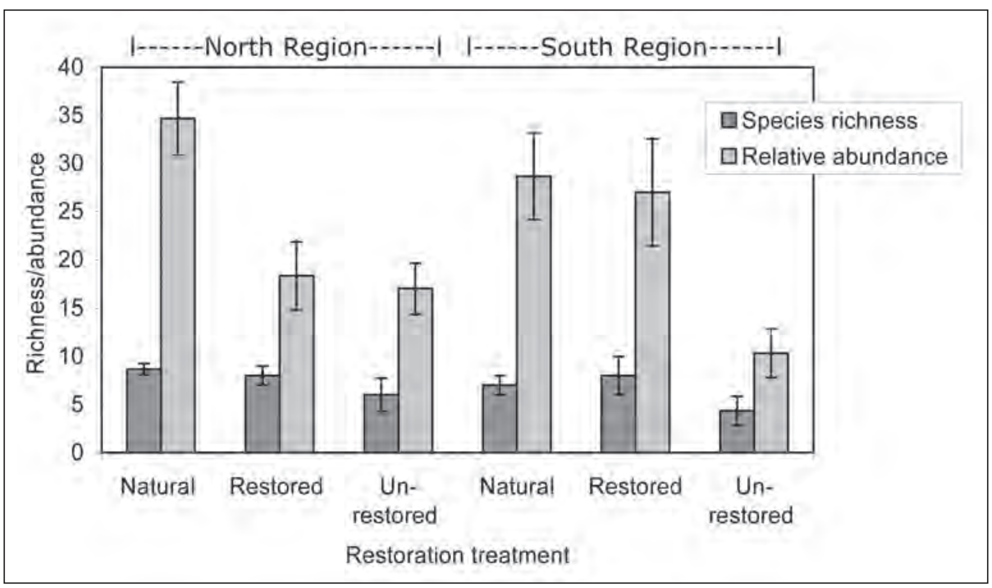


Figure 2. Mean species richness and relative abundance of anurans (from 3 replicates) within each restoration category by sample region documented via audible-call surveys (error bars represent standard deviation). Richness is not significantly different when examined by region, but the treatments are different ($P = 0.007$) when the 2 regions are combined. Relative abundance is significantly different among treatments, both by region and when data is combined across regions ($P < 0.001$).

was indicated (F = 7.4; df = 2,12; P = 0.008) (Fig. 2). The simple main effects analysis showed significant differences among treatments when separated into region for both the north (F = 19.08; df = 2,12; P < 0.05) and the south (F = 20.26; df = 2,12; P = 0.05). In the north, the natural treatment sites had higher relative abundance than the restored or un-restored treatments (HSD Test: P < 0.05), but the restored and un-restored treatments were not significantly different (HSD Test: P > 0.05). In the south, the restored and natural treatment sites were not significantly different from each other in relative abundance (HSD Test: P > 0.05), but both were significantly higher than the un-restored treatment (HSD Test: P < 0.05).

The results of the MDS ordination show distinct groupings among the natural wetlands, restored, and un-restored sites (Fig. 3). The sites in the north and south regions separated within the natural and un-restored groups, while the regions were mixed within the restored sites group. The stress value of the MDS ordination equaled 0.17, which indicates a potentially useful image (Clarke and Warwick 2001). The results of the cluster analysis using the SIMPROF permutation test did not reveal any statistically significant groupings (P < 0.05).

The two-way nested ANOSIM of the treatments (restored, un-restored, and natural) and regions (north and south) indicated significant differences between restoration treatments (R = 0.58, P = 0.001) but not between regions (R = 0.074, P = 0.6). The results of the SIMPER analysis (Dixon 2009) indicate that the anuran species assemblages collected from natural wetland sites were the most similar to each other (73% similarity), followed by the

