AQUATIC FAUNAL ASSESSMENT OF PHASE 2A (1) & 2B (2, 3 & 4) IN KING'S BAY

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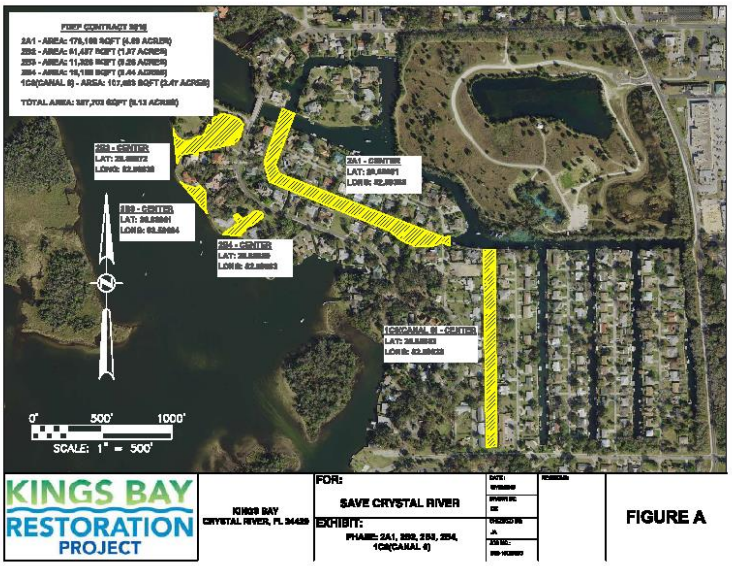

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1.0 INTRODUCTION:

The Kings Bay estuarine ecosystem is a complex mixture of freshwater springs, tidal creeks, artificial canals, and wetland riparian habitats subjected to wet season stormwater runoff from the adjacent urban land uses (**Figure 1**). This aquatic faunal assessment was conducted to establish the baseline biological conditions prior to any restoration activities and to collect time-zero data immediately following completion of the de-mucking operations. The Florida Department of Environmental Protection (DEP) has developed methods for assessing freshwater streams but not for spring-fed tidal estuarine systems like Kings Bay. *"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."* (DEP bioassessment website 2018). The project is designed to used DEP established and standardized methods where practical but some of these methods are applied research adapted to diverse conditions found in Kings Bay.

Prior to de-mucking and planting of submerged aquatic vegetation (SAV) in exclosures, a baseline assessment of fish and macroinvertebrate communities was conducted. Following the de-mucking of Phase 2A and Phase 2B, a time-zero assessment of fish and macroinvertebrate communities was again conducted using the same methods. SAV was assessed during the benthic assessment baseline and time-zero monitoring events. SAV was also assessed visually throughout the study areas. Basic water chemistry data, including temperature, salinity, and dissolved oxygen were also collected from Phase 2A and 2B before and after de-mucking by Sea and Shoreline, LLC. This report summarizes the biological sampling results for fishes and macroinvertebrates from the baseline and time-zero sampling events from both Phase 2A and Phase 2B.

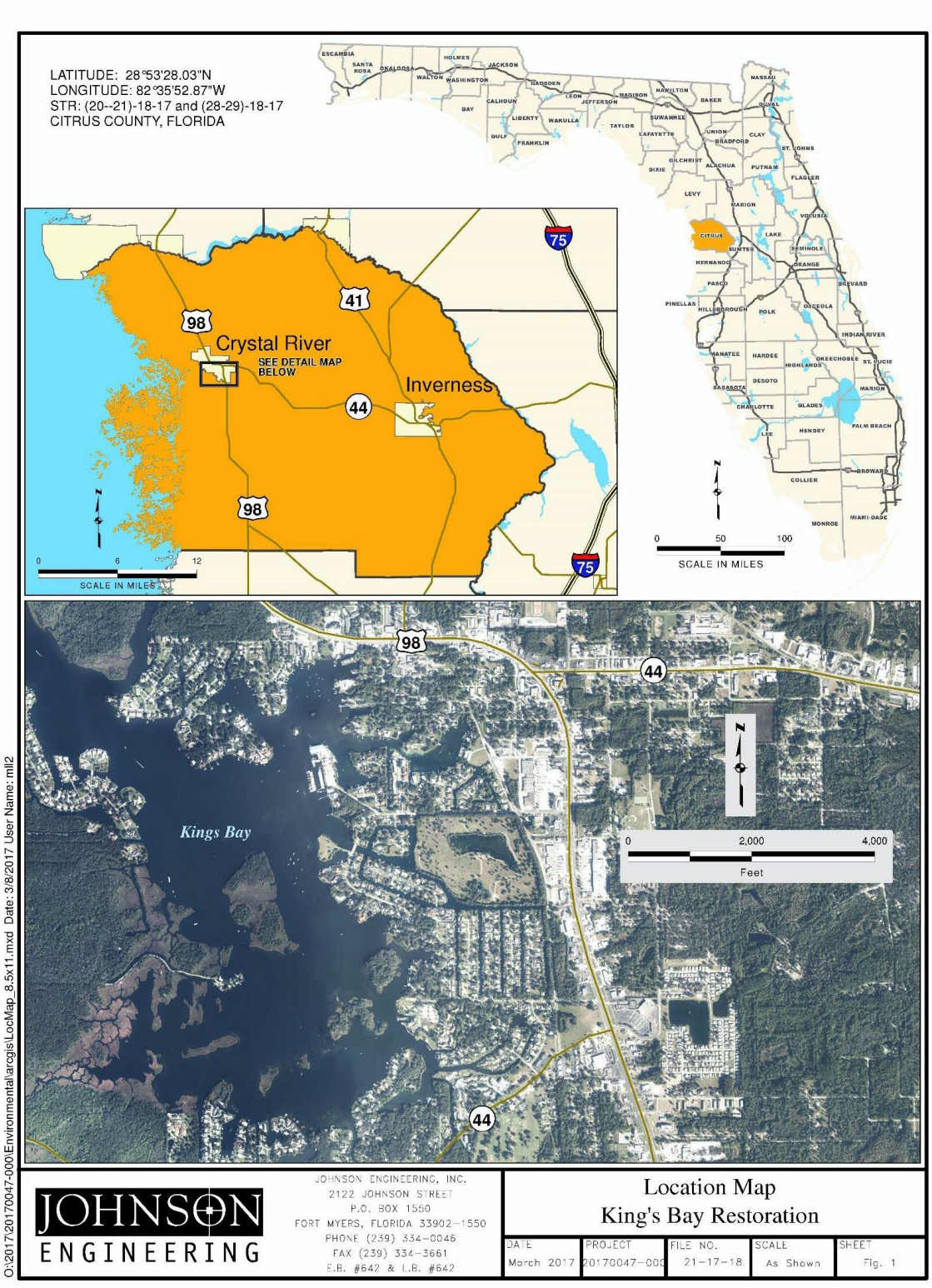

Figure 1. General Location Map of King's Bay Restoration Project

2.0 METHODS

Fish Surveys

Fish community assemblages can be difficult to assess in open water systems due to their motility and natural flight response to predators and humans working in or above the water column. Therefore, the baseline fish sampling event was planned to avoid periods of high human activity, such as scallop season in Summer and peak tourism season in Winter. Baseline fish surveys were conducted in mid-September and early October 2019 with time-zero surveys conducted in late June through July 2020. Fish communities were sampled using a variety of trapping methods including mesh (umbrella traps), plastic funnel traps (Breder 1960), and modified crayfish traps, along with visual observations during underwater vegetation transects and other surface observations from boats. This combination of passive traps and visual sampling techniques were used to evaluate baseline and time-zero fish communities. Seining was not feasible due the canal configuration and depth, along with an abundance of docks, moored vessels, and rocky substrate. An otter trawl was used to experimentally sample the deep canal to supplement other methods.

Figure 2. Breder (1960) traps ready for deployment in (left) and crayfish trap (right).

A total of ten (10) Breder traps and ten (10) crayfish traps were deployed throughout Phase 2A and allowed to colonize for one hour and overnight, respectively (**Figure 2**). After corresponding colonization periods, traps were retrieved for fish identification and enumeration.

Visual assessments were completed over approximated 50 meter transects via a diver with mask and snorkel and abundance was estimated for all species sighted.

Field water quality parameters (salinity, specific conductance, temperature, and dissolved oxygen) and atmospheric conditions (air temperature, wind speed and direction, and cloud cover) were collected in conjunction with baseline fish sampling. Data sheets were used to record species richness and abundance from all sampling techniques and were later transcribed into an Excel™ database. Data were managed in Excel and univariate diversity metrics were determined using PRIMER v6 (Clarke and Gorley 2006).

Macroinvertebrate Surveys

Aquatic macroinvertebrate communities were sampled using artificial substrates (EPA Hester-Dendy) to quantify community structure and assess biological conditions before and immediately after restoration activities were completed. A minimum of three (3) Hester-Dendy (HD) substrates were deployed in both Phase 2A and 2B and retrieved after a 28-day colonization period (**Figure 3**). Locations for the Hester-Dendy substrates were positioned in a stratified manner along the Phase 2A canal and suspended under docks in Phase 2B. Additional substrates were deployed to ensure that at least three would survive the 28-day colonization period unmolested by human activity. After the colonization period, samplers were processed for collection and preservation of epi-fauna using 80% ethanol in Nalgene bottles for later identification. Baseline HD substrates were deployed on September 19, 2019, and retrieved and processed on October 17, 2019, for later identification. Time-zero HD substrates were deployed on June 30, 2020, and retrieved and processed on July 28, 2020, for later identification. Dip nets (**Figure 3**) were used to sample littoral zone vegetation during the baseline and time-zero sampling events. Samples were field sorted using a shallow white sorting tray with forceps and eyedroppers with organisms preserved in 4 oz. Nalgene jars using 80% ethanol as preservative. Dip netting and field sorting was conducted in all available shoreline habitat until the asymptote of the species accumulation curve was reached for each area sampled. Generally, this required at least one hour of sampling and field sorting by an aquatic ecologist with extensive experience surveying macroinvertebrates in all types of aquatic habitats. All macroinvertebrates collected were identified to the lowest practical taxa and enumerated. Chironomid midge larvae

and oligochaete worms were not mounted on microscope slides but identified to the lowest practical taxa using a 10x-60x stereo-zoom microscope. 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 using State verified voucher specimens provided by Robert Rutter, retired DEP macroinvertebrate taxonomist. 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.

