FINAL REPORT
South Carolina State Wildlife Grant F21AF03426-00
South Carolina Department of Natural Resources
October 1, 2021 – September 30, 2022

Project Title: Developing Genetic Sequences for benthic prey resources of migratory Piping Plover and Red Knot

Objectives: The goal was to support the sustainable management of Piping Plover and Red Knot by increasing the genetic library of their benthic prey species. The specific objectives were to:

(1) Create a list of target Piping Plover and Red Knot prey species that are lacking genetic sequences in GenBank.
(2) Collect twenty or more intertidal marine invertebrate taxa from the target list.
(3) Identify and photo document 1-3 specimens from each target taxa.
(4) Generate genetic sequences for each specimen to be included in GenBank for use in Piping Plover and Red Knot diet analysis.

Accomplishments: A list of unique prey species identified in the 2015 SC SWAP Intertidal Marine Invertebrates Guild (SCDNR 2015) was generated. This list was then compared to the library of genetic sequences already included in GenBank to determine where gaps in the available data exist. The target taxa selected are known or likely Piping Plover or Red Knot prey items not already represented in GenBank. The organisms targeted for collection based on this analysis included polychaete worms (~1-5 cm), small bivalves (~1-2 cm), amphipods (~2-5 mm), and larger burrowing organisms including hemichordate acorn worms. A search of SCDNR databases was conducted to obtain intertidal locations where these potential Piping Plover and Red Knot prey species have been found in previous studies.

Specimens were collected from eight locations in the coastal zone of Charleston County representing a range of intertidal habitats where target prey species have been collected in past studies including front beach, coastal inlet and tidal creek sites (Figure 1.1). Intertidal sediment samples were collected using a shovel, sediment core, or slurp gun (for larger burrowing organisms). Sediment samples were typically retained in a cooler and processed in the laboratory or occasionally processed in the field when appropriate. Sediment samples were filtered through a 500uM sieve to separate benthic organisms from the sediment. The live specimens were identified to the species level using various taxonomic resources and then, if they were target taxa, were preserved in 95% ethanol and assigned a specimen number.

A total of 23 taxa were selected for inclusion in the new sequencing efforts. Between one and three specimens of each selected taxa were photographed to show the distinguishing characteristics necessary for identification using either a compound or dissecting microscope (Figure 1.2). Photographs were archived as a voucher collection at SCDNR and are available for future review. The identified and photographed specimens were transferred to the SCDNR Population Genetics group for DNA extraction, amplification, and genetic sequencing.

Total DNA was extracted from the whole body of each specimen using a Promega Wizard^{tm} SV Genomic DNA Purification system in 200 µL of digestion solution composed of 145.5µL of nuclei lysis solution, 36.36µL of 0.5M EDTA, 14.55µL of 20mg/mL proteinase K,

and 3.64µL of nuclease free water. Hard tissues were pulverized in the digestion solution with a sterile pestle in a 1.5ml microcentrifuge tube. Digestions were incubated at 55°C for at least 1.5 hours.The cytochrome c oxidase subunit I (COI) region was sequenced for all specimens. Folmer's (1994) universal invertebrate primers were used with the following PCR cycling protocol: 35 cycles at 94ºC for thirty seconds, 51ºC for thirty seconds, 72ºC for one minute, with final extension at 72ºC for 5 minutes. PCR was conducted in 25µL reactions with 1.9mM MgCl2, 0.25mM each dNTPs, 1x Accustart II PCR buffer (Quantabio), 0.5uM forward and reverse primers, 0.275 units of Accustart II Taq DNA polymerase (Quantabio), and 1uL of template DNA. Amplicons were verified via gel electrophoresis and quantified using a Quantifluor® ONE dsDNA system (Promega) before sequencing. PCR amplicons were sequenced by Eurofins Genomics. The resultant sequence files were trimmed to remove low quality bases and blasted against the GenBank database to confirm sister taxa identity. The sequence reads were uploaded to NCBI's GenBank for future use.

A total of 56 individual organisms were sequenced and 33 unique haplotypes were submitted to the GenBank representing 19 of the 23 taxa collected (Table 1.1). Despite several attempts, sequencing failed for two species – *Sphenia fragilis* and *Tubicolixa chaetopterana*. Additionally, two sequences are currently being rejected from GenBank due to the lack of similarity with sequences from the same genera – *Ameroculodes miltoni* and *Barnea truncata*. We are in communications with NCBI's staff and will continue to work through the accession process for these two sequences. Accession numbers for submitted sequences are listed in Table 1.1.

The additional taxa sequences submitted to GenBank were used to support a SCDNR and USFWS study titled, "Identifying optimal foraging habitat characteristics to inform Piping Plover and Red Knot habitat management." This effort collected and genetically analyzed fecal samples from Piping Plover using the GenBank sequencing added by this study.

