AQUATIC FAUNAL ASSESSMENT OF SUBMERGED AQUATIC VEGETATION (SAV) HABITATS IN THE CRYSTAL RIVER ECOSYSTEM

June 28, 2017

prepared for:

Save Crystal River, Inc.
Post Office Box 2020
Crystal River, Florida 34423

Prepared by:

JOHNSON ENGINEERING
2122 Johnson Street
Fort Myers, Florida 33901
(239) 334-0046
E.B. # 642
www.johnsonengineering.com

Job #20170047

TABLE OF CONTENTS

Page

LIST OF TABLES

Number

LIST OF FIGURES

Number

TABLE OF CONTENTS CONTINUED

Page

LIST OF FIGURES

APPENDICES

EXECUTIVE SUMMARY

A biological assessment was initiated in February and completed in May 2017 to survey the aquatic faunal communities of restored and unrestored habitats within the King's Bay area of Crystal River. Petite Ponar dredge, Hester-Dendy (HD) substrates and qualitative dip net sampling of macroinvertebrate communities were conducted along with fish trapping and visual surveys. This report may serve as a baseline assessment for unrestored canal habitat, as well as time-zero assessment for Phase 1A, and first annual assessment of the Pilot Project (Area 3). A total of 63 macroinvertebrate species were collected and identified, representing six (6) classes, 17 orders, and 33 families. Univariate and multivariate statistical approaches were used to evaluate quantitative macroinvertebrate samples. Restored habitats had higher species richness and diversity than unrestored sites. Multivariate analysis of community structure was useful in identifying several potential indicator species for restoration monitoring in the future. Generally, bivalves and gastropods were common or abundant in restored habitats but rare or absent in benthic samples from unrestored sites. In both Ponar and HD samples, the restored and unrestored sites were 73% dissimilar in community structure, and in HD substrates they formed two significantly different ($p<0.05$) groups. Several taxa were present only at restored sites and these organisms may serve as indicators of restoration success for future assessments.

A total of sixteen species of fishes were collected representing 10 families and 13 genera using a combination of visual transects, activity traps, and dip net sampling techniques. A total of 16 species were documented at restored canal sites while only 12 species were recorded at control sites. Visual surveys were more effective than activity traps for collecting fishes during this study. The most noteworthy fish sampling results were obtained from visual transects and video documentation of spawning and nest protection by largemouth bass (and other sunfishes) only in the restored habitat. This spawning resulted in the production of young of the year largemouth bass and Lepomis sunfishes that had spread to other areas in King's Bay by the May sampling event. Future fish surveys should consider the impacts of heavy tour-boat traffic and snorkelers on fish behavior.

1.0 INTRODUCTION

A biological assessment was initiated in February and completed in May 2017 to survey the aquatic faunal communities of restored and unrestored habitats within 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 (or tape grass), (*Vallisneria Americana*). The introduction of non-native plant species (e.g. *Hydrilla*), in conjunction with sedimentation and eutrophication have contributed to massive losses of eel grass in the system and a general degradation of aquatic habitats, especially in the canals where mucky sediments have accumulated. A phased restoration project is currently underway and consists of de-mucking the waterways and replanting of eel grass.

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. A biological assessment was requested by Save Crystal River, Inc. to identify aquatic faunal communities that inhabit areas restored under a pilot study in 2015, a Phase 1A restoration area in progress, and unrestored control sites to establish baseline conditions. The objectives of this study will be to clearly document the aquatic biological diversity of restored and unrestored submerged aquatic vegetation (SAV) habitat. The focus will be on comparing vascular plant communities (*Vallisneria americana, Hydrilla verticilatta, Najas spp.*) with filamentous algal communities (*Lyngbya* sp.) within the same spring-fed river ecosystem. The biological assessment primarily consists of surveying the fish and macroinvertebrate communities within restored *V. americana* habitat and in pre-selected unrestored habitats dominated by filamentous algae of Crystal River. The study will include quantitative assessments of aquatic faunal communities including macroinvertebrates and fishes at two distinct habitat types and will be completed over two seasonal time periods.

Under ideal conditions, there would be a baseline, time-zero, and post restoration biological assessments at all locations. However, in this case the assessment includes a baseline assessment of unrestored canal habitat, a time-zero assessment of the Phase 1A restoration, and first annual assessment of the pilot restoration area. It was not in the budget or scope of these preliminary biological assessments to conduct intensive faunal surveys of all three phases with numerous replicates in each treatment. This aquatic faunal assessment represents biological snapshots in time for February through May 2017 for three different phases of the King's Bay de-mucking and submerged aquatic vegetation (SAV) restoration project.

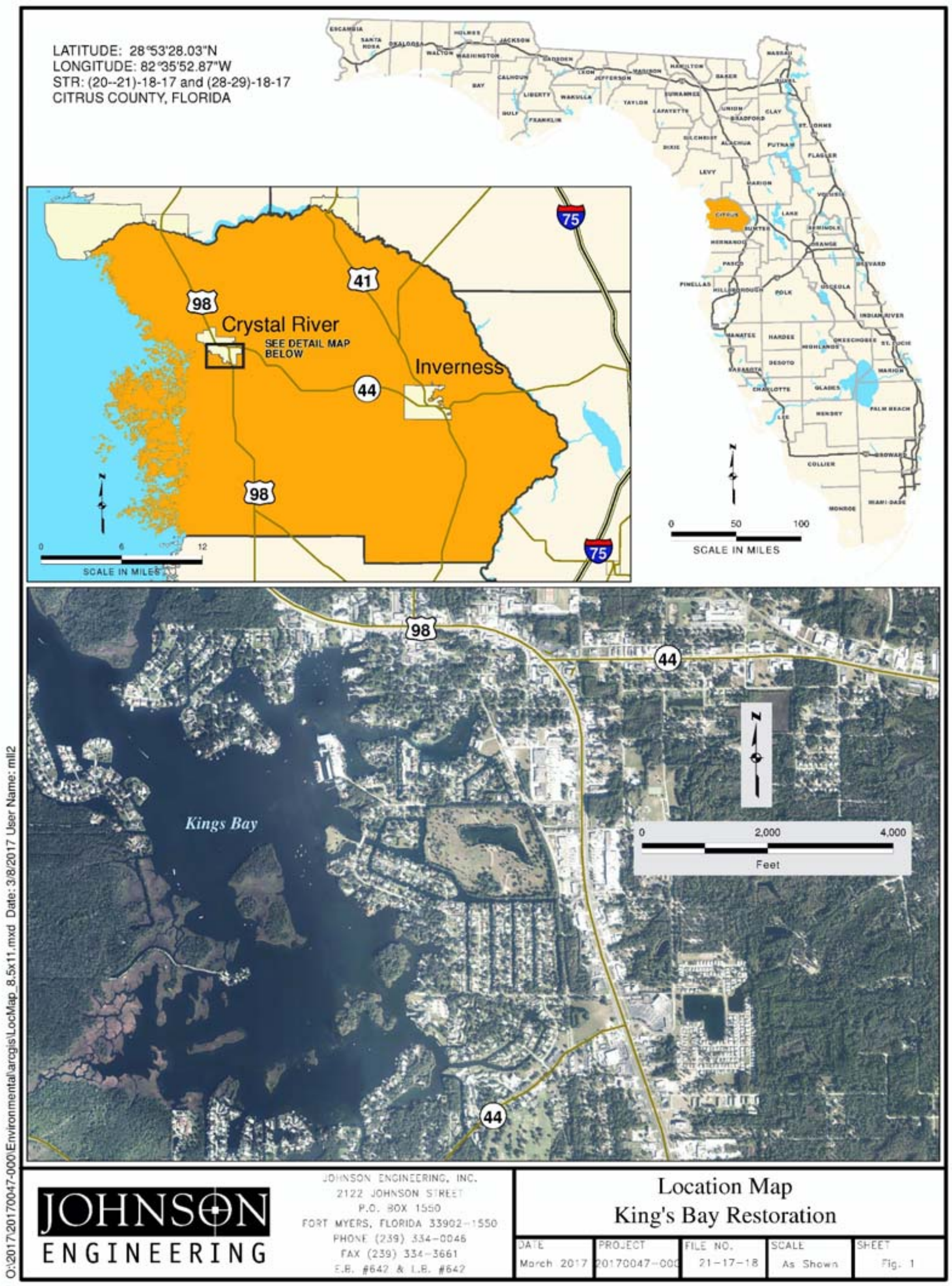


Figure 1. Location Map of King's Bay Restoration Area

A secondary objective of this study is to document wildlife utilization in restored and unrestored habitats through qualitative observations (visual, tracks) and photographic recording of reptiles, amphibians, birds and mammals during the field survey process for other lower taxonomic groups. Ecotourism and outdoor recreational opportunities are expected to increase with increased wildlife diversity and abundance in restored aquatic habitats. Representative study sites would be selected from restored (Pilot and Phase 1A) and unrestored sections of the waterways prior to sampling. This study required reconnaissance surveys by boat and SCUBA/snorkeling to assess bottom conditions, water depth, bottom contour, and accessibility. Site selection was coordinated with Save Crystal River (SCR) representatives to ensure representative sites are selected for sampling.

2.0 METHODS

Macroinvertebrate Communities

The winter aquatic sampling period was conducted from February 22-24, 2017. Aquatic macroinvertebrate communities were sampled using three different techniques to obtain a robust and more complete sample of community structure. D-frame aquatic dip nets, a petite Ponar dredge, and artificial substrates (EPA Hester-Dendy) were used to quantify and compare community structure between treatments (restored and unrestored SAV habitat). Sampling locations were stratified among the restored and unrestored canals to collect data from typical habitats present at representative locations (Figure 2).