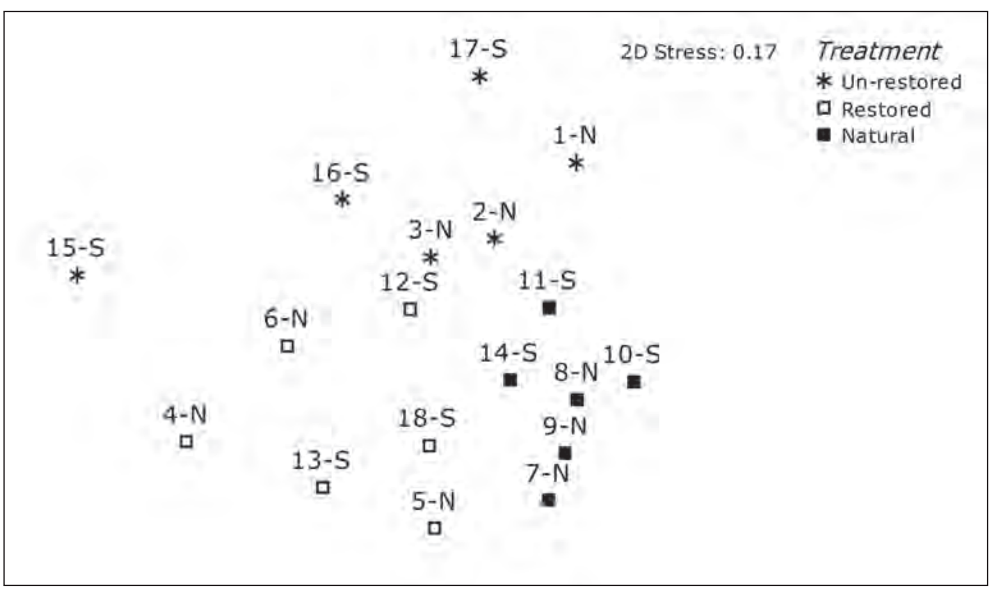


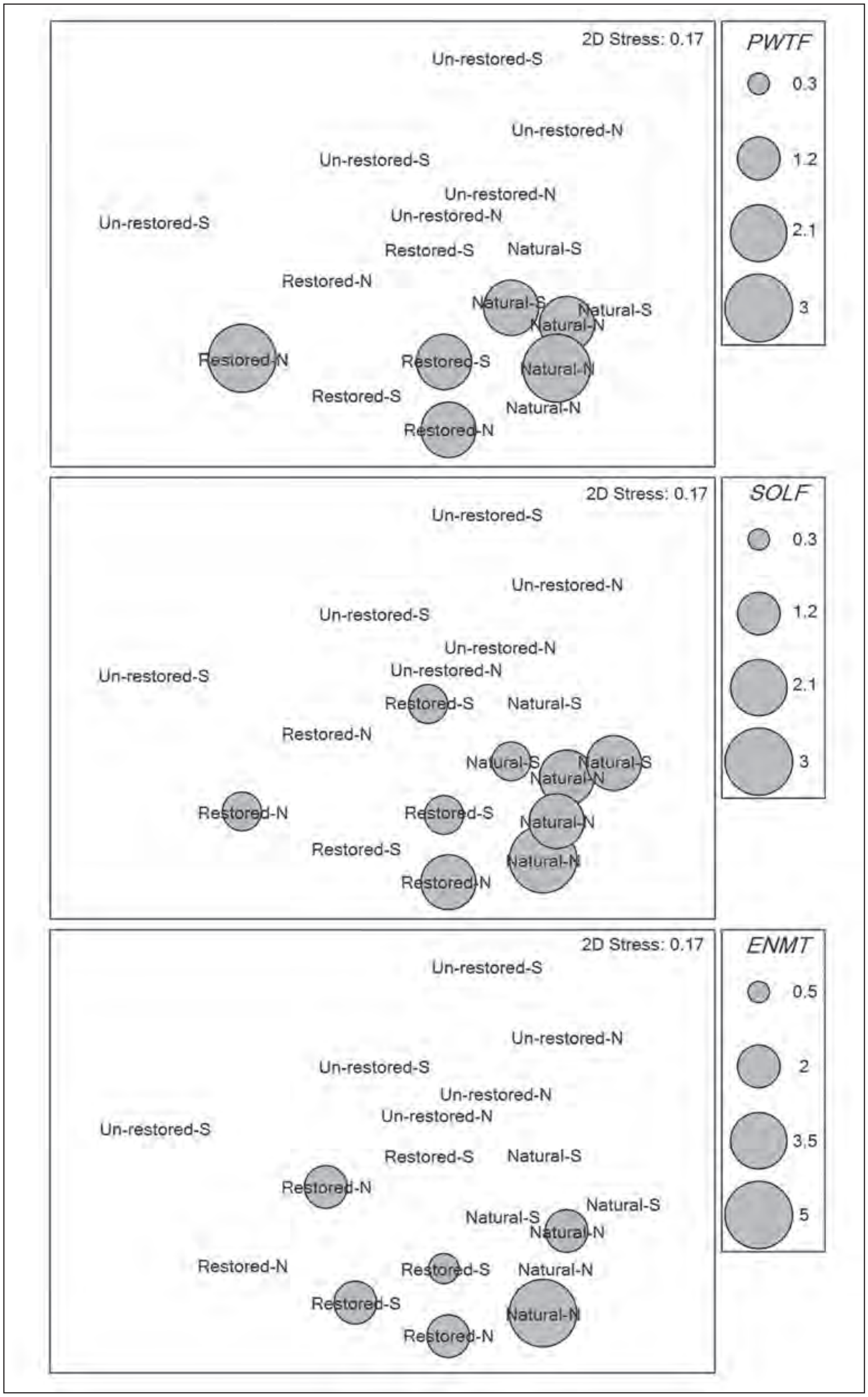
Figure 3. MDS ordination by restoration treatment, sample site, and sample region using Bray-Curtis similarity from audible-call abundance codes. Reference to north region (N) and south region (S) are provided after each site number.