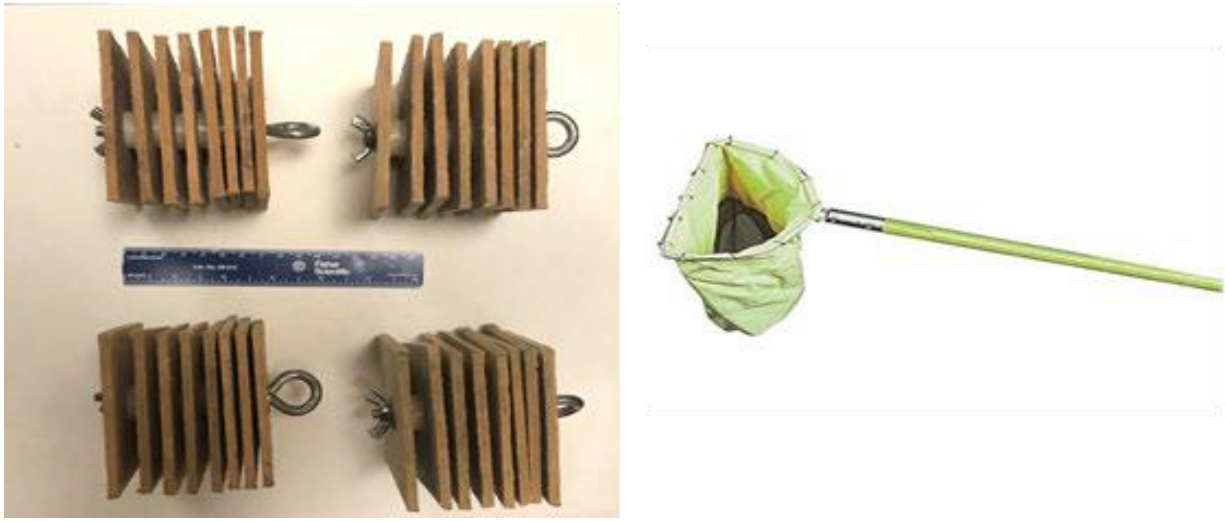

Figure 3. Hester-Dendy (HD) substrates (left) and standard D-frame dip net (right) for sampling macroinvertebrate communities.

Submerged Aquatic Vegetation (SAV)

During the period of September 16 through 18, 2019 the baseline submerged aquatic vegetation (SAV) habitats were selected for monitoring. Baseline conditions along the littoral zone of Phase 2A were assessed using a standard 0.5 x 0.5-meter quadrat (**Figure 4**). Five quadrats were sampled in each Phase at stratified distances along the littoral zone, but not in the deep center of the canal of Phase 2A or bay of Phase 2B. Vascular plant species and algae presence/absence and percent cover were photographed for assessment with representative samples collected for identification. Following the de-mucking, time-zero conditions were also assessed at stratified locations in both phases. Underwater videos were taken along with aerial drone photos and videos. Shallow SAV quadrats were assessed along the steep littoral zone in canal in Phase 2A.

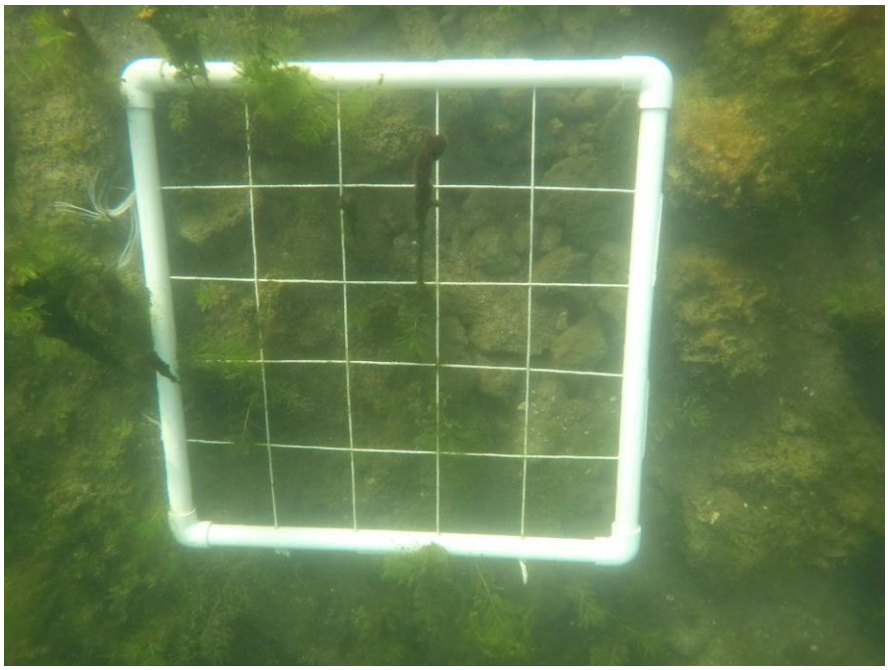

Figure 4. Quadrat used to assess SAV percent cover and frequency of occurrence in subplots.

3.0 RESULTS

Fishes

Fish communities were sampled using a variety of trapping methods including mesh (umbrella traps), plastic (Breder), and modified crayfish traps, along with visual observations during underwater vegetation transects and other surface observations from boats. Breder traps were found to be less effective than other methods for collecting fishes in deeper habitats of Phase 2. Breder traps are highly effective in shallow, densely vegetated habitats of both fresh (Ceilley 2008) and salt marshes (Sargent and Carlson 1987). Data from all methods were composited for a comparison between baseline and time-zero monitoring events in 2019 and 2020 respectively. **Table F1** lists total fish species collected from Phase 2A and 2B and their overall abundance. The table also includes species known from to inhabit King's Bay spring-fed habitats but may not have been collected or observed during these sampling events. For example, gulf killifish, *Fundulus grandis*, Florida gar, *Lepisosteus platyrhincus*, Atlantic stingray, *Hypanus sabinus*, and Atlantic needlefish, *Strongylura marina* have all been observed in King's Bay Phase 1 but have not yet been observed in Phase 2A or 2B restoration sites. These fish species are expected to be collected or observed in future monitoring events as restoration efforts continue.

In Phase 2A baseline samples a total of ten (10) fish species, representing six (6) families were collected or directly observed (Table F2). Immediately after restoration represents "time-zero" conditions (**Table F1**). At time-zero a total of thirteen (13) fish species, representing eight (8) families were collected or observed. In Phase 2B baseline samples a total of five (5) species, representing four (4) families were collected or observed during sampling while time-zero sampling produced twelve (12) fish species, representing 10 families. **Table F2** indicates that all univariate diversity metrics increased in both Phase 2A and 2B following restoration activities. Margalef's richness increased from 1.617 to 2.294 in Phase 2A and from 0.709 to 2.345 in Phase 2B after restoration activities. Shannon diversity (H') increased from 1.358 to 1.777 in Phase 2A and from 0.240 to 1.473 in Phase 2B following restoration activities. The increase in fish diversity is attributed to the removal of muck deposits and layers of *Microseira (Lyngbya) wollei.* The only univariate metric that decreased was total abundance. The tolerant rainwater killifish, *Lucania parva* was very abundant during the baseline sampling event but declined during time-zero sampling. Pielou's evenness J' increased from 0.5896 to 0.6928 in Phase 2A and from 0.1492 to 0.5929 in Phase 2B because of a more diverse and evenly distributed abundance of fish species, rather than the dominance of one or two species (**Table F2**).

Table F1. Fish species collected by all methods during baseline and time-zero sampling of Phases 2A and 2B							
Family	**Genus**	**Species**	**Common Name**	**2A 2019**	**2A 2020**	**2B 2019**	**2B 2020**
Lepisosteidae	*Lepisosteus*	*platyrhincus*	Florida Gar	0	0	0	0
Poeciliidae	*Heterandria*	*formosa*	Least Killifish	0	1	0	0
	Gambusia	*holbrooki*	Eastern Mosquitofish	0	0	0	1
Fundulidae	*Lucania*	*goodei*	Bluefin Killifish	50	68	1	0
	Lucania	*parva*	Rainwater Killifish	148	46	269	65
	Fundulus	*seminolis*	Seminole Killifish	2	0	0	0
	Fundulus	*grandis*	Gulf Killifish	0	0	0	0
Cyprinodontidae	*Cyprinodon*	*variegatus*	Sheepshead Minnow	0	1	0	0
Centrarchidae	*Lepomis*	*punctatus*	Spotted Sunfish	31	20	2	2
	Lepomis	*macrochirus*	Bluegill	0	0	0	1
	Lepomis	*microlophus*	Redear Sunfish	6	26	0	0
	Lepomis	*gulosus*	Warmouth	12	8	0	0
	Micropterus	*salmoides*	Largemouth Bass	0	7	0	2
Centropomidae	*Centropomus*	*undecimalis*	Common Snook	0	0	0	0
Dasyatidae	*Hypanus*	*sabinus*	Atlantic Stingray	0	0	0	0
Ariidae	*Ariopsis*	*felis*	Hardhead Catfish	0	0	0	1
Ictaluridae	*Ameiurus*	*natalis*	Yellow Bullhead	0	2	0	0
Gerridae	*Eucinostomus*	*harengulus*	Tidewater Mojarra	4	3	7	8
Gobiidae	*Gobiosoma*	*bosc*	Naked Goby	0	1	0	15
	Microgobius	*gulosus*	Clown Goby	4	3	3	0
Megalopidae	*Megalops*	*atlanticus*	Tarpon	0	0	0	1
Atherinopsidae	*Menidia*	*beryllina*	Inland Silverside	0	1	0	3
Mugilidae	*Mugil*	*cephalus*	Striped Mullet	0	0	0	5
Archiridae	*Trinectes*	*maculatus*	Hogchoker	1	0	0	0
Belonidae	*Strongylura*	*marina*	Atlantic Needlefish	0	0	0	0
Sparidae	*Archosargus*	*probatocephalus*	Sheepshead	3	0	0	0
	Lagodon	*rhomboides*	Pinfish	0	0	0	5

Table F2. Univariate diversity metrics for Phase 2a and 2B baseline (2019) and time-zero (2020) fish sampling events where S = species richness, N = total abundance, d = Margalef richness, J'= Pielou's eveness, H'= Shannon diversity, and 1-Lambda = Simpson's index.

