Georgia Journal of Science

Volume 81 *Scholarly Contributions from the Membership and Others* Article 7

2023

Regional Aquatic Macroinvertebrate Biodiversity

Sally Sir
Georgia College & State University, Milledgeville, GA 31061, sally.sir@bobcats.gcsu.edu

Kris N. White Ph.D.
Georgia College & State University, kristine.white@gcsu.edu

Regional Aquatic Macroinvertebrate Biodiversity

Acknowledgements

Funding was provided by Georgia College & State University's Aquatic Sciences Center Summer Fellowship. Georgia College & State University's Department of Biological and Environmental Sciences provided support in the form of space and equipment. Finally, a special thank you to Heidi Mead for instructing the authors on the use of the imaging system.

REGIONAL AQUATIC MACROINVERTEBRATE BIODIVERSITY

Sally Sir[1]* and Kristine N. White[1]
[1]Georgia College & State University, Department of Biological and Environmental Sciences, Milledgeville, GA 31061 U.S.A
*Corresponding author: sally.sir@bobcats.gcsu.edu

ABSTRACT

Macroinvertebrates are sensitive to environmental conditions, giving them the ability to be utilized in studies indicating the health of their environment. Additionally, macroinvertebrates are an important food source for vertebrates and invertebrates. Since macroinvertebrates play such significant roles in the ecosystem, it is important to identify and document their presence. In order to begin documenting the macroinvertebrates in central Georgia, aquatic macroinvertebrates were collected from ten sampling sites along a transect of central Georgia. Live specimens were imaged, preserved, identified, and sequenced. COI mitochondrial DNA and 18s nuclear DNA and 3D images of the organisms were made publicly available. The newly documented biodiversity of aquatic macroinvertebrates included organisms from the classes Branchiopoda, Gastropoda, Insecta, Malacostraca, and Ostracoda.

Keywords: central Georgia, bioindicator species, 18S nuclear DNA, COI mitochondrial DNA, water quality

INTRODUCTION

In aquatic systems, macroinvertebrates have been noted for their ability to alter the cycling and production of nutrients in bodies of water and for their potential to indicate stream health (Anderson and Sedell 1979; Uherek and Gouveia 2014). While macroinvertebrates are recognized for numerous functions, perhaps their most crucial role is that of a primary consumer. Since macroinvertebrates are in a lower trophic level, they are food sources for a number of aquatic organisms, especially fish (Wallace and Webster 1996). Given the high diversity of aquatic organisms that rely on macroinvertebrates in Georgia, the success of those macroinvertebrates is crucial for ecosystem sustainability. (Georgia Department of Natural Resources 2023). Documenting the presence of aquatic macroinvertebrates is important as it can indicate the health and overall success of the body of water from which they were collected, and the anthropogenic effects on valuable freshwater resources. This concept is thoroughly explored by Karr (1999), who credits biological monitoring as fundamental in assessing the integrity of rivers. While some orders or families of organisms are considered generally sensitive to pollutants, human influence, or other environment-altering factors, certain species within those orders or families can be more tolerant than other species within the same order or family. Failure to identify organisms to species level can provide an incorrect perception of an environments' health. Despite this, most of the available literature contains identification keys to order or family level as the morphological

characteristics used to identify to species level can be hard to see or to distinguish between species.

Molecular methods in biological monitoring have made significant progress in recent years. The progress of molecular methods in biological monitoring is reflected in environmental DNA (eDNA), where organismal DNA is detected in water, sediment, soil, or air samples taken from their environment. It is possible to detect this DNA because organisms tend to leave behind genetic material in their environment, such as excrement or the shedding of carapaces (Pilliod et al. 2013). Thus, by taking a water sample and using techniques like metagenomics, which identifies all or most of the organisms that left genetic material in that sample, it is possible to determine the organisms that recently habited that body of water without collecting the organisms themselves. Nanopore sequencing is a cost-effective and adaptable technique to sequence bacterial microbiota in river water samples for monitoring (Urban et al. 2021), suggesting that this technology will be used more commonly in future studies as it becomes increasingly accessible and continues to improve. In order to use eDNA methods, the DNA sequence information for properly identified species has to be first made available for comparison.

This research is the first attempt to document aquatic macroinvertebrate diversity in central Georgia using both morphological and molecular methods. This study is not meant to conclude the health of any of the sampled water systems. The sites which were sampled were not sampled extensively enough to draw definitive conclusions nor should the sites be compared against one another as time spent sampling and collecting techniques was not consistent amongst locations. Instead, the goal of this project was to serve as a preliminary baseline of central Georgia macroinvertebrates to use in future studies. This goal included sampling from as much of Central Georgia as possible within the budget and time constraints for the project. Sampling sites were selected throughout central Georgia based on ease of access to create a baseline which would provide insight into which of these sampling sites could be useful for comprehensive surveys. In addition to these goals, we set out to increase the available DNA sequences of macroinvertebrates and create a freely available resource for identification of the organisms by both scientists and laypeople.