Figure 2. King's Bay Restoration Sample Sites

1. 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 3).**

Figure 3. Standard D-Frame Aquatic Dip-net used for aquatic faunal sampling; fishes and macroinvertebrates.

2. Three (3) Hester Dendy (HD) substrates were also deployed as replicates at a recently restored canal in Phase 1A and an unrestored sampling site and allowed to colonize with invertebrates for 28 days **(Figure 4).** To quantify the macroinvertebrate community structure provided by restored eel grass beds, researchers deployed HD substrates inside of planted exclosure cages in the Phase 1A restoration area. After the colonization period, samplers were retrieved and processed for collection and preservation of epi-fauna using 80% ethanol and labeled and archived for identification in the laboratory. HD substrate samples were processed, sorted, and all macroinvertebrates identified to the lowest practical taxonomic level. The macroinvertebrate species richness and diversity will be calculated for each site for comparison between sites and treatments.

Figure 4. EPA Hester-Dendy Artificial Substrates for aquatic macroinvertebrate sampling.

3. In addition to the dip net and HD samples collected, three petite Ponar samples were collected from representative study site locations from the Pilot restoration area and an unrestored control site **(Figure 2)** in February and again in May 2017. 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 (lower right) being used to collect replicate sediment samples from restored canal habitat in King's Bay Pilot Project (Area 3).

Petite Ponar dredge, Hester-Dendy 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). The fish community surveys were conducted in conjunction with macroinvertebrate assessments when possible.

1. 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. Still

photos and video recordings are included as an attachment to this report on a portable data storage disk (flash drive).

2. 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” for sampling fish communities in shallow waters.

3. Three modified crayfish traps (Fisher International, Tampa, FL.) at each treatment site for a period of 24 hours before pulling traps and identifying and enumerating fishes collected from each site **(Figure 7)**.

Figure 7. Modified crayfish 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 February fish sampling events, there was heavy tour-boat traffic with large groups of snorkelers in the waters around the sampling sites which probably impacted fish behavior patterns. This may have also impacted the Breder trap fish sampling effectiveness. Follow up fish surveys were conducted in May 2017 when tour-boat activity was expected to be greatly reduced. During the May sampling tour-boat traffic was reduced but remained problematic for fish sampling.

3.0 RESULTS

Aquatic Macroinvertebrates

Macroinvertebrates from the petite Ponar dredge samples have been separated from the sediments using Standard Sieves and identified to the lowest practical taxonomic level. HD substrates were retrieved on March 24, 2017 and processed for identification. Additional petite Ponar dredge samples and dip net samples were collected from restored and unrestored habitats in May 2017.

The results from all the macroinvertebrate sampling methods are presented in **Table 1**. Macroinvertebrate identifications were performed by David W. Ceilley, M.S., CSE, Senior Aquatic Ecologist at Johnson Engineering Inc. and by Robert Rutter, Macroinvertebrate Identification Services. Mr. Ceilley has decades of experience in Florida conducted aquatic and wetland faunal assessments. Mr. Rutter is a retired Florida Department of Environmental Protection macroinvertebrate expert taxonomist who was primarily responsible for identification of oligochaete worms and chironomidae collected by Ponar and HD substrates.

Table 1. Macroinvertebrate Species Collected by Petite Ponar, Hester-Dendy Substrate, and Dip Net Sampling at Restored and Unrestored (Control) Sites in King's Bay, Florida February and May 2017.

				Feb. Ponar Dredge		May Ponar Dredge						HD1	HD2	HD3	HD1	HD2	HD3	D-Net	D-Net
Taxa	**Order**	**Family**	**Genus Species**	**Restored**	**Control**	**Restored 1**	**Restored 2**	**Restored 3**	**Control 1**	**Control 2**	**Control 3**	**Restored-1**	**Restored-2**	**Restored-3**	**Control-1**	**Control-2**	**Control-3**	**Restored**	**Control**
Annelida	Rhyncobdellida	Hirudinea	*Helobdella elongata*	1	1													2	3
			Helobdella stagnalis							1							1	5	4
			Myzobdella sp.													1			
	Oligochaeta	Naididae	*Limnodrilus hoffmeisteri*	92	1	2	1	4											
			L. claparedeianus	16															
			Ilyodrilus tempeltoni	13															
			Nais variabilis										1		1				
		Lumbriculidae	*Lumbriculidae*							2	3								
Nematoda	Nematoda	Nematoda	*Nematoda*	2	2														
Platyhelminthes	Tricladida	Planariidae	*Planariidae*	1											2	3		1	6
Crustacea	Amphipoda	Talitridae	*Hyalella azteca grp.*	54			12	28	13	3		16	9	32	1			17	17
		Gammaridae	*Gammarus sp.*		7				5			10	12	10	16	1	10		
			Crangonyx sp.						1										4
		Aoridae	*Grandidierella bonnierodes*		8										1	1	27		
	Isopoda	Sphaeromidae	*Cassidinidea ovalis*									2	6	2	20	5	6	3	3
		Asellidae	*Caecidotea sp.*															7	
	Tanaidacea	Leptocheliidae	*Hargeria rapax*												1		2		
	Mysida	Mysidacae	*Taphromysis lousianae*															16	
	Decopoda	Panopeidae	*Rhithropanopeus harisii*														1		
Mollusca	Gastropoda	Thiaridae	*Melanoides tuberculata*	108		11	10	8	30	5	4	3	1					9	12
		Physidae	*Haitia (Physa) cubensis*									2		3	10	4		14	
		Planorbidae	*Planorbella duryi*	6								1	2	3				5	
			Planorbella scalaris			1	1	2											
			Micromenetus floridensis					4							1				
		Hydrobiidae	*Pyrogophorus platyrhicus*			3		12	11	5			1		35	7	41	1	4
		Amnicolidae	*Amnicola dalli*	2															
		Ancylidae	*Hebetancylus excentricus*					1					1						
	Pelecypoda	Sphaeriidae	*Musculium (lacustre)*	21				3	1		1							3	9
			Eupera cubensis												2	1	1		1
		Corbiculidae	*Corbicula fluminea*	50														3	
Insecta	Odonata	Libelullidae	*Epicordulia princeps*	1															

Table 1. Macroinvertebrate Species Collected by Petite Ponar, Hester-Dendy Substrate, and Dip Net Sampling at Restored and Unrestored (Control) Sites in King's Bay, Florida February and May 2017 (cont.).

				Feb. Ponar Dredge			**May Ponar Dredge**					**HD1**	**HD2**	**HD3**	**HD1**	**HD2**	**HD3**	**D-Net**	**D-Net**
Taxa	**Order**	**Family**	**Genus Species**	**Restored**	**Control**	**Restored 1**	**Restored 2**	**Restored 3**	**Control 1**	**Control 2**	**Control 3**	**Restored-1**	**Restored-2**	**Restored-3**	**Control-1**	**Control-2**	**Control-3**	**Restored**	**Control**
			Libellula auripennis															1	1
		Gomphidae	*Aphylla williamsoni*															1	
		Coenagrionidae	*Enallagma sp.*															1	
			Ischnura ramburii															1	
	Ephemeroptera	Baetidae	*Callibaetis sp.*																2
	Heteroptera	Corixidae	*Trichocorixa sp.*	1														1	
			Synaptonecta issa										1					1	
		Gerridae	*Neogerris hesione*															1	5
	Tricoptera	Polycentropidae	*Cyrnellus fraternus*										1		8	2	3		
		Hydroptilidae	*Oxythira sp.*											1	13	6	6		
		Leptoceridae	*Leptoceridae*			4													
	Coleoptera	Haliplidae	*Peltodytes sp.*															5	1
		Elmidae	*Stenelmis sp.*															1	
	Diptera	Chironomidae	*Ablabesmyia rhamphe grp.*										1	1	1	1	1		
			Chironominae							1	1								
			Cryptochironomus sp.											1					
			Beardius truncatus									1		2					
			Glyptotendipes meridionalis											2					
			Asheum beckae									13	22	16		1	1		
			Chironomus decorus grp.									3	4	2					
			Procladius sp. I Rutter	1	1		1	1					1						
			Einfeldia natchitocheae		6														
			Dicrotendipes modestus		2														
			Dicrotendipes neomodestus		1							8	2	3					
			Dicrotendipes simpsoni									3		1					
			Psuedochironomus sp.									5	1						
			Polypedilum scalaenum grp.														1		
			Goeldichironomus cf. natans									1							
			Tanytarsus sp. K										1						
			Tanytarsus sp. g complex									2	1	1					
			Tanytarsus hastatus									1		3					
			Cricotopus sp.										2	1					

Univariate Analyses

A total of 63 macroinvertebrate species were collected and identified, representing six (6) classes, 17 orders, and 33 families. 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 2**. Highest values for each metric are shown in bold font. The highest species richness (22 taxa) was found in the qualitative dip net sampling conducted in restored habitat while the highest abundance (369) was found in composited Ponar samples from restored habitat in February. Margalef richness was also highest (4.57) in the dip net sample from restored habitat with the next three highest values (4.237, 3.611, 3.284) from Hester-Dendy substrates collected from restored habitats where the substrates were placed inside of *Vallisneria americana* exclosure cages to both protect them from boat anchor entanglements and to represent the SAV habitat being restored throughout King's Bay. Pielou's evenness (J') is an indication of how equal each species abundance is in a sample but has very limited value ecologically. Pielou's evenness was highest (.8992) in a control Ponar sample but only six (6) species and only 17 total organisms were collected there. 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 (2.592) in the dip net sample collected from restored habitat in May 2017 (**Table 2**). Simpson's diversity index (1-Lambda) is another metric based on species richness and evenness of abundance. Simpson's index was also highest (0.9085) in the dip-net sample collected from restored habitat.