Sample	**S**	**N**	**d**	**J'**	**H'(loge)**	**1-Lambda'**
2A 2020	13	187	2.294	0.6928	1.777	0.7767
2B 2020	12	109	2.345	0.5929	1.473	0.6198
2A 2019	10	261	1.617	0.5896	1.358	0.6267
2B 2019	5	282	0.709	0.1492	0.240	8.96E-2

Multivariate statistical analysis was conducted with Primer v6 (Clarke and Gorley 2006). All fish data were square root transformed to down-weight the importance of very abundant species. This relatively mild data transformation also increases the importance of rare species. Square-root transformation is an appropriate pretreatment of data for community assessments such as Bray-Curtis similarity matrices and subsequent analyses (Clarke and Gorley 2006, Clarke and Warwick 2001). A hierarchical cluster analysis (based on Bray-Curtis similarity) with similarity profile (SIMPROF) global significance test identified two significant groupings ($p<0.05$) (**Figure F1**). Fish communities in Phase 2B showed significant change from the baseline conditions with increased species richness and overall diversity following restoration actions. Phase 2A baseline and time-zero fish communities shared >70% similarity and were not significantly different from each other. Phase 2A samples were 50% similar with, and not significantly different to Phase 2B baseline but significantly different from Phase 2B time-zero (**Figure F1**). **Figure F2** represents a multi-dimensional scaling (MDS) ordination based on Bray-Curtis similarity showing the relative distances between each of the fish communities of Phases 2A and 2B before (baseline) and after (time-zero) restoration. The multidimensional distances between points are compressed into 2-dimensional space for illustration with the Stress level of 0.0, indicating *"an excellent representation with no prospect of misleading interpretation"* (Clarke and Warwick 2001, Chapter 5, p 5-6). Figure F2 includes an overlay at 49% similarity from the SIMPROF significance test to further illustrate that fish communities are different but only Phase 2B time-zero was significantly different at the 95% confidence level.

Another useful tool to better understand why fish communities are different is the similarity percentage test (SIMPER) that identifies the contributions that each species has to the similarity within a group or dissimilarity between groups. SIMPER results are included in the Appendix to this report and were used to identify major contributors to the percent dissimilarity in the cluster analysis and distance of separation in the MDS. The very abundant rainwater killifish was the most important contributor with 21.6% to the dissimilarity between baseline and time-zero groups. More important ecological contributors or indicators may be those species that were not present until after restoration and the MDS ordination can be overlaid with the abundance of

these species to illustrate the contribution. These include the largemouth bass, *Micropterus salmoides* (6.4%) and the naked goby, *Gobiosoma bosc* (7.9%) that were not present in the baseline samples but showed up in the time-zero sampling (**Figure F3**). Inland silversides, *Menidia beryllina* (4.3%) and striped mullet, *Mugil cephalus*, (3.7%) (**Figure F4**) along with pinfish, *Lagodon rhomboides* (3.7%) were also important contributors that were not present until after restoration. Another species identified as important indicators in Phase 1A restoration (JEI 2018) was the redear sunfish, *Lepomis microlophus.* Redear sunfish contributed 7.4% to the dissimilarity between baseline and time-zero groups because it increased in abundance in Phase 2A following restoration. The small rainwater killifish declined in abundance in both Phase 2A and 2B (**Figure F5**) while several predaceous species increased in abundance.

Macroinvertebrates

Macroinvertebrate sampling was conducted during 2019 prior to restoration (baseline) and shortly after restoration activities (time-zero) in Phases 2A and 2B of the King's Bay Restoration Project. Methods included replicate Hester-Dendy (HD) artificial substrates and qualitative dip-net sampling. Petite Ponar sampling was conducted during the Phase 1A restoration monitoring but was determined to be ineffective for quantification of invertebrates due to the clogging of standard sieves by *Microseira* (*Lyngbya*) *wollei*. We observed the same clogging of dip-nets during the baseline sampling event. Therefore, the macroinvertebrate sampling for the baseline was restricted primarily to HD substrates suspended in the water column above the benthos and *Microseira* layer. Time-Zero sampling consisted of both HD substrates and qualitative dip-net sampling. Results from HD substrates from the baseline were then compared with HD substrates collected immediately after restoration activities were completed.

A total of 2,626 individual macroinvertebrates were collected and identified to the lowest practical taxonomic level during the baseline and time-zero sampling events, representing five (5) Classes, 20 Orders, 27 Families and 44 species (**Table M1**). HD substrates deployed during the baseline in Phase 2A produced 28 species and 1,086 individual macroinvertebrates While species richness (28) and Margalef richness (3.863) was higher during the baseline at Phase 2A, but Pielou's evenness and Shannon diversity was higher in Phase 2A time-zero samples with 0.7728 and 2.518 respectively (**Table M2**). In Phase 2B, the species richness increased from a

baseline of 15 taxa to 17 taxa in time-zero with a corresponding increase in Margalef richness from 2.574 to 2.682. Total abundance also increased from 230 to 390 individual organisms based on HD samples alone. Pielou's evenness and Shannon diversity decreased slightly from the baseline to time-zero sampling events in Phase 2B (**Table M2**). Simpson's index values varied from a low of 0.8357 in Phase 2B time-zero to 0.8901 in the Phase 2B baseline. Both Simpson's and Shannon's diversity indices are used to quantify biodiversity and are based on the number of species present as well as the abundance of each species. Shannon diversity of macroinvertebrate communities is listed by FDEP in Florida Administrative Code 62-303 for the protection of water quality and identification of "Impaired Waters".

Table M2 also includes qualitative dip-net sample results from Phases 2A and 2B for the time-zero sampling event. No baseline dip-net samples were collected due to *Microseira wollei* clogging dip-nets and sieve samples. The time-zero dip-net samples will serve as starting point for future comparisons as restoration progresses through time. A total of 12 taxa were collected and identified in Phase 2A and seven (7) taxa were collected from Phase 2B. Several motile species are typically collected using dip-nets that would not normally be found in artificial substrates, including certain Heteropterans, (Corixidae, Mesovelidae), Odonates and Coleopterans (**Table M1**).

Table M1. Macronvertebrate taxa collected during baseline (2019) and time-zero (2020) sampling events by Hester-Dendy (HD) and dip-net (Dnet) methods.

Taxon	2A.HD Base	2B.HD.Base	2A.HD.TZ	2B.HD.TZ	2A.Dnet.TZ	2B.Dnet.TZ
Cernotina sp.	0	0	2	0	0	0
Anopsilana browni	0	5	0	1	0	0
Corophium louisianum	0	0	3	25	0	0
Caenis sp.	19	0	66	0	0	0
Callibaetis sp.	15	0	2	0	0	3
Cassidinidea ovalis	94	0	184	1	0	0
Ceratopogonidae	27	16	3	0	0	0
Chironominae	97	69	145	95	0	2
Enallagma pollutum	2	0	0	0	0	0
Enallagma sp.	3	0	1	0	1	0
Gammarus sp.	50	36	14	3	2	0
Grandidierella bonnierodes	4	5	63	0	1	0
Hargeria rapax	3	23	3	19	0	0
Hesperocorixa sp.	0	0	0	0	8	0
Hyalella azteca grp.	330	0	9	0	0	0
Hydroptila sp.	0	12	3	0	0	0
Ischnura ramburii	0	1	0	0	0	0
Mesovelia mulsanti	0	0	0	0	5	5
Mooreobdella sp.	3	0	0	0	0	0
Munna reynoldsi	2	32	101	78	0	1
Mytilopsis leucophaeata	0	1	49	61	0	0
Orthotrichia sp.	9	0	32	0	1	0
Oxythira sp.	1	0	0	0	0	0
Planorbella scalaris	67	0	3	0	0	0
Planorbella triv. int.	1	1	0	0	0	0
Planorbella sp.	1	0	49	0	0	0
Pyrogophorus platyrhicus	3	0	0	0	0	0
Physella spp.	202	1	6	0	0	1
Rhithropanopeus harisii	2	5	16	19	2	0

Table M1. Macronvertebrate taxa collected during baseline (2019) and time-zero (2020) sampling events by Hester-Dendy (HD) and dip-net (Dnet) methods.

Synaptonecta issa	3	0	0	0	0	0
Tanypodinae	43	22	59	9	0	0
Taphromysis lousianae	0	0	0	0	6	1
Trepobates sp.	0	0	0	0	1	0
Eupera cubensis	0	0	8	0	0	0
***Balanus* sp.**	0	0	0	71	0	0
Chironomidae	0	0	20	1	0	0
Cladoceran	1	0	23	0	0	0
Coenagrionidae imm.	0	0	4	1	0	0
Corduliidae imm.	1	0	0	0	0	0
Gammaridae	0	0	0	1	0	0
Harpacticoid	1	0	0	1	1	0
Hydrobiidae	100	0	4	2	1	5
Ostracoda	2	1	0	0	0	0
Acariformes	0	0	0	2	1	0
Species Richnesss	**28**	**15**	**26**	**17**	**12**	**7**

Table M2. Univariate diversity metrics for Phase 2a and 2B baseline (2019) and time-zero (2020) macroinvertebrate sampling events where S = species richness, N = total abundance, d = Margalef richness, J'= Pielou's eveness, H'= Shannon diversity, and 1-Lambda = Simpson's index.

