BIOLOGICAL ASSESSMENT OF PHASE 1A RESTORATION OF KINGS BAY: FISHES, MACROINVERTEBRATES AND SUBMERGED AQUATIC VEGETATION

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EXECUTIVE SUMMARY

This biological assessment was conducted for Save Crystal River, Inc. to identify aquatic faunal (fishes and macroinvertebrates) communities that inhabit areas restored under a Phase 1A restoration project completed in 2017. In addition, a submerged aquatic vegetation (SAV) transect was established to monitor the growth and reproduction of *Vallisneria americana* through time and seasons. The SAV transect included five (5) 1.0 meter^2 quadrats in the restored Phase 1A habitat and five (5) 1.0 meter^2 quadrats in the adjacent east-west canal of the Pilot Project area. Grazing pressure has been well documented as a limiting factor preventing recovery of unprotected eel grass (wild celery, tape grass) in both Kings Bay (Hauxwell et al. 2004a and 2004b) and in the Caloosahatchee River and estuary (Ceilley et al. 2013 and Johnson Eng. 2018). Seasonal sampling of SAV communities (*Vallisneria, Hydrilla, Najas, Ruppia*) and algal cover (*Lyngbya* and filamentous green algae) was conducted to document variations in SAV cover in the peak of the dry season (May 2018) following months of grazing pressure by manatees, and near the end of the growing season (October 2018) when most manatees are gone and *Vallisneria* growth was expected to be near a maximum. Fish communities were also surveyed in May and October 2018 in conjunction with SAV monitoring.

Macroinvertebrates were sampled only once using three replicate artificial substrates (EPA Hester-Dendy substrates) and three replicate dredge samples (petite Ponar) in November to December 2017 and January 2018 respectively. Hester-Dendy and petite Ponar sampling, sample processing, and macroinvertebrate identification is labor intensive, requires slide mounting of specimens and identification using high-power compound microscopes. Qualitative dip-net sampling was also conducted to survey the macroinvertebrate communities of the shallow littoral zone of Phase 1A. Macroinvertebrate identifications were conducted by aquatic entomologist, Robert Rutter and aquatic ecologist David W. Ceilley using stereozoom and compound microscopes. Identification of snails, worms, midges and crustaceans were confirmed using a State of Florida Department of Environmental Protection (FDEP) verified collection currently in the possession of Johnson Engineering Inc.

Vallisneria americana cover was patchy in Phase 1A, but relatively dense where it was found. As anticipated, *Vallisneria* cover nearly tripled from May to October 2108. Overall cover increased

from 12% in May 2018 to 35% cover in October 2018. *Hydrilla* cover also increased from 1% in May to 12% in October. The Braun-Blanquet SAV cover class average increased from 1.6 to 4.2 during the same time frame. In the adjacent canal connecting Phase 1A to the Pilot Project, *Vallisneria* cover increased from 35% to 89% from May to October 2018 and female (pistillate) flowers were abundant throughout all quadrats. *Hydrilla* and other species of vascular SAV disappeared from this area as of the October 2018 monitoring event, possibly being crowded out by *Vallisneria* growth. The average Braun-Blanquet cover class, based on *Vallisneria* alone, doubled from May to October 2018 from 2.4 to 4.8 respectively. The October 2018 monitoring was conducted prior to arrival of large numbers migrating manatees seeking thermal refugia during the winter months. Subsequent qualitative observations conducted in early December 2018 revealed that intensive grazing by manatees has greatly reduced the biomass and cover of *Vallisneria* in the Phase 1A area. Longer term monitoring of *Vallisneria* (and other SAV species) on a seasonal basis will be necessary to determine if SAV restoration goals can be achieved and maintained.

In Phase 1A, a total of 1,461 macroinvertebrate were collected and identified, representing five (5) classes, 14 orders, and 25 families and at least 42 species. Aquatic macroinvertebrates communities appear to be a reliable biological indicator of habitat restoration and for monitoring recovery through time. Both quantitative sampling techniques, petite Ponar and Hester-Dendy artificial substrates demonstrated significant changes ($p<0.05$) in aquatic macroinvertebrate communities are occurring as a result of the benthic habitat and SAV restoration actions. Multivariate statistical analyses demonstrated how community structure has been changing in response to restoration and several potential indicator taxa were identified. The most important species responsible for separating restored and unrestored sites were the amphipod *Hyalella azteca* (grp.) which was extremely abundant in restored habitat samples but almost absent in unrestored habitat samples. *Hyalella azteca* (grp.) is an important prey item for many small fishes and was responsible for 20% of the dissimilarity between restored and unrestored samples. At least 12 other aquatic macroinvertebrate species were identified as potential indicators of restoration success using multivariate analysis of community structure based on Bray-Curtis similarity, together with similarity profile (SIMPROF) and similarity percentage (SIMPER) tests. In addition, some potential indicators of a degraded habitat dominated by *Lyngbya* were also identified since they only occurred in unrestored (impaired) areas. Rapid biological assessment using timed dip-net

samples appear to be valuable tool for assessing shallow wadable habitats. The combination of Ponar sampling and Hester-Dendy (HD) substrates provide a basis for quantitative comparisons of benthic habitat and water quality conditions respectively. Once indicator taxa have been confirmed for identification of restoration success, qualitative rapid assessment techniques may be more cost effective than Ponar dredge and HD substrates which can be labor intensive and expensive in comparison.

Fish community health overall is critical to ecosystem integrity and something the public can readily understand as an important ecosystem service provided by healthy aquatic habitats. Fish communities were found to be responding positively to the restoration activities in Phase 1A and several species were identified as indicators of restoration success. Sport fishes, especially largemouth bass and sunfishes, are beginning to recover in Kings Bay (based on the monitoring program) because flocculent organic substrates were unsuitable for successful spawning by most sunfish species including largemouth bass, bluegill, and redear and spotted sunfish. The relative abundance of several sunfish species, and the presence of demersal species like the hogchoker are positive indicators of habitat restoration. Multiple fish sampling methods are recommended, including visual surveys for larger fish species that avoid traps and other sampling methods. Creel surveys may also be an important tool for future fisheries assessments. Further investigation on fish community structure in Kings Bay is needed to understand seasonality and habitat use, as well as the behavioral responses of fishes to fluctuations in tides, SAV cover, and human activities.

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1.0 INTRODUCTION

A biological assessment was initiated in December 2017 and completed in October 2018 to survey the aquatic faunal communities of restored habitats within Phase 1A the King's Bay area of the Crystal River ecosystem located in Citrus County Florida (**Figure 1**). Kings Bay is the headwaters to Crystal River that discharges into the Gulf of Mexico and is an oligohaline, tidally-influenced complex of freshwater springs with several anthropogenic canals. The watershed consists of native habitats and mixed-use urban development. King's Bay/Crystal River is also a water-based ecotourism destination because of the numerous springs which serve as winter thermal refuge for the federally-threatened manatee (*Trichechus manatus*) (FFWCC 2017). The submerged aquatic vegetation (SAV) in King's Bay historically consisted of native freshwater species, primarily eel grass (also known as tape grass and wild celery), *Vallisneria americana.* The introduction of non-native plant species (e.g. *Hydrilla*), in conjunction with anthropogenic eutrophication, environmental stochastic events, and increased grazing pressure by herbivores (manatees, turtles, crabs, fish and invasive apple snails) have contributed to massive losses of eel grass in the system. This has led to a general degradation of aquatic habitats, especially in the canals where mucky organic sediments have accumulated. A phased restoration project is currently underway and consists of de-mucking the waterways and replanting of eel grass, *Vallisneria americana*. Eel grass (also called tape grass, or wild celery) beds provide habitat for at least 44 species of fishes as well as many crustaceans, mollusks and other macroinvertebrates (Robbins 2005) which serve as trophic linkages to higher level consumers in the estuary.

This biological assessment was conducted for Save Crystal River, Inc. to identify aquatic faunal (fishes and macroinvertebrates) communities that inhabit areas restored under a Phase 1A restoration project completed in 2017. In addition, a submerged aquatic vegetation (SAV) transect was established to monitor the growth and reproduction of *Vallisneria americana* through time and seasons. Grazing pressure has been well documented as a limiting factor preventing recovery of unprotected eel grass (wild celery, tape grass) in both Kings Bay (Hauxwell et al. 2004a and 2004b) and in the Caloosahatchee River and estuary (Ceilley et al. 2013 and Johnson Eng. 2018). The restoration of Kings Bay aquatic habitat is incomplete and continued monitoring of the aquatic fauna and SAV communities was requested by Save Crystal River Inc. to track restoration success and identify ecological indicators that may inform decision makers in the future.

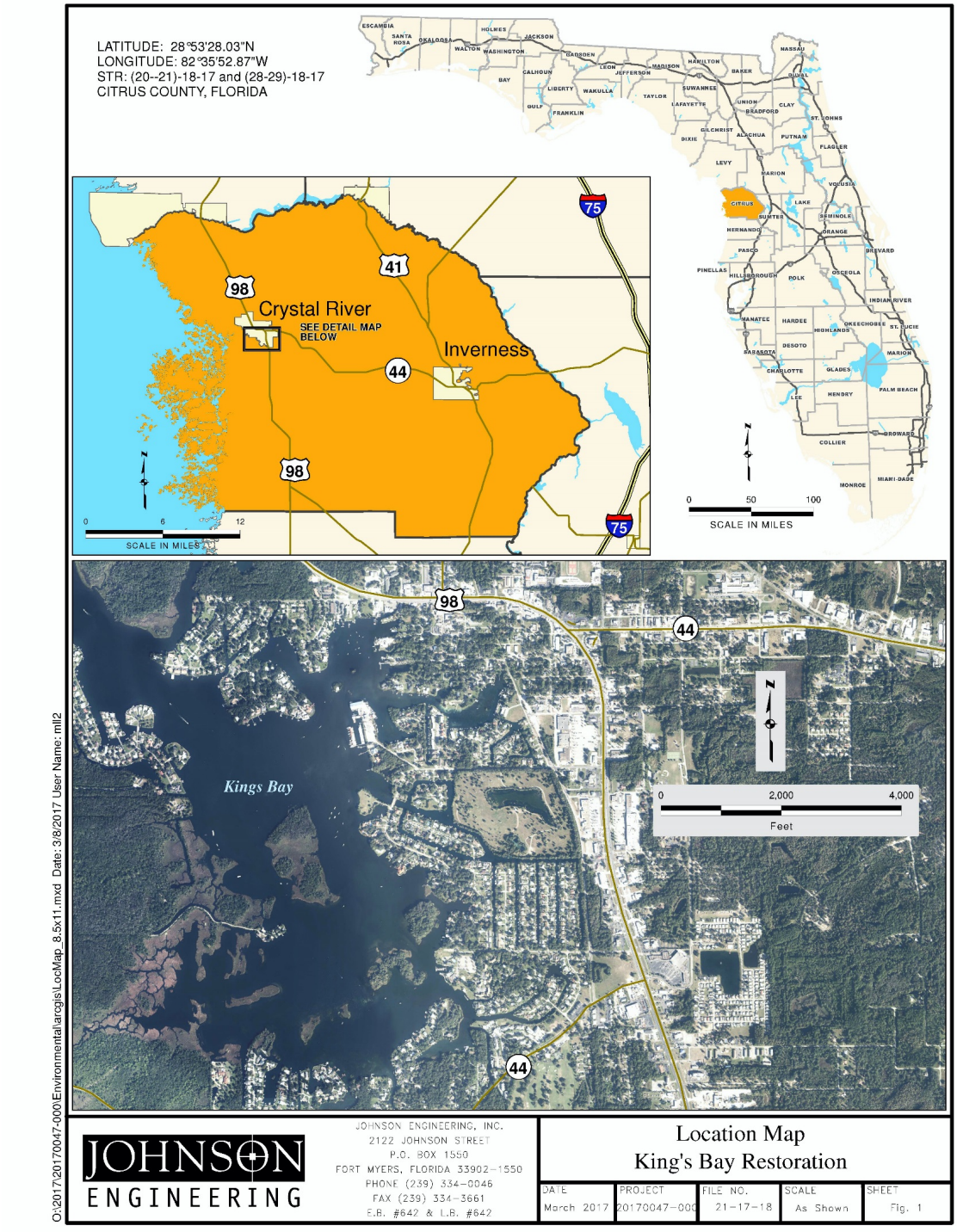

Figure 1. General Location Map of King's Bay Restoration Area, Citrus

2.0 METHODS

Macroinvertebrate Communities

The Florida Department of Environmental Protection (FDEP) has been at the leading edge of science with respect to assessing aquatic communities through the use of benthic macroinvertebrate communities. Several assessment tools have been developed over the past three decades that use macroinvertebrates to assess the biological condition of freshwater lakes, rivers, and wadable streams. These include the Lake Condition Index (LCI), Stream Condition Index (SCI) and Biological Reconnaissance Field Method (BioRecon). However, these methods have limitations when it comes to tidal systems that are very dynamic in terms of water levels, salinity regimes, nutrient loading and sources, and are inhabited by euryhaline, brackish, and freshwater species. "*DEP has conducted exploratory studies and workshops for the development of estuarine and marine bioassessment tools. While the previous attempts have not yielded practical results, DEP is currently planning studies for further development, potentially including an evaluation of epi-benthic taxa and fish in a variety of habitat types*." (FDEP 2018). This biological monitoring program is based on applied research, existing FDEP protocols, and adaptive assessment methods (Ceilley 2008) to develop practical bioassessment tools for monitoring restoration success/failure in the King's Bay ecosystem. The program employs quantitative sampling techniques (FDEP 2017) in combination with standardized qualitative observations of ecosystem conditions and univariate and multivariate statistics to analyze community structure and response to restoration activities.

Aquatic macroinvertebrate communities were sampled using a petite Ponar dredge and artificial substrates (EPA Hester-Dendy) to quantify community structure and assess biological conditions one year after restoration activities were completed. In addition, qualitative dip net sampling was conducted within the shallow vegetated habitat. Samples were field sorted from debris in a shallow white pan using forceps and vials and preserved in 80% ethanol for laboratory identification by a taxonomic expert.