Table 2. Macroinvertebrate species richness (S), abundance (N), Margalef richness (d), Pielou's evenness (J'), Shannon diversity (H') and Simpson's index (1-Lambda).

Sample	Method	S	N	d	J'	H'	1-Lambda
Feb. Restored	Ponar (N=3)	15	**369**	2.369	0.6937	1.879	0.8079
Feb. Control	Ponar (N=3)	9	29	2.376	0.8454	1.858	0.8374
May Restored	Ponar 1	5	21	1.314	0.8086	1.301	0.6905
May Restored	Ponar 2	5	25	1.243	0.6866	1.105	0.63
May Restored	Ponar 3	9	63	1.931	0.7621	1.674	0.7501
May Control	Ponar 1	6	61	1.216	0.7407	1.327	0.6842
May Control	Ponar 2	6	17	1.765	**0.8992**	1.611	0.8235
May Control	Ponar 3	4	9	1.365	0.8764	1.215	0.75
Restored	HD 1	15	71	3.284	0.8487	2.298	0.8821
Restored	HD 2	19	70	4.237	0.7731	2.276	0.8522
Restored	HD 3	17	84	3.611	0.736	2.085	0.8058
Control	HD1	14	112	2.755	0.7578	2	0.8298
Control	HD 2	12	33	3.146	0.887	2.204	0.8939
Control	HD 3	13	101	2.6	0.6781	1.739	0.7525
Restored	D-Net	**22**	99	**4.57**	0.8386	**2.592**	**0.9085**
Control	D-Net	14	72	3.04	0.8776	2.316	0.8873
Highest values for each		**22**	**369**	**4.57**	**0.8992**	**2.592**	**0.9085**

Multivariate Analyses: Ponar Samples

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. Excel ™ and Primer v6 (Clarke and Gorley 2006) software were used to organize and analyze the macroinvertebrate data respectively. 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. The cluster analysis and SIMPROF test did not show significant differences based on petite Ponar samples collected during the May 2017 sampling event (**Figure 8**).

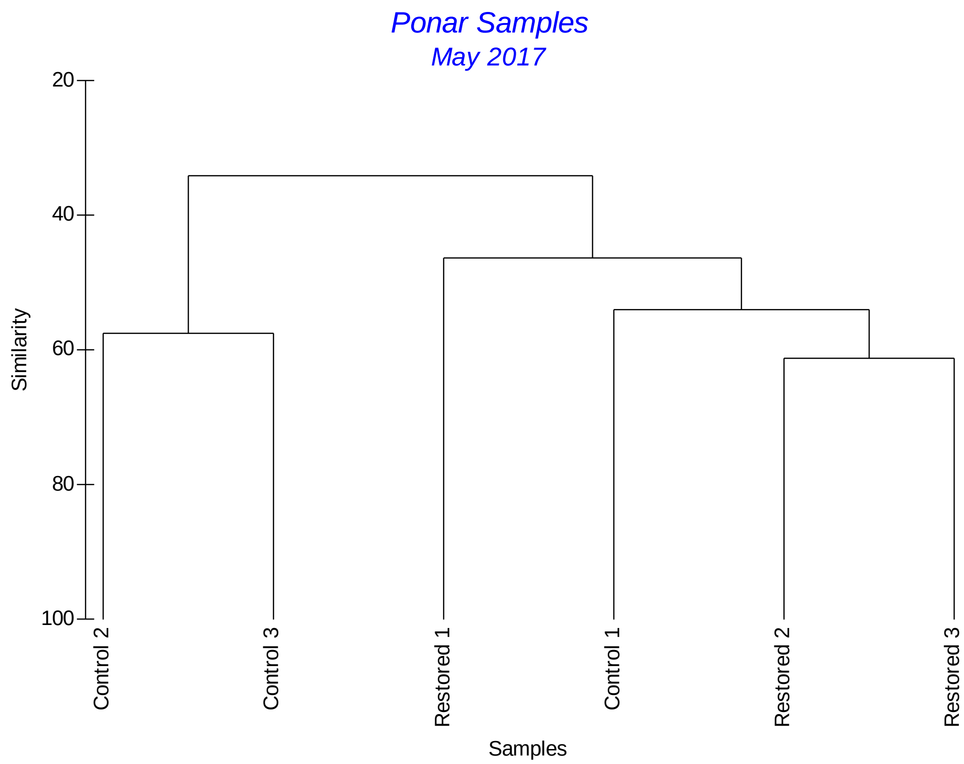


Figure 8. Cluster analysis of Ponar samples of macroinvertebrates collected in May 2017.

This appears to be partly due to the variability in samples collected from sediments within the restored and control sites. Restored 1 (Ponar) sample was collected near the dead end of the canal and contained a large amount of silty clay sediment (**Appendix B**) while the Control 1 (Ponar) sample contained less muck and *Lyngbya* than the other Control 2 and 3 samples, indicating that there may be a natural vent or spring in the area. The MDS ordination shows dissimilarity between control and restored sites represented as distances between communities (**Figure 9**).

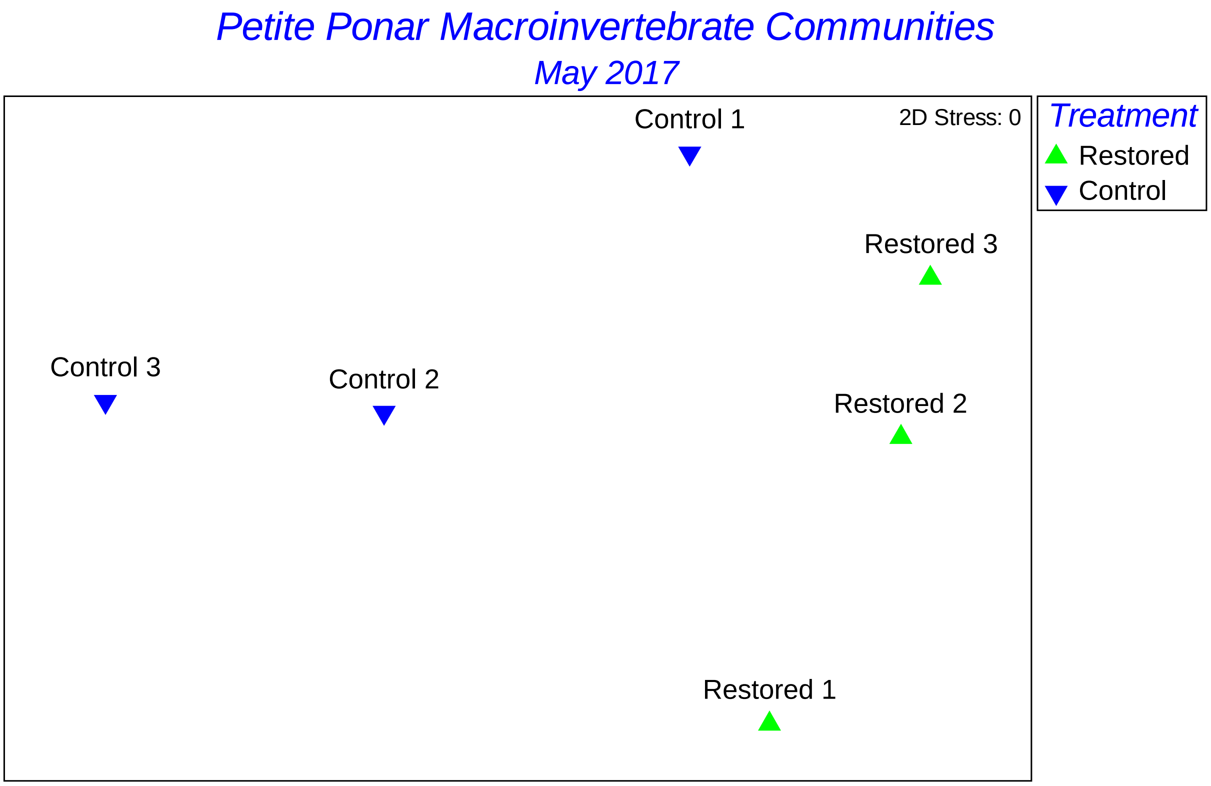


Figure 9. MDS ordination of Ponar macroinvertebrate samples from May 2017.

To further assess the benthic communities represented in the Ponar samples, we composited the data from three samples and compared the February and May macroinvertebrate communities using MDS (Figure 5). The composited Ponar samples from restored and control sites for both seasons combined were 73.2% dissimilar (**Appendix A**).