Sample	S	N	d	J'	H'(loge)	1-Lambda'
2A.HD Base	28	1086	3.863	0.6638	2.212	0.8411
2B.HD.Base	15	230	2.574	0.7711	2.088	0.8416
2A.HD.TZ	26	872	3.692	0.7728	2.518	0.8901
2B.HD.TZ	17	390	2.682	0.7084	2.007	0.8357
2A.Dnet.TZ	12	30	3.234	0.8562	2.128	0.8736
2B.Dnet.TZ	7	18	2.076	0.8922	1.736	0.8431

Multivariate statistical analyses of macroinvertebrate communities were based on a Bray-Curtis similarity matrix using square root transformed abundance of each taxa to down-weight the importance of extremely abundant species and increase the importance of rare, and potentially ecologically significant species. A more radical transformation, such as fourth root or presence/absence was not deemed appropriate for HD substrate samples or dip-net samples collected in King's Bay based on field observations and reviewing univariate statistical results. A hierarchical cluster analysis, using the similarity profile significance test (SIMPROF) was used to illustrate the percent similarity between groups of samples and their significance (Clarke and Gorley 2006). **Figure M1** is a cluster analysis of composited HD and dip-net samples by Phase and periods (baseline and time-zero). There were two significant groupings ($p<0.05$) showing a separation of sampling methods (HD vs. dip-net) at less than 15% similarity. **Figure M2** compared samples only from HD substrates from both Phases and periods. There was no significant difference in the communities based on the SIMPROF global significance test. There is a separation of Phases at 37% similarity indicating that Phase (location) was more important in forming the cluster than period (baseline vs. time-zero) that showed 49% similarity (**Figure M2**). An MDS ordination of the HD macroinvertebrate communities further illustrates the dissimilarity between Phases and periods (**Figure M3**). The multidimensional distance between points is compressed into 2-dimensions for illustration with the Stress level of 0.0, indicating *"an excellent representation with no prospect of misleading interpretation"* (Clarke and Warwick 2001, Chapter 5, p 5-6). The conclusion here is that there are clear differences (based on Bray-

Curtis similarity) between Phases 2A and 2B and clear differences between baseline and time-zero communities, albeit not significant at the 95% confidence level using SIMPROF.

The similarity percentage test (SIMPER) was then used to identify the contributions that each macroinvertebrate species has to the similarity within a group or dissimilarity between groups. SIMPER results were used to identify the taxa contributions to the dissimilarity in the cluster analysis and distance of separation in the MDS. Typically, the most abundant species rise to the top in terms of contributions to similarity within a group or dissimilarity between groups, even when abundance data are square-root transformed. The amphipod, *Hyalella azteca* group was the most important contributor (7.51% contribution) to the dissimilarity between baseline and time-zero groups mainly because it was extremely abundant in the baseline and less abundant in time-zero. The filter-feeding, Conrad's false mussel, *Mytilopsis leucophaeata* was the second most important contributor (7.07%) and was rare in the baseline samples but very abundant in time-zero. A bubble overlay of the abundance of Conrad's false mussel was displayed on the MDS ordination from **Figure M3** to illustrate relative abundance (**Figure M4**). Another filter-feeder that was an important contributor (5.12%) was the common barnacle, *Balanus* sp. that was absent during the baseline but relatively abundant in the time-zero samples from Phase 2B where salinity levels were consistently higher. The amphipod, *Corophium louisianum* was absent in the baseline samples but abundant in time-zero samples and important contributor to the dissimilarity between groups (**Figure M5**). Immature damselfly nymphs in the Coenagrionidae family were absent in the baseline samples but present in time-zero samples for both Phases 2A and 2B (**Figure M5**). The oligohaline mud crab, *Rhithropanopeus harissii* was present during both baseline and time-zero but increased in abundance following restoration activities (**Figure M6**).

Submerged Aquatic Vegetation (SAV)

A total of five SAV quadrats were surveyed along the littoral zone of Phase 2A and 2B during the baseline sampling. Photos of Phase 2A quadrats are included in the Appendix to this report. Filamentous green algae was present in all quadrats averaging 45% cover. Eurasian watermilfoil, (*Myriophyllum spicatum*) was present in two quadrats with overall average cover of 3% and *Hydrilla verticillata* was present in only one quadrat with <1% cover (**Appendix**).

Both species are non-native and invasive and were removed during the de-mucking operations. The native SAV was mostly confined to the eastern end of Phase 2A near the low bridge where tape grass (eel grass), *Vallisneria americana* began to colonize the canal from previous plantings near Three Sister's Spring. This colonization preceded the restoration activities but continued into July of 2020. The Benthic Assessment Report (JEI 1/2021) included results from ten (10) core samples each collected from Phase 2A and 2B before and after restoration. No SAV were collected in any of the core samples during the baseline or time-zero sampling events which was not surprising. SAV (*V. americana*) plantings were confined to exclosure cages as of July 2020 and to the eastern end of the Canal in Phase 2A. On July 1, 2020, the de-mucking at Phase 2B was still in operation apparently as the result of a ruptured sediment bag at the staging area that discharged back into waterway behind turbidity screens.

Water Chemistry

Water chemistry data were collected during fish and macroinvertebrate sampling events in September 2019 and July 2020. Dissolved oxygen levels were less than 0.5 mg/l (anoxic) near the bottom during the baseline sampling (Table 1). Removal of the flocculent organic matter and layer of *Microseira wollei* resulted in a corresponding increase in D.O. level throughout the water column with mid-depth D.O. concentrations increasing in both Phase 2A and 2B (Table 1).

Table 1. Water Chemistry Data: King's Bay Restoration Phase 2A and 2B

Phase	Date	Sample	Depth m	Temp. C°	Salinity ppt	D.O. mg/l
2A Base	9/18/2019	Top	0.2	25.0	0.2	4.1
2A Base	9/18/2019	Mid	2.75	24.1	0.5	3.6
2A Base	9/18/2019	Bottom	5.75	24.3	0.6	0.4
2A TZ	6/30/2020	Mid	2.5	27.5	0.3	6.4
2B Base	9/17/2019	Top	0.2	26.3	0.6	6.4
2B Base	9/17/2019	Mid	1.8	26.0	0.6	6.1
2B Base	9/17/2019	Bottom	3.5	24.0	0.8	0.3
2B TZ	6/29/2020	Mid	1.8	30.4	1.6	8.3
2B TZ	7/1/2020	Mid	1.8	29.4	1.5	6.8

4.0 SUMMARY AND DISCUSSION

Fish and macroinvertebrate sampling was conducted before (baseline), and immediately following (time-zero) the habitat restoration activities performed by Sea and Shoreline LLC. This report summarizes the aquatic faunal data collections from baseline and time-zero sampling. Some de-mucking activities were still ongoing in July 2020 during the time-zero sampling at Phase 2B as the result of a ruptured sediment container or "Geo-bag" at the staging area resulting in organic muck spilling back into the water. A Benthic Assessment Report was submitted in January 2021 that included the results of sediment core sampling before and after de-mucking (JEI 2021). Water chemistry in Phase 2B was typical of oligohaline tidal habitats with salinity ranging from 0.5 to 2.0 ppt. Salinity was consistently lower in Phase 2A overall because of its proximity to, and hydrologic connection with Three Sisters Spring. Dissolved oxygen concentrations were very low (indicative of anoxia) near the bottom of Phases 2A and 2B during the baseline monitoring and unsuitable for most invertebrates and fishes. Removal of flocculent organic material, *Microseira* and muck, and restoration of SAV will improve conditions over time. Aquatic fauna showed an almost immediate response to de-mucking activities. Fish species richness increased from 10 species and six (6) families during the baseline to 13 species and eight (8) during time-zero. In Phase 2B baseline samples, a total of five (5) species, representing four (4) families were collected or observed during sampling while time-zero sampling produced twelve (12) fish species, representing 10 families. This 30% increase in fish species in Phase 2A and 240% increase in Phase 2B richness is attributed mostly to the improvement in physical benthic habitat. Univariate diversity metrics increased across the board, except for total number of fishes. The tolerant and euryhaline rainwater killifish declined in abundance while other species generally increased. The decreased abundance of tolerant species is a positive indication that restoration activity is improving aquatic habitat (Loeb and Spacie 1994, Simon 2004).

The cluster analysis with SIMPROF random permutation tests for significance illustrated the dissimilarity between time periods and Phases. This multivariate analysis of the fish data identified a significant ($p<0.05$) change in fish community structure in Phase 2B from the increased species richness and diversity observed in the time-zero samples. The fish community of Phase 2A increased in diversity in the time-zero sampling but the increase was not significant at the 95% confidence level.

The Bray-Curtis similarity matrix, with similarity percentage test (SIMPER) identified in rank order the most important fish species contributing to the dissimilarity between baseline and time-zero fish communities. Largemouth bass were not collected during the baseline sampling, but several fingerling largemouth bass were collected in Phase 2A with a few also collected in Phase 2B. Naked gobies also were not collected during baseline sampling but were present in Phase 2A and abundant in Phase 2B time-zero samples. The gobies are primarily bottom-dwelling fishes and restoration of the benthos in both Phases increased habitat suitability for naked gobies and for many sunfish species that are demersal spawners. Naked gobies are primarily "*a marine species that enters rivers, creeks, lakes and canals and is usually found over sand or rocky substrates*." (Robins et al. 2018 pp. 414). Inland silversides were only collected in time-zero samples as well and were important contributors to the dissimilarity between baseline and time-zero communities in both 2A and 2B. Striped mullet and pinfish were also important in Phase 2B since they were only collected or observed in time-zero sampling. King's Bay is an open and tidally influenced ecosystem. The increases in fish diversity documented in Phase 2A and 2B are attributed to the physical removal of organic floc and muck that was unsuitable habitat. The fish communities in both areas are likely to continue to change over time as SAV coverage increases and extends outside of the exclosures that were planted by Sea and Shoreline LLC.

Aquatic macroinvertebrates have been used by humans to diagnose the impacts of pollution for more than 100 years (Rosenberg and Resh 1993), and for assessing the condition of streams and lakes for decades (USEPA 1989). The methods used to sample the aquatic macroinvertebrates in King's Bay have been adapted for use in a spring-fed tidal ecosystem from methods used from other areas in Florida. Artificial HD substrates were used to collect small macroinvertebrates that can colonize hard substrates relatively quickly and are generally good indicators of water quality. Dip net sampling is more a qualitative method for collecting invertebrates from existing substrates in wetlands and littoral shoreline habitats. Both methods were used in combination during the baseline and time-zero sampling events. However, since the filamentous *Microseira wollei* was clogging dip nets during the baseline, it was not an effective method for collecting invertebrates until these areas were de-mucked. A total of 2,626 individual macroinvertebrates were collected and identified to the lowest practical taxonomic level during the baseline and time-zero sampling events, representing five (5) Classes, 20 Orders, 27 Families and 44 species.