Three (3) Hester-Dendy substrates were deployed in Phase 1A and retrieved after a 28 days colonization period. After the colonization period, samplers were processed for collection and preservation of epi-fauna using 80% ethanol in Nalgene bottles for later identification. Three replicate petite Ponar samples were also collected from Phase 1A. Samples were processed

following FDEP (2017) Standard Operating Procedures (SOP FS4000/FS7400). Biological samples were processed following FDEP protocols (LT7700) for processing and identification. All macroinvertebrates collected will be identified to the lowest practical taxa and enumerated. Chironomid midge larvae and oligochaete worms were mounted on microscope slides using CMC-10 clearing media and allowed to dry for several days prior to identification. While FDEP protocols were followed wherever practicable, macroinvertebrate samples were collected, processed, and identified using proven methods for the research and assessment of aquatic habitats in southwest Florida and Everglades Restoration respectively. Macroinvertebrate identifications were confirmed by Mr. Robert Rutter, aquatic entomologist and retired FDEP macroinvertebrate taxonomist, with voucher specimens prepared for future documentation. Bench sheets for macroinvertebrate identifications and recording were written by hand and then used to transcribe data into Excel database for presentation in tables and statistical analysis. Mr. Rutter's FDEP verified voucher specimens for midges, worms, snails, and bivalves were purchased by Johnson Engineering Inc. in 2018 and will be retained for specimen identification and confirmation for any future studies.

Sampling locations for the Hester-Dendy substrates are shown in **Figure 2**. Sampling locations were selected based on representative habitats available but field located to avoid heavy boat traffic patterns, manatee tour-boat operations, private dockage, and ongoing maintenance activities. The sites are believed to be representative of the overall Phase 1A habitat conditions. EPA round Hester-Dendy substrates, along with anchors and floats used in this study are shown in **Figure 3**.

Figure 2. Phase 1A King's Bay Restoration Hester-Dendy and petite Ponar Sample Sites.

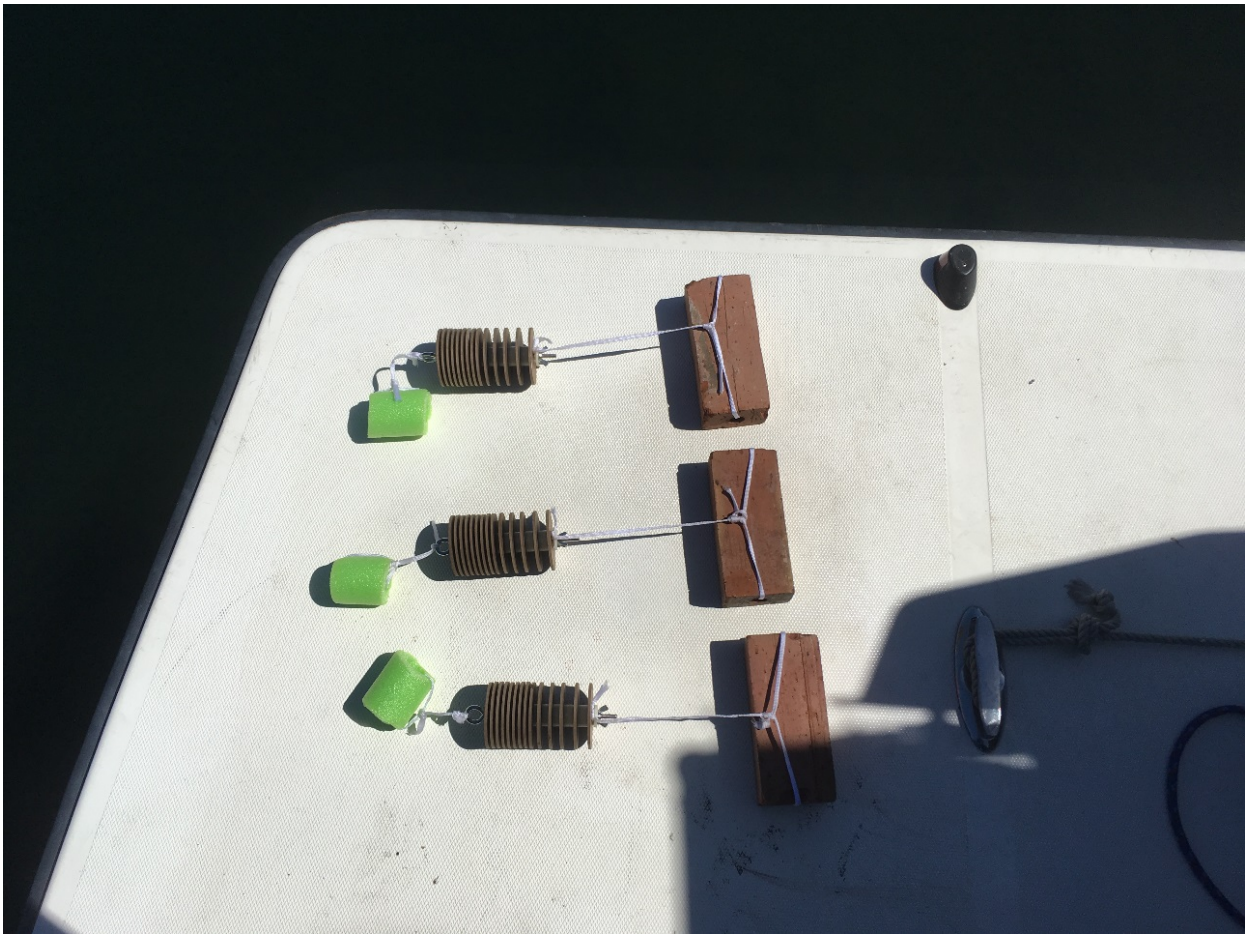

Figure 3. EPA Hester-Dendy Artificial Substrates for aquatic macroinvertebrate sampling.

D-frame dip net sampling based on methods recommended by the Florida Department of Environmental Protection (R. Frydenborg personal communication 2003) but modified based on field conditions and recommendations by USFWS Habitat Evaluation Team biologists (GEER 2010) and methods used for the Baseline Assessment of the Picayune Strand Restoration Project (Ceilley 2008). This includes active dip net sampling in wadable waters using a 1000 micron mesh standard D-frame dip net with field sorting in a shallow white pan for a period of one hour at each treatment site. Organisms are sorted from debris and collected in small jars and vials and preserved in 80% ethanol **(Figure 4).**

Figure 4. Standard D-Frame Aquatic Dip-net used for aquatic faunal sampling; fishes and macroinvertebrates.

In addition to the dip net and HD sampling, three (3) petite Ponar samples were collected from representative study site locations in Phase 1A on January 2, 2018 **(Figure 2)**. Samples were processed following FDEP (2017) Standard Operating Procedures (SOP FS4000/FS7400) **(Figure 5).** Biological samples were processed following FDEP protocols (LT7700) for processing and identification.

Figure 5. Petite Ponar dredge (left) being used to collect replicate sediment samples (right) from restored canal habitat in King's Bay Phase 1A.

All petite Ponar dredge, Hester-Dendy substrate and dip net samples were processed and the macroinvertebrates collected were identified to the lowest practical taxonomic level and are listed in the results section.

Fish Communities

Fish community structure can be difficult to quantify in open water systems due to the motility of fishes and natural flight response to predators and humans working in the water column or in the vicinity above it. Fish community assessments consisted of qualitative visual assessments, dip net sampling, and activity trap sampling using two trap types in each of the treatment areas (Ceilley et al. 2013, Ceilley 2008). Visual fish community surveys were conducted in conjunction with macroinvertebrate sampling and during the deployment of activity traps (funnel, Breder, and crayfish traps) during May 2018 and October 2018. The timing of quantitative fish sampling events was planned in order to 1. Collect seasonal variations in fish communities from late Spring and early Fall to coincide with dry and wet season fluctuations; and 2. Avoid periods of high human activity such as scallop season opening in Summer and peak tourism and manatee watching season of Winter. Prior to the biological assessments, underwater visual surveys of fishes were conducted by divers using mask and snorkel and underwater slates and video cameras to record fish usage in the areas around the study sites. Visual transects recorded species richness and relative abundance (present, common or abundant) for comparison between treatment sites.

Ten replicate Breder (1960) traps were deployed at each treatment site and allowed to colonize for one hour and retrieved for fish identification and enumeration. Fish collections from both locations were identified to species level and enumerated with voucher specimens retained for future reference **(Figure 6).**

Figure 6. Clear plastic "Breder Traps" being deployed sampling fish communities in shallow waters of Phase 1A in October 2018.

Three modified funnel traps (Fisher International, Tampa, FL.) were deployed overnight at locations outside of the main boat channels and heavy traffic areas for a period of approximately 24 hours before pulling traps and identifying and enumerating fishes collected from each site **(Figure 7)**.

Figure 7. Modified funnel traps for overnight sampling of fish communities

The overnight fish sampling was repeated on a second night at a different location within the restored and unrestored habitats. During the May fish sampling events, there was moderate tour-boat traffic with groups of snorkelers in the waters around the sampling sites that may have impacted fish behavior patterns. Follow up fish surveys were conducted in October 2018 when *Vallisneria americana* growth was expected to be at peak biomass for the year and tour-boat activity was expected to be reduced. In addition to the three black funnel traps (**Figure 7**) a total of ten crayfish traps were deployed overnight for a period of approximately 20-24 hours in a stratified pattern across Phase 1A and retrieved the following day. Crayfish traps were deployed in May and October 2018 to obtain seasonal samples of demersal fishes utilizing SAV habitats (**Figure 8**).

Figure 8. A crayfish trap being prepared for deployment in May 2018.

Visual observations of large species of fish were also recorded throughout the study and recorded. These larger species are transient and typically not collected in the funnel, crayfish, or Breder traps. To supplement visual observations, a 30-meter bag seine was used in October 2018 to sample the shallow eel grass beds near Hunter Spring in the center of Phase 1A. Three seine hauls (approximately 50 meters in length) were conducted over shallow (≤ 1 meter) eel grass beds to the east and west of Hunter Springs Park (**Figure 9**).

Figure 9. Seining of shallow eel grass beds at Hunter Springs Park.

Submerged Aquatic Vegetation (SAV)

The relative cover and condition of *Vallisneria americana* plantings and other vascular plant species is an important part of the overall restoration project. SAV species composition, percent coverage, and estimated shoot density were assessed using a modified Braun-Blanquet coverage class method. A permanent transect was established in Phase 1A to represent restored conditions, marked with submerged PVC pipes and GPS located for long term monitoring and tracking. The transect consist of five (5) stratified 1 m^2 sampling plots in Phase 1A (**Figure 10**). For comparison, and additional five stratified 1 m^2 sampling plots were established along the canal connecting Phase 1A to the Pilot Project where restoration planting were established in the preceding year. SAV data from these plots were collected twice during the study period, May and October 2018 to represent seasonal variations in coverage and condition. Grazing pressure by manatees is known to be problematic and potentially a limiting factor for growth and recovery of *Vallisneria americana* in the King's Bay ecosystem without protection by exclosures (Hauxwell et al. 2004) Other known grazers include waterfowl, turtles, fishes, crabs, crayfish, and invasive non-native apple snails of the genus *Pomacea* (Johnson Eng. 2018).

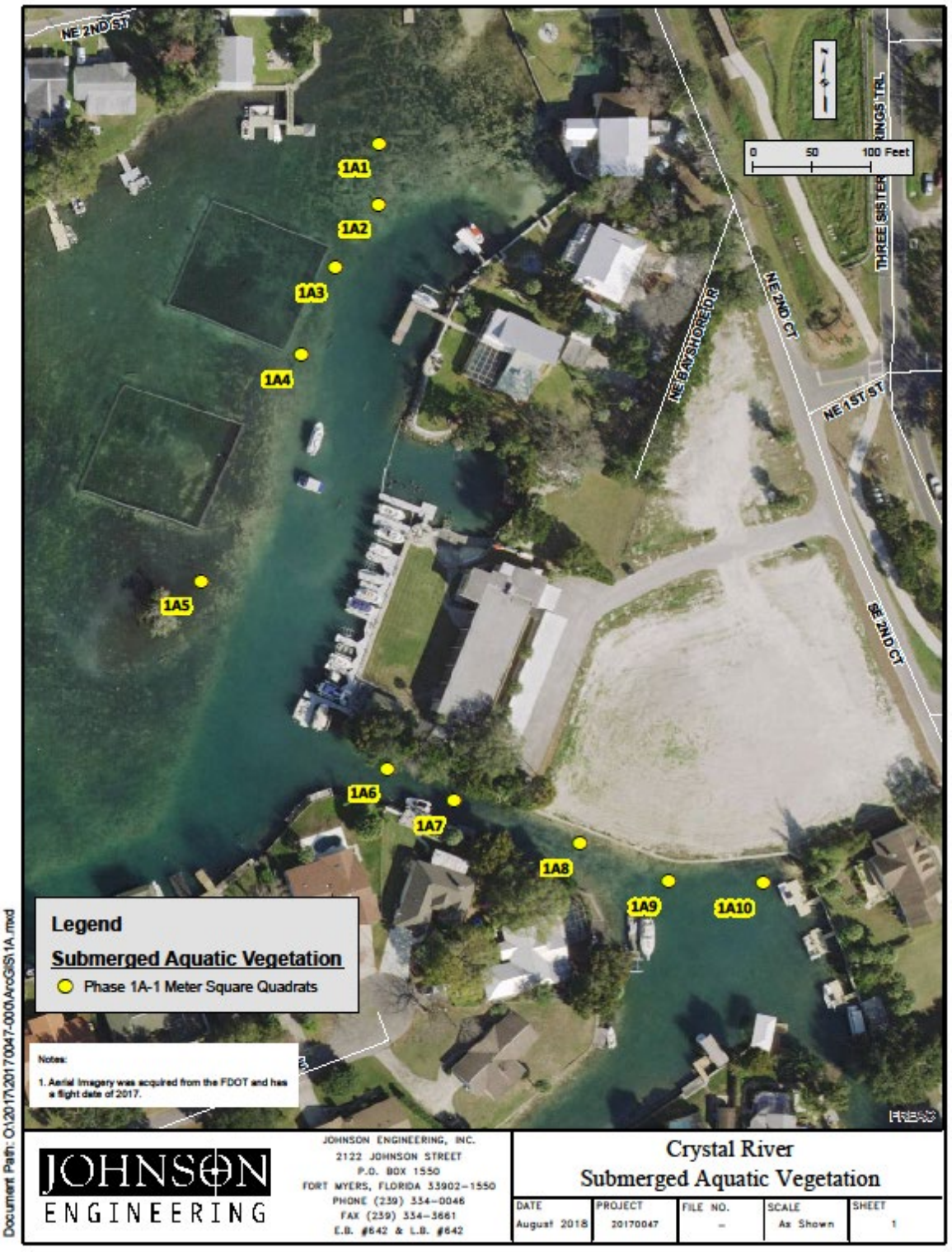

Figure 10. Submerged aquatic vegetation (SAV) monitoring transect and location of quadrats in Phase 1A and adjacent canal to Pilot Project area.

3.0 RESULTS

The results of this biological monitoring program are presenting in the following three sections beginning with the submerged aquatic vegetation (SAV) results from May 2018 and October 2018 sampling events. The restoration project is primarily about restoring functional benthic habitat and native aquatic vegetation (*Vallisneria americana*), which is the foundation of the ecosystem and serves as primary productivity for higher level consumers. This section is followed by the aquatic macroinvertebrate sampling results, and last but certainly not least, the fish sampling results.