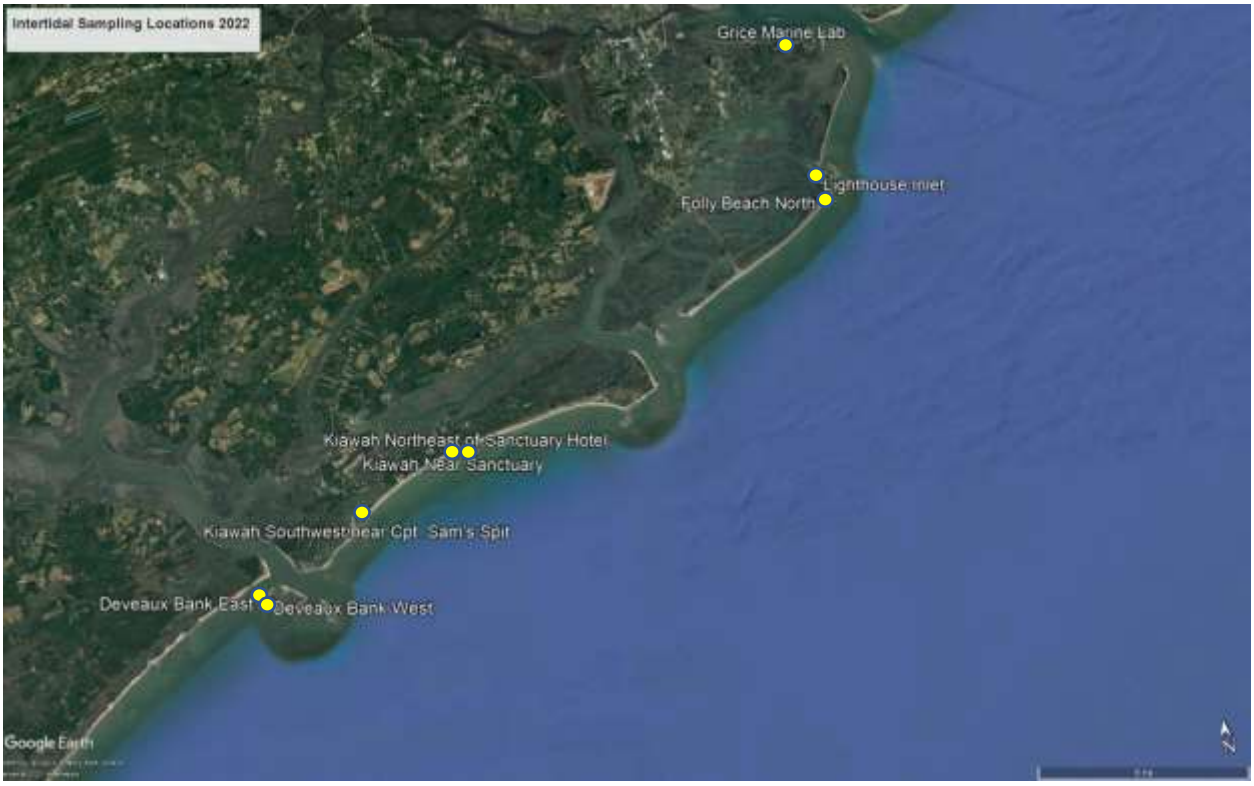

Figure 1.1: Location of the 8 intertidal sites sampled.

Haustorius canadensis

20221001@4X: Peraeopod 7, Article 4, distolateral margin with spines along posterior ¼

20221001@4X: Peraeopod 6, Article 5, distal margin straight, anterodistal angle rounded

20221001@4X: Antenna 1, accessory flagellum 3-5 articulate

20221001@10X: Mandible, palp article 3 with median row of comb setae

Figure 1.2: An example set of voucher photographs archived at SCDNR.

Table 1.1: Species names and NCBI's Genbank accession numbers for sequences submitted for the current project.

Organism Type	Family	Scientific Name	Accession Numbers
polychaete	Paraonidae	*Paraonis fulgens*	OQ225095, OQ225096, OQ225097
	Capitellidae	*Mediomastus ambiseta**	OQ271797, OQ271798
	Cirratulidae	*Tharyx acutus**	OQ236233
	Capitellidae	*Notomastus latericeus*	OQ224686, OQ224687
	Glyceridae	*Glycera americana*	OQ223396, OQ223397
	Lumbrineridae	*Scoletoma tenuis**	OQ236229
amphipod	Haustoriidae	*Acanthohaustorius millsi**	OQ224982
	Haustoriidae	*Neohaustorius schmitzi*	OQ224733
	Haustoriidae	*Lepidactylus dytiscus*	OQ224386, OQ224387
	Haustoriidae	*Protohaustorius wigleyi**	OQ236226, OQ23227, OQ236228
	Haustoriidae	*Parahaustorius longimerus**	OQ236224, OQ236225
	Haustoriidae	*Haustorius canadensis*	OQ223406, OQ223407
	Haustoriidae	*Parahaustorius holmesi**	OQ236221, OQ236222
	Haustoriidae	*Acanthohaustorius intermedius**	OQ236219
	Oedicerotidae	*Ameroculodes miltoni*	Accession Pending
	Bathyporeiidae	*Amphiporeia virginiana**	OQ236217, OQ236218
	Ischyroceridae	*Cerapus tubularis**	OQ236220
decapod	Bodotriidae	*Spilocuma watlingi**	OQ236230, OQ236231, OQ236232
isopod	Sphaeromatidae	*Exosphaeroma diminutum**	OQ236223
	Harrimaniidae	*Saccoglossus kowalevskii**	OQ225141
bivalve	Veneridae	*Barnea truncata*	Accession Pending

*Species formerly not represented in GenBank

Significant Deviations:

No significant deviations occurred.

Literature Cited:

Folmer, O., et al. "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates." Molecular Marine Biology and Biotechnology 3.5 (1994): 294-299.

SCDNR, South Carolina Department of Natural Resources. 2015. South Carolina State Wildlife Action Plan and Supplemental Volume. www.dnr.sc.gov/swap

U.S. Fish and Wildlife Service (USFWS). 2009. Piping plover, 5-year review: summary and evaluation. http://www.fws.gov/midwest/endangered/recovery/5yr_rev/pdf/PipingPlover5yr2 009.pdf

Total Federal Cost:

$44,909.45 (fully expended)

Recommendations:

Close the grant.