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

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Progress Report 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

EXECUTIVE SUMMARY

An independent biological assessment was initiated in February 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. This progress report includes a description of the standard methods employed to assess the aquatic faunal communities along with some preliminary results from the February 2017 monitoring events. When completed, the study will include quantitative assessments of aquatic faunal communities (macroinvertebrates and fishes) at two distinct habitat types and will be completed over two seasons to reflect seasonal differences.

Three different sampling techniques were employed to assess both macroinvertebrate and fish assemblages to increase sample size and minimize sampling biases. Fish traps, dip nets and visual transects were used to assess fishes while a petite Ponar dredge, Hester-Dendy substrates and dip net were used to sample macroinvertebrates.

At least 13 species of aquatic invertebrates, representing 13 families and 10 orders were collected from restored canal habitats in King's Bay. The unrestored control site samples are still being processed but several important indicator taxa are missing, including the benthic bivalves (*Musculium* and *Corbicula*), snails (Gastropoda) and riffle beetles of the genus *Stenelmis*. These missing benthic taxa indicate that sediments are unsuitable for filter feeding bivalves due to thick layers of organic muck and anoxic conditions.

West Indian manatees were very abundant during the February monitoring event and they were observed grazing on planted eel grass, *Vallisneria americana*, both inside and outside exclosure cages. Grazing pressure appeared to regulating eel grass bed size, height and density throughout the King's Bay system yet small rosettes were found scattered in much of the restored canals. Heavy tour-boat traffic with large groups of snorkelers in the waters around the sampling sites most likely impacted fish behavior patterns. However, a total of eleven (11) fish species were documented from restored habitats, with nine (9) species in unrestored areas. It was noteworthy that largemouth bass and spotted sunfish were only observed at restored canal sites where spawning activity was documented over hard bottom (sand, gravel) habitats. Unrestored areas do not support native sunfish spawning success because of the deep muck accumulations (30-40 cm) and anoxic conditions present in the benthos.

TABLE OF CONTENTS

		Page
EXECUTIVE SUMMARY		i
1.0	INTRODUCTION	1
2.0	METHODS	3
	Macroinvertebrate Communities	3
	Fish Communities	7
3.0	PRELIMINARY RESULTS	10
	Aquatic Macroinvertebrates	10
	Fish Species Collections	11
4.0	FUTURE WORK	12
	Deliverables	13
5.0	REFERENCES CITED	14

LIST OF TABLES

Number	
1	Macroinvertebrates collected by dip net and Ponar dredge combined.
2	Fish species observed and collected from restored and unrestored canal sites.

LIST OF FIGURES

Number	
1	Location Map
2	King's Bay Restoration Sample Site Map
3	Standard D-Frame Aquatic Dip-net used for aquatic faunal sampling; fishes and macroinvertebrates.
4	EPA Hester-Dendy Artificial Substrates for aquatic macroinvertebrate sampling.
5	Petite Ponar dredge (lower right) being used to collect replicate sediment samples from restored canal habitat in King's Bay Area 3.
6	Clear plastic "Breder Traps" for sampling fish communities in shallow waters.
7	Modified crayfish traps for overnight sampling of fish communities.

ATTACHMENTS

A	Save Crystal River Aquatic Fauna Data Sheet
B	USFWS HET Working Group Macroinvertebrate Field Sheet

1.0 INTRODUCTION

A biological assessment was initiated in February 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-endangered 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 species, 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, *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. 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.