restored sites (60% similarity). The un-restored sites had the lowest similarity to each other (54%). *Hyla cinerea* (Green Treefrog), *Anaxyrus terrestris* (Southern Toad), and *Osteopilus septentrionalis* (Cuban Treefrog) contributed a total of 46% of the total dissimilarity between the un-restored and natural wetland treatments, which were overall 48% dissimilar. The Green Treefrog, *Hyla squirella* (Squirrel Treefrog), and *Lithobates grylio* (Pig Frog) accounted for 44% of the total dissimilarity between the un-restored and natural wetland treatments, which were 49% dissimilar. The Green Treefrog, Squirrel Treefrog, Pig Frog, and Cuban Treefrog accounted for 61% of the total dissimilarity between the restored and natural wetland treatments, which were 43% dissimilar. Although *Lithobates sphenocephalus utricularius* (Southern Leopard Frog), *Gastrophryne carolinensis* (Eastern Narrow-mouthed Toad), and *Hyla femoralis* (Pine Woods Treefrog) were not the most important contributors to the dissimilarity between the different restoration treatments, each of these species were only detected at the natural wetlands and restored sites, and not at any of the un-restored sites (Fig. 4).

Dip netting

A total of 7 anuran larvae species were documented by dip netting (Table 1) in the restored and natural sites. No anuran larvae were collected within any of the un-restored sites. The natural sites contained a total of 6 species, the restored Phase I sites contained a total of 3 species, and the restored Phase II sites contained a total of 4 species (together, a total of 5 species of anuran larvae were collected from all restored sites). Eleven of the 12 restored and natural sites were found to have at least 1 species of anuran larvae. Only 7 sites (3 restored sites (6-N, 12-S, and12-S) and 4 natural sites (7-N, 8-N, 10-S, and 11-S)) produced 2 or more species of anuran larvae. Larvae of the Cuban Treefrog, Southern Toad, *Anaxyrus quercicus* (Oak Toad), and Eastern Narrow-mouthed Toad were collected from both restored and natural sites. Larval Green Treefrogs were collected from three southern restored sites (12-S, 13-S, and 18-S) but not from the natural sites, while Squirrel Treefrog larvae were only collected at 1 natural site (11-S). The ANOSIM tests identified larval assemblages at restored and natural sites to be significantly different than the un-restored sites (with no larvae), but restored and natural sites were not significantly different above the 90% confidence level. Further exploration of the dip-net dataset using cluster analysis with SIMPROF tests, MDS ordinations, and SIMPER analysis revealed no clear patterns or significant groupings of sites or treatments based on anuran larvae richness and abundance. Please note that these results must be reviewed in the context that only a small, non-