MATERIALS & METHODS

In May of 2023, we collected samples from 10 sites within Georgia. These sites included the Ocmulgee River, Lake Oconee, Lake Sinclair, the Oconee River, the Ohoopee River, the Ogeechee River, and Sapelo Island (Figure 1, *Table I*). At each location, we used a YSI meter to measure salinity, water temperature, dissolved oxygen levels, and pH levels. Live samples were collected using a variety of techniques: D-nets were used for epifaunal invertebrates (7 sweeps per site), a bait pump (yabbi pump) was used for infaunal invertebrates (25 attempts per site), and plankton nets were used for pelagic invertebrates (2 minutes per tow). Two Lake Oconee samples were collected from Old Salem Park (samples 1 and 2) where the freshwater was slow moving and the shoreline was heavily

vegetated with a variety of shrubbery and trees and is a public access area with a boat ramp. A third Lake Oconee sample was collected from the Lawrence Shoals Recreational Area, about 13 km from Old Salem Park with habitat and usage (sample 3). All Lake Oconee sites remain submerged year-round. The Ocmulgee River samples were from the Ocmulgee Heritage Trail Canoe Launch (sample 4) and the Jay Hill Memorial Canoe launch (sample 5). These sites had high freshwater flow and were heavily polluted with trash and plastic, both of which experience changes in water height depending on water flow. The Ocmulgee Heritage Trail Canoe Launch had little vegetation and muddy water, while the Jay Hill Memorial Canoe launch had clear water and more trees lining the shoreline. Three Lake Sinclair freshwater samples were taken by boat along the shoreline, but further from boat ramps and human influence. The vegetation and water flow were similar to the Lake Oconee sites, remaining submerged year-round. Sapelo Island saline water samples (9 and 10) were collected subtidally from Cabretta beach, which is undeveloped and free of vegetation with high wave energy, and by scraping fouling organisms from a boat dock on a moderately saline creek (11 and 12). One Ogeechee River freshwater sample was collected from vegetation at a boat ramp with moderately high and clear water flow (sample 13). One Ohoopee river freshwater sample was collected under a bridge (sample 14). This site had high muddy water flow and appeared to remain submerged year-round. It was heavily vegetated with aquatic plants, while the shoreline was lined with trees. One freshwater sample was collected from a South Oconee River boat ramp with high muddy water flow and sparse vegetation (sample 15). This site appeared to experience high fluctuation in water level with changes in water flow.

Live specimens were identified into morphospecies and imaged using a portable macro-rail stacked imaging system. Once imaged, we preserved the organisms in 99.5% ethanol. We then identified the specimens morphologically to species level using a variety of taxonomic keys (Haney et al. 2013; Bouchard 2004; Bright 2013; Bruno et al. 2005; Dillon et al. 2019; Epler 2001, 2006; Fall 1922; Gustafson and Miller 2015; Murray et al. 2018; Taylor 1991; Thorp 1991). Thirty-one species were identified within our time frame, and DNA was extracted from three specimens each of the identified species using the guanidine DNA extraction method (Sinniger et al. 2010). We amplified COI mitochondrial DNA and 18S nuclear DNA using methods of White et al. (2016). PCR purification was done using ExoSAP-IT™ for COI and QIAquick Gel Extraction Kits for 18S. Eurofins Genomics completed DNA sequencing. We used BioEdit 7.2.5 (Hall 1999) to assemble consensus sequences, which were aligned in SeaView 5.0.4 (Gouy et al. 2010) for comparison. Alignments contained 575 base pairs for COI and 760 base pairs for 18S. Sequences were compared to available sequences in GenBank.