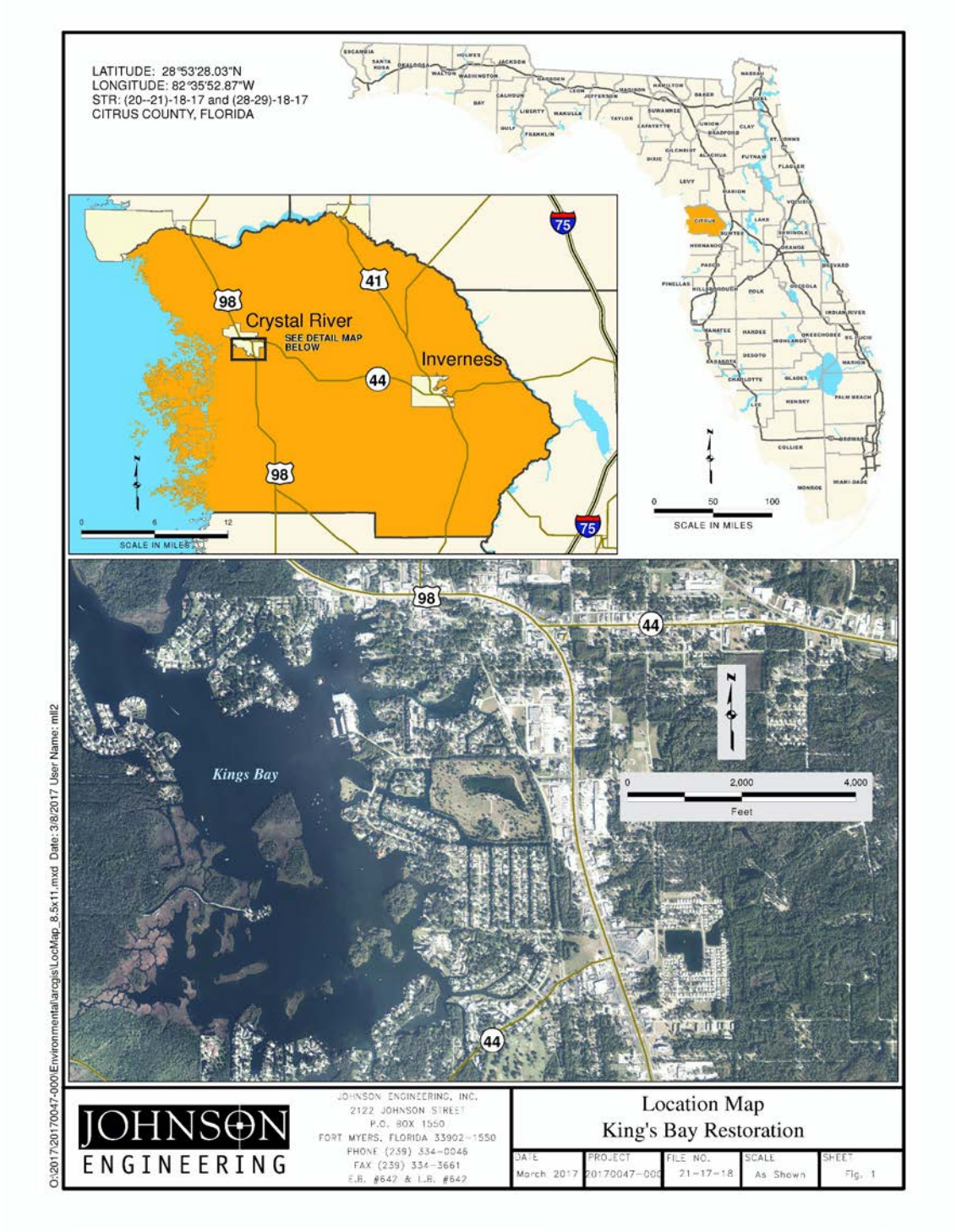

Figure 1. Location Map of King's Bay Restoration Area

A secondary objective of this study is to document wildlife utilization in restored and unrestored habitats through qualitative observations (visual, tracks, audible, scat, sheds) 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 and unrestored sections of the waterways prior to sampling. This 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. GPS coordinates will be collected and mapped for figures in the final report.

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 20 minutes 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 substrates were also deployed as replicates at restored and unrestored sampling sites and allowed to colonize with invertebrates for 28 days **(Figure 4).** After the colonization period, samplers will be retrieved and processed

for collection and preservation of epi-fauna using 80% ethanol and labeled and archived for identification in the laboratory. Hester-Dendy substrate samples will be processed, sorted and all macroinvertebrates identified to the lowest practical taxonomic level. The macroinvertebrate species richness and density will be calculated for each site for comparison between sites, treatments, and through time.