HD substrates deployed during the baseline in Phase 2A produced 28 species and 1,086 individual macroinvertebrates While species richness was slightly higher during the baseline sampling at Phase 2A, Pielou's evenness and Shannon diversity was higher in time-zero samples. In Phase 2B, the species richness increased from a baseline of 15 taxa to 17 taxa in time-zero with a corresponding increase in Margalef richness. Total abundance also increased from 230 to 390 individual organisms based on HD samples alone. Overall, there appeared to be a trophic shift in the macroinvertebrate communities from the baseline to time-zero events with a decline in grazers and an increase in filter-feeders and some predators including damselfly nymphs. The grazing amphipod *Hyalella azteca* decreased in abundance following de-mucking and removal of filamentous algae. The filter-feeding, Conrad's false mussel was the second most important contributor and was rare in the baseline samples but very abundant in time-zero. The changes in macroinvertebrate community structure at both Phases was not significant at the 95% confidence level. However, the MDS ordination of macroinvertebrate communities showed large distances between baseline and time-zero samples. The MDS showed a Stress level of 0.0 (no distortion of the ordination) when compressed into 2-dimensional space. Using the SIMPER test, several taxa were identified as important contributors to the separation between baseline and time-zero communities. Follow up sampling of macroinvertebrates using HD substrates and dip net sampling is needed after SAV has become established to track the colonization and recovery of aquatic macroinvertebrates. The disturbances caused by de-mucking are likely to be temporary and post-restoration sampling following the establishment of SAV beds is recommended to document biological recovery.

5.0 REFERENCES

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APPENDIX

Fish Figures

Macroinvertebrate Figures

Photos of SAV Quadrats in Phase 2A

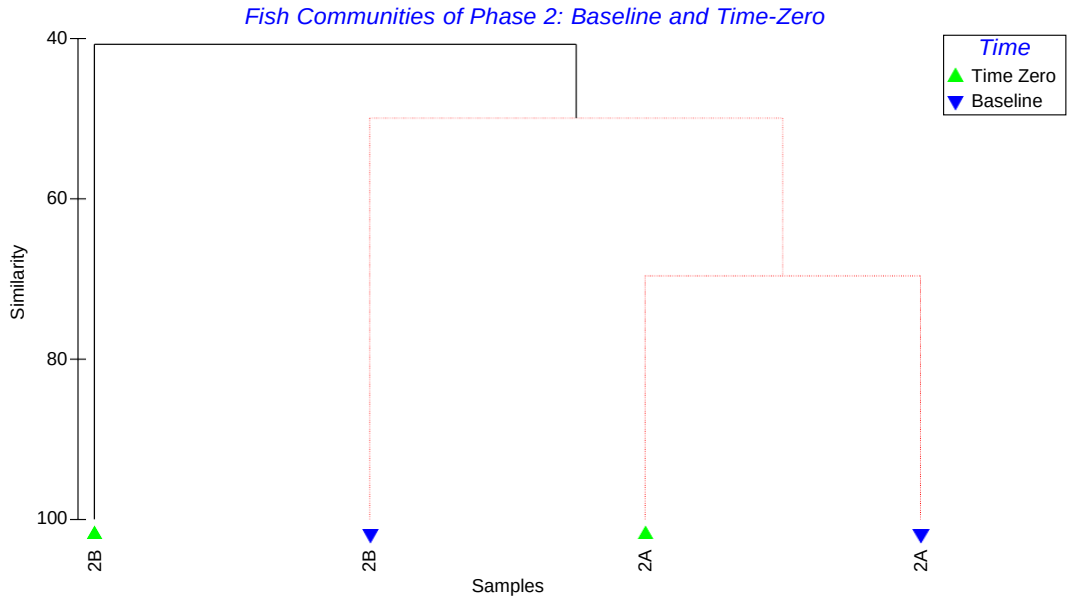


Figure F1. Cluster diagram of Bray-Curtis similarity of fish communities in Phase 2A and 2B during baseline and time-zero monitoring events showing time-zero 2B significantly different from baseline.

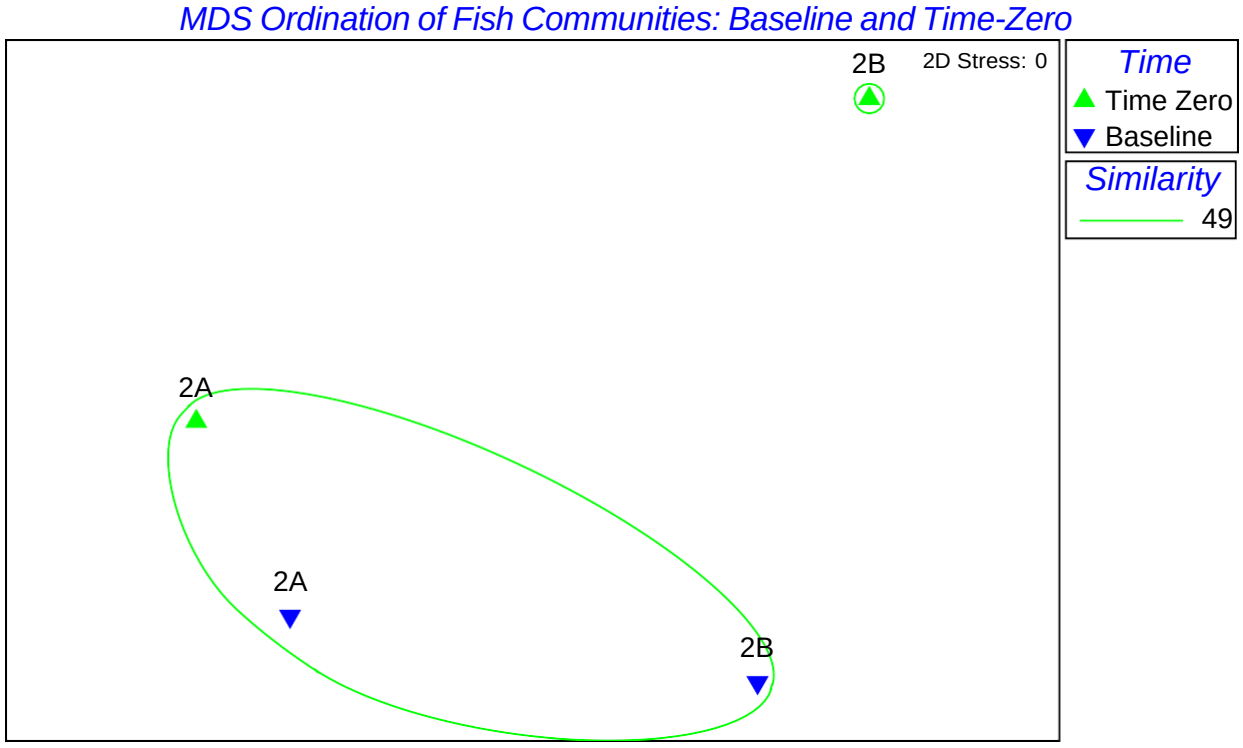


Figure F2. Multidimensional Scaling (MDS) ordination of Bray-Curtis similarity of fish communities with overlay of significant groups from Similarity Profile (SIMPROF) test.