Figure 4 (opposite page). MDS ordination by restoration treatment with superimposed average calling abundance for Southern Leopard Frog (SOLF), Eastern Narrow-mouthed Toad (ENMT), and Pine Woods Treefrog (PWTF). Reference to north region (N) and south region (S) are provided after each sites restoration treatment label.



2D Stress: 0.17
Un-restored-S
Un-restored-N
Un-restored-S
Un-restored-N
Un-restored-N
Un-restored-S
Restored-S
Natural-S
Restored-N
Natural-S
Natural-S
Natural-N
Restored-N
Restored-S
Natural-N
Restored-S
Natural-N
Restored-N
PWTF
0.3
1.2
2.1
3
2D Stress: 0.17
Un-restored-S
Un-restored-N
Un-restored-S
Un-restored-N
Un-restored-N
Un-restored-S
Restored-S
Natural-S
Restored-N
Natural-S
Natural-S
Natural-N
Restored-N
Restored-S
Natural-N
Restored-S
Natural-N
Restored-N
SOLF
0.3
1.2
2.1
3
2D Stress: 0.17
Un-restored-S
Un-restored-N
Un-restored-S
Un-restored-N
Un-restored-N
Un-restored-S
Restored-S
Natural-S
Restored-N
Natural-S
Natural-S
Natural-N
Restored-N
Restored-S
Natural-N
Restored-S
Natural-N
Restored-N
ENMT
0.5
2
3.5
5

random representative sample was collected, which could have underestimated species richness.

Discussion

Based upon the audible-call data, it appears that the hydrologic restoration within the PSRP has recovered some native anuran habitat, which is reflected in significantly increased species richness and relative abundance within some restored sites relative to un-restored sites, as indicated by both univariate (Fig. 2) and multivariate analyses (Figs. 3, 4). However, the significant interaction between region and treatment for the relative abundance data appears to be driven by lower abundance in the earlier restored northern sites, which are not significantly different than the un-restored sites and are lower than the more recently restored southern sites (Fig. 2). The explanation for this difference may be that there is a time lag associated with restoration efforts. The communities in the southern region may have not fully responded to the hydrologic restoration at the time that sampling occurred, since the sampling was conducted immediately following the plugging of Prairie Canal. An overlap of pre-restoration communities (still dominated by exotic anurans) and post-restoration communities (including initial recovery of native anurans) could have resulted in higher richness and/or abundance in this transition state for the more recent, southern restoration sites. This scenario would indicate the need for long-term monitoring and support the use of multivariate approaches that maintain the species-specific information in tracking recovery of communities post-restoration.

Greater variation in community structure has been tied to ecological stress (Burns et al. 2008, Tolley et al. 2006), including in other aquatic faunal communities in the PSRP (Ceilley 2008). Our results indicated the highest similarity (least variation) among the natural sites, followed by the restored sites, with the lowest similarity (highest variation) among the un-restored sites. This is well illustrated in the MDS ordination (Fig. 3). Ceilley (2008) reported the same type of pattern for fish and macroinvertebrate species assemblages in the PSRP baseline assessment; very high similarity was observed among communities from natural "reference" sites, while very low similarity (high dissimilarity) was observed at the hydrologically impacted sites. These patterns were expected due to the corresponding habitat qualities in the natural and restored sites, which are of higher value than within the un-restored sites.

Although we documented with the audible-call surveys anurans attempting to breed at the un-restored sites, we could not confirm that breeding was successful through the dip-net sampling for larvae. No anuran larvae were collected at any of the un-restored sites, but larvae were collected at least once within all the restored sites and all but one of the natural sites, which support the conclusion that anurans are successfully responding to restoration activities.