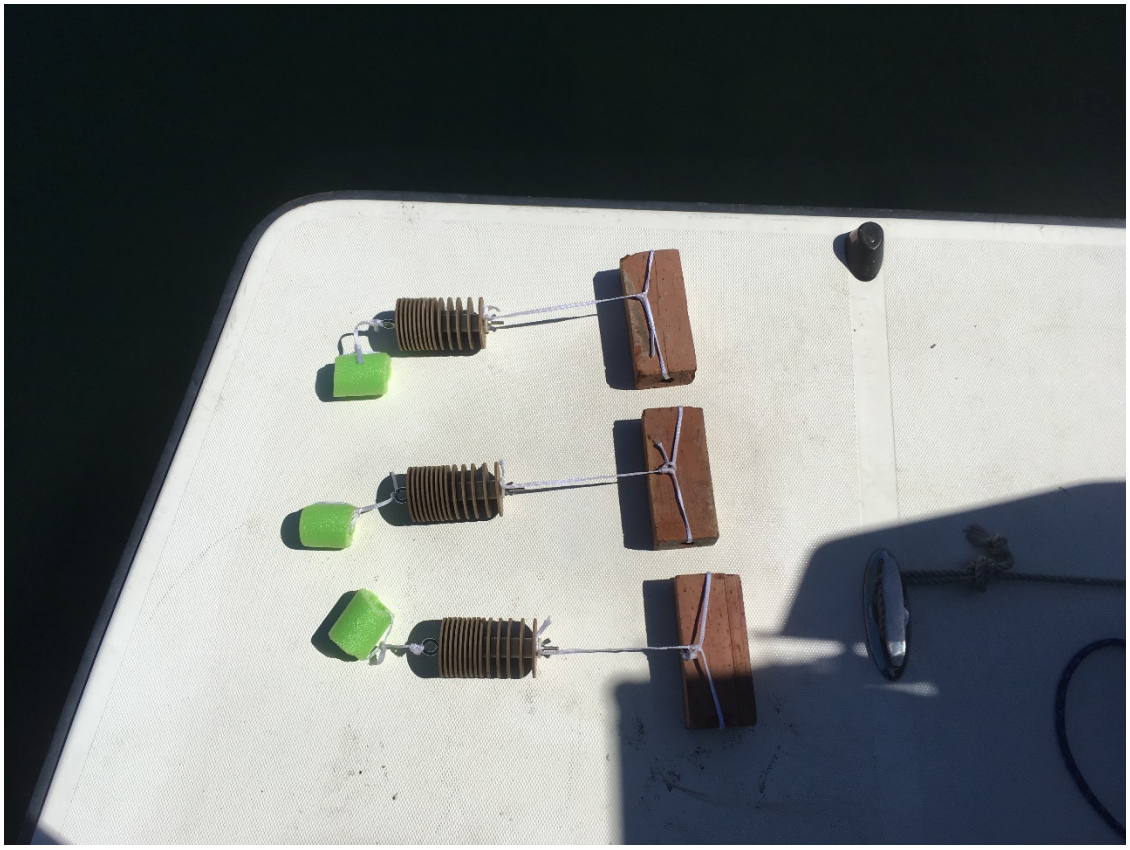

Figure 4. EPA Hester-Dendy Artificial Substrates for aquatic macroinvertebrate sampling.

3. In addition to the dip net and Hester-Dendy samples collected, three petite Ponar samples were collected from representative study site locations **(Figure 5).** Samples were processed following FDEP (2017) Standard Operating Procedures (SOP FS4000/FS7400). 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 Area 3.

Petite Ponar dredge and dip net samples have been processed and many of the macroinvertebrates collected have been identified and are listed in the following results section. However, the results are preliminary and not complete since the Hester-Dendy substrates have not been retrieved and some taxa collected by dip nets and Ponar dredge are still being identified.