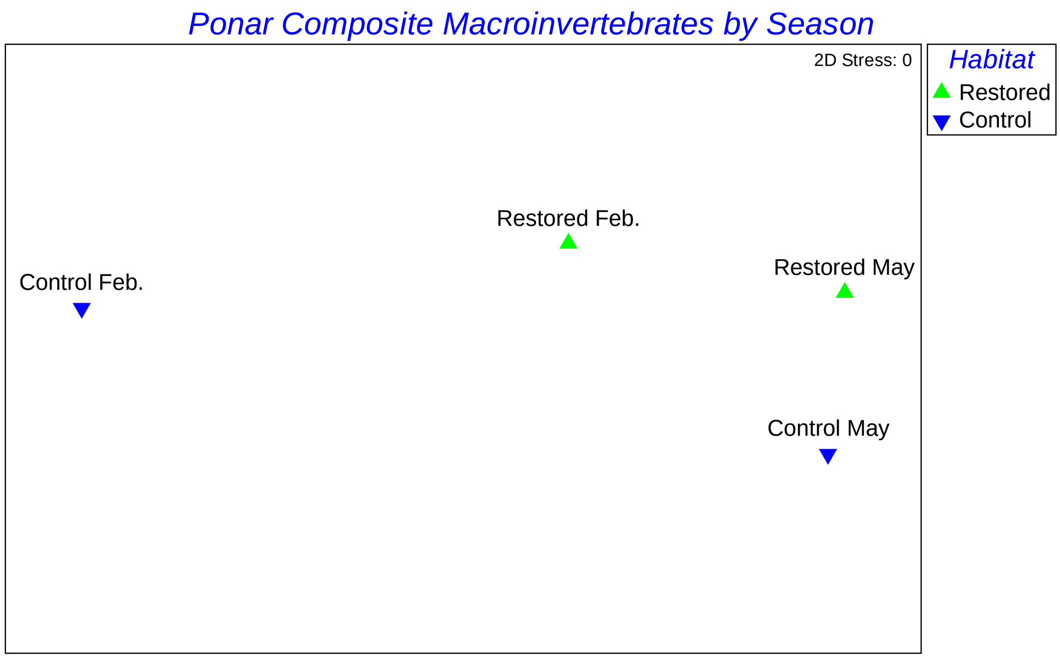


Figure 10. Ponar composite samples labeled by habitat and season for 2017.

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 three most important species in separating restored and control sites were the non-native snail (*Melanoides tuberculate*), the amphipod (*Hyalella Azteca*), grp. and oligochaete worm (*Limnodrilus hoffmeisteri*) (**Figure 11**). All three were contributed more than 11% to the dissimilarity and were extremely abundant in the restored group while uncommon or rare at the control sites. The tiny native fingernail clam (*Musculium lacustre*), was more abundant in restored habitat the control samples and contributed 5.11% to the dissimilarity between the communities. *Corbicula fluminea* was relatively abundant in the restored areas but absent in the unrestored (control) canal Ponar samples. Three other native snail species (*Planorbella scalaris, P. duryi*, and *Micromenetus floridensis*), the limpet, (*Hebetancylus excenticus*) were collected from restored canal sites but were not found in any of the control Ponar samples. Conversely, benthic samples from the control sites contained *Gammarus* sp. amphipods but they were absent in samples collected from the restored canals. The following series of figures illustrates the relative abundance of the most important species contributing to the dissimilarity between restored benthic habitat and control (unrestored) habitats as identified by the SIMPER analysis.

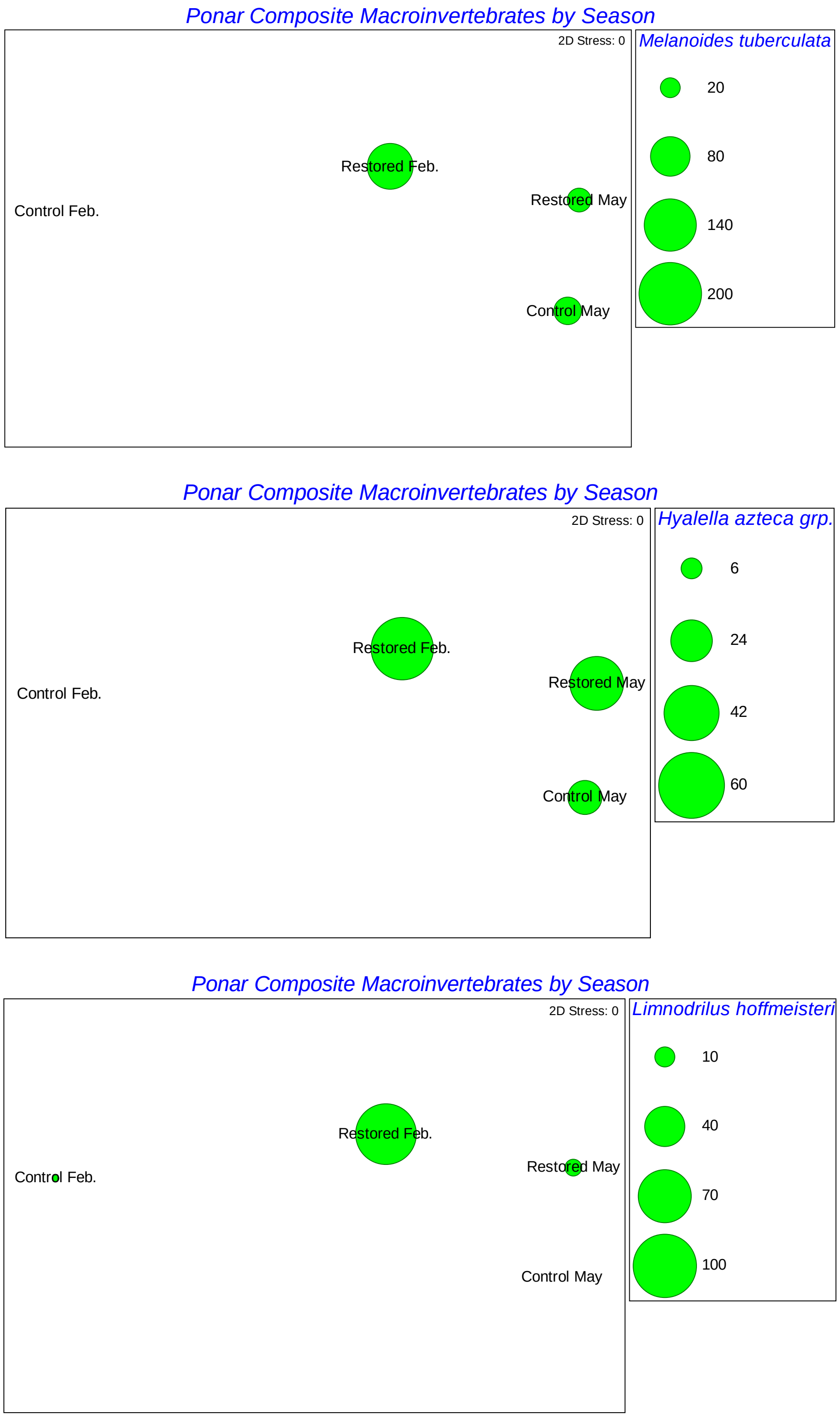
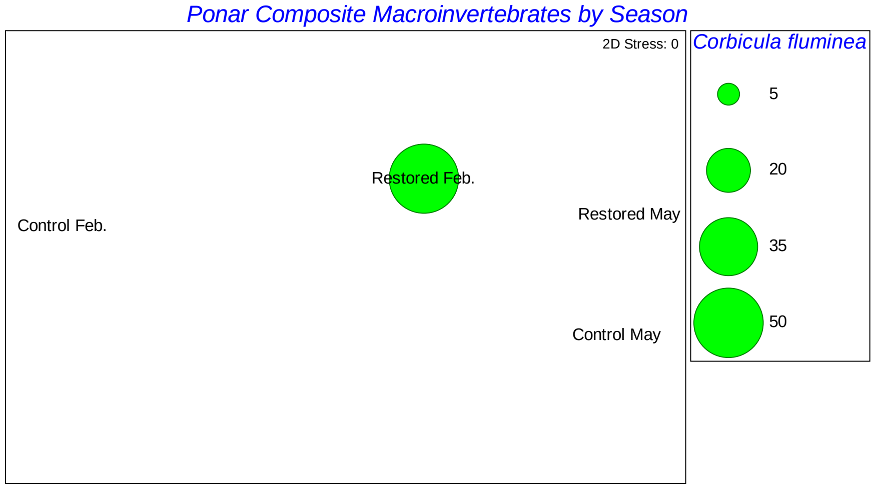
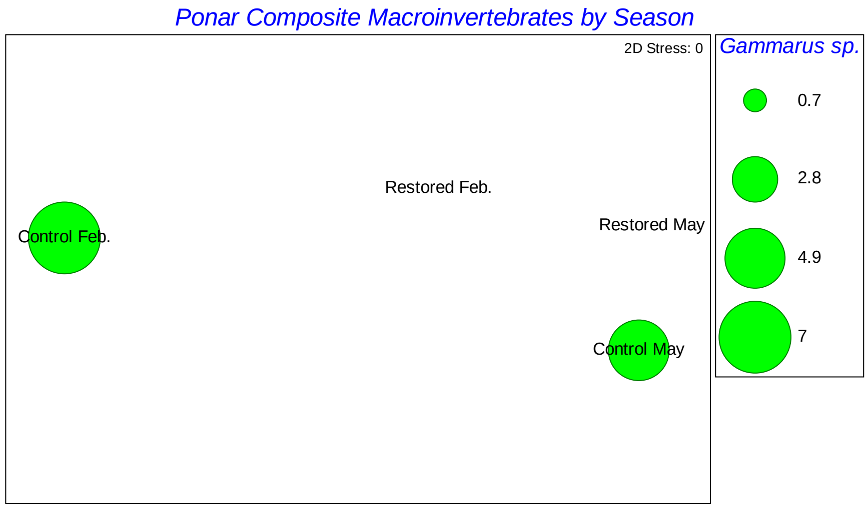


Figure 11. MDS ordination of Ponar composite samples with bubble overlays of the abundance the three most important taxa, each contributing >11% to the dissimilarity between restored and control sites (Appendix A).