Together, the Southern Leopard Frog, Eastern Narrow-mouthed Toad, and Pine Woods Treefrog were heard calling at all but one of the natural and restored sites, but were absent at the un-restored sites (Fig. 4). In addition, the dip-net sampling produced no larval anurans of these species. Their habitat and breeding requirements (shallow and ephemeral wetlands) are mostly associated with the restored and natural areas of the PSRP; and they are not associated with the existing un-restored canal due to the deep, permanent, open water that flows throughout the year. Mazzotti et al. (2008) determined that in southwest Florida, the highest hydroperiod suitability was between 2.1–10.1 months for the Southern Leopard Frog, between 0.1–3.1 months for the Eastern Narrow-mouthed Toad, and between 0.1–2.1 months for the Pine Woods Treefrog. Southern Leopard Frogs can be found in all shallow freshwater habitats, the Eastern Narrow-mouthed Toads only breed in temporary wetlands, and Pinewoods Treefrogs have a habitat requirement for sandy soils as found in mesic and hydric pine flatwoods and wet prairies (Mazzotii et al. 2008). These life-history traits of the 3 indicator species may help explain why they were only identified within the natural and restored sites. When viewed as a suite, these 3 taxa can be used as indicators of restoration success because of their sensitivity to particular environmental factors and because together they can be detected in a wider variety of habitats and hydroperiods. Having an association of species that are complementary of each other rather than related has more merit when used as indicators (Sewell and Griffiths 2009).

The seasonal wet/dry nature of ephemeral ponds is important to amphibians because it creates an inhospitable environment for many species of predacious fish and some macroinvertebrates (Means 2008). Some anuran species breed principally or exclusively in ephemeral ponds (Means 2008). Temporary wetlands generally contain highly productive and species-rich larval amphibian communities, while permanent wetlands typically contain relatively depauperate amphibian communities (Baber et al. 2005). The seasonal hydroperiod of the natural sites most likely contributed to the higher species richness and relative abundance values, possibly due to low predator prevalence.

All of the "pools" that were created as a result of the filling of Prairie Canal held water throughout the year. Permanent wetlands are not as suitable for amphibians as ephemeral wetlands because of the greater abundance of predatory fish (Baber et al. 2005, Mazzotti et al. 2008, Semlitsch 2000a). The higher abundance of fish in the pools from the former Prairie canal likely contributed to the decreased species richness and relative abundance in the restored sites, compared against the natural sites. However, since the restored sites contained ephemeral wetlands between the pools, the restoration has increased the extent and quality of amphibian habitat, relative to the un-restored sites. We expect that the amphibian populations will continue to improve as the restored areas mature ecologically, providing refugia for anuran larvae and adults as vegetation continues to naturally recruit. In addition, the hydrologic restoration is expected to return the entire landscape to a historic pattern of seasonal sheet flow, improving amphibian habitat surrounding these human-created pools.

The un-restored sites had the lowest species richness and relative abundance, and no anuran larvae were collected there. This result was not unexpected due to the known impact that canals can have on amphibian habitats. Ditching is detrimental to pond-breeding amphibians due to hydroperiod alteration and facilitation of predacious fish movement (Means 2008). The 4 major canals of PSRP were found to harbor large predaceous sunfishes (Centrarchidae) and at least 2 non-native predaceous cichlids, with a fish community assemblage that was significantly different from natural wetlands of the region (Ceilley 2007). In addition to these detrimental effects, the water within the existing canal also flows (sometimes very rapidly) to the south and eventually to saltwater habitats.

It appears the altered hydrology, presence of predators, and water-flow patterns contributed to the low anuran species richness and relative abundance values within the un-restored sites. These variations in habitat quality among un-restored, restored, and natural sites demonstrate the impacts resulting from previous habitat alteration (canalization) and the habitat improvements targeted by the restoration plan. This study indicates that anuran communities are responding to the habitat changes resulting from the implementation of this plan. The combination of species composition and proportion of each habitat occupied at a certain point in time form specific communities defined by their environmental factors; therefore, if these communities can be accurately defined and measured, restoration success can be evaluated, restoration targets can be established, and restoration alternatives can be compared within the Everglades (USGS 2004). It is hoped that amphibian monitoring will also assist the Florida Division of Forestry with post-restoration land-management practices including prescribed fire treatment, selective silviculture, and the protection of upland buffers around wetland habitats within the PSRP.