Submerged Aquatic Vegetation

Results from the submerged aquatic vegetation transect surveys are summarized for the May 2018 sampling in **Table 1**. *Vallisneria* coverage in the Phase 1A transect ranged from 0 to 30% cover with an average (mean) cover of 12%. Short shoot counts ranged from 0 to 75 in the 25 cm^2 plots with an average of 32 shoots (512 shoots/m^2). Braun-Blanquet cover ranged from 0 to 3 with an average of 1.6. *Lyngbya* cover ranged from 0 to 70% cover with an average of 19%. Unidentified filamentous green algae was also present in two of the quadrats with an average of 19%. Other SAV species included *Hydrilla verticillata* (1%), *Najas* sp. (*guadalupensis*) (2%) and *Ruppia maritima* (2%).

In the Pilot Project canal directly east of, and connected to Phase 1A, the *Vallisneria* cover ranged from 16% to 85% with an average cover of 35%. *Vallisneria* short-shoot counts ranged from 32 to 150 shoots with an average of 76 shoots per 25cm^2 subplot. *Lyngbya* cover ranged from 0 to 17% with an average of 5%. Green algae was relatively abundant with cover ranging from 0 to 80% (average of 35%). *Hydrilla* was also present in three of the quadrats with an average cover of 5%. Braun-Blanquet cover classes for SAV ranged from 2 to 3 with an average of 2.4.

During the October SAV monitoring there was an obvious increase in SAV cover overall and dominated by *Vallisneria*. Female (pistillate) flowers were present to abundant in all quadrats that contained *Vallisneria* (**Figure 11**).

Table 1. Phase 1A Submerged Aquatic Vegetation Cover and Braun-Blanquet Cover Class; May 2, 2018

		Percent (%) Coverage by Species							
	Plot	**Vallisneria**	**Hydrilla**	**Najas**	**Ruppia**	**Lyngbya**	**Green Algae**	**Vallisneria SS**	**SAV Braun-Blanquet**
Phase 1A	1	**12**	1	3	0	0	40	12	**2**
	2	**17**	1	1	3	0	55	72	**2**
	3	**30**	1	3	3	12	0	75	**3**
	4	**0**	0	0	0	12	0	0	**0**
	5	**0**	0	2	5	70	0	0	**1**
Mean		**12**	1	2	2	19	19	32	**1.6**
Pilot Canal	6	**85**	8	0	0	7	3	150	**3**
	7	**30**	0	0	0	17	0	125	**2**
	8	**16**	0	0	0	0	22	37	**2**
	9	**30**	17	0	0	0	80	32	**3**
	10	**15**	2	0	0	0	20	36	**2**
Mean		**35**	5	0	0	5	25	76	**2.4**

Vallisneria SS = short shoot count in 1/4 meter square subsample

SAV Braun-Blanquet for vascular plant cover only

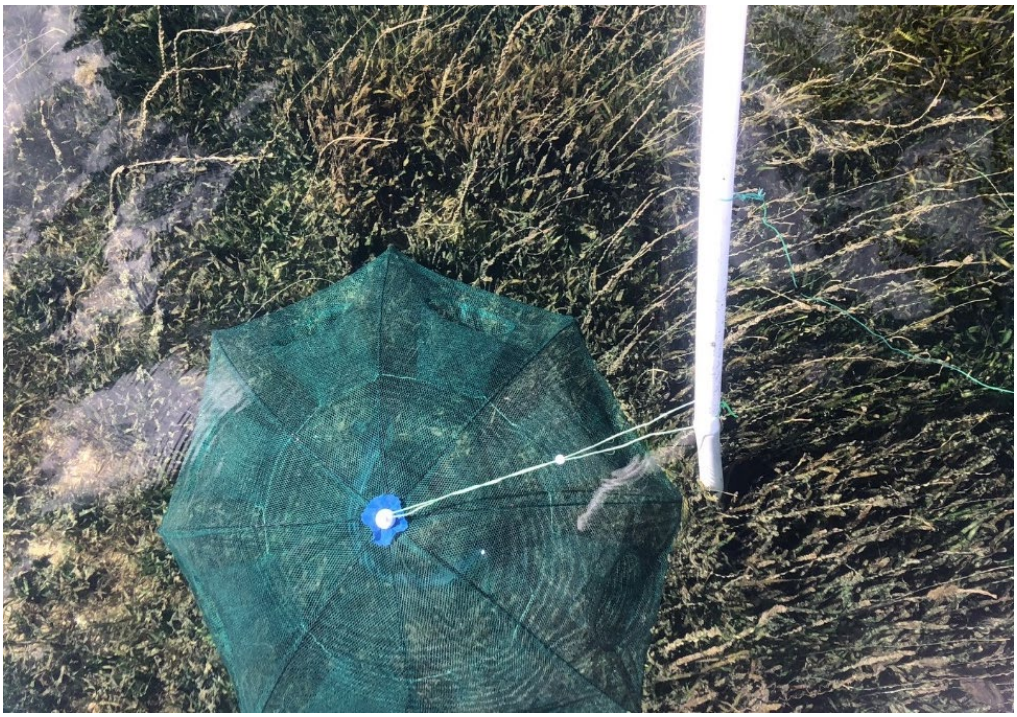

Figure 11. Dense *Vallisneria americana* beds documented during the October 2018 monitoring of SAV transect and fish communities. Note the abundance of female (pistillate) flower stalks.

In the Phase 1A quadrats, overall *Vallisneria* cover ranged from 0 to 80% cover and increased 31% from May 2018 to an average of 43% cover. *Vallisneria* short-shoot counts ranged from 0 to 280 shoots with an average of 98 shoots per 25cm^2 subplot to an average of 43% cover in October 2018 (**Table 2**). *Lyngbya* was also abundant, ranging from 0-100% cover with an average of 41%. Green algae was present in only two quadrats with an overall average 15%. Other SAV species included *Hydrilla* and *Najas* with average cover of 12% and 14% respectively. *Ruppia* was not found in any of the quadrats in Phase 1A during October, which may reflect seasonal changes in freshwater flow and salinity fluctuations. Braun-Blanquet cover for SAV ranged from 3 to 5 with an average of 4.2 (**Table 2**).

Table 2. Phase 1A Submerged Aquatic Vegetation Cover and Braun-Blanquet Cover Class; October 16, 2018

		Percent Coverage by Species							
	Plot	**Vallisneria**	**Hydrilla**	**Najas**	**Ruppia**	**Lyngbya**	**Green Algae**	**Vallisneria SS**	**SAV Braun-Blanquet**
Phase 1A	1	**60**	40	3	0	10	0	60	5
	2	**80**	10	20	0	0	20	280	5
	3	**75**	0	3	0	15	55	150	5
	4	**0**	5	25	0	80	0	0	3
	5	**0**	5	20	0	100	0	0	3
Mean		**43**	12	14	0	41	15	98	**4.2**
Pilot Canal	6	**60**	0	0	0	45	0	100	4
	7	**90**	0	0	0	20	0	300	5
	8	**100**	0	0	0	0	15	300	5
	9	**95**	0	0	0	0	25	300	5
	10	**100**	0	0	0	0	25	300	5
Mean		**89**	0	0	0	13	13	260	**4.80**

Vallisneria SS = short shoot count in 1/4 meter square subsample

SAV Braun-Blanquet for vascular plant cover only

In the east-west canal connecting Phase 1A to the Pilot Project areas *Vallisneria* cover more than doubled from the May sampling event. *Vallisneria* cover ranged from 60% to 100% with an average of 89% cover. Short shoot counts were too high (≥ 300) in four of the subplots to get precise numbers and so a best estimate of 300 shoots was recorded which averaged out to 260 shoots per $25cm^2$ (**Figure 11**). There were no other SAV species observed in the five quadrats. Braun-Blanquet cover class for *Vallisneria* alone ranged from 4 to 5 with an average of 4.8. *Lyngbya* was present in two quadrats with overall average of 13% cover. Green algae was present in three quadrats and also averaged 13% cover.

Macroinvertebrate Communities

Macroinvertebrate communities were assessed from three Hester-Dendy substrates, three petite Ponar grabs and qualitative dip net samples collected from Phase 1A habitats (**Figure 2**). The following results begin with univariate statistics and diversity metrics followed by multivariate community analyses for comparisons between sampling methods and comparisons with time-zero restoration samples and unrestored habitat samples collected in 2017.

Univariate Analyses

In Phase 1A, a total of 1,461 macroinvertebrate were collected and identified, representing five (5) classes, 14 orders, and 25 families and at least 42 species. A complete list of species collected from all sampling methods in 2018 is included in **Appendix A**. Univariate diversity metrics were calculated for each sample collected and include species richness (S), abundance (N), Margalef richness (d), Pielou's evenness (J'), Shannon diversity (H') and Simpson's index (1-Lambda). The results of the univariate diversity analysis are presented in **Table 3**.

Table 3. Univariate diversity metrics for Phase 1A samples from 2018 data and unrestored "control' samples collected and identified in 2017. [Species richness (S), abundance (N), Margalef richness (d), Pielou's evenness (J'), Shannon diversity (H')]

Sample	S	N	d	J'	H'(loge)
Phase 1A Ponars	14	142	2.62	0.5205	1.37
Phase 1A HDs	21	**570**	3.15	0.4725	1.44
Ponar control	9	29	2.38	0.8454	1.86
HD control	19	246	3.27	0.7208	2.12
D-net	**22**	99	**4.57**	0.8386	**2.59**
D-net control	14	72	3.04	0.8776	2.32

Highest values for each metric are shown in bold font while lowest values are underlined. The highest species richness of 22 taxa was found in the qualitative dip net sampling conducted in restored habitat followed closely by 21 taxa collected from Hester-Dendy substrates deployed in Phase 1A. Species richness (9) and Margalef richness (2.38) was lowest in the benthic samples collected by petite Ponar (Ponar control) from unrestored benthic habitats of Canal 7. The highest abundance (570 organisms) was found in composited Ponar samples from restored habitat of Phase 1A. Pielou's eveness (J') is an indication of how equal each species abundance is in a sample but has very limited value ecologically. Since each sampling method has a unique bias, it is best to compare samples collected by the same method from control and restored site samples. In this case, there is an increase from nine (9) species to 14 species in Ponar samples collected from control and restored sites. There is a corresponding increase in Hester-Dendy species from control sites (19 taxa) to restored sites (21 taxa) and from dip-net control (14 taxa) to dip net restored (22 taxa). Shannon diversity (H') is generally considered as a standard measure of macroinvertebrate diversity and is codified in Florida Statutes (FAC Chapter 62-302) for the protection of surface waters from degradation (FDEP 2010). Shannon diversity was highest (2.59) in the dip net sample collected from restored habitat. Quantitative Hester-Dendy and Ponar samples along with qualitative dip net sample results are summarized in **Table M1**.

Table M1. Aquatic macroinvertebrates collected and identified from Phase 1A restored habitat.							
Taxa	**Order**	**Family**	**Genus species**	**Ponars**	**HDs**	**Ponar +HD**	**D-net Qual**
Annelida	Rhyncobdellida	Hirudinea	*Gloiobdella elongata*	3	9	12	2
			Helobdella stagnalis	2	5	7	5
			Myzobdella sp.	0	0	0	0
	Oligochaeta	Naididae	*Limnodrilus hoffmeisteri*	18	0	18	0
			L. claparedeianus	0	0	0	0
			Eclipidrilus sp.	1	0	1	0
			Ilyodrilus tempeltoni	0	0	0	0
			Nais variabilis	0	0	0	0
		Lumbriculidae	*Lumbriculidae*	0	0	0	0
Nematoda	Nematoda	Nematoda	*Nematoda*	0	0	0	0
Platyhelminthes	Tricladida	Planariidae	*Planariidae*	0	0	0	1
Crustacea	Amphipoda	Talitridae	*Hyalella azteca grp.*	93	319	412	17
		Gammaridae	*Gammarus mucronotus*	0	9	9	0
			Crangonyx sp.	0	0	0	0
		Aoridae	*Grandidierella bonnierodes*	0	0	0	0
	Isopoda	Sphaeromidae	*Cassidinidea ovalis*	0	104	104	3
		Anthuridae	*Cyathura polita*	3	0	3	0
		Asellidae	*Caecidotea sp.* *	0	1	1	7
	Tanaidacea	Leptocheliidae	*Hargeria rapax*	0	0	0	0
	Mysida	Mysidacae	*Taphromysis lousianae*	0	0	0	16
	Decopoda	Panopeidae	*Rhithropanopeus harisii*	0	0	0	0
Mollusca	Gastropoda	Thiaridae	*Melanoides tuberculata*	2	1	3	9
		Physidae	*Haitia (Physa) cubensis*	0	0	0	14
		Planorbidae	*Planorbella duryi*	0	0	0	5
			Planorbella scalaris	0	0	0	0
			Gyralus parvus	3	0	3	0
			Micromenetus floridensis	0	0	0	0
		Hydrobiidae	*Hydrobiid sp. A*	0	1	1	0
			Pyrogophorus platyrhicus	3	2	5	1
		Amnicolidae	*Amnicola dalli*	0	0	0	0
		Ancylidae	*Hebetancylus excentricus*	0	0	0	0
	Pelecypoda	Sphaeriidae	*Musculium (lacustre)*	0	0	0	3
			Eupera cubensis	0	0	0	0
		Corbiculidae	*Corbicula fluminea*	0	0	0	3