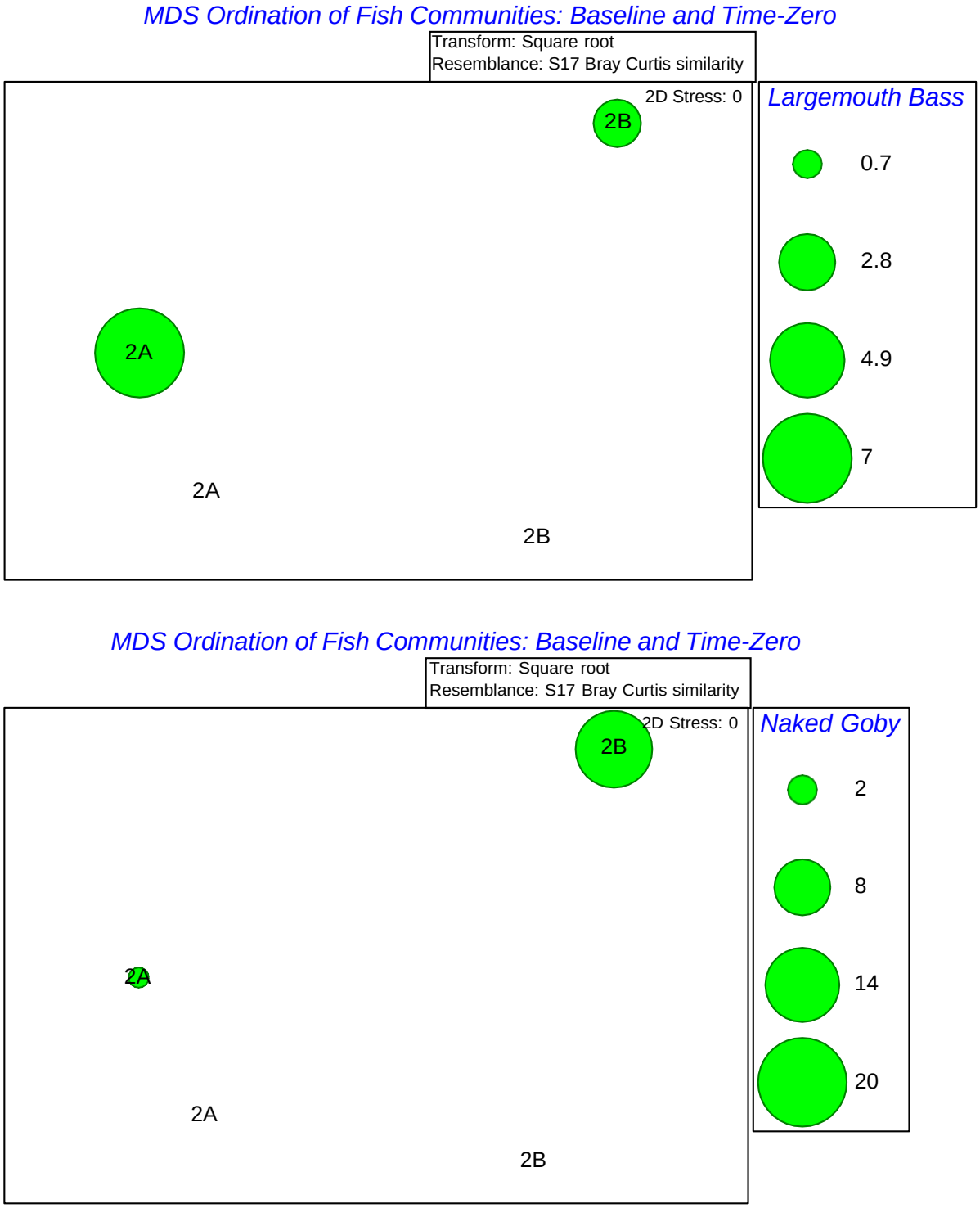
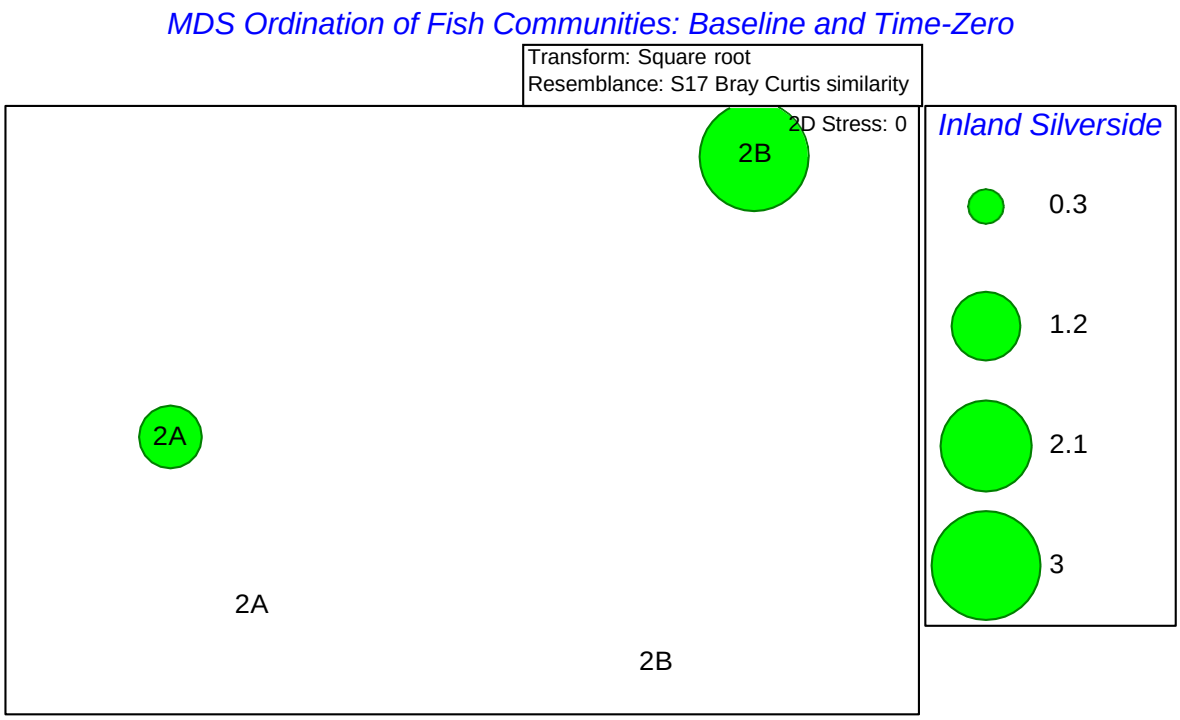


Figure F3. MDS from Figure F2 with overlay of square root abundance of largemouth bass (top) and naked goby (bottom). Both species were absent in baseline samples but present in time-zero samples.



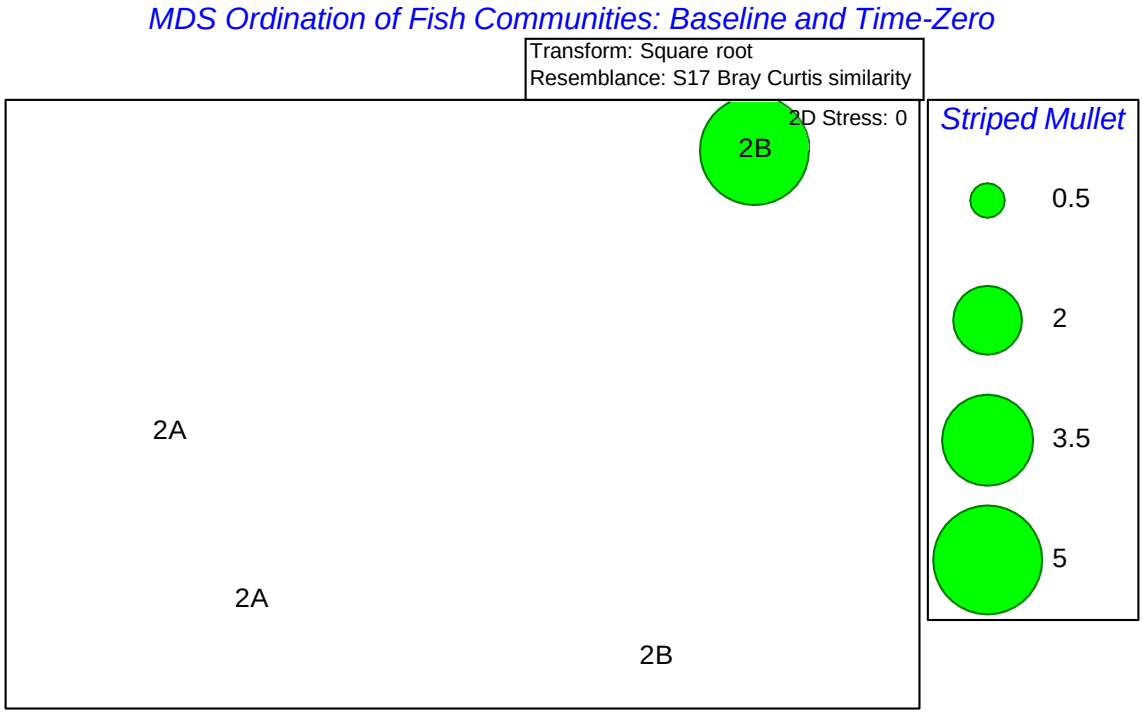


Figure F4. MDS ordination from Figure F2 with overlay of square root abundance and striped mullet (bottom). Note the appearance of inland silversides in Phases 2A and 2B and the appearance of striped mullet in Phase 2B in time-zero samples.

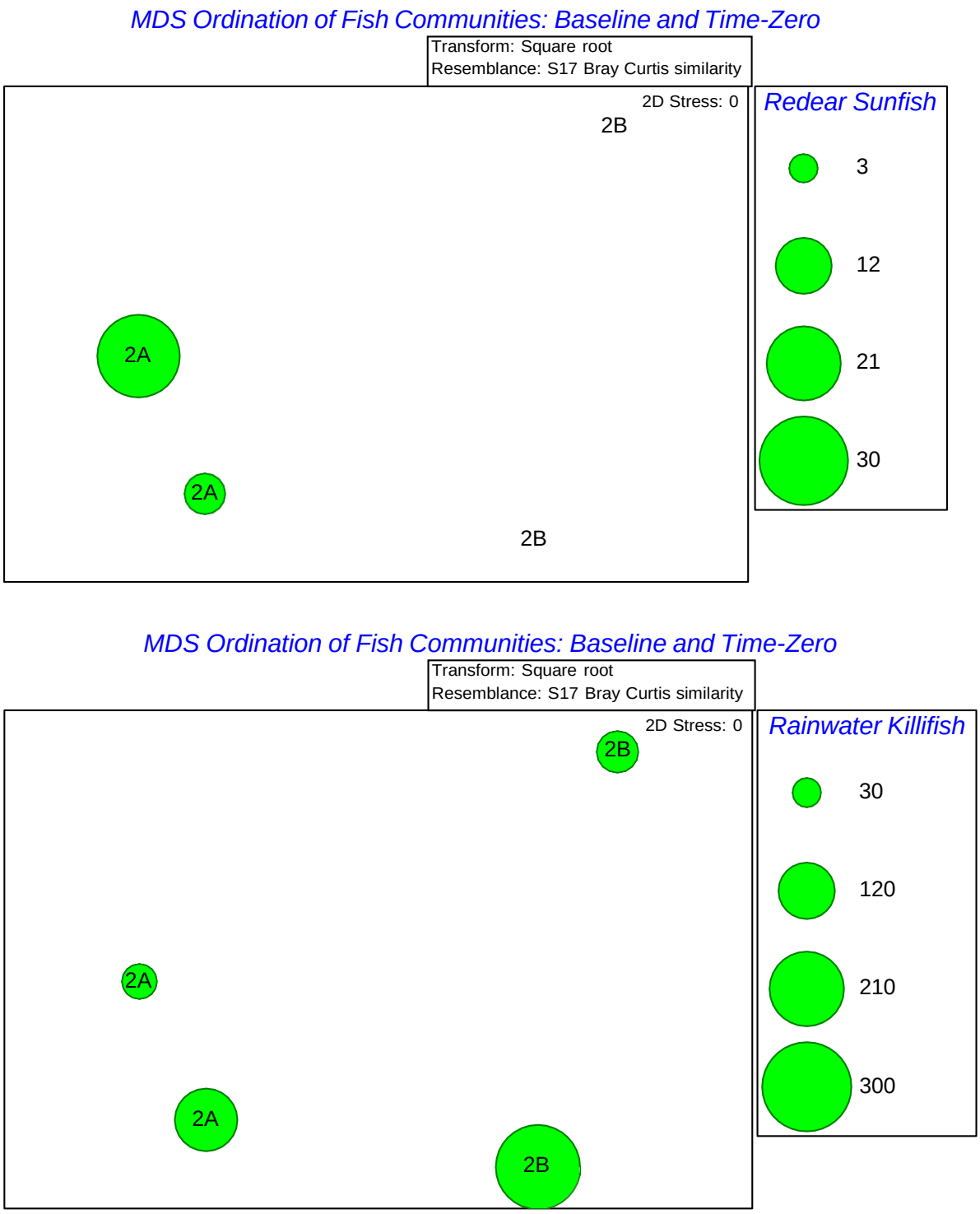


Figure F5. MDS ordination from Figure F2 with square root abundance of redear sunfish (top) and rainwater killifish (bottom). Note increase in redear and decrease in rainwater killifish in time-zero samples.