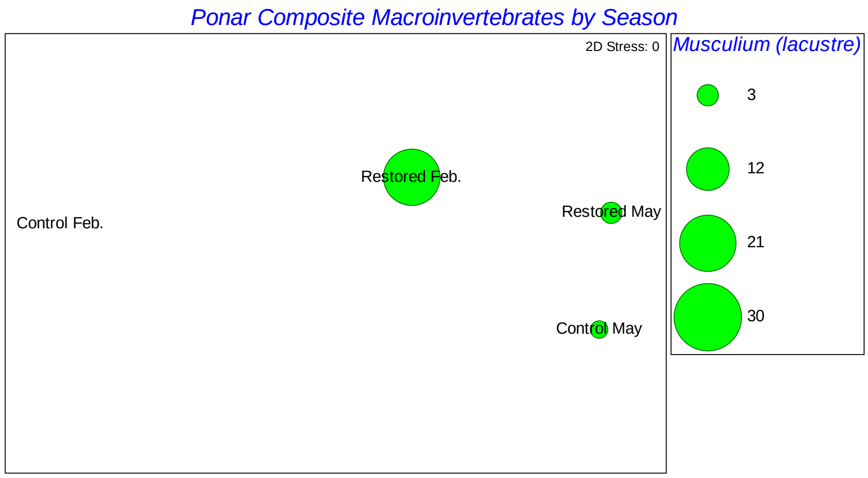


Figure 12. MDS ordination of Ponar composite samples with bubble overlays of the abundance *of Corbicula*, *Gammarus*, and *Musculium* which were important contributors to the dissimilarity between restored and control sites.

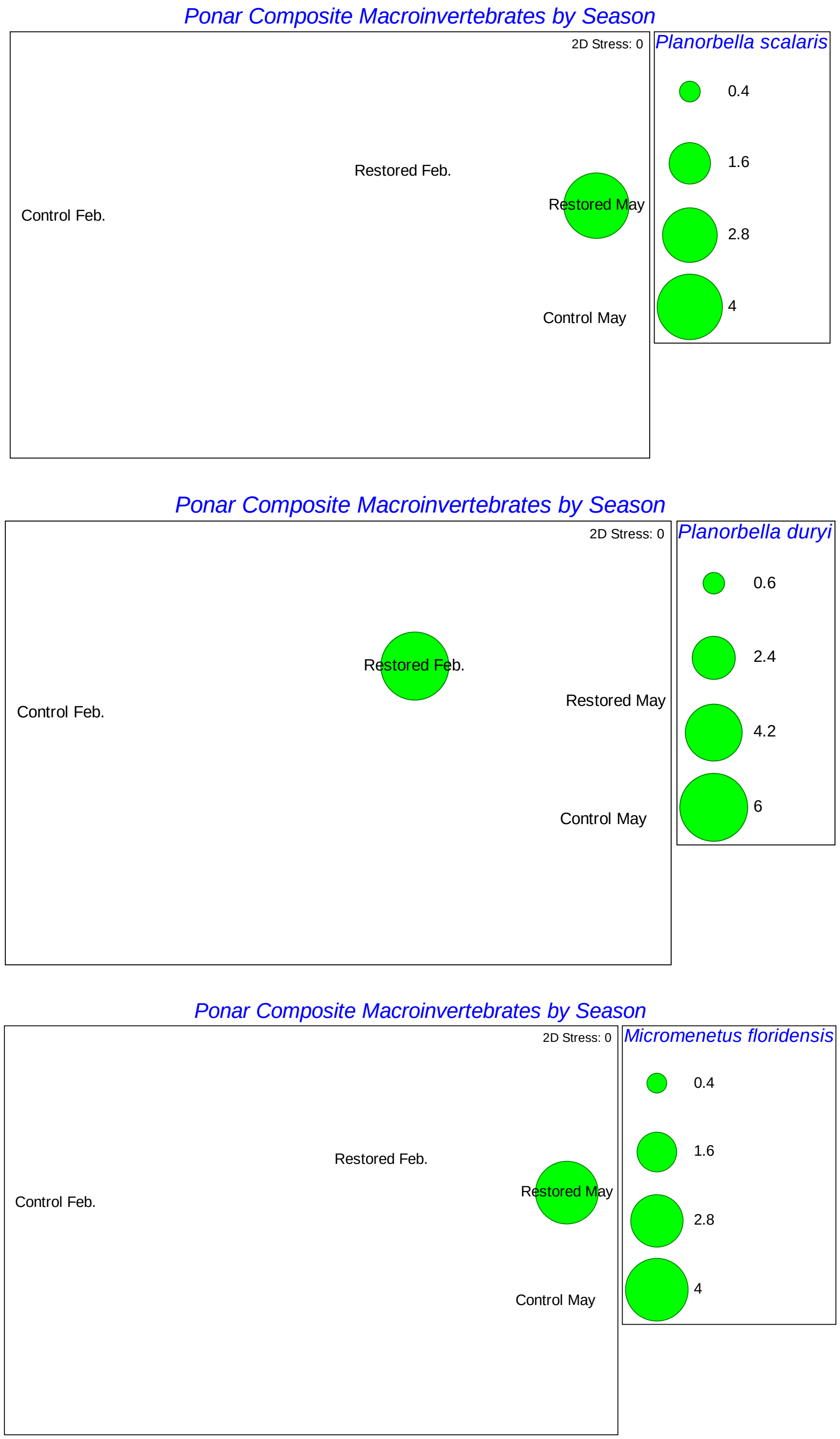


Figure 13. MDS ordination with bubble overlay of the abundances of three native snail species that were relatively abundant at restored sites but absent at control sites.

The control sites sampled typically consisted of 30-40 cm of organic muck with a top layer of algae, primarily *Lyngbya*. This dense mucky habitat is does not support a diverse benthic invertebrate (or plant) community that is considered biologically healthy. Apparently, *Gammarus* is a tolerant organism and they were foraging on the surface of the algal mats. The bivalves *Corbicula fluminea* and *Musculium lacustre* filter large volumes of water and remove particulates and nutrients and serve as prey for higher level consumers. Native snail species (*Planorbella scalaris*, *P. duryi* and *Micromenetus floridensis*) and the limpet (*Hebetancylus excentricus*) are grazers that feed primarily on green algae and diatoms on submerged aquatic vegetation and hard substrates. These grazers were only found in restored habitat benthic samples and appear to indicators of restoration success.

Multivariate Analyses: Hester-Dendy Substrates

Hester-Dendy (HD) substrate results help to identify whether water quality above the substrate is suitable for supporting healthy macroinvertebrate communities. HD substrates deployed inside eel grass beds protected by exclosure cages were intended to represent SAV habitat being restored throughout the system. Univariate statistical analysis of HD substrate data is included in Appendix A. Mean species richness for the restored habitat was 17 species (range = 15-19) while control sites averaged 13 species (range = 12-14). A cluster analysis based on Bray-Curtis similarity, paired with the SIMPROF test identified that significant differences exist between macroinvertebrate communities collected from restored eel grass and control (unrestored) sites **(Figure 14).** The restored and control sites were very dissimilar (73%) and form two significantly different groups ($p<0.05$) that share 60% similarity within each group. **Figure 14** represents significance in the cluster with black lines while red lines identify samples that are not significantly different from each other. There is a clear break at 27% similarity between the two treatments and each group shows high similarity within treatments at >60% **(Figure 14).**

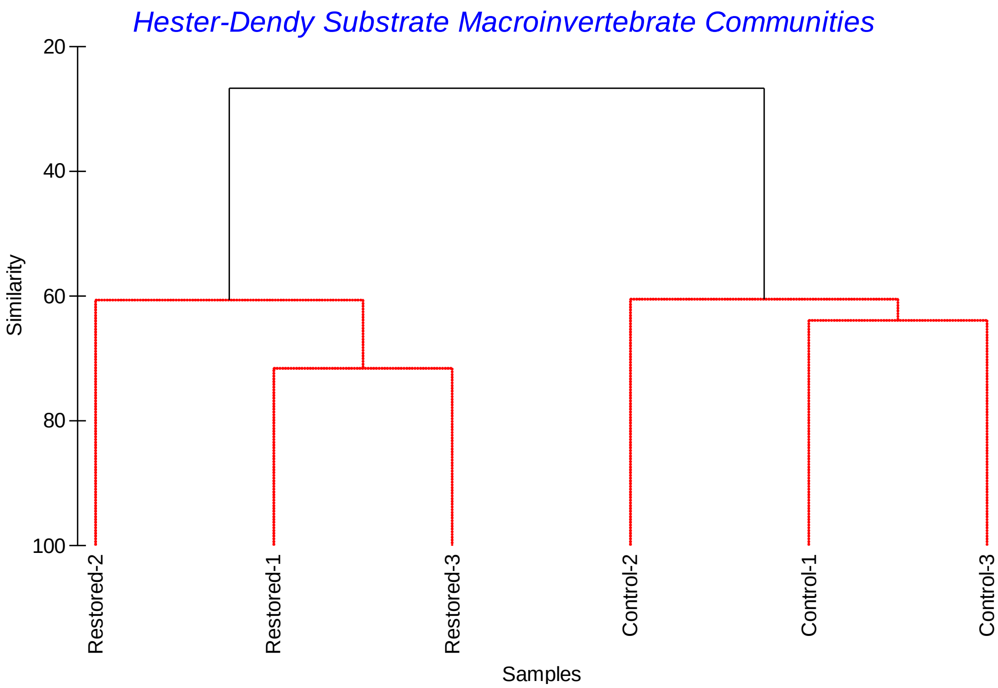


Figure 14. Cluster analysis of Hester-Dendy macroinvertebrate communities with significant groups ($p<0.05$) identified by black lines.