Table M1. Aquatic macroinvertebrates collected and identified from Phase 1A restored habitat.							
Taxa	**Order**	**Family**	**Genus species**	**Ponars**	**HDs**	**Ponar +HD**	**D-net Qual**
Insecta	Odonata	Libelullidae	*Epicordulia princeps*	0	0	0	0
			Libellula auripennis	0	0	0	1
		Gomphidae	*Aphylla williamsoni*	0	0	0	1
		Coenagrionidae	*Enallagma sp.*	0	0	0	1
			Ischnura hatata	0	1	1	0
			Ischnura ramburii	0	0	0	1
	Ephemeroptera	Baetidae	*Callibaetis sp.*	0	0	0	0
		Caenidae	*Caenis sp.*	0	3	3	0
	Heteroptera	Corixidae	*Trichocorixa sp.*	0	0	0	1
			Synaptonecta issa	0	0	0	1
		Gerridae	*Neogerris hesione*	0	0	0	1
	Tricoptera	Polycentropidae	*Cyrnellus fraternus*	0	0	0	0
		Hydroptilidae	*Orthotrichia sp.*	0	8	8	0
			Oxythira sp.	0	0	0	0
		Leptoceridae	*Triaenodes sp.* *	0	0	0	0
	Coleoptera	Haliplidae	*Peltodytes sp.*	0	0	0	5
		Elmidae	*Stenelmis sp.*	0	0	0	1
	Diptera	Chironomidae	*Ablabesmyia rhamphe grp.*	0	3	3	0
			Chironominae	0	0	0	0
			Cryptochironomus sp.	1	0	1	0
			Beardius truncatus	0	4	4	0
			Glyptotendipes meridionalis	0	0	0	0
			Asheum beckae	0	0	0	0
			Chironomus decorus grp.	0	0	0	0
			Procladius sp. I Rutter	0	0	0	0
			Einfeldia natchitocheae	0	0	0	0
			Dicrotendipes modestus	4	86	90	0
			Dicrotendipes neomodestus	1	5	6	0
			Dicrotendipes simpsoni	0	0	0	0
			Paralauterborniella nigrohalteralis	7	0	7	0
			Psuedochironomus sp.	0	2	2	0
			Polypedilum bekae	0	2	2	0
			Polypedilum scalaenum grp.	0	0	0	0
			Goeldichironomus cf. natans	0	0	0	0
			Tanytarsus sp. K	0	0	0	0
			Tanytarsus sp. g complex	0	0	0	0
			Tanytarsus buckleyi	0	1	1	0
			Tanytarsus hastatus	0	0	0	0
			Clinotanypus sp.	1	0	1	0
			Corynoneura sp.	0	3	3	0
			Cricotopus sp.	0	1	1	0
			Species Richness	**14**	**21**	**28**	**22**

Multivariate Analyses

While univariate diversity metrics have value they also have severe limitations for assessing ecosystem level responses to disturbances or restoration activities by simplifying the complex trophic-level interactions into a single number. For this reason, it is preferable to evaluate the community-level response using multivariate statistical tools that are better suited for complex data sets and can help to identify trajectories of change (temporally and spatially) and identify indicator species of restoration success. The MS Excel ™ software program was used to enter and manage the macroinvertebrate database (and SAV and fish data) and Primer v6 (Clarke and Gorley 2006) was used to conduct the multivariate analyses. Data were square root transformed to down-weight the importance of very abundant taxa prior to analyses. Bray-Curtis similarity was used to compare macroinvertebrate communities from restored and unrestored habitats and for each of the sampling methods. Similarity percentage tests (SIMPER) were used to identify species that were most important in contributing to the similarity within groups and dissimilarity between restored and unrestored communities. Multi-dimensional scaling ordinations and hierarchical agglomerative cluster analysis with similarity profile (SIMPROF) significance test were applied to the Bray-Curtis similarity results to visually display community structure and identify significance, respectively. Results of the SIMPER analysis are included in **Appendix A**.

Figure M1 represents a hierarchical agglomerative cluster analysis based on Bray-Curtis similarity of samples collected by Hester-Dendy substrate, petite Ponar grabs, and qualitative dip net from samples from control (unrestored) time-zero (in process 2017) and restored Phase 1A (2018). In this case, individual Hester-Dendy and Ponar samples from 2018 were compared with composite samples of all three along with composite samples from the control site and time-zero conditions of Phase 1A. Dip net samples from control and restored conditions were also included to assess similarity in community structure between sampling methods and treatments (control, time-zero restored. Dip net samples from control and restored treatments group closely together and were significantly different ($p<0.05$) from other methods. This is likely due to the fact that dip net samples were taken from shallow shoreline habitats (littoral zone) that receive plenty of sunlight, have more diversified habitat and are well oxygenated, thus providing more suitable habitat for most aquatic organisms. Control Hester-Dendy and control Ponar samples group together (albeit with low similarity) and significantly different than all other samples. Restored Hester-Dendy sample HD2 grouped in with the restored Ponar samples. This is explained by the fact that HD2

was deployed in an area approximately 4 meters deep (**Figure 2**). The water pressure at this depth slowly caused the floatation device to compress during the 28-day colonization period and the substrate sank to rest on the bay bottom where it was colonized by benthic fauna, similar to those collected by Ponar samples. When the qualitative dip net samples were removed from the Bray-Curtis similarity matrix and cluster analysis, there emerged some separation of HD2 from the restored Ponar samples. However, HD2 remained significantly different from the restored HD samples HD1, HD2 and the composite of all three (**Figure M2**). Significant groupings ($p<0.05$) are illustrated by the black lines in the cluster analysis and control sites remain significantly different from both time-zero samples and all restored samples which clearly indicates that the aquatic macroinvertebrate communities are changing through time and in response to habitat restoration activities.

To further investigate the changes in macroinvertebrate community, samples were analyzed separately by sampling method beginning with the petite Ponar samples from restored conditions in 2018, time-zero condition in 2017 and unrestored habitat in 2017. A cluster analysis of Ponar macroinvertebrate samples identified three significantly different groups (**Figure M3**). All restored Ponar samples showed 50% similarity and were significantly different than control (unrestored) and time-zero samples. This is also illustrated in the 2-dimensional MDS ordination plot where the distance between points on the plot represents relative dissimilarity in community structure with an overlay of the significant groupings from the cluster analysis and SIMPROF test (**Figure M4**). Petite Ponar samples clearly show that macroinvertebrate community structure is changing in response to restoration through time. However, Ponar sample collection and processing is labor intensive and samples consist primarily of small aquatic fauna like amphipods, leeches, worms and midge larvae. Taxonomic expertise, slide mounts, and compound microscopes are required to identify midges and worms which is time consuming and expensive. It is not a rapid biological assessment tool but may be appropriate for documenting baseline and post restoration conditions for benthic habitat restoration projects.

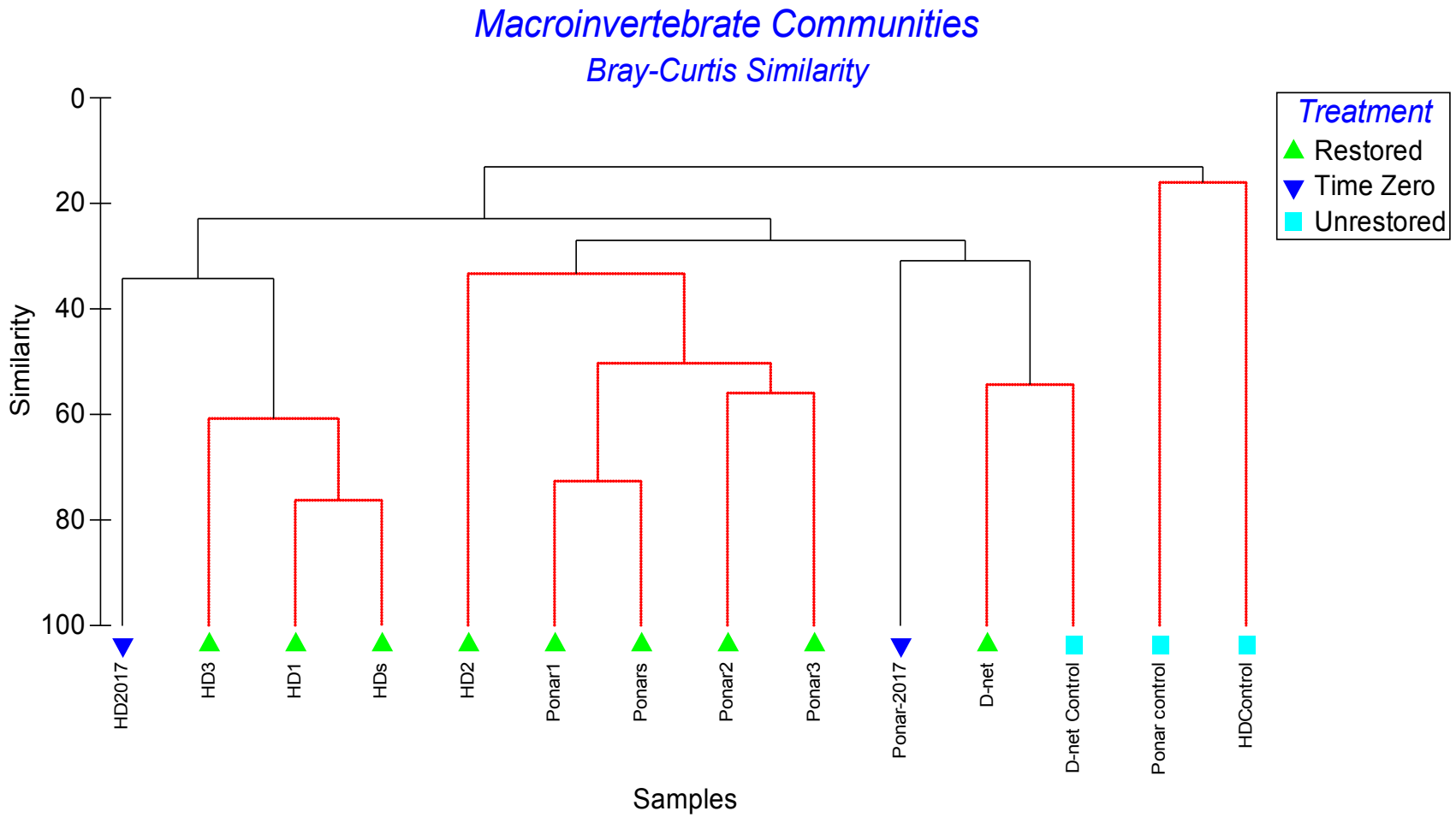

Figure M1. Macroinvertebrate community comparison between Phase 1A restored (2018), Phase 1A time-zero (2017), and unrestored habitats in Kings Bay, Citrus County Florida. Black lines indicate significant groupings ($p<0.05$) using SIMPROF test while red lines indicate no significant difference at 95% confidence level. Note restored HD2 groups with restored Ponar sediment samples. HD2 was deployed at depth of 12 feet with foam float that gradually compressed (due to water pressure over 28 day colonization period) causing HD substrate to sink to bottom where it was colonized by benthic invertebrates found in the sediments. Restored communities were significantly different ($p<0.05$) from unrestored and time-zero communities except for D-net samples.

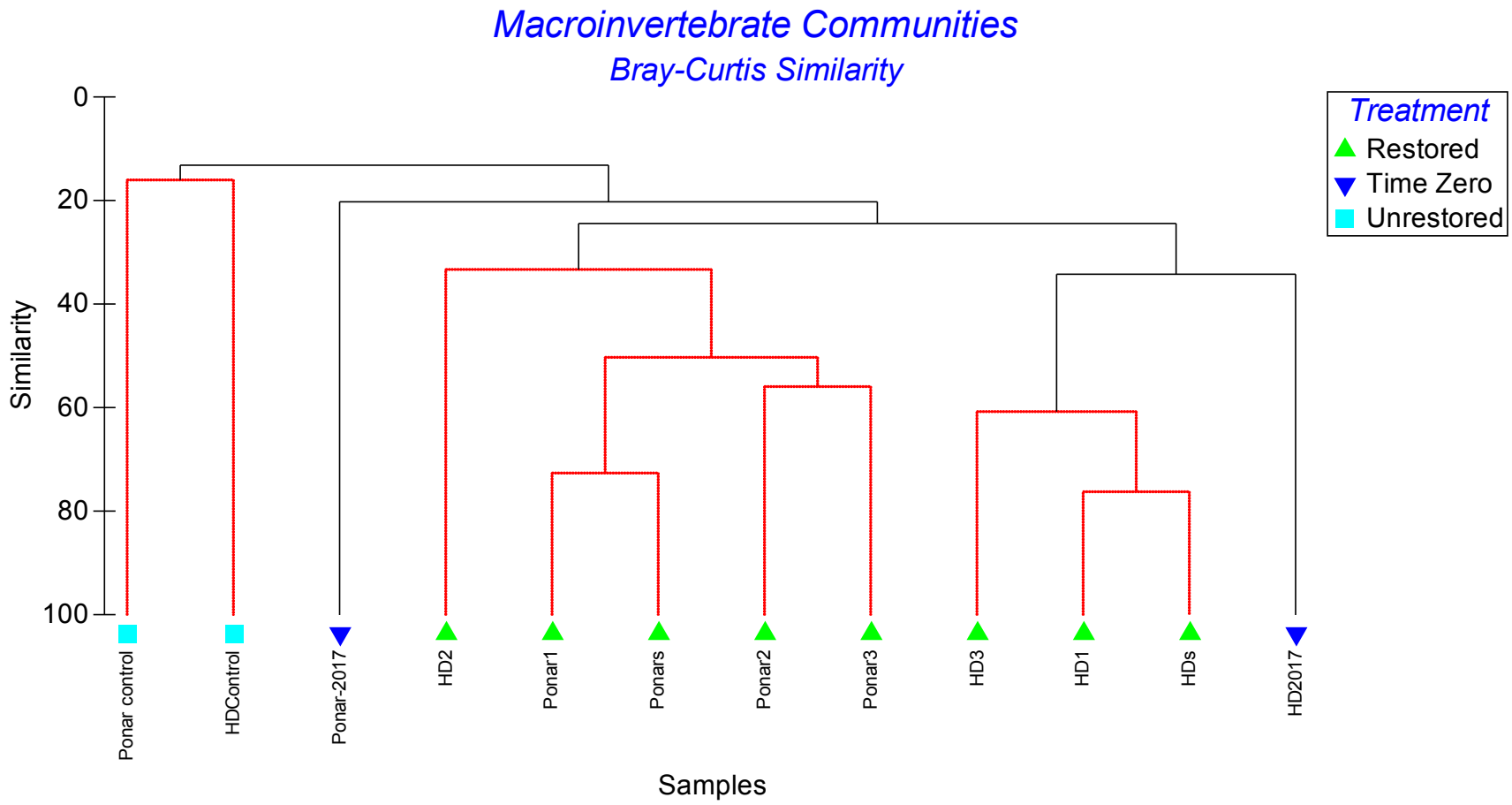

Figure M2. Macroinvertebrate community comparison between Phase 1A restored (2018), Phase 1A time-zero (2017), and unrestored habitats in Kings Bay, Citrus County Florida. Black lines indicate significant groupings ($p<0.05$) using SIMPROF test while red lines indicate no significant difference at 95% confidence level. Note restored HD2 groups with restored Ponar sediment samples as mentioned in Figure M1. With D-net samples removed, Restored communities were significantly different ($p<0.05$) from both methods of time-zero communities and unrestored (controls), while time-zero Ponars and time-zero HDs were significantly different from each other and unrestored communities (Ponars and HDs).