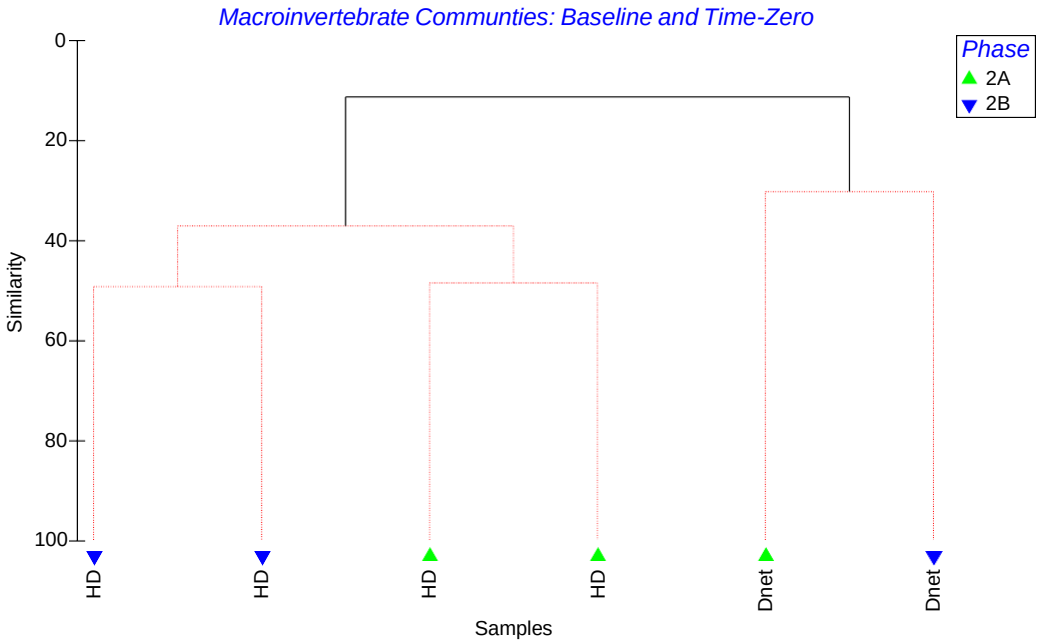


Figure M1. Cluster analysis based on Bray-Curtis similarity of macroinvertebrate communities from Phase 2A and 2B baseline and time-zero sampling of Hester-Dendy with time-zero dip net results.

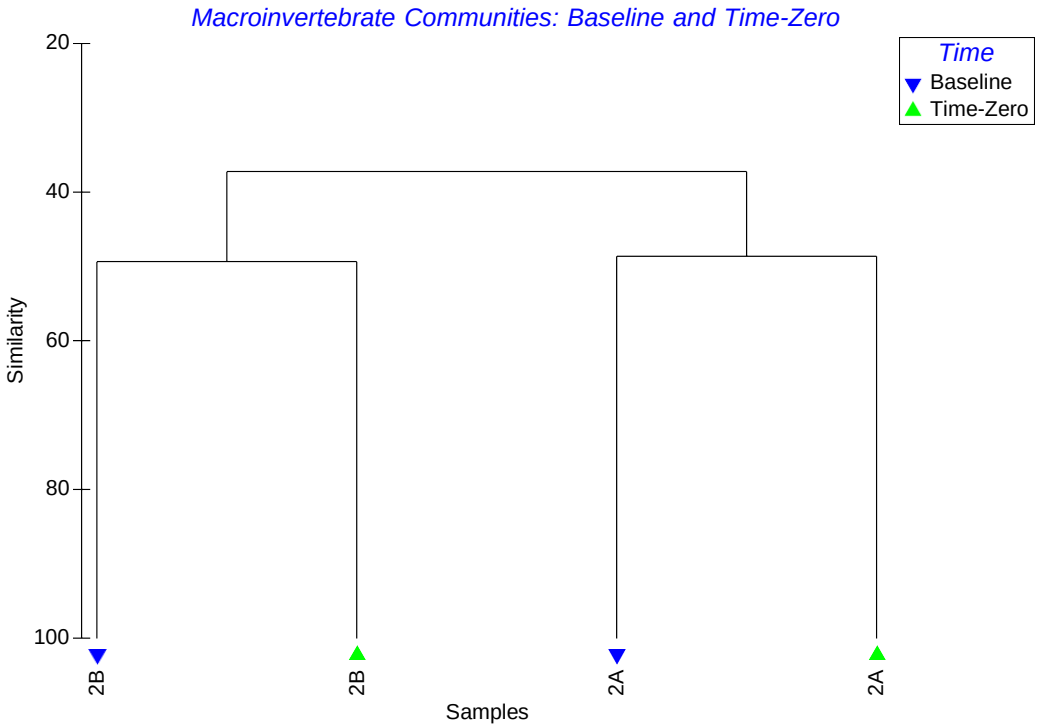


Figure M2. Cluster analysis of Hester-Dendy (HD) macroinvertebrate communities from Phases 2A and 2B, baseline and time-zero samples.

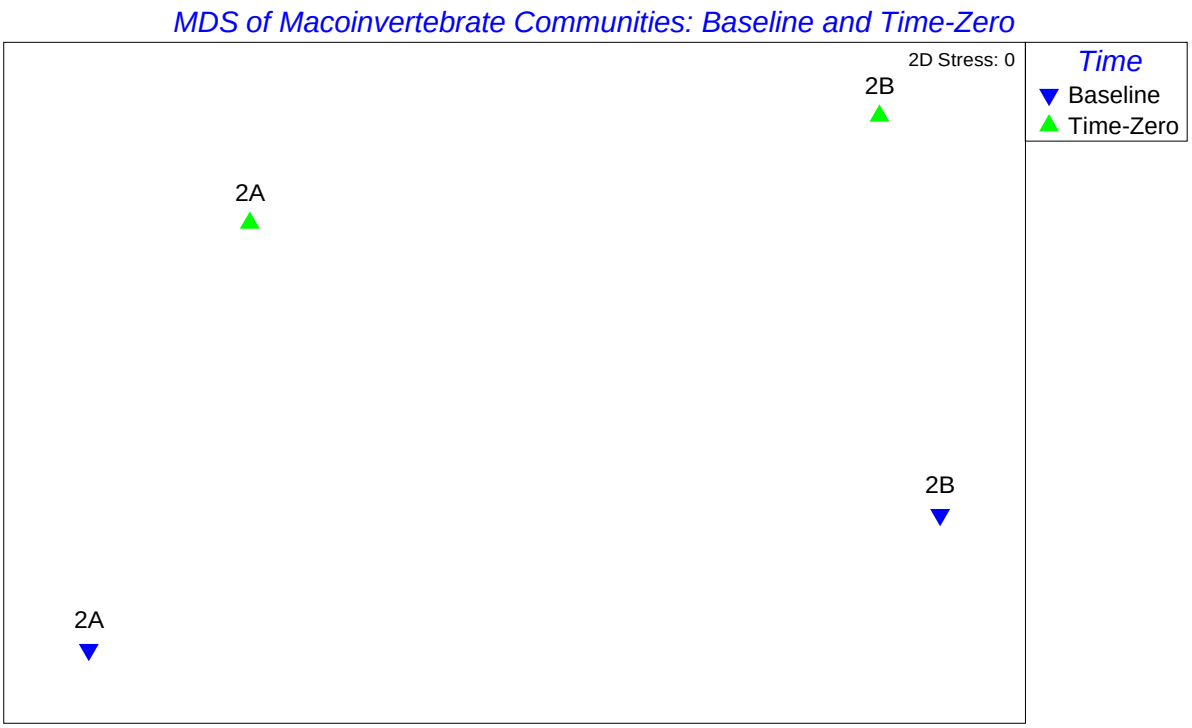


Figure M3. MDS ordination of Hester-Dendy macroinvertebrate communities from Phases 2A and 2B, baseline and time-zero samples.