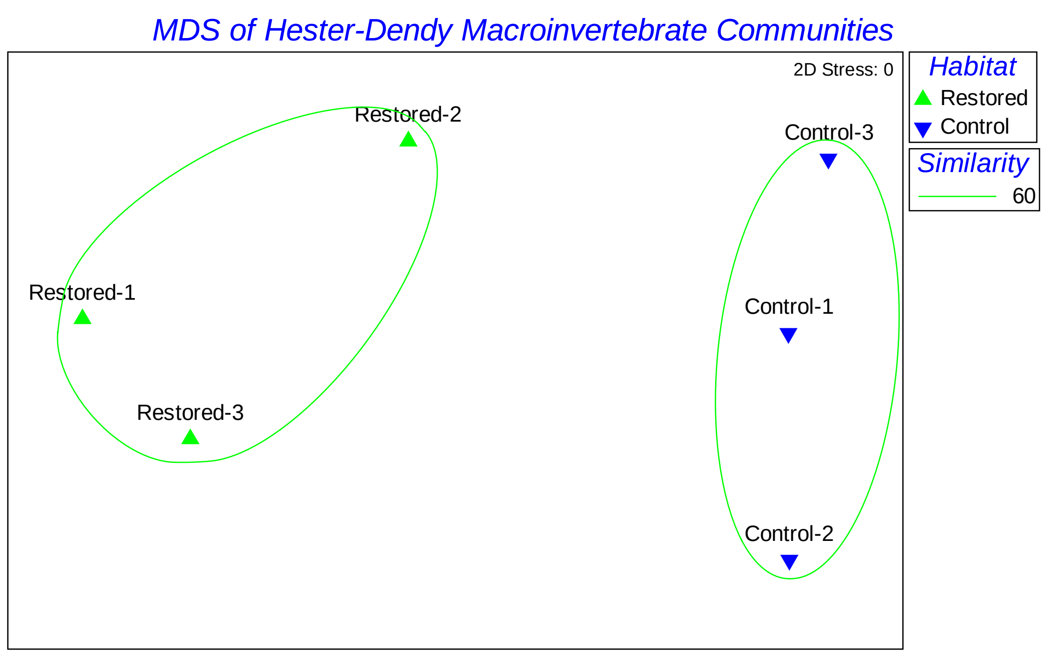


Figure 15. MDS ordination of Hester-Dendy macroinvertebrate communities.

Results of the SIMPER analysis of HD substrate samples are included in **Appendix A**. A total of eleven (11) species were present in the restored site samples but were absent at control sites while only four (4) species were present at control sites but absent in the restored site samples. The tiny hydrobiidae crownsnail (*Pyrogophorus platyrhicusi*) was extremely abundant on the control HD substrates but rare on the restored HD substrates and contributed the most (10.82%) to the dissimilarity between communities. In contrast, the amphipod (*Hyalella azteca* and chironomid *Asheum beckae*) were abundant in restored HDs but rare on unrestored control HD substrates. The native snail (*Planorbella duryii),* non-native snail, *Melanoides tuberculata* along with seven chironomidae larvae (*Tanytarsus* sp. g complex, *Tanytarsus hastatus, Psuedochironomus* sp*., Dicrotendipes simpsoni, Beardius truncates, Cricotopus sp. and Glyptotendipes meridionalis*) were present on restored HD substrates but absent at the unrestored control site. Two crustaceans; the gammaroid amphipod (*Grandidierella bonnierodes*) and tanaidacean (*Hargeria rapax*) along with a *Planariidae* flatworm and non-native clam (*Eupera cubensis*) were collected on control HD substrates but not from the restored HD substrates. These taxa may serve as future indicators of SAV restoration success or degraded conditions respectively depending on presence absence and relative abundance.

<u>Fish Species Collections</u>

A total of sixteen species of fishes were collected and/or visually identified in the Baseline Biological Assessment. This includes ten (10) families and 13 genera **(Table 3).** A total of eleven (11) species were documented in the February 2017 sampling events with an additional four (4) species collected during the May 2017 sampling events using a combination of visual transects, activity traps, and dip net sampling techniques. A total of sixteen species were documented at restored canal sites using a combination of techniques. Twelve species were documented at control sites using those same methods.

Table 3. Fish species collected by Breder trap and Crayfish Trap in restored and unrestored canals, February and May 2017.

Family	Genus	Species	Common Name	Restored Breder Traps	Restored Crayfish Traps	Restored Visual	Control Breder Trap	Control Crayfish Traps	Control Visual
Lepisosteidae	*Lepisosteus*	*osseus*	Longnose Gar			P			P
Poeciliidae	*Heterandria*	*formosa*	Least Killifish	1		P			
	Gambusia	*holbrooki*	Eastern Mosquitofish	39		A			
Fundulidae	*Lucania*	*goodei*	Bluefin Killifish	6	8	C	71	71	A
	Lucania	*parva*	Rainwater Killifish		3	P	99	11	A
	Fundulus	*seminolis*	Seminole Killifish			C	1		
Centrarchidae	*Lepomis*	*punctatus*	Spotted Sunfish		1	C			
	Lepomis	*macrochirus*	Bluegill			C		1	P
	Lepomis	*gulosus*	Warmouth			P		1	P
	Micropterus	*salmoides*	Largemouth Bass			C	3	3	P
Gerridae	*Eucinostomus*	*argenteus*	Spotfin Mojarra			C			C
Gobiidae	*Gobiosoma*	*bosci*	Naked Goby			P			C
Mugilidae	*Mugil*	*cephalus*	Striped Mullet			C			C
Soleidae	*Trinectes*	*maculatus*	Hogchoker			P			P
Belonidae	*Strongylura*	*marina*	Atlantic Needlefish			P			P
Sparidae	*Archosargus*	*probatocephalus*	Sheepshead			P			P
			Species Richness	**3**	**3**	**16**	**4**	**5**	**12**

Abundance Codes:	P = present
	C = common
	A = abundant

No elaborate multivariate statistical analysis was applied to the fish sampling data set primarily because activity traps were much less effective than expected and qualitative observations and visual transects were more representative of conditions in the field.

Two (2) native centrarchid sunfish species; largemouth bass and spotted sunfish were observed or collected from restored canal sites but were absent from unrestored canal samples in February 2017. Fingerling, young of the year (YOY) largemouth bass (*Micropterus salmoides*) were collected at control sites in May 2017 but no adult bass were collected or observed during the February or May 2017 sampling events. Juvenile bluegill (*Lepomis macrochirus*) and warmouth (*L. gulosus*) were collected at one location in the control site but not at the restored site. The presence of fingerling bass and juvenile sunfishes indicates that the habitat may be suitable as nursery habitat for these sunfish species but spawning habitat is mostly lacking. The abundance of *Gammarus* amphipods on the surface of *Lyngbya* beds may serve as forage for small fishes such as bluefin and rainwater killifish and in turn are forage for larger piscivorus fishes.

In addition, we documented spawning activity and nest protection by largemouth bass and spotted sunfish in the restored canal site only. Spawning beds and nest protection by largemouth bass was recorded by underwater video on February 24, 2017. We also video documented aggregations of spotted sunfish and potential spawning beds in the same Area 3. No native sunfishes were observed or collected from the unrestored control sites in February 2017 and muck substrates are unsuitable for successful spawning by most sunfish species including largemouth bass and spotted sunfish.

4.0 DISCUSSION

Benthic macroinvertebrate communities appear to be a reliable biological indicator of habitat restoration and habitat quality in King's Bay. A total of 63 macroinvertebrate species were collected and identified, representing six (6) classes, 17 orders, and 33 families. The highest species richness (22 taxa) was found in the qualitative dip net sampling conducted in restored habitat while the highest abundance (369) was found in composited Ponar samples from restored habitat in February. 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.

Virtually all univariate statistical metrics (species richness, Margalef richness, Shannon diversity and Simpsons diversity) identified higher macroinvertebrate diversity in restored habitats. Pielous' evenness was highest in one control Ponar, but only six (6) species and 17 total organisms were present there. Multivariate analysis of community structure was useful in identifying several potential indicator species for restoration monitoring in the future. Several indicator taxa were present in the restored areas that were absent entirely or found in very low numbers from unrestored control sites. The three most important species in separating restored and control sites were the non-native snail (*Melanoides tuberculate*), the amphipod (*Hyalella Azteca*), grp. and oligochaete worm (*Limnodrilus hoffmeisteri*). All three were contributed more than 11% to the dissimilarity and were extremely abundant in the restored group while uncommon or rare at the control sites. The tiny native fingernail clam (*Musculium lacustre*) was more abundant in restored habitat the control samples and contributed 5.11% to the dissimilarity between the communities. *Corbicula fluminea* was relatively abundant in the restored areas but absent in the unrestored (control) canal Ponar samples. Three other native snail species (*Planorbella scalaris, P. duryi*, and *Micromenetus floridensis*), the limpet (*Hebetancylus excenticus*) were collected from restored canal sites but were not found in any of the control Ponar samples. Conversely, benthic samples from the control sites contained *Gammarus* sp. amphipods but they were absent in samples collected from the restored canals. The deep organic muck (30-40 cm) with a coating of *Lyngbya* at the surface is completely unsuitable habitat for most native snails and for larger specimens of the non-native *Melanoides tuberculata* which appear to sink into the soft sediments and die. Methane and/or hydrogen sulfide odors emitted from sediments during the sampling events indicate anoxic conditions persist throughout most of the sediment in unrestored areas.