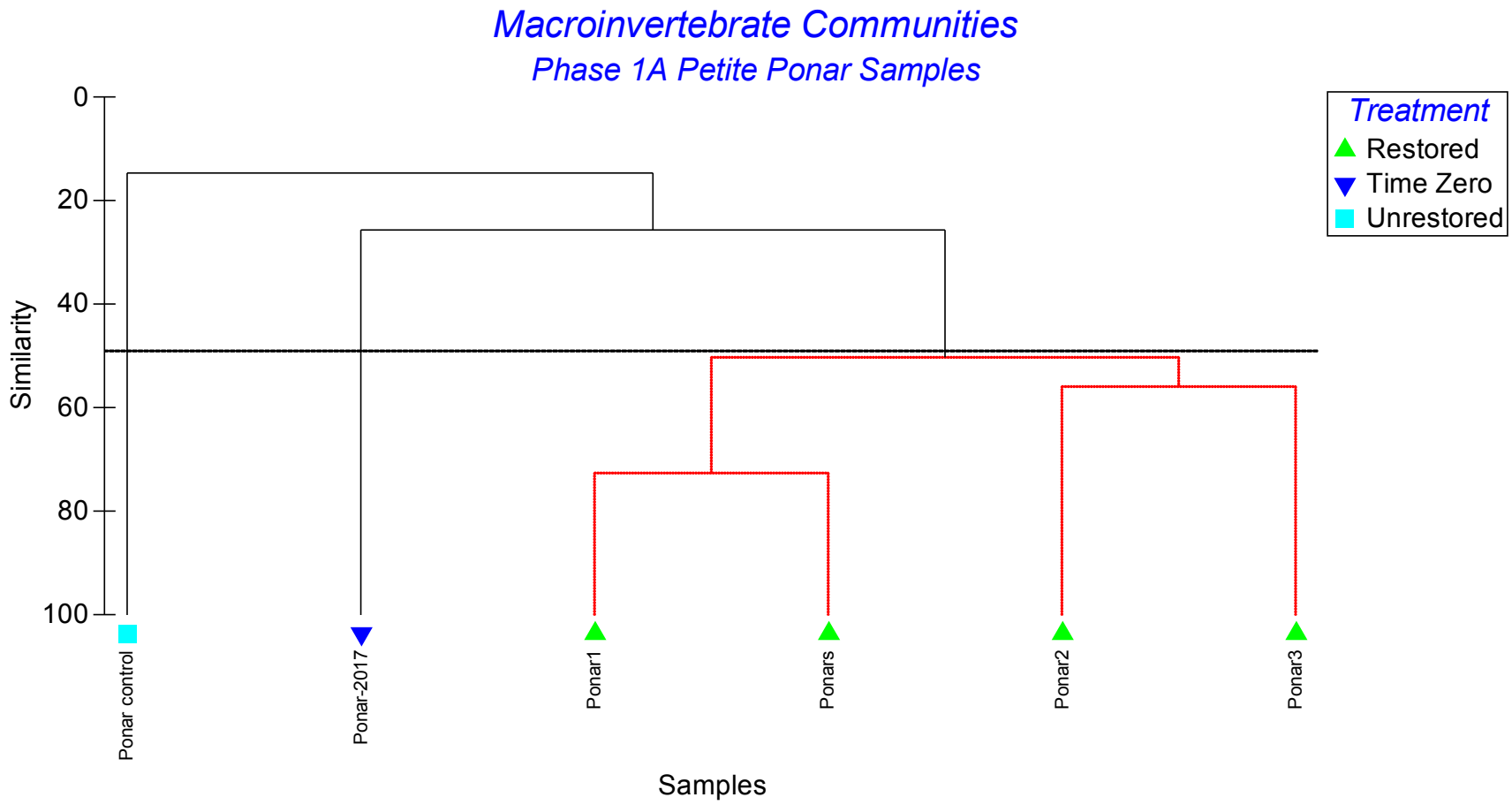

Figure M3. Ponar sample comparison within 2018 samples (HD1, HD2, HD3 and combined HDs) and between unrestored habitat and Phase 1A time-zero (2107) and restored (2018) habitat. Restored sample replicates were not significantly different ($p<0.05$) from each other with slice at 50% similarity indicating significant grouping. Restored, time-zero and unrestored communities were significantly different from each other indicating a significant change through time in benthic community structure post-restoration.

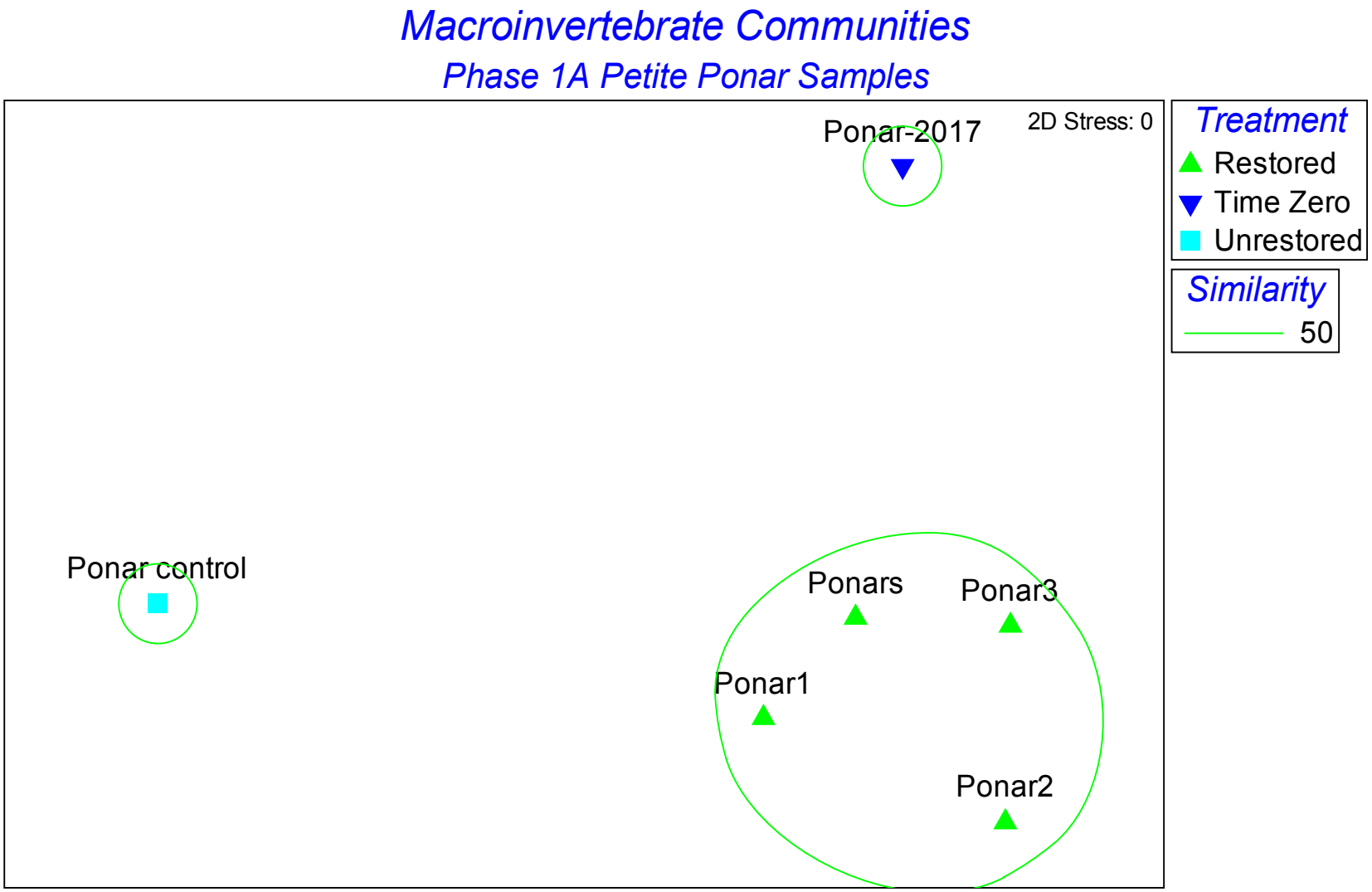

Figure M4. MDS ordination of petite Ponar samples with overlay of significant groups from SIMPROF test in cluster analysis showing separation of unrestored, time-zero and restored benthic macroinvertebrate communities. Note 2-Dimensional Stress of 0.00 indicates an excellent ordination with no prospect of misleading interpretation of the ordination based on Bray-Curtis similarity.

Hester-Dendy substrate samples were similarly analyzed using Bray-Curtis similarity, cluster analysis with SIMPROF significance test, followed by MDS ordinations. **Figure M5** is a cluster analysis that identified significant groups. As mentioned previously, the HD2 sample was an outlier due to equipment failure causing the substrate to sink down and rest on the sediments. The remaining HD1 and HD3 substrates, and the composite sample, HDS all group together at >60% similarity and were significantly different from the control and time-zero composite Hester-Dendy samples. The 2 dimensional MDS ordination shows the clear separation between treatments (with the exception of outlier HD2) and the overlay of significant groups from the SIMPROF test at 60% similarity. With a Stress level of 0.00, the MDS represents an excellent ordination with no real prospect of misinterpreting the results (Clarke and Gorley 2006). What the MDS also shows is a directional vector of change in community structure from control (unrestored) to time-zero to restored conditions represented by the arrow overlaid on the graph (**Figure M6**).

Hester-Dendy substrates were an effective method for sampling aquatic fauna and results demonstrated clear differences in macroinvertebrate community structure between restored, time-zero and restored habitats. Hester-Dendy substrates have limitation since they are artificial habitat that may have a sampling bias. They are best suited for freshwater systems where water quality is the primary concern. In brackish or marine environments they become encrusted by barnicles and do not function well for assessing diversity. They require a minimum of 4 weeks deployment for colonization by aquatic macroinvertebrates, followed by processing, sorting fauna, and laboratory identification using similar techniques used for Ponar samples. They are not a rapid bioassessment and can be time consuming and expensive to deploy, process, sort and identify taxa.

In order to better understand what organisms are driving the major changes in macroinvertebrate community structure, the similarity percentage (SIMPER) test was applied to square root transformed data. The composited data from Hester-Dendy and Ponar samples from restored and unrestored areas were compared to each other. The results of the SIMPER test identify the most important taxa contributing to the similarity within a group of samples and the dissimilarity between groups. In this case, restored and unrestored macroinvertebrate samples were compared in an effort to identify indicator taxa of restored habitat conditions and possibly some indicator taxa of degradation or habitat impairment.

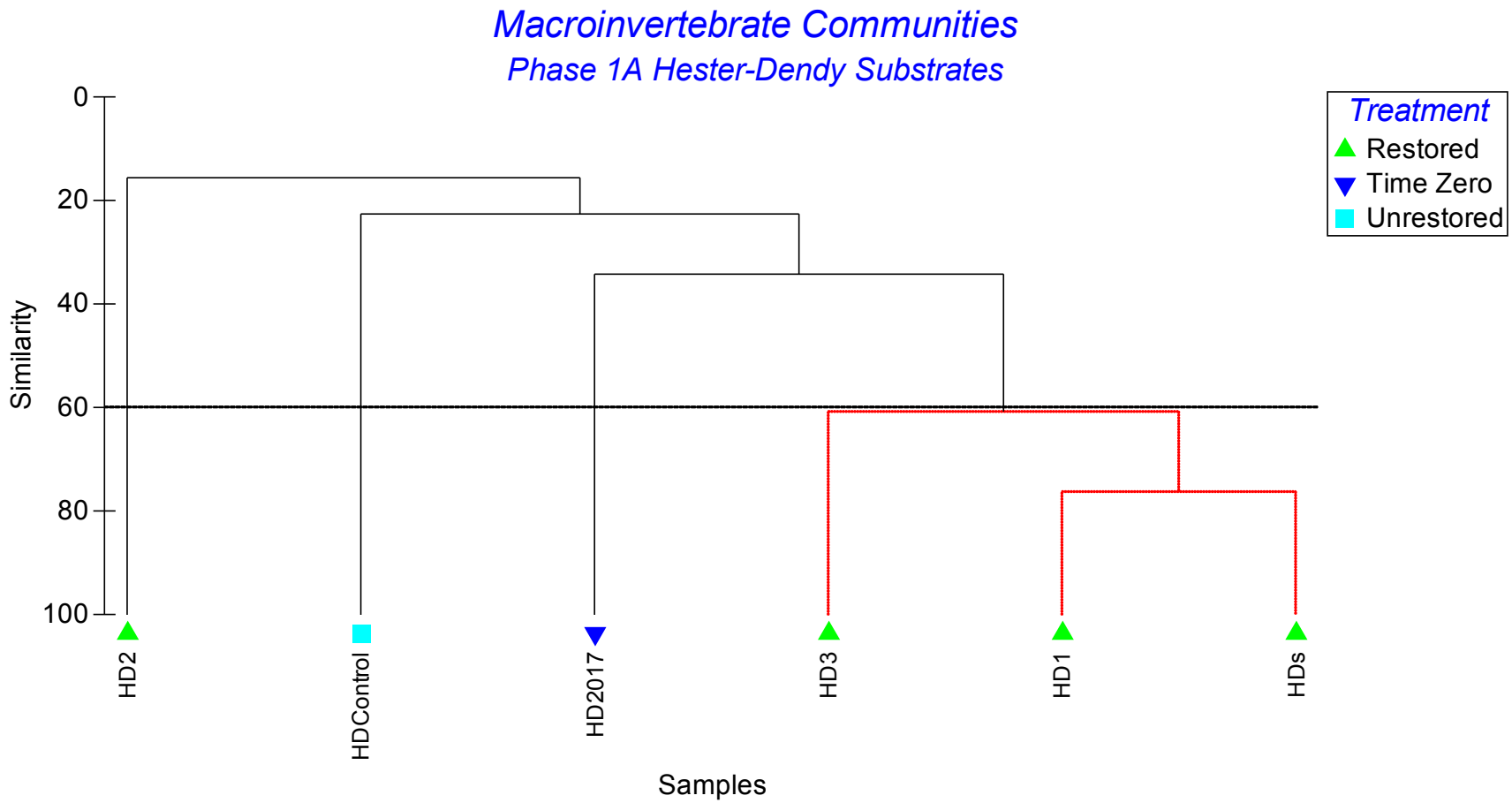

Figure M5. Hester-Dendy (HD) sample comparison within 2018 samples (HD1, HD2, HD3 and combined HDs) and between unrestored (control) habitat and Phase 1A time-zero (2107) and restored (2018) samples. Restored HD sample replicates, except HD2 were not significantly different ($p<0.05$) from each other with slice at 60% similarity indicating significant grouping. HD2 was deployed at depth and float compressed over time causing HD substrate to lie on the bottom and become colonized by benthic community from sediment. Restored, time-zero and unrestored communities were significantly different from each other indicating a significant change through time in benthic community structure post-restoration.