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. Timed visual transects will record species richness and relative abundance for comparison between treatment sites. Still photos and video recordings will be provided along with the written biological assessment report.
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

This overnight fish sampling was repeated on a second night at a different location within the restored and unrestored habitats. Note: 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 will be conducted in May 2017 when tour-boat activity is expected to be greatly reduced.

3.0 PRELIMINARY RESULTS

Aquatic Macroinvertebrates

Macroinvertebrates from the petite Ponar dredge samples have been separated from the sediments using Standard Sieves and are in the process of being identified. Hester-Dendy substrates will be retrieved on March 24, 2017 and will be processed, identified and quantified as to density per square meter of substrate for both restored and unrestored habitats. Preliminary macroinvertebrate sampling results from dip-net samples and dredge samples are presented in **Table 1**.

Table 1. Macroinvertebrates Collected by dip net and Ponar dredge combined.

Taxa	Order	Family	Genus spp.	Restored	Control
Annelida	Oligochaeta	Naididae	Pending ID	x	x
	Oligochaeta	Lumbriculidae	Pending ID	x	
	Rhynchobdellida	Hirundinea	*Helobdella sp.*	1	
Crustacea	Amphipoda	Spaeromidae	*Exosphaeroma sp.*	2	
	Amphipoda	Talitridae	*Hyalella azteca*	46	x
	Mysida	Mysidacae	*Taphromysis lousianae*	1	
Insecta	Odonata	Libellulidae	*Epicordulia princeps*	1	
	Heteroptera	Corixidae	*Trichocorixa sp. imm.*	1	
	Diptera	Chironomidae	Pending ID		
	Coleoptera	Elmidae	Stenelmis sp.	8	0
Mollusca	Gastropoda	Thiaridae	*Melanoides tuberculata**	100+	0
	Gastropoda	Planorbidae	*Planorbella duryi*	6	0
	Pelecypoda	Sphaeriidae	*Musculium lacustre*	5	0
	Pelecypoda	Corbiculidae	*Corbicula fluminea**	50+	0
			Species Richness	13	2
*** = Non-native taxa**					

A minimum of 13 species of aquatic invertebrates, representing 13 families, and 10 orders have been collected from restored canal habitats in King's Bay. The unrestored control site samples are still being processed but several important indicator taxa are missing, including the benthic bivalves (*Musculium* and *Corbicula*), snails (Gastropoda)

and riffle beetles of the genus *Stenelmis*. These missing taxa indicate that sediments are unsuitable for filter feeding bivalves due to thick layers of organic muck and anoxic conditions. The control sites sampled typically consisted of 30-40 cm of organic muck with a top layer of algae, primarily *Lyngbya* and other filamentous species. This dense mucky habitat is does not support a diverse benthic invertebrate (or plant) community that is considered biologically healthy. Bivalves (even non-native *Corbicula fluminea*) filter large volumes of water and remove particulates and nutrients and serve as prey for higher level consumers. Hester-Dendy substrate results are pending but will help identify whether water quality above the substrate is suitable for supporting healthy benthic invertebrate communities.

Fish Species Collections

A total of eleven (11) species were documented in the February 2017 sampling events using a combination of visual transects, activity traps, and dip net sampling techniques. This includes nine (9) families and ten (10) genera **(Table 2).** Two native centrarchid sunfish species; largemouth bass and spotted sunfish were observed and collected only from restored canal sites but were absent from unrestored canal samples. In addition, we documented spawning activity and nest protection by largemouth bass in the restored canal site identified as “Area 3” in the permit drawings provided by Gator Dredging Inc. 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 and muck substrates are unsuitable for successful spawning by most sunfish species including largemouth bass and spotted sunfish.

Table 2. Fish species observed and collected from restored and unrestored canal sites.