We believe that anuran species richness and relative abundance can be used as a performance measure of restoration success within the Greater Everglades because they responded positively to hydrologic/habitat restoration. More specific to the PSRP, we believe that the Southern Leopard Frog, Eastern Narrow-mouthed Toad, and Pine Woods Treefrog can be indicator species of restoration success when viewed as a suite. We expect these 3 species to expand their range across the wet prairies, hydric flatwoods, and cypress strands of PSRP as restoration work continues. We also found that nocturnal audible-call surveys and dip netting are highly effective, repeatable, and low-cost methods that can be used to document anuran breeding activity and reproduction, respectively.

Further research is needed to: determine if anuran populations continue to display positive responses to the restoration as the restored areas mature ecologically, substantiate the use of the 3 indicator species identified, see if other anuran species begin to show a shift in their distribution, and gather additional data toward the development of specific performance measures of restoration success. Future research can be improved by increasing sampling frequencies and duration, expanding seasonal sampling, and sampling at additional sample locations, including reference native habitats within the adjacent Fakahatchee Strand Preserve State Park and Florida Panther National Wildlife Refuge. The establishment of reference wetland habitats with undisturbed hydroperiods will be necessary to set restoration targets for restored and natural wetlands within the PSRP area.

Continued monitoring is a critical component of restoration coordination and verification (RECOVER) that is needed to achieve the ever adapting goals of CERP (Chuirazzi 2009) and monitoring amphibian communities within the Everglades is an important aspect of the adaptive assessment process (USGS 2004). The results of this study can be used to complement past, present, and future studies within the PSRP, and in combination, these studies can be used to evaluate trends in anuran composition over time as one measure of the success of Everglades restoration.

Acknowledgments

We would like to genuinely thank Les Alderman and Jim Alderman with Florida Panther Conservation Bank, LLC for providing the funds for sample materials and vehicle gas expenses; the Florida Division of Forestry for providing a swamp buggy with an experienced chauffeur when the water levels were too high to access the sampling stations with a vehicle; the SFWMD for donating gas for the swamp buggy; Passarella and Associates, Inc. for their geographic information system mapping assistance; and Charles Gunnels for his help in interpreting the results of the GLM. In addition, this manuscript was greatly improved through the thoughtful comments of David Chalcraft from East Carolina University and 2 anonymous reviewers. This project would not have been possible without these generous aids and assistance.

Literature Cited

Addison, D.S., M.J. Barry, I.A. Bartoszek, D.W. Ceilley, J.R. Schmid, and M.J. Schuman. 2006. Pre-restoration wildlife surveys in the Southern Golden Gate Estates (2001–2004). Final Report for South FloridaWater Management District, West Palm Beach, FL. Contract No. C-13259.

Altig, R. 1970. A key to the tadpoles of the Continental United States and Canada. Herpetologica 26(2):180–207.

Baber, M.J., K.J. Babbitt, F. Jordan, H.L. Jelks, and W.M. Kitchens. 2005. Relationships among habitat type, hydrology, predator composition, and distribution of larval anurans in the Florida Everglades. *In* W.E. Meshaka and K.J. Babbitt Amphibians and Reptiles: Status and Conservation in Florida. Krieger Publishing Company. Malabar, FL.

Bartoszek, I.A, M.J. Schuman, D.S. Addison, K.B. Worley, and J.R. Schmid. 2007. Biological monitoring of aquatic and terrestrial fauna for the Picayune Strand Restoration Project (2005–2007). Final Report for South FloridaWater Management District, West Palm Beach, FL. Contract No. PC600891.

Bray, J.R., and J.T. Curtis. 1957 An ordination of upland forest communities of Southern Wisconsin. Ecological Monographs 27:325–349.

Burns, D.A., K. Riva-Murray, R.W. Bode, and S. Passy. 2008. Changes in stream chemistry and biology in response to reduced levels of acid deposition during 1987–2003 in the Neversink River Basin, Catskill Mountains. Ecological Indicators 8:191–203.