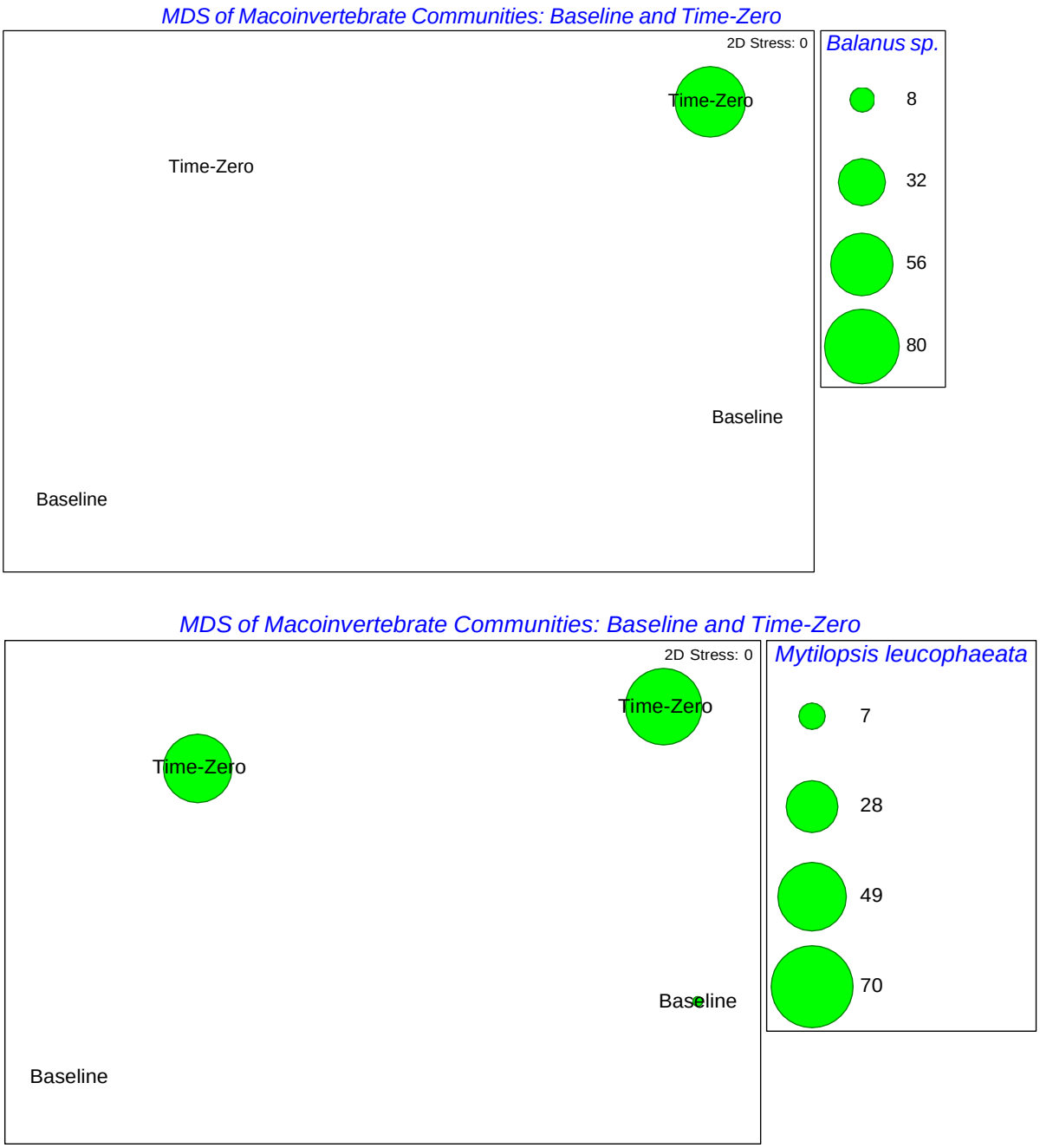


Figure M4. MDS ordination with overlay of square root abundance of two filter-feeders with the barnacle, *Balanus* sp. (top) and Conrad's false mussel, *Mitilopsis leucophaeata* (bottom).

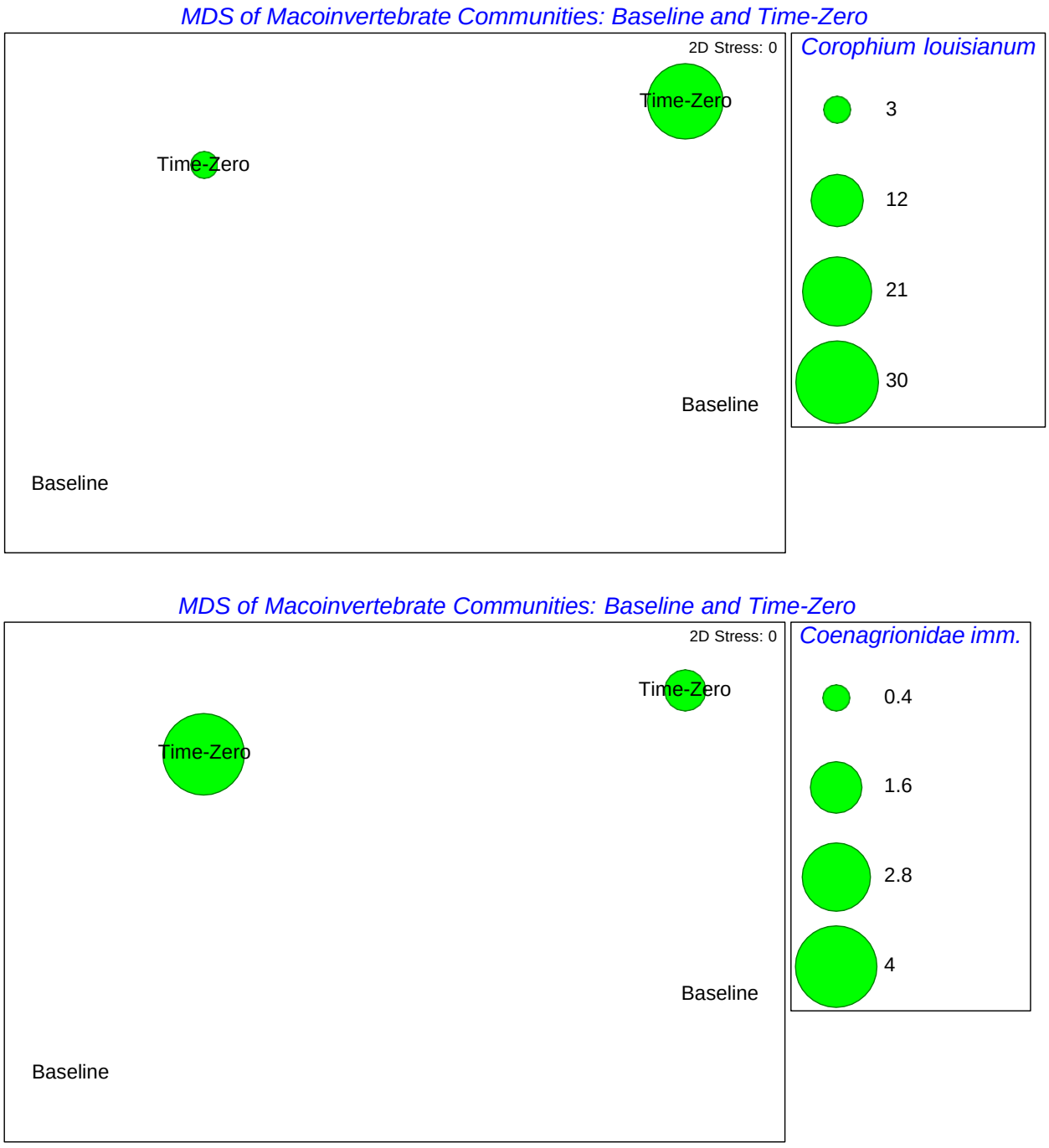


Figure M5. MDS ordination with overlay of square root abundance of the amphipod, *Corophium louisianum* (top) and immature damselfly nymph of the family Coenagrionidae (bottom).

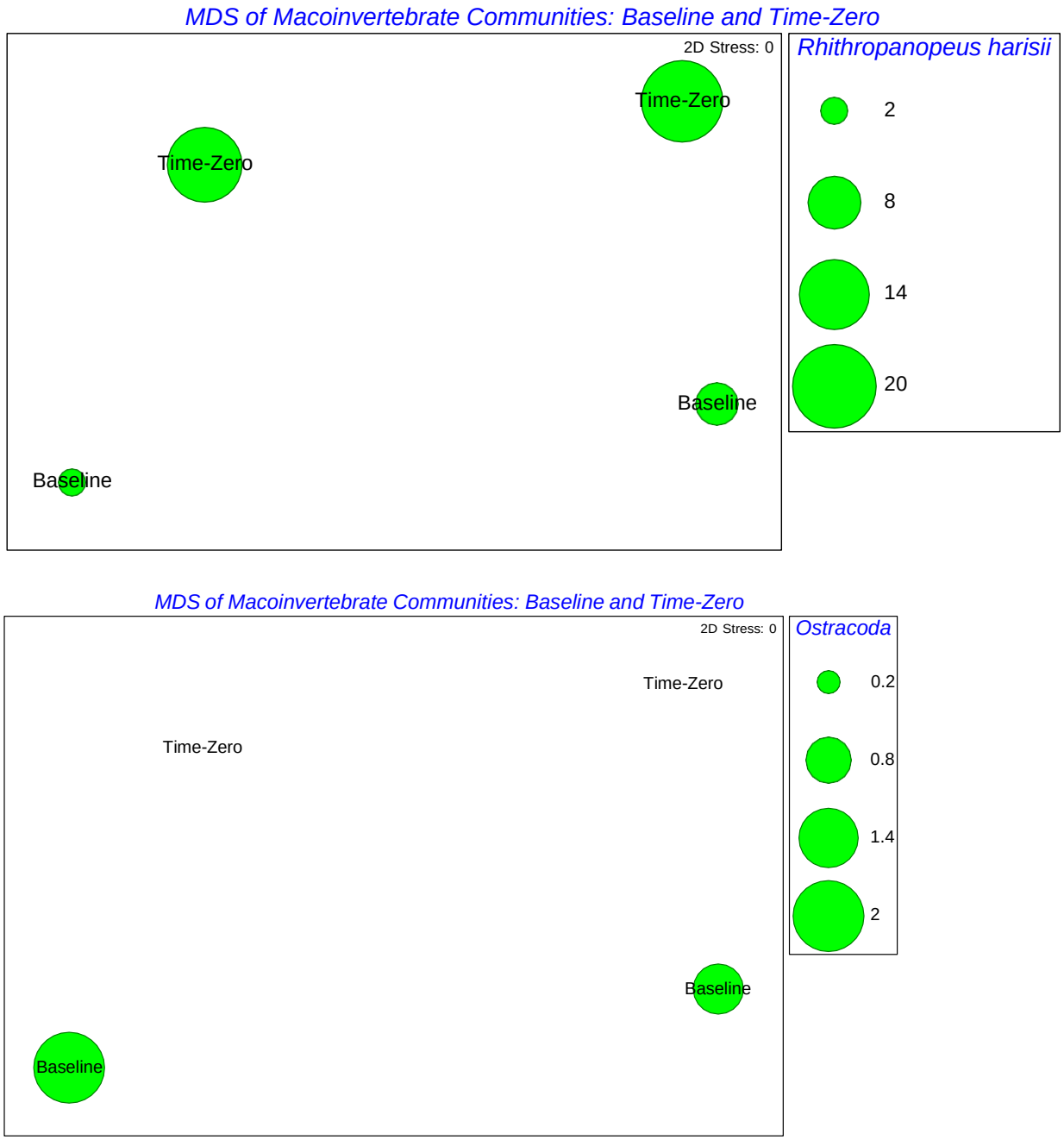


Figure M6. MDS ordination with overlay of square root abundance of the mud crab, *Rithropanopeus harissii* (top) and unidentified Ostracoda (bottom).