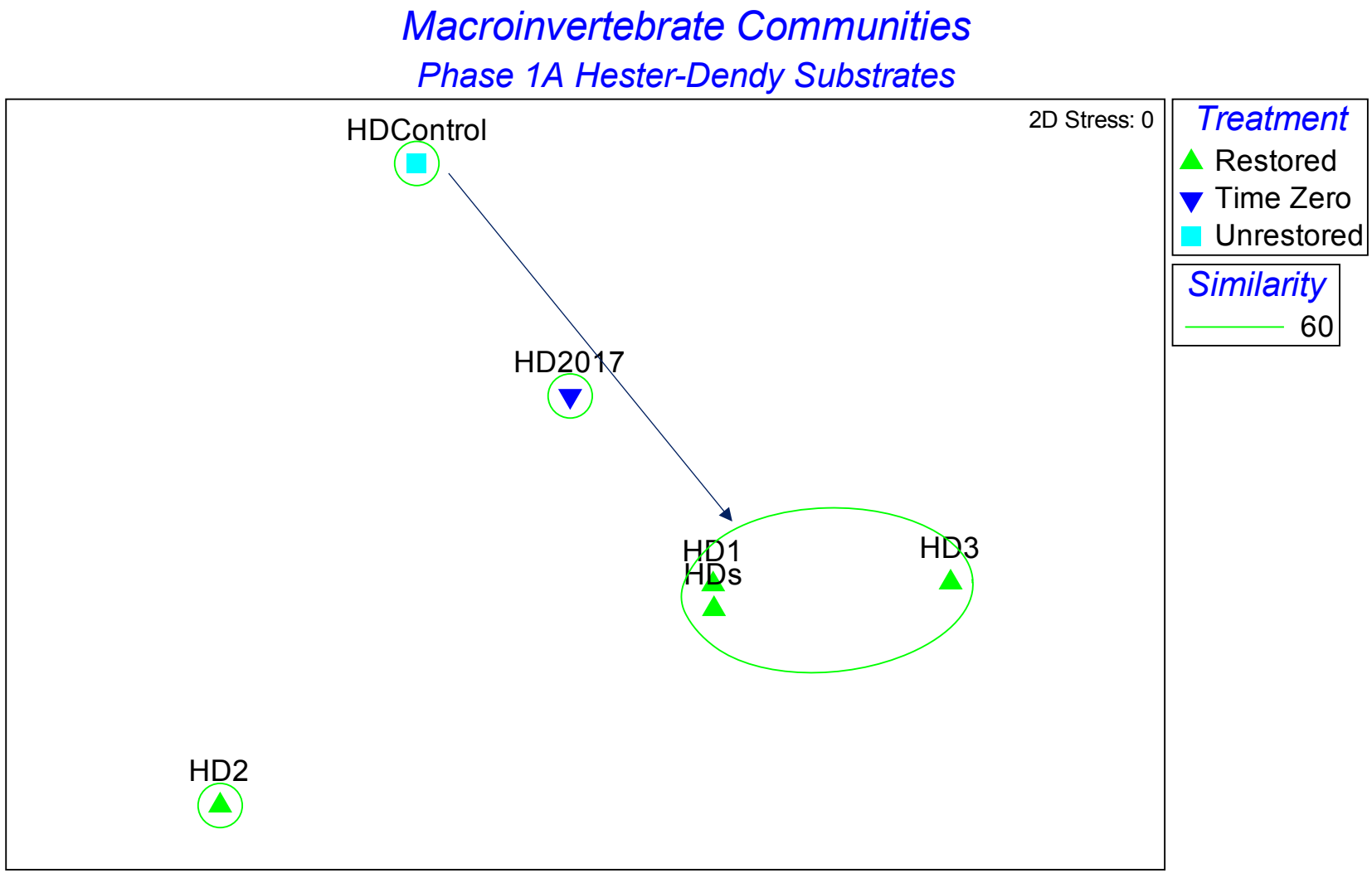

Figure M6. MDS ordination of Hester-Dendy macroinvertebrate samples with overlay of significant groupings from the SIMPROF test from the cluster analysis. The 2-Dimensional Stress of 0.00 indicates an excellent ordination with no real prospect of a misleading interpretation based on Bray-Curtis similarity. Vectors of change (arrow) in community structure from unrestored to time-zero to restored conditions are evident from the MDS.

All quantitative (HD and Ponar) samples from restored sites in Phase 1A were compared with the same combined samples from unrestored habitat in canals collected in 2017. Overall, macroinvertebrate communities from restored Phase 1A were 83.9% dissimilar to macroinvertebrate communities from unrestored habitats. Several important indicator taxa were present in the restored areas that were absent entirely or found in very low numbers from unrestored (control) sites. The most important species responsible for separating restored and unrestored sites were the amphipod *Hyalella azteca* grp. which was responsible for 19.7% of the dissimilarity. *Hyalella azteca* was extremely abundant in restored habitat samples but almost absent in unrestored habitat samples. Conversely, the amphipod *Gammarus mucronotus* was abundant in unrestored habitats but relatively uncommon in restored areas and contributed to 4.1% of the dissimilarity. Other important taxa that were much more abundant in restored habitats and major contributors to the dissimilarity were the isopod *Cassidinidea ovalis* (6.7%), the midge *Dicrotendipes modestus* (6.2%), the snail *Pyrogophorus platyrhicus* (6.1%) and the oligochaete worm *Limnodrilus hoffmeisteri* (3.9%). Several other species may also serve as indicators of restoration success since they were absent in unrestored habitat but present or common in restored areas of Phase 1A. These include; the midges *Paralauterborniella nigrohalteralis*, *Beardius truncatus,* and *Cryptochironomus* sp., the snails *Melanoides tuberculata* and *Gyralus parvus*, the mayfly *Caenis* sp., and caddisfly *Orthotrichia* sp.. The life history requirements of each of these taxa apparently were not being met in *Lyngbya* dominated system with a layer of anoxic flocculent organic covering the bay bottom. For example, the anthurid isopod, *Cyathura polita* burrows into sand and mud sediments of oligohaline and mesohaline habitats and requires two years to complete its life cycle. This species often occurs in large numbers, is an important part of the food chain (Heard 1982) and was only found in restored habitats of Phase 1A (**Appendix A**). *Cyathura polita* is a clear indicator of restoration success and contributed 1.7% to the dissimilarity between restored and unrestored habitats.

Some potential indicators of a degraded habitat dominated by *Lyngbya* include the following that were present in unrestored habitats but absent in the restored samples from Phase 1A; the pond snail *Haitia (Physa) cubensis*, the amphipod *Grandidierella bonnierodes*, midges *Einfeldia natchitocheae* and *Procladius* sp. I Rutter, and few other species. The complete SIMPER analysis and species contributions are included in **Appendix A**. In summary, SIMPER analysis serves as a quantitative measure of species contributions to the differences between restored and unrestored

habitats which is important benchmark for assessment. Of equal importance is understanding the life history requirements of all macroinvertebrates, including uncommon or rare species which may only be found in healthy ecosystems and are significant ecological indicators.

Fish Communities

A total of species of fishes were collected and/or visually identified in the Baseline Biological Assessment. A total of 18 fish species were documented during the Phase 1A sampling events combined. This includes 11 families and 15 genera **(Table F1).** A total of 11 species were documented in the May 2018 sampling events. A total of sixteen species were documented were collected or visually documented in the October 2018 sampling events. The most abundant species overall, in rank order were bluefin killifish (*Lucania goodei*, 449), rainwater killifish (*L. parva*, 163), largemouth bass (*Micropterus salmoides*, 74), spotted sunfish (*Lepomis punctatus*, 49), striped mullet (*Mugil cephalus*, 47), tidewater mojarra (*Eucinostomus argenteus*, 42), Atlantic needlefish (*Stongylura marina*, 29) and bluegill (*Lepomis macrochirus*, 26). There were some shifts in species presence and community structure from May to October, especially in the centrarchid (sunfishes) family. Only bluegills and largemouth bass were collected or visually documented in the May sampling events and most of them were young of the year.

In October sampling, the bluegills decreased in abundance overall while three new sunfishes appeared, including the spotted sunfish (49), redear sunfish (*Lepomis microlophus*, 7), and the warmouth (*Lepomis gulosus*, 2). The appearance of these new sunfish species is a positive indicator of a more diverse and healthy aquatic ecosystem. Redear sunfish are also known as shellcrackers for their preference for feeding on snails and their presence indicates a thriving snail population exists in Phase 1A, thus providing a trophic link between fishes the algal epiphytes found in *Vallisneria* beds. The "warmouth" is true to its name as a predator of smaller fishes and aquatic insects. The spotted sunfish inhabits pools of creeks, and small to large rivers, ponds, lakes, and swamps (Robins et al. 2018) but is also known from artificial canals, borrow pits, and drainage ditches (Ceilley 2008). Redear, warmouth, and spotted sunfish are considered as sportfish, along with bluegill and largemouth bass. During the May 2018 sampling events, there were many young of the year largemouth bass. Since largemouth bass nest only on the bottom in well oxygenated hard bottom or sandy substrates, the abundance of juveniles is an excellent indication that successful reproduction is occurring in the immediate area of Phase 1A.

Table F1. Phase 1A Fish Survey Results for Breder Trap, Crayfish Traps, Umbrella Traps and Visual Surveys

				May 2-31, 2018					October 15-16, 2018					
Family	**Genus**	**Species**	**Common Name**	**Funnel**	**Breder**	**Crayfish**	**Visual**	**Total**	**Funnel**	**Breder**	**Crayfish**	**Visual**	**Seine**	**Total**
Lepisosteidae	*Lepisosteus*	*osseus*	Longnose Gar				1	**1**						**0**
Poeciliidae	*Heterandria*	*formosa*	Least Killifish					**0**						**0**
	Gambusia	*holbrooki*	Eastern Mosquitofish		7	1		**8**		5				**5**
Fundulidae	*Lucania*	*goodei*	Bluefin Killifish	75	4	193		**272**	20	8	148		1	**177**
	Lucania	*parva*	Rainwater Killifish	2	73	55		**130**	2	1	29		1	**33**
	Fundulus	*seminolis*	Seminole Killifish					**0**						**0**
	Fundulus	*grandis*	Gulf Killifish					**0**					4	**4**
Cyprinodontid	*Cyprinodon*	*variegatus*	Sheepshead Minnow										2	**2**
Centrarchidae	*Lepomis*	*punctatus*	Spotted Sunfish					**0**	12		37			**49**
	Lepomis	*macrochirus*	Bluegill				25	**25**			1			**1**
	Lepomis	*microlophus*	Redear Sunfish					**0**	2		5			**7**
	Lepomis	*gulosus*	Warmouth					**0**	1	1				**2**
	Micropterus	*salmoides*	Largemouth Bass	3		10	50	**63**				10	1	**11**
Centropomida	*Centropomus*	*undecimalis*	Common Snook				2	**2**						**0**
Ariidae	*Ariopsis*	*felis*	Hardhead Catfish				0	**0**			5			**5**
Gerridae	*Eucinostomus*	*argenteus*	Tidewater Mojarra					**0**			10		32	**42**
Gobiidae	*Gobiosoma*	*bosci*	Naked Goby					**0**						**0**
Mugilidae	*Mugil*	*cephalus*	Striped Mullet				25	**25**				22		**22**
Soleidae	*Trinectes*	*maculatus*	Hogchoker			2		**2**			7			**7**
Belonidae	*Strongylura*	*marina*	Atlantic Needlefish				10	**10**				16	3	**19**
Sparidae	*Archosargus*	*probatocephalus*	Sheepshead				10	**10**				4		**4**
Crustaceans														
Palaemonidae	*Palaemonetes*	sp.	Grass Shrimp			10					8			
Cambaridae	*Procambarus*	sp.	Crayfish			1					1			
Portunidae	*Callinectus*	*sapidus*	Blue Crab			3					4		1	
Reptiles														
Kinosternidae	*Sternothorus*	*odoratus*	Musk Turtle			2					5			

Univariate Analyses

Table F2 represents univariate diversity metrics calculated based on a combined data from all fish sampling data collected in May and October and combined data from 2018. Sampling methods and level of effort increased from previous studies conducted in 2017 and therefore data were not considered comparable to time-zero and control sites.

Table F2. Univariate diversity metrics for fish sampling conducted in May and October 2018.
(Species richness = S, Abundance = N, Margalef richness = d, Pielou's evenness = J', Shannon diversity = H' and Simpson's index = 1-Lambda' with highest values for each metric in bold)

Sample	S	N	d	J'	H'(loge)	1-Lambda'
May	11	548	1.586	0.6171	1.48	0.6803
Oct	16	390	**2.514**	**0.6844**	**1.898**	**0.7538**
2018	**18**	**938**	2.484	0.6256	1.808	0.7256

There was an increase is fish species documented from the May to October sampling periods. This is partly due to the increase in sunfishes but also a result of seining that picked up two new species from the shallow grass beds near Hunter Springs Park. The sheepshead minnow (*Cyprinodon variegatus*) and gulf killifish (*Fundulus grandis*) are brackish to marine species that appear to be transients that likely move back and forth depending on tidal influences on Kings Bay. The hogchoker (*Trinectes maculatus*) increased in abundance in trap samples from only two (2) in May to seven (7) in October 2018. The hogchoker is a sole (Archidae) that looks similar to a flounder and is a demersal fish species that depends on well oxygenated benthic conditions with a sandy bottom. The increase in hogchoker abundance is another positive ecological indicator.

Multivariate Analyses

A hierarchical cluster analysis of fish sampling results in Phase 1A was conducted based on season (Spring and Fall) and methods including Breder traps (Breder 1960), funnel traps (Fisher International), crayfish traps, seine net, and visual observations. Data were square root transformed to down-weight the importance of abundant species. Bray-Curtis similarity and the SIMPROF significance test identified (see black lines in clustering) that visual methods showed a

65% similarity between Spring and Fall surveys and visual methods were significantly different ($p < 0.05\%$) than various trapping methods, as well as the overall composited data from Spring and Fall (**Figure F1**). Crayfish traps produced results with greatest similarity to overall composited data for fish community structure. Crayfish traps attracted many small species of fish. However, in overnight sampling the nylon mesh crayfish traps also attracted the piscivorous hardhead catfish (*Ariopsis felis*) which then preyed upon trapped fish and also became entangled in the mesh of the crayfish traps.