Ceilley, D.W. 2007. Enhancing the recovery of threatened and endangered species in South Florida through aquatic refugia: Supplemental data analyses and discussion. Cooperative agreement # 1448-40181-01 G47, US Fish and Wildlife Service, Vero Beach, FL.

Ceilley, D.W. 2008. Picayune Strand restoration project: Baseline assessment of inland aquatic fauna. Final report to the South Florida Water Management District's Everglades Acceler8 Program, West Palm Beach. FL.

Chuirazzi, K.J. 2009. South Florida environmental report. appendix 7B: RECOVER activities update. South Florida Water Management District. West Palm Beach, FL.

Chuirazzi, K.J., and M.J. Duever. 2008. South Florida environmental report. appendix 7A-2: Picayune Strand Restoration Project baseline. South Florida Water Management District, West Palm Beach, FL.

Clarke, K.R., and R.N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E Ltd. Plymouth, UK.

Clarke, K.R., and R.M. Warwick. 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd Edition. PRIMER-E Ltd. Plymouth, UK.

Dixon, A.D. 2009. Anuran use of natural wetlands, created pools, and existing canals within the Picayune Strand Restoration Project. M.Sc. Thesis. Florida Gulf Coast University, Fort Myers, FL.

Dodd, C.K., Jr. 2003. Monitoring amphibians in Great Smoky Mountains National Park. US Geological Circular 1258. US Geologocal Survey, Denver, CO.

Doren, R.F., J.C. Trexler, A.D. Gottlieb, and M.C. Harwell. 2009. Ecological indicators for system-wide assessment of the Greater Everglades ecosystem restoration program. Ecological Indicators 9(6):S2–S16.

Duellman, W.E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill, Inc., New York, NY.

Ehrenfeld, J.G. 2000. Evaluating wetlands within an urban context. Ecological Engineering 15:253–265.

Heyer, R.W., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster. 1994. Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. Smithsonian Books, Washington, DC.

Integrated Taxonomic Information System (ITIS). 2010. On-line Database. Available online at http://www.itis.gov. Accessed 23 May 2010.

Keppel, G. 1991. Design and Analysis: A Researcher's Handbook. Prentice-Hall, Inc. Englewood Cliffs, NJ.

Knutson, M.G., J.R. Sauer, D.A. Olson, L.M. Mossman, L.M. Hemesath, and M.J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology 13:1237–1446.

Mazzotti, F.J., R.G. Harvey, L.G. Pearlstine, A.D. Daugherty, L.A. Brandt, K.L. Chartier, K.G. Rice, J.H. Waddle, D.W. Ceilley, and M.J. Duever. 2008. Stressor-response model for Southwest Florida amphibians. Report for ecological modeling support for the evaluation of alternatives for the Southwest Florida feasibility study. University of Florida. Gainesville, FL.

Mazzotti, F.J., R.G. Best, L.A. Brandt, M.S. Cherkiss, B.M. Jeffery, and K.G. Rice. 2009. Alligators and crocodiles as indicators for restoration of Everglades Ecosystems. Ecological Indicators 9(6):S137–S149.

Means, R. 2008. Management strategies for Florida's ephemeral ponds and pond-breeding amphibians. Florida Fish and Wildlife Conservation Commission, Tallahassee, FL. Final Report.

Meshaka, W.E., W.F. Loftus, and T. Steiner. 2000. The herpetofauna of Everglades National Park. Florida Scientist 63:84–103.

McKinney, M.L. 2002. Urbanization, biodiversity, and conservation. Bioscience 52:88–890.

Pieterson, C.E., L.M. Addison, J.N. Agobian, B. Brooks-Solveson, J. Cassani, and E.M. Everham III. 2006. Five years of the Southwest Florida frog-monitoring network: Changes in frog communities as an indicator of landscape change. Florida Scientist 69:117–126.