Family	Genus	Species	Common Name	Restored	Unrestored
Lepisosteidae	*Lepisosteus*	*osseus*	Longnose Gar	P	P
Poeciliidae	*Heterandria*	*formosa*	Least Killifish	P	P
Fundulidae	*Lucania*	*goodei*	Bluefin Killifish	A	A
	Lucania	*parva*	Rainwater Killifish	C	C
Centrarchidae	*Lepomis*	*punctatus*	Spotted Sunfish	C	
	Micropterus	*salmoides*	Largemouth Bass	C	
Gerridae	*Eucinostomus*	*argenteus*	Spotfin Mojarra	C	A
Gobiidae	*Gobiosoma*	*bosci*	Naked Goby	C	C
Mugilidae	*Mugil*	*cephalus*	Striped Mullet	C	C
Soleidae	*Trinectes*	*maculatus*	Hogchoker	P	P
Belonidae	*Strongylura*	*marina*	Atlantic Needlefish	P	P
			Species Richness	**11**	**9**
Abundance Codes:	P = present				
	C = common				
	A = abundant				

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 began running at sunrise and continued throughout the days of Wednesday through Friday, February 22-24, 2017. 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.

4.0 FUTURE WORK

Macroinvertebrates are still in the process of being collected, identified, enumerated for statistical analysis, and archived for future reference. Hester-Dendy substrates will be retrieved by the end of March 2017 for processing and identification.

Fish and aquatic macroinvertebrate sampling will be repeated in May to early June for inclusion in the final technical report to compare the restored and unrestored (control)

habitats and biological integrity. Qualitative and quantitative sampling approaches are being employed concurrently to improve efficiency and provide empirical statistical data respectively. Underwater video and photographic evidence will be included in the final report along with voucher specimens.

Data will be analyzed by sampling method and treatment using univariate (species richness, Shannon diversity, Pielou's eveness, Simpson's Index) and multivariate techniques based on Bray-Curtis similarity including hierarchical agglomerative cluster analysis, multi-dimensional scaling (MDS) ordination, similarity profile (SIMPROF), similarity percentage (SIMPER) tests, and analysis of similarity (ANOSIM) which is a multivariate analog of ANOVA (Clarke and Gorley 2006). Data will be managed in Excel™ and analyzed using univariate diversity metrics and multivariate approaches for evaluating community structure and indicator taxa using PRIMER v6. Restoration indicator taxa will be identified using SIMPER. The final report will also include recommendations for future biological assessments related to sample site selection, survey methodologies, frequency and timing of sampling events, and discussion/conclusions.

Deliverables

- A Final Technical Report that includes results, analyses and summary of findings of the aquatic faunal assessment that compares the fish and macroinvertebrate communities from restored and unrestored aquatic habitats. This will include both univariate and multivariate statistical analyses and discussion of any significant differences between the communities -and- identification of potential indicator species for assessment of restoration success or impairment.

- Voucher specimens of macroinvertebrate taxa will be provided for future reference and verification.

- The biological sampling will consist of two sampling events in 2017 to represent seasonal differences between February and May/June 2017. An additional 21 days following the second sampling will be needed to complete the assessment with a final report due no later than June 28 2017.

- A professional presentation to the client in the form of PowerPoint slide show summarizing the results followed by a question/answer session (at a location to be determined in the State of Florida).

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ATTACHMENT A

Save Crystal River Aquatic Fauna Data Sheet

SAVE CRYSTAL RIVER AQUATIC FAUNA DATA SHEET

Site ID_____
Date_____
Field Crew_____
_____
Sampling Start Time_____
Sampling Finish Time_____

Sky Condition_____
Air Temp:_____
Wind:_____
Inverts Collected: Yes No
Invert Start_____ Breder Start_____
Invert Finish_____ Breder Finish_____

Trap No.	FLUCS	Depth (cm)	Species	Count	Total

Dipnet & Visuals for fish/amphibians

Species	Number	Commments

Comments:_____

ATTACHMENT B

USFWS HET Working Group Macroinvertebrate Field Sheet

FIELD SHEET

Time Limited Dipnet Qualitative Sample for Field Identifiable Freshwater Wetland Macroinvertebrates