Hester-Dendy substrate results help to identify whether water quality above the substrate is suitable for supporting healthy macroinvertebrate communities. A cluster analysis based on Bray-Curtis similarity, paired with the SIMPROF test identified that significant differences exist between macroinvertebrate communities collected from restored SAV habitat in Phase 1A and the control (unrestored) site. The restored and control sites were 73.27% dissimilar and form two significantly different ($p<0.05$) groups; restored group and control group. Hester-Dendy substrate results supports the findings from other sampling methods that there are significant changes in community structure with increased species richness and abundance of macroinvertebrates at restored sites compared to unrestored control sites. Based on HD substrate collections alone, eleven species, including seven chironomid species were present in the Phase 1A restored eel grass beds that were absent from the unrestored control site. Several species were identified that may serve as indicators of restoration success in future biological assessments. These results are based on three replicate Hester-Dendy substrates deployed in the water columns above the benthos at one restored area and one unrestored control area. Therefore, results should be considered as a preliminary baseline for these two sites only. However, HD substrate data supports the findings from other sampling methods and that there are significant changes in community structure and increased species richness and abundance of macroinvertebrates at restored sites compared to unrestored control sites.

Fish sampling using activity traps (Breder traps and modified crayfish traps) was likely effected negatively by the intense tour-boat traffic and the large numbers of snorkelers swimming in the study areas. Tour boats activity and large numbers of snorkelers had impacts on fish behavior that is difficult to separate and quantify. Visual surveys were more effective than activity traps for collecting fishes in these high traffic canals and in King's Bay. The most noteworthy fish sampling results were obtained from visual transects and video documentation of spawning and nest protection by largemouth bass and other sunfishes only in the restored habitats. Additional experimentation with fish sampling methods is highly recommended in order to quantify habitat use and seasonal fish movements within and between habitats in the King's Bay ecosystem.

Manatees were also relatively abundant during the study period and they appear to have greatly reduced the distribution, abundance and biomass of eel grass, *Vallisneria americana* planted in the restored canals. Small rosettes of eel grass were present and scattered throughout the restored canals. Quantifying eel grass coverage and density was not a part of the current study but is recommended for future biological investigations related to monitoring restoration success.

5.0 REFERENCES

Breder, C. M. 1960. Design for a fish fry trap. Zoologica 45:155-160.

Ceilley, D.W, L. Brady-Herrero, K. Niemec, K. M. Ross. J. A. Ferlita, and E. M. Everham, III. 2013. Fish community structure of streams and canals at Babcock Ranch, Charlotte and Lee Counties, FL. Florida Scientist Vol. 76 (2): 198-215.

Ceilley, D.W. 2008. Picayune Strand Restoration Project: Baseline Assessment of Inland Aquatic Fauna ORSP File #05112 South Florida Water Management District, West Palm Beach, FL. 97 pp.

Ceilley, D.W., I. Bartoszek, M. Schuman, R.E. Schroeder, J. Schmid, and K. Worley. 2007. Enhancing the Recovery of Threatened and Endangered Species in the South Florida Ecosystem through Aquatic Refugia. Final Report for Cooperative Agreement No. 1448-40181-01-G-47, U.S. Fish and Wildlife Service, Vero Beach, FL. 272 pp.

Ceilley, D.W. 2007. Supplemental Data Analyses and Discussion for: Enhancing the Recovery of Threatened and Endangered Species in South Florida through Aquatic Refugia. Cooperative Agreement # 1448-40181-01-G-47, US Fish and Wildlife Service, Vero Beach, Florida. 53 pp.

Ceilley, D.W., I. Bartoszek, G. G. Buckner, and M.J. Schuman. 2003. Tape grass (*Vallisneria americana*) restoration feasibility study. Final Report to the South Florida Water Management District, 3300 Gun Club Rd. West Palm Bch, FL. 16 pp.

Clarke, K. R. and R. N. Gorley. 2006. PRIMER User Manual/Tutorial, Version 6, PRIMER-E Ltd., Plymouth, United Kingdom.

Florida Department of Environmental Protection (FDEP). 1993 Draft. Standard Operating Procedures Manual - Benthic macroinvertebrate sampling and habitat assessment methods: 1. Freshwater streams and rivers. Contract #WM385. FDER, Tallahassee, Florida.

Florida Department of Environmental Protection (FDEP). Accessed January 4, 2017. http://www.dep.state.fl.us/water/sas/sop/sops.htm

Hauxwell, J., C.W. Osenberg, and T.K. Frazer. 2004. Conflicting management goals: manatees and invasive competitors inhibit restoration of a native macrophyte. Ecological Applications, 14(2), 2004, pp. 571-586 by the Ecological Society of America, Washington, D.C.

Johnson Engineering Inc. and Sea and Shoreline. 2016. Tape Grass (*Vallisneria americana*) Restoration and Seed Stock Enhancement in the C-43 (Caloosahatchee River) With Plantings and Exclosure Cages. First Annual Report to the South Florida Water Management District, West Palm Bch. FL. 40 pp.

Main, M.B., D.W. Ceilley, and P.A. Stansly. 2007. Freshwater fish assemblages in Isolated South Florida Wetlands. Southeastern Naturalist 6(2): 343-350.

Moore, K.A., E.C. Shields, and J.C. Jarvis. 2010. The role of habitat and herbivory on the restoration of tidal freshwater submerged aquatic vegetation populations. Restoration Ecology Vol.18, No. 4, pp. 596-604.

Robbins, B.D. 2004. Habitat use of *Vallisneria americana* beds in the Caloosahatchee River: Final Report to the South Florida Water Management District. Mote Marine Lab. Tech Rpt; No.993.

APPENDIX A

Macroinvertebrate Statistical Analysis

SIMPER

Similarity Percentages - species contributions

One-Way Analysis

Data worksheet

Name: Ponar Composite Samples

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	Habitat
Restored Feb.	Restored
Restored May	Restored
Control Feb.	Control
Control May	Control

Group Restored

Average similarity: 40.09

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Hyalella azteca grp.	6.84	14.84	#######	37.01	37.01
Melanoides tuberculata	7.89	12.63	#######	31.52	68.53
Limnodrilus hoffmeisteri	6.12	6.21	#######	15.48	84.01
Musculium (lacustre)	3.16	4.06	#######	10.14	94.15

Group Control

Average similarity: 11.68

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Gammarus sp.	2.44	11.68	#######	100.00	100.00

Groups Restored & Control

Average dissimilarity = 73.22

Species	Group Restored Av.Abund	Group Control Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Melanoides tuberculata	7.89	3.12	8.45	1.40	11.54	11.54
Hyalella azteca grp.	6.84	2.00	8.39	1.67	11.46	23.00
Limnodrilus hoffmeisteri	6.12	0.50	8.21	1.88	11.21	34.22
Corbicula fluminea	3.54	0.00	4.67	0.86	6.37	40.59
Gammarus sp.	0.00	2.44	4.23	2.98	5.78	46.36
Musculium (lacustre)	3.16	0.71	3.74	1.57	5.11	51.47
Pyrogophorus platyrhicus	1.94	2.00	3.55	0.83	4.85	56.32
L. claparedeianus	2.00	0.00	2.64	0.86	3.61	59.93
Grandidierella bonnierodes	0.00	1.41	2.63	0.82	3.59	63.52
Ilyodrilus tempeltoni	1.80	0.00	2.38	0.86	3.25	66.77
Einfeldia natchitocheae	0.00	1.22	2.27	0.82	3.11	69.87
Planorbella scalaris	1.00	0.00	2.12	0.86	2.90	72.77
Micromenetus floridensis	1.00	0.00	2.12	0.86	2.90	75.67
Leptoceridae	1.00	0.00	2.12	0.86	2.90	78.57
Lumbriculidae	0.00	1.12	1.77	0.83	2.42	80.99
Planorbella duryi	1.22	0.00	1.62	0.86	2.21	83.20
Dicrotendipes modestus	0.00	0.71	1.31	0.82	1.79	84.99
Nematoda	0.71	0.71	1.26	0.80	1.72	86.71
Procladius sp. I Rutter	1.21	0.50	1.23	1.09	1.68	88.39
Chironominae	0.00	0.71	1.12	0.83	1.53	89.92
Hebetancylus excentricus	0.50	0.00	1.06	0.86	1.45	91.37

SIMPER

Similarity Percentages - species contributions

One-Way Analysis

Data worksheet

Name: **Hester-Dendy Substrates**

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	Habitat
Restored-1	Restored
Restored-2	Restored
Restored-3	Restored
Control-1	Control
Control-2	Control
Control-3	Control

Group Restored

Average similarity: 64.37

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Asheum beckae	4.10	12.24	23.14	19.02	19.02
Hyalella azteca grp.	4.22	10.93	5.59	16.99	36.01
Gammarus sp.	3.26	10.37	55.57	16.11	52.12
Chironomus decorus grp.	1.72	4.99	7.42	7.75	59.87
Dicrotendipes neomodestus	1.99	4.99	7.89	7.75	67.61
Cassidinidea ovalis	1.76	4.64	55.57	7.20	74.82
Planorbella duryi	1.38	3.72	5.23	5.78	80.60
Tanytarsus sp. g complex	1.14	3.28	55.57	5.09	85.69
Haitia (Physa) cubensis	1.05	1.55	0.58	2.41	88.11
Melanoides tuberculata	0.91	1.11	0.58	1.72	89.83
Psuedochironomus sp.	1.08	1.11	0.58	1.72	91.55