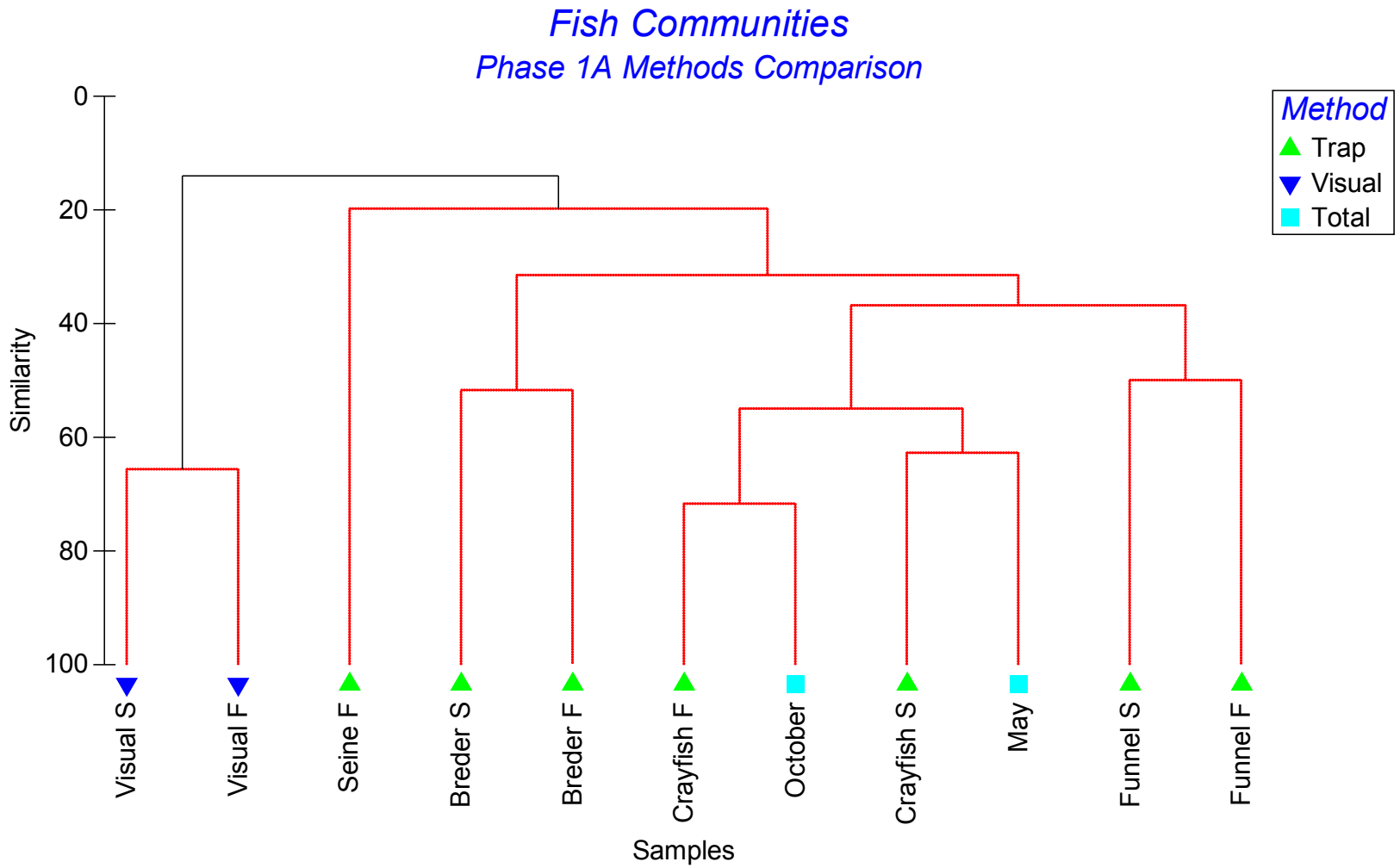

Figure F1. Hierarchical Cluster Analysis of fish sampling results (square root transformed) in Phase 1A based on season (Spring and Fall) and methods including Breder traps (Breder 1960), funnel traps (Fisher International), crayfish traps, seine net, and visual observations. Bray-Curtis similarity and the SIMPROF significance test identified (see black lines in clustering) that Visual methods showed a 65% similarity between Spring and Fall surveys and visual methods were significantly different ($p < 0.05\%$) than various trapping methods, as well as the overall composited data from Spring and Fall. Crayfish traps produced results with greatest similarity to overall composited data for fish community structure.

Another cluster analysis with SIMPROF test was conducted on the same dataset but with fourth root transformed data because of the extreme abundance of bluefin killifish and rainwater killifish in both samples from May and October (**Figure F2**). The composited samples from May group closely with Visual sampling results for both periods and are significantly different ($p < 0.05\%$) than Spring and Fall trapping results and combined Fall samples. A slice was added at 32% to illustrate the significant groupings. Visual surveys were similar between seasons but twice as many species were visually detected in the May sampling which may be a reflection of improved water clarity in the late dry season of 2018.

In an effort to identify which season is best to sample for fishes if time and budgets are limiting factors in future assessments, a cluster analysis based on Bray-Curtis similarity of communities was conducted comparing May and October sampling results with overall combined data for both events. This was based on fourth root transformed data and all methods combined for both sampling events (**Figure F3**). The cluster analysis, with SIMPROF significance test identified that the October fish sampling of community structure was >87% similar to the combined fish sampling from May and October 2018. The May results was nearly 70% similar to combined data and to October results but remained significantly different.

The conclusions from the fish methods testing and seasonal sampling are the following:

1. Fish communities are responding positively to the restoration activities in Phase 1A and several species were identified as indicators of restoration success.
2. Multiple fish sampling methods should be employed and visual detection of larger fish species is an important consideration to obtain a more complete species list.
3. Crayfish traps function as artificial reefs for attracting small fishes and had the highest species richness for all traps tested. They also attracted predaceous estuarine catfish in overnight sampling which became problematic.
4. October sampling results were most similar to overall combined results from May and October. Further investigation on fish community structure in Kings Bay is needed to understand seasonality and behavioral responses of fishes to fluctuations in tides, SAV cover, and human activities.

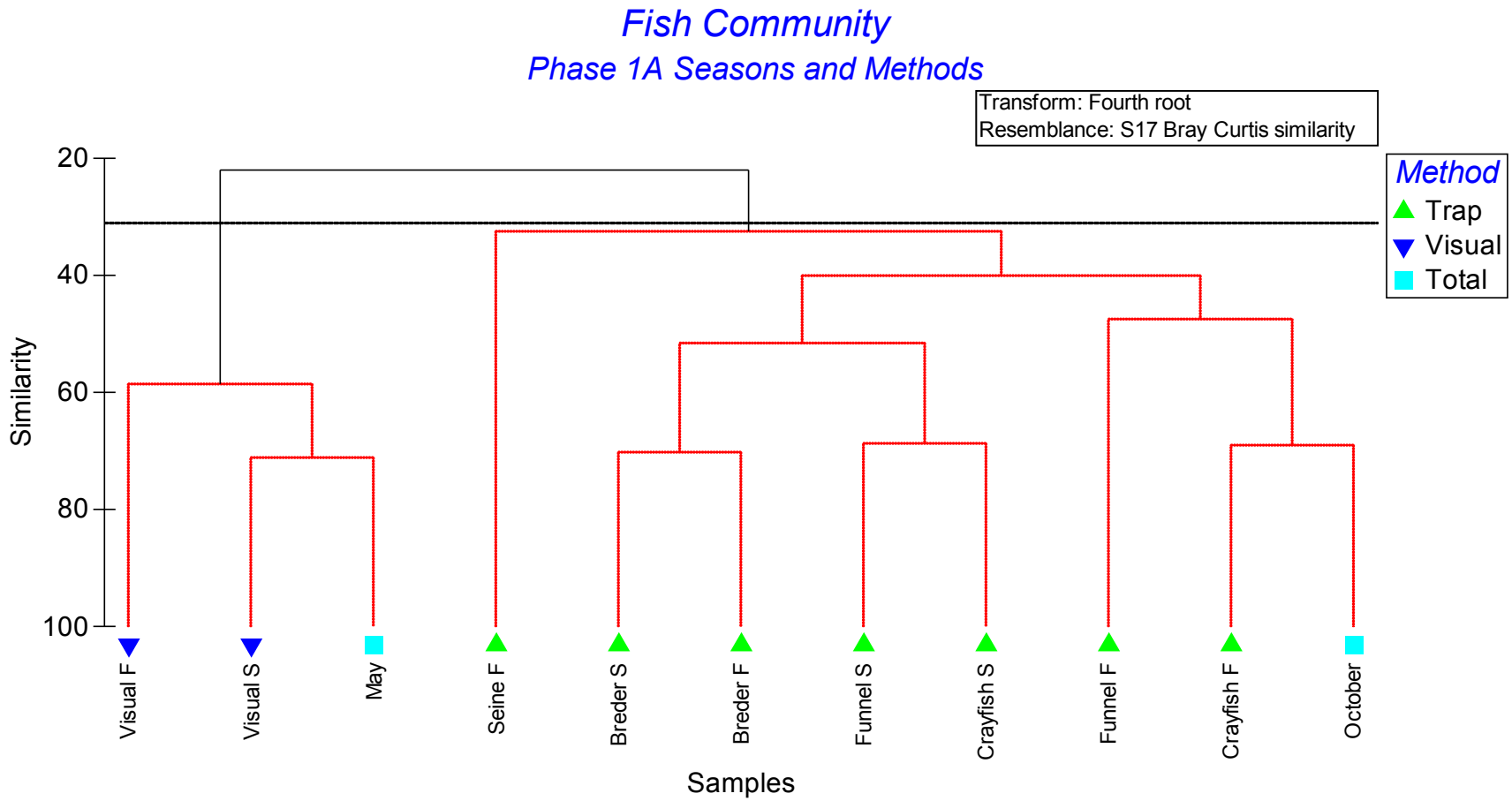

Figure F2. A fourth-root transformation of the data was conducted to down-weight the importance of extremely abundant species including bluefin killifish, *Lucania goodei* and rainwater killifish, *L. parva*. The composited samples from May group closely with Visual sampling results for both periods and are significantly different ($p < 0.05\%$) than Spring and Fall trapping results and combined Fall samples.

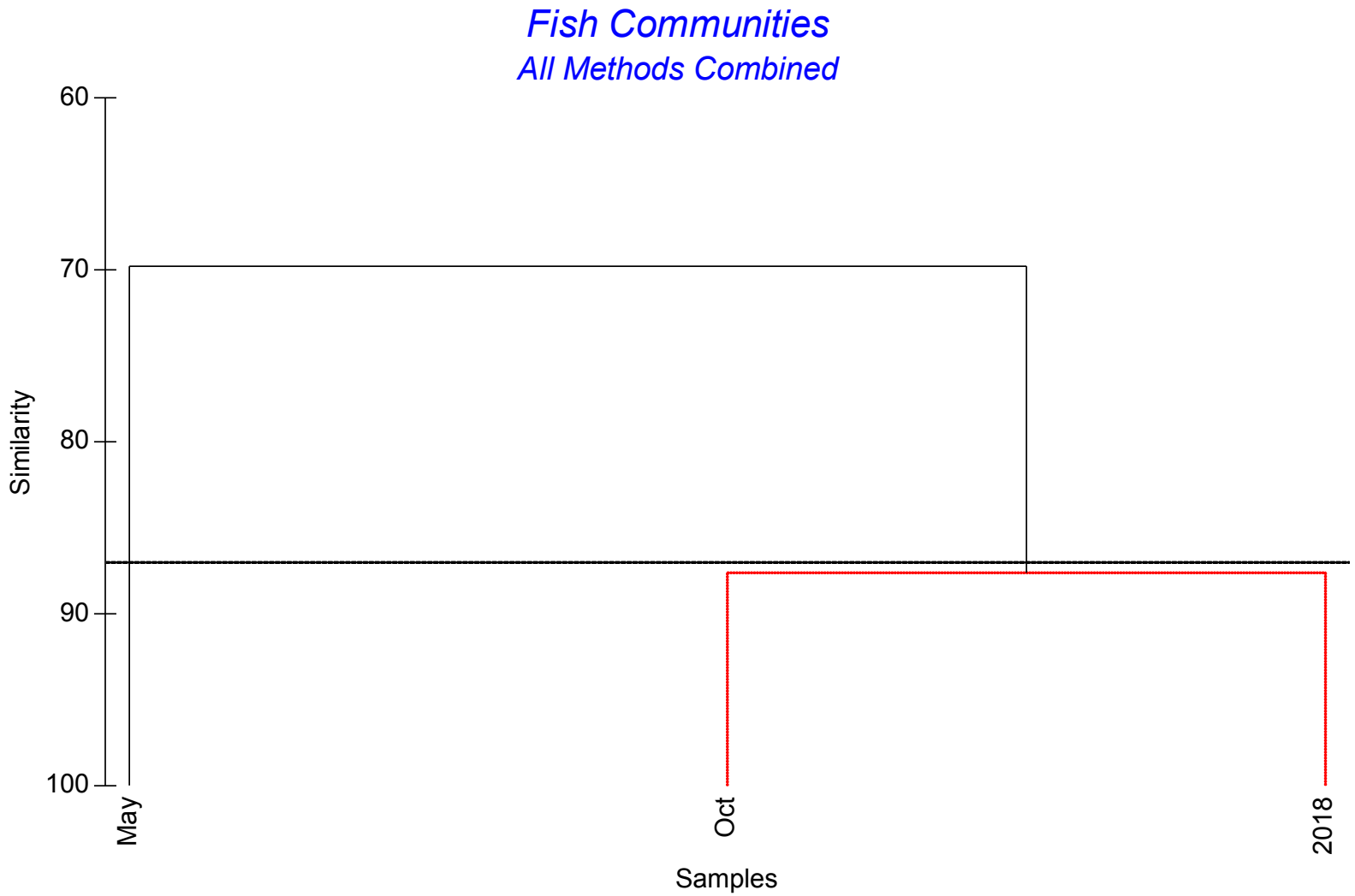

Figure F3. A seasonal comparison of fish communities (Spring and Fall) with overall combined fish species and abundances (fourth-root transformed) using Bray-Curtis Similarity and SIMPROF Significance test. October 2018 fish community and the overall combined 2018 fish community were >87% similar and significantly different ($p < 0.05\%$) than Spring 2018 fish community, but only separating at 70% similarity.

4.0 DISCUSSION

Submerged Aquatic Vegetation

In summary, *Vallisneria* cover increased in Phase 1A from 12% in May 2018 to 35% cover in October 2018. *Hydrilla* cover also increased from 1% in May to 12% in October. The Braun-Blanquet SAV cover class average increased from 1.6 to 4.2 during the same time frame. In the adjacent canal connecting Phase 1A to the Pilot Project, *Vallisneria* cover also increased from 35% in May 2018 to 89% in October 2018. Hydrilla and other species of vascular SAV disappeared from this area as of the October 2018 monitoring event (based on results of the five quadrats). The average Braun-Blanquet cover class, based on *Vallisneria* alone, doubled from May to October 2018 from 2.4 to 4.8 respectively. The October 15 and 16, 2018 monitoring was conducted prior to arrival of large numbers migrating manatees seeking thermal refugia during the winter months. Subsequent qualitative observations conducted in December 2018 revealed that intensive grazing by manatees has greatly reduced the biomass and cover of *Vallisneria* in the Phase 1A area. Longer term monitoring of *Vallisneria* (and other SAV species) on a seasonal basis will be necessary to determine if restoration goals can be achieved and maintained.