Rice, K.G., J.H. Waddle, B.M. Jeffery, and F.H. Percival. 2004. Herpetofaunal inventories of the National Parks of South Florida and the Caribbean: Volume 1. Everglades National Park. US Geological Survey, Ft. Lauderdal, FL.

Rice, K.G., J.H. Waddle, B.M. Jeffery, A.N. Rice, and F.H. Percival. 2005. Herptofaunal inventories of the National Parks of South Florida and the Caribbean: Volume 3. Big Cypress National Preserve. US Geological Survey, Ft. Lauderdal, FL.

Rice, K.G., J.H. Waddle, M.E. Crockett, C.D. Bugbee, B.M. Jeffery, and F.H. Percival. 2007. Herptofaunal Inventories of the National Parks of South Florida and the Caribbean: Volume 4. Biscayune National Park. US Geological Survey, Ft. Lauderdal, FL.

Rocha, C.F.D., M. Van Sluys, M.A.S. Alves, H.G. Bergallo, and D. Vrcibradic. 2001. Estimates of forest floor-litter frog communities: A comparison of methods. Austral Ecology 26:14–21.

Rubbo, M.J., and J.M. Kiesecker. 2005. Amphibian breeding distribution in an urbanized landscape. Conservation Biology 19(2):504–511.

Semlitsch, R.D. 2000a. Critical elements of biologically based recovery plans of aquatic-breeding amphibians. Conservation Biology 16:619–629.

Semlitsch, R.D. 2000b. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management 64:615–631.

Sewell, D., and R.A. Griffiths. 2009. Can a single amphibian species be a good biodiversity indicator? Diversity 1:102–117.

Tolley, S.G., A.K. Volety, M. Savarese, L.D. Wells, C. Linardich, and E.M. Everham III. 2006. Impacts of salinity and freshwater inflow on oyster-reef communities in Southwest Florida. Aquatic Living Resources 19: 371–387.

Tuberville, T.D., J.D. Willson, M.E. Dorcas, and J.W. Gibbons. 2005. Herpetofaunal species richness of southeastern national parks. Southeastern Naturalist 4(3):537–569.

Turner, R.E., I.A. Mendelssohn, K.L. McKee, R. Costanza, C. Neill, J.P. Sikora, W.B. Sikora, and E. Swenson. 1988. Backfilling canals in coastal Louisiana. Proceedings of the National Wetlands Symposium: mitigation of impacts and losses. Association of State Wetland Managers, Inc., New Orleans. October, 1986:135–141.

Turner, R.E., J.M. Lee, and C. Neill. 1994. Backfilling canals as a wetland restoration technique in coastal Louisiana. OSC Study MMS 94-0026. US Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA.

US Army Corps of Engineers and South Florida Water Management District (USACE and SFWMD). 1999. Central and Southern Florida project comprehensive review study. Final integrated feasibility report and programmatic environmental impact statement. Jacksonville, FL.

USACE and SFWMD. 2004. Comprehensive everglades restoration plan Picayune Strand Restoration (formerly Southern Golden Gate Estates ecosystem restoration) final integrated project implementation report and environmental impact statement. Jacksonville, FL.

US Geological Survey (USGS). 2004. Use of amphibians as indicators of ecosystem restoration. Fact Sheet 2004-3106. Davie, FL.

USGS. 2009. North American amphibian monitoring program. Protocol description. Available on-line at http://www.pwrc.usgs.gov/naamp/index.cfm?fuseaction=app.protocol. Accessed 18 July 2009.

Vitt, L.J, J.P. Caldwell, H.M. Wilber, and D.C. Smith. 1990. Amphibians as harbingers of decay. BioScience 40:418.

Waddle, J.H. 2006. Use of amphibians as ecosystem indicator species. Ph.D. Dissertation. University of Florida, Gainesville, FL.

Watanabe, S., N. Nakanishi, and M. Izawa. 2005. Seasonal abundance in the floor-dwelling frog fauna on Iriomote Island of the Ryukyu Archipelago, Japan. Journal of Tropical Ecology 21:85–91.

Welsh, H.H.J., and L.M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: A case study from California's redwoods. Ecological Applications 8:1118–1132.