Group Control
Average similarity: 61.71

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Pyrogophorus platyrhicus	4.99	13.57	2.77	21.99	21.99
Oxythira sp.	2.83	9.29	7.57	15.05	37.04
Cassidinidea ovalis	3.05	8.71	11.55	14.11	51.15
Gammarus sp.	2.72	6.13	1.71	9.94	61.09
Cyrnellus fraternus	1.99	5.71	23.31	9.25	70.34
Grandidierella bonnierodes	2.40	3.79	7.57	6.14	76.48
Eupera cubensis	1.14	3.79	7.57	6.14	82.63
Ablabesmyia rhamphe grp.	1.00	3.79	7.57	6.14	88.77
Haitia (Physa) cubensis	1.72	2.60	0.58	4.21	92.98

Groups Restored & Control
Average dissimilarity = 73.27

	Group Restored	Group Control				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Pyrogophorus platyrhicus	0.33	4.99	7.93	2.99	10.82	10.82
Hyalella azteca grp.	4.22	0.33	6.93	2.75	9.45	20.28
Asheum beckae	4.10	0.67	6.00	6.07	8.19	28.47
Oxythira sp.	0.33	2.83	4.36	3.74	5.95	34.42
Grandidierella bonnierodes	0.00	2.40	4.13	1.19	5.63	40.06
Dicrotendipes neomodestus	1.99	0.00	3.54	2.76	4.83	44.89
Chironomus decorus grp.	1.72	0.00	3.04	5.16	4.15	49.03
Cyrnellus fraternus	0.33	1.99	2.84	2.32	3.88	52.91
Planorbella duryi	1.38	0.00	2.44	4.06	3.33	56.23
Haitia (Physa) cubensis	1.05	1.72	2.35	1.47	3.21	59.44
Cassidinidea ovalis	1.76	3.05	2.24	1.30	3.06	62.50
Tanytarsus sp. g complex	1.14	0.00	2.02	4.27	2.76	65.26
Eupera cubensis	0.00	1.14	1.99	8.18	2.71	67.97
Gammarus sp.	3.26	2.72	1.98	0.97	2.71	70.68
Psuedochironomus sp.	1.08	0.00	1.93	1.08	2.64	73.32
Planariidae	0.00	1.05	1.92	1.24	2.63	75.94
Melanoides tuberculata	0.91	0.00	1.63	1.18	2.23	78.17
Dicrotendipes simpsoni	0.91	0.00	1.62	1.18	2.22	80.39
Tanytarsus hastatus	0.91	0.00	1.61	1.20	2.19	82.58
Beardius truncatus	0.80	0.00	1.42	1.27	1.94	84.52
Cricotopus sp.	0.80	0.00	1.41	1.25	1.92	86.45

Hargeria rapax	0.00	0.80	1.32	1.26	1.80	88.25
Glyptotendipes meridionalis	0.47	0.00	0.82	0.66	1.12	89.37
Nais variabilis	0.33	0.33	0.76	0.83	1.04	90.41

DIVERSE

Univariate Diversity indices

Data worksheet

Name: Hester-Dendy Substrates

Data type: Abundance
Sample selection: All
Variable selection: All

Sample	S	N	d	J'	H'(loge)	1-Lambda'
Restored-1	15	71	3.284	0.8487	2.298	0.8821
Restored-2	19	70	4.237	0.7731	2.276	0.8522
Restored-3	17	84	3.611	0.736	2.085	0.8058
Control-1	14	112	2.755	0.7578	2	0.8298
Control-2	12	33	3.146	0.887	2.204	0.8939
Control-3	13	101	2.6	0.6781	1.739	0.7525

APPENDIX B

Photographs of Macroinvertebrate and Fish Sampling

Restored Site Ponar Sample “R-1” showing clay and silt that was not present in other samples collected from the restored canal site.

Control (unrestored) Site Ponar Sample “C-3” with dense Lyngbya and several dead snails, *Melanoides tuberculata.*

Ponar Sample processing and preservation station using standard seives to sort benthic macroinvertebrates from sediments and debris.

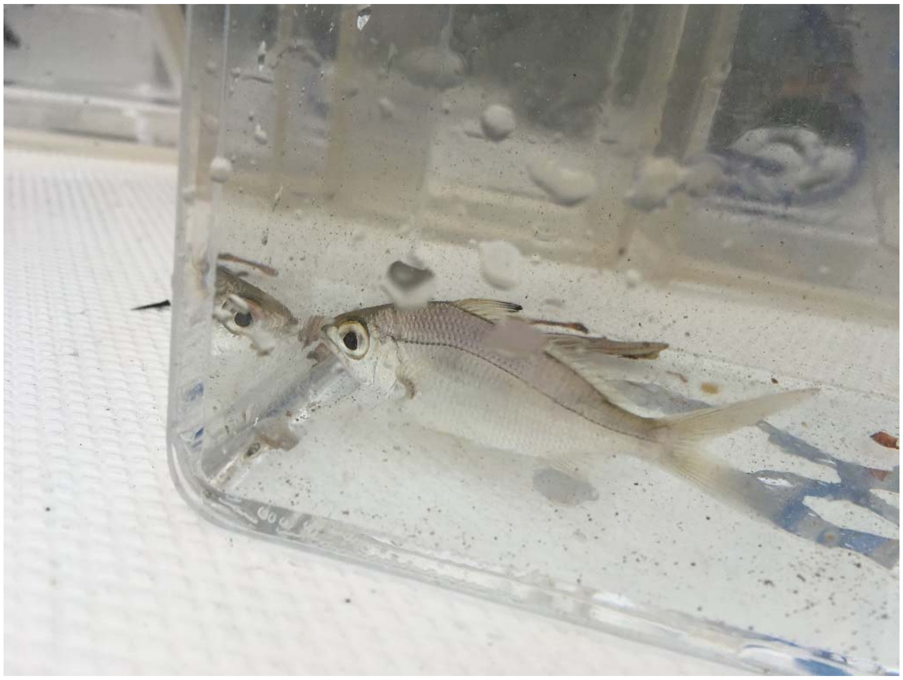
Two standard sieves used to sort petite Ponar benthic samples with sieve #10 (2 mm) on left and sieve #30 (500 micron) on right.

Sorted and preserved petite Ponar benthic macroinvertebrate samples prepared for identification.

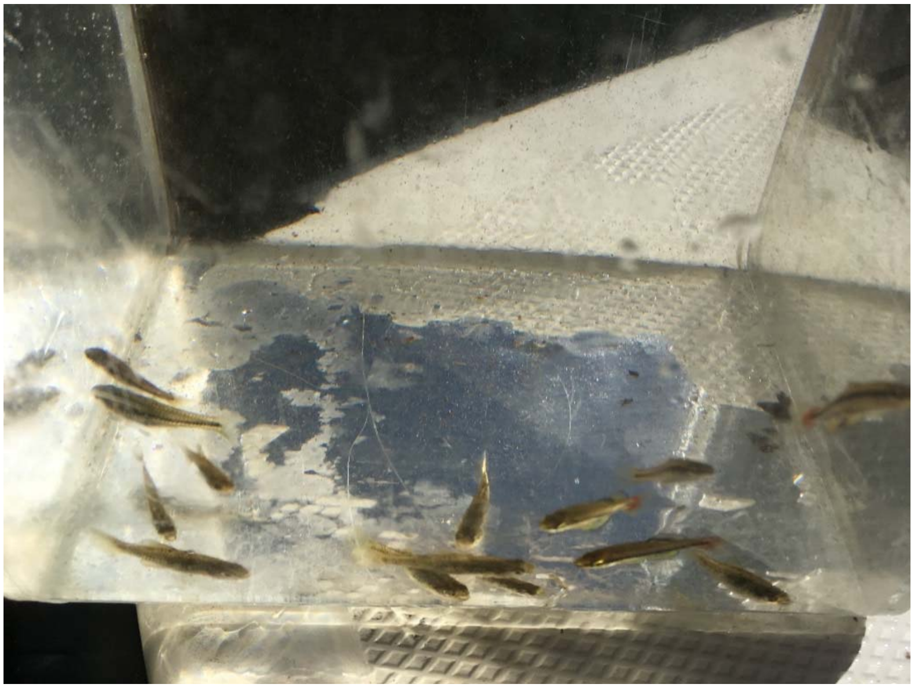
Fish sampling using visual transect and crayfish traps on May 18, 2017 in unrestored canal of King's Bay.

Fish collection from crayfish trap in unrestored canal. Note: small forage fishes and juvenile sunfish were confined to shallow edge habitat at base of seawalls where there was less muck and *Lyngbya*.

Bluefin killifish, *Lucania goodei* and spotted sunfish, *Lepomis punctatus* collected from restored canal habitat in February, 2017. Spawing and nest defense behaviors by spotted sunfish and largemouth bass, *Micropterus salmoides* were documented in the restored canal “pilot project” but not in control (unrestored) canals.



Typical one-hour Breder trap fish sample from restored canal site with rainwater and bluefin killifish common to abundant in most shallow areas.



Spotfin mojarra, *Eucinostomus argenteus,* collected in Breder trap at restored canal site in February 2017.