Macroinvertebrates

Aquatic macroinvertebrates communities appear to be a reliable biological indicator of habitat restoration and for monitoring recovery through time. Both quantitative sampling techniques, petite Ponar and Hester-Dendy artificial substrates demonstrated significant changes in aquatic macroinvertebrate communities are occurring as a result of the benthic habitat and SAV restoration actions. Of all the univariate diversity metrics, species richness appears to be most important. Multivariate statistical analyses demonstrated how community structure has been changing in response to restoration and several potential indicator taxa were identified. None of the macroinvertebrates collected in Ponar or Hester-Dendy samples are considered as Sensitive taxa by FDEP. This was not unexpected because Kings Bay is a complex tidally influenced system with fluctuating salinities, numerous freshwater springs, mixed land use and variable nutrient loading from both human and natural sources (including hundreds manatees). The most important species responsible for separating restored and unrestored sites were the amphipod *Hyalella azteca* grp. which was responsible for nearly 20% of the dissimilarity between restored and unrestored habitats. *Hyalella azteca* was extremely abundant in restored habitat samples but almost absent in

unrestored habitat samples. Conversely, the amphipod *Gammarus mucronotus* was abundant in unrestored habitats but relatively uncommon in restored areas and contributed to 4.1% of the dissimilarity. Other important taxa that were much more abundant in restored habitats and major contributors to the dissimilarity were the isopod *Cassidinidea ovalis*, the midge *Dicrotendipes modestus* the snail *Pyrogophorus platyrhicus* and the oligochaete worm *Limnodrilus hoffmeisteri*.

Several other species may also serve as indicators of restoration success, including the midges *Paralauterborniella nigrohalteralis*, *Beardius truncatus,* and *Cryptochironomus* sp., the snails *Melanoides tuberculata* and *Gyralus parvus*, the mayfly *Caenis* sp., and caddisfly *Orthotrichia* sp.. The life history requirements of each of these taxa apparently were not being met in *Lyngbya* dominated system with a layer of anoxic flocculent organic covering the bay bottom. For example, the anthurid isopod, *Cyathura polita* burrows into sand and mud sediments of oligohaline and mesohaline habitats and requires two years to complete its life cycle. *Cyathura polita* is a clear indicator of restoration success because of its life history requirements.

Some potential indicators of a degraded habitat dominated by Lyngbya include the following that were present in unrestored habitats but absent in the restored samples from Phase 1A; the pond snail *Haitia (Physa) cubensis*, the amphipod *Grandidierella bonnierodes*, midges *Einfeldia natchitocheae* and *Procladius* sp. I Rutter, and few other species. Rapid biological assessments using timed dip net samples appear to be valuable tool for assessing shallow wadable habitats while a combination of Ponar sampling and Hester-Dendy substrates provide a basis for quantitative comparisons of benthic habitat and water quality conditions respectively. Once indicator taxa have been confirmed for identification of restoration success, qualitative rapid assessment techniques may be more cost effective than Ponar dredges and HD substrates which can be labor intensive and expensive in comparison. It is recommended that rapid bioassessment methods similar to BioRecon be developed to enhance the cost-effectiveness of biological assessment of restoration projects where time constraints are real and a rapid response is needed to inform decision makers.

Fishes

Fish communities are responding positively to the restoration activities in Phase 1A and several species were identified as indicators of restoration success. Multiple fish sampling methods should be employed and visual detection of larger fish species is an important consideration to obtain a

more complete species list. Crayfish traps function as artificial reefs for attracting small fishes and had the highest species richness for all traps tested. They also attracted predaceous estuarine catfish in overnight sampling which became problematic. October sampling results were most similar to overall combined results from May and October. Further investigation on fish community structure in Kings Bay is needed to understand seasonality and behavioral responses of fishes to fluctuations in tides, SAV cover, and human activities.

Fish community health overall is critical to ecosystem integrity and something the public can readily understand as an important ecosystem service provided by healthy aquatic habitats. Sport fishes, especially largemouth bass and sunfishes, are beginning to recover in Kings Bay based on the monitoring program. In comparison with the unrestored habitat fish surveys conducted in 2017 (Johnson Eng. 2017) No native sunfishes were observed or collected from the unrestored (control) sites in 2017 because flocculent organic substrates are unsuitable for successful spawning by most sunfish species including largemouth bass, bluegill, and redear and spotted sunfish. The relative abundance of sunfishes, and the presence of demersal species like the hogchoker are positive indicators of restoration.

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APPENDIX A

SIMPER RESULTS

Macroinvertebrate Results SIMPER

Similarity Percentages - species contributions

One-Way Analysis

Data worksheet
Name: Data8
Data type: Abundance
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 90.00%

Factor Groups

Sample	Treatment
Ponars	Restored
HDs	Restored
Ponar control	Unrestored
HDControl	Unrestored

Group Restored
Average similarity: 36.28

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Hyalella azteca grp.	13.75	19.22	#######	52.98	52.98
Dicrotendipes modestus	5.64	3.99	#######	10.99	63.96
Gloiobdella elongata	2.37	3.45	#######	9.51	73.48
Helobdella stagnalis	1.83	2.82	#######	7.77	81.24
Pyrogophorus platyrhicus	1.57	2.82	#######	7.77	89.01
Melanoides tuberculata	1.21	1.99	#######	5.49	94.51

Group Unrestored
Average similarity: 15.99

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Grandidierella bonnierodes	4.11	8.26	#######	51.67	51.67
Gammarus mucronotus	3.92	7.73	#######	48.33	100.00

Groups Restored & Unrestored
Average dissimilarity = 83.85

Species	Group Restored Av.Abund	Group Unrestored Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Hyalella azteca grp.	13.75	0.50	16.49	3.01	19.67	19.67
Cassidinidea ovalis	5.10	2.78	5.65	1.09	6.73	26.40
Dicrotendipes modestus	5.64	0.71	5.17	1.29	6.16	32.56
Pyrogophorus platyrhicus	1.57	4.56	5.07	1.69	6.05	38.61
Grandidierella bonnierodes	0.00	4.11	5.03	3.73	6.00	44.61
Gammarus mucronotus	1.50	3.92	3.47	1.25	4.14	48.75
Limnodrilus hoffmeisteri	2.12	0.50	3.26	1.02	3.89	52.64
Oxythira sp.	0.00	2.50	2.48	0.84	2.96	55.60
Paralauterborniella nigrohalteralis	1.32	0.00	2.18	0.80	2.59	58.19
Gloiobdella elongata	2.37	0.50	2.11	5.01	2.52	60.71
Einfeldia natchitocheae	0.00	1.22	2.04	0.81	2.43	63.14
Haitia (Physa) cubensis	0.00	1.87	1.86	0.84	2.21	65.36
Helobdella stagnalis	1.83	0.50	1.80	1.45	2.15	67.50
Cyrnellus fraternus	0.00	1.80	1.79	0.84	2.13	69.64
Melanoides tuberculata	1.21	0.00	1.67	1.75	1.99	71.63
Orthotrichia sp.	1.41	0.00	1.43	0.84	1.71	73.34
Cyathura polita	0.87	0.00	1.42	0.80	1.70	75.04
Gyralus parvus	0.87	0.00	1.42	0.80	1.70	76.73
Nematoda	0.00	0.71	1.18	0.81	1.41	78.14
Dicrotendipes neomodestus	1.62	0.50	1.12	1.41	1.34	79.48
Planariidae	0.00	1.12	1.11	0.84	1.32	80.80
Ablabesmyia rhamphe grp.	0.87	0.87	1.03	0.87	1.22	82.02
Beardius truncatus	1.00	0.00	1.01	0.84	1.21	83.23
Eupera cubensis	0.00	1.00	0.99	0.84	1.18	84.42
Caenis sp.	0.87	0.00	0.88	0.84	1.05	85.46
Corynoneura sp.	0.87	0.00	0.88	0.84	1.05	86.51
Hargeria rapax	0.00	0.87	0.86	0.84	1.02	87.54
Procladius sp. I Rutter	0.00	0.50	0.83	0.81	0.99	88.53
Eclipidrilus sp.	0.50	0.00	0.82	0.80	0.98	89.51
Cryptochironomus sp.	0.50	0.00	0.82	0.80	0.98	90.49

SIMPER
Similarity Percentages - species contributions

One-Way Analysis

Data worksheet
Name: Data4
Data type: Abundance
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 90.00%

Factor Groups

Sample	Season
Funnel S	Spring
Breder S	Spring
Crayfish S	Spring
Visual S	Spring
May	Spring
Funnel F	Fall
Breder F	Fall
Crayfish F	Fall
Visual F	Fall
Seine F	Fall
October	Fall

Group Spring
Average similarity: 41.80

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bluefin Killifish	2.43	13.13	1.00	31.41	31.41
Rainwater Killifish	2.04	11.34	0.98	27.13	58.55
Largemouth Bass	1.71	8.06	1.11	19.28	77.82
Eastern Mosquitofish	0.86	2.92	0.59	6.99	84.82
Bluegill	0.89	1.23	0.32	2.95	87.77
Striped Mullet	0.89	1.23	0.32	2.95	90.73

Group Fall
Average similarity: 31.66

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Bluefin Killifish	1.99	9.11	1.10	28.78	28.78
Rainwater Killifish	1.32	6.48	1.17	20.46	49.24
Spotted Sunfish	1.16	2.47	0.47	7.80	57.05
Tidewater Mojarra	1.12	2.28	0.47	7.21	64.26
Atlantic Needlefish	0.90	2.22	0.45	7.02	71.27
Warmouth	0.53	1.80	0.41	5.70	76.97
Largemouth Bass	0.77	1.78	0.45	5.62	82.60
Redear Sunfish	0.72	1.55	0.47	4.91	87.51
Striped Mullet	0.72	0.78	0.26	2.46	89.97
Eastern Mosquitofish	0.50	0.57	0.26	1.80	91.77

Groups Spring & Fall

Average dissimilarity = 65.44

Species	Group Spring Av.Abund	Group Fall Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Bluefin Killifish	2.43	1.99	7.95	1.37	12.15	12.15
Rainwater Killifish	2.04	1.32	6.81	1.25	10.40	22.56
Largemouth Bass	1.71	0.77	6.05	1.34	9.24	31.80
Spotted Sunfish	0.00	1.16	4.48	0.90	6.85	38.65
Striped Mullet	0.89	0.72	4.43	0.82	6.77	45.42
Atlantic Needlefish	0.71	0.90	4.37	0.94	6.68	52.10
Tidewater Mojarra	0.00	1.12	4.25	0.85	6.49	58.59
Eastern Mosquitofish	0.86	0.50	4.01	0.96	6.12	64.71
Bluegill	0.89	0.33	3.60	0.91	5.49	70.21
Sheepshead	0.71	0.47	3.35	0.89	5.12	75.33
Redear Sunfish	0.00	0.72	2.79	0.89	4.26	79.59
Hogchoker	0.48	0.54	2.71	0.95	4.14	83.72
Warmouth	0.00	0.53	2.59	0.83	3.96	87.68
Gulf Killifish	0.00	0.47	1.81	0.61	2.76	90.44

SIMPER
Similarity Percentages - species contributions

One-Way Analysis

Data worksheet
Name: Data8
Data type: Abundance
Sample selection: All
Variable selection: All

Parameters
Resemblance: S17 Bray Curtis similarity
Cut off for low contributions: 90.00%

Factor Groups

Sample	Treatment
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HDs	Restored
Ponar control	Unrestored
HDControl	Unrestored

Group Restored
Average similarity: 36.28

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
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Dicrotendipes modestus	5.64	3.99	#######	10.99	63.96
Gloiobdella elongata	2.37	3.45	#######	9.51	73.48
Helobdella stagnalis	1.83	2.82	#######	7.77	81.24
Pyrogophorus platyrhicus	1.57	2.82	#######	7.77	89.01
Melanoides tuberculata	1.21	1.99	#######	5.49	94.51

Group Unrestored
Average similarity: 15.99

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Grandidierella bonnierodes	4.11	8.26	#######	51.67	51.67
Gammarus mucronotus	3.92	7.73	#######	48.33	100.00

Groups Restored & Unrestored
Average dissimilarity = 83.85

Species	Group Restored Av.Abund	Group Unrestored Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
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Grandidierella bonnierodes	0.00	4.11	5.03	3.73	6.00	44.61
Gammarus mucronotus	1.50	3.92	3.47	1.25	4.14	48.75
Limnodrilus hoffmeisteri	2.12	0.50	3.26	1.02	3.89	52.64
Oxythira sp.	0.00	2.50	2.48	0.84	2.96	55.60
Paralauterborniella nigrohalteralis	1.32	0.00	2.18	0.80	2.59	58.19
Gloiobdella elongata	2.37	0.50	2.11	5.01	2.52	60.71
Einfeldia natchitocheae	0.00	1.22	2.04	0.81	2.43	63.14
Haitia (Physa) cubensis	0.00	1.87	1.86	0.84	2.21	65.36
Helobdella stagnalis	1.83	0.50	1.80	1.45	2.15	67.50
Cyrnellus fraternus	0.00	1.80	1.79	0.84	2.13	69.64
Melanoides tuberculata	1.21	0.00	1.67	1.75	1.99	71.63
Orthotrichia sp.	1.41	0.00	1.43	0.84	1.71	73.34
Cyathura polita	0.87	0.00	1.42	0.80	1.70	75.04
Gyralus parvus	0.87	0.00	1.42	0.80	1.70	76.73
Nematoda	0.00	0.71	1.18	0.81	1.41	78.14
Dicrotendipes neomodestus	1.62	0.50	1.12	1.41	1.34	79.48
Planariidae	0.00	1.12	1.11	0.84	1.32	80.80
Ablabesmyia rhamphe grp.	0.87	0.87	1.03	0.87	1.22	82.02
Beardius truncatus	1.00	0.00	1.01	0.84	1.21	83.23
Eupera cubensis	0.00	1.00	0.99	0.84	1.18	84.42
Caenis sp.	0.87	0.00	0.88	0.84	1.05	85.46
Corynoneura sp.	0.87	0.00	0.88	0.84	1.05	86.51
Hargeria rapax	0.00	0.87	0.86	0.84	1.02	87.54
Procladius sp. I Rutter	0.00	0.50	0.83	0.81	0.99	88.53
Eclipidrilus sp.	0.50	0.00	0.82	0.80	0.98	89.51
Cryptochironomus sp.	0.50	0.00	0.82	0.80	0.98	90.49