Region:	**County:**	**System:**	
Site Name:	**Sample ID:**	**N Lat:**	**W Long:**
Date:	**Collected by:**	**Assistant:**	**Start time:** **End time:**
Hydroperiod: ☐ Known to be wet 30+ days ☐ Unknown ☐ Other:			
Habitats/depths sampled and comments:			
Observations:			

Abundance categories: Present (1-3), Common (4-10), Abundant (11-100), Dominant (>100)

Taxa	Tally	AC	Taxa	Tally	AC	Taxa	Tally	AC
Oligochaeta			**Hemiptera**			**Coleoptera (Cont.)**		
Naidinae			Abedus/Belostoma sp.			Hydrophilidae		
Lumbriculidae			Benacus/Lethocerus sp.			Laccophilus sp.		
Tubificinae			Buenoa sp.			Noteridae		
			Corixidae			Pelonomus obscurus		
Hirudinea			Gerridae			Peltodytes sp.		
Mooreobdella sp.			Hebridae/Microvelia sp.			Scirtidae (L)		
Helobdella stagnalis			Hydrometra sp.			Suphisellus sp.		
Hirudinea Unidentified			Limnogonus franciscanus			Suphis inflatus		
			Limnoporus canaliculatus			Thermonectus sp.		
Amphipoda			Mesovelia sp.			Tropisternus sp.		
Crangonyx sp.			Micronecta ludibunda					
Gammarus sp.			Neogerris hesione					
Hyalella azteca complex			Notonecta sp.			**Diptera**		
			Pelocoris sp.			Ceratopogonidae		
Isopoda			Platyvelia brachialis			Chironomidae: Tanypodinae		
Caecidotea sp.			Pleidae			Chironomidae: Chironominae		
			Ranatra sp.			Culicidae:		
Decapoda			Saldidae			Culicidae: Anopheles		
Palaemonetes sp.			Steinovelia stagnalis			Psychodidae		
Procambarus sp.			Synaptonecta issa			Stratiomyidae		
						Syrphidae		
Acariformes						Tabanidae		
Blue/Green			**Trichoptera**			Tipulidae		
Red			Cernotina/Polycentropus sp.					
			Hydroptila sp.			**Gastropoda**		
Ephemeroptera			Nectopsyche sp.			Ancylidae		
Baetidae			Oecetis sp.			Biomphalaria havensis		
Caenis sp.			Orthotrichia sp.			Haitia sp.		
Callibaetis sp.			Oxyethira sp.			Hydrobiidae		
Choroterpes basalis			Triaenodes sp.			Marisa comuarietus		
						Melanoides tuberculata		
Odonata - Anisoptera			**Lepidoptera**			Gyraulus/Micromenetus sp.		
Aeschnidae			Crambidae			Planorbella sp.		
Anax sp.						Planorbidae		
Aphylla williamsoni			**Coleoptera**			Pomacea paludosa		
Celithemis sp.			Berosus sp.			Pomacea – exotic		
Coryphaeschna sp.			Carabidae			Pomacea sp.		
Crocothemis/Erythemis sp.			Celina sp.			Pseudosuccinea sp.		
Gomphidae			Chrysomelidae					
Libellulidae			Copelatus sp.			**Bivalvia**		
Macromia sp.			Coptotomus sp.			Eupera cubensis		
Nasiaeschna pentacantha			Curculionidae			Pisidiidae (=Sphaeriidae)		
Pachydiplax longipennis			Cybister fimbriolatus					
Pantala/Tramea sp.			Derallus altus			**Miscellaneous**		
			Desmopachriá sp.			Porifera		
			Dineutus sp.			Planariidae		
Odonata - Zygoptera			Dytiscidae			Prostoma sp.		
Argia sp.			Enochrus sp.			Ectoprocta (=Bryozoa)		
Coenagrionidae			Gyrinus sp.			Eulimnadia sp.		
Enallagma sp.			Haliplus sp.					
Ischnura sp.			Helobata striata					
Lestes sp.			Hydaticus sp.					
			Hydrocanthus sp.					

[Rutter with Ceilley edits for the HET Invertebrate Working Group, Version 22 April 2010]