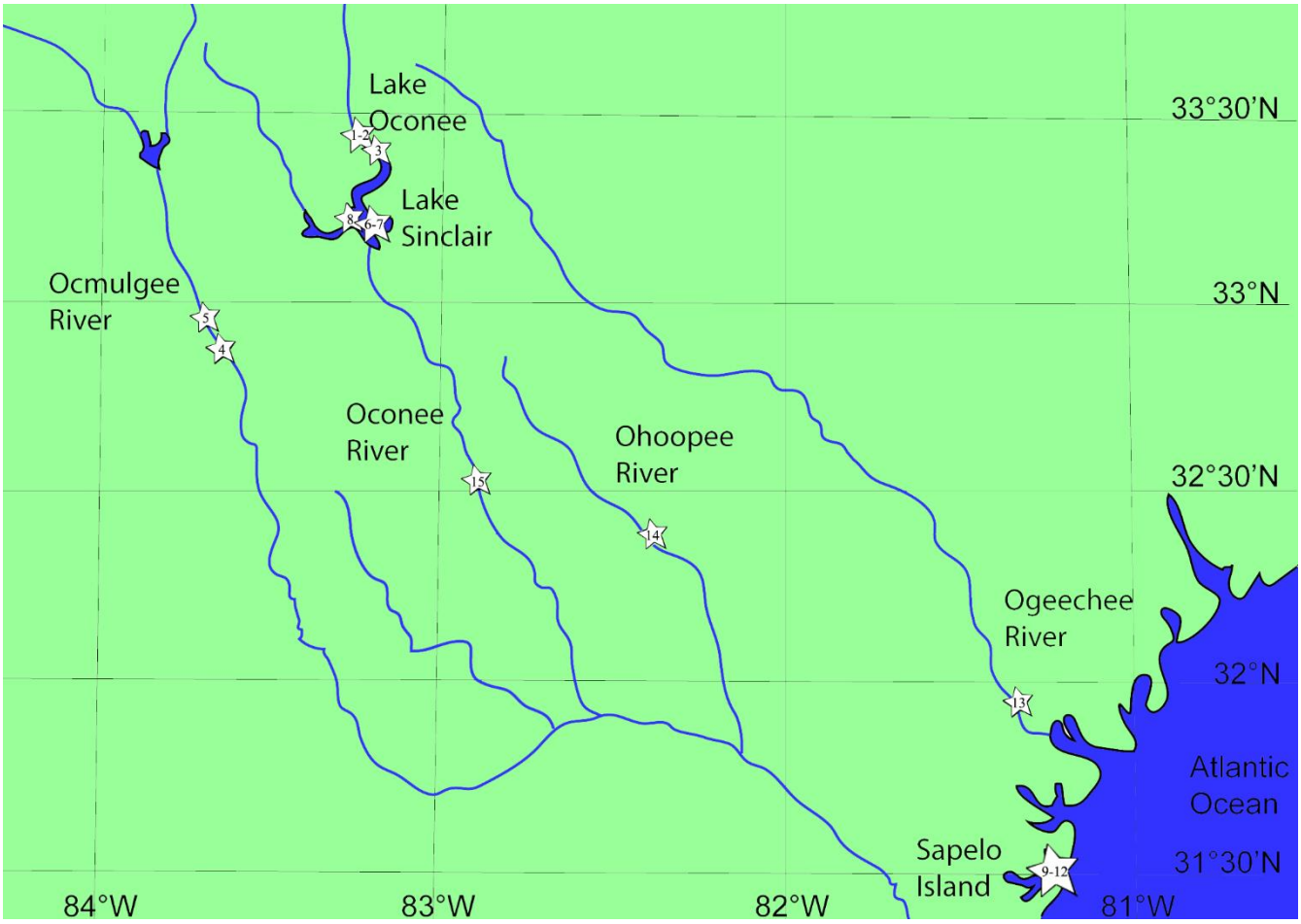

Figure 1. A Map of the sampling sites in central Georgia. Numbers on stars refer to samples in Table I.

Table I. Sampling sites and collecting data

Samples	Location	Date	Lat Lon	Elevation (m)	Temperature (°C)	Dissolved Oxygen (Mg/L)	Conductivity (Ms/cm)	Salinity	pH	Method
1	Lake Oconee, Old Salem Park	9-May-22	N 33°26.035', W 083°94.958'	138.38	21.5	6.77	0.055	0.03	7.06	plankton tow
2	Lake Oconee, Old Salem Park	9-May-22	N 33°26.035', W 083°94.958'	138.38	21.5	6.77	0.055	0.03	7.06	D-net
3	Lake Oconee, Lawrence Shoals Rec Area	9-May-22	N 33°21.286', W 083°09.962'	138.68	23.05	9.37	0.055	0.03	8.38	D-net
4	Ocmulgee River, Ocmulgee Heritage Trail	16-May-22	N 32°50.738', W 083°37.648'	103.63	22.85	6.5	0.095	0.04	7.16	D-net
5	Ocmulgee River, Jay Hill Memorial Canoe Launch	16-May-22	N 32°52.898', W 083°39.478'	93.88	23.43	5.22	0.091	0.04	7.17	D-net
6	Lake Sinclair (site 1)	19-May-22	N 33°09.543', W 083°11.324'	111.862	26.97	1.87	0.06	0.03	8.8	plankton tow
7	Lake Sinclair (site 2)	19-May-22	N 33°09.712', W 083°11.506'	106.68	27.24	1.75	0.06	0.83	8.66	D-net
8	Lake Sinclair (site 3)	19-May-22	N 33°10.514', W 083°15.184'	109.12	26.78	1.88	0.059	0.03	8.64	D-net
9	Sapelo Island, Cabretta	28-May-22	N 31°26.005', W 081°13.948'	-2.44	31.82	2.76	50.84	29.7	8.01	yabbi pump
10	Sapelo Island, Cabretta	28-May-22	N 31°26.005', W 081°13.948'	-2.44	31.82	2.76	50.84	29.7	8.01	plankton tow
11	Sapelo Island, DNR Dock	28-May-22	N 31°25.932', W 081°16.968'	-14.63	27.9	4.6	38.39	22.91	7.25	algae on buoy
12	Sapelo Island, DNR Dock	28-May-22	N 31°25.932', W 081°16.968'	-14.63	27.9	4.6	38.39	22.91	7.25	plankton tow
13	Ogeechee River	30-May-22	N 31°58.699' W 081°17.287'	1.21	27.26	5.7	0.1	0.04	6.8	D-net
14	Ohoopee River	30-May-22	N 32°23.496' W 082°18.826'	43.28	23.45	7.78	0.055	0.03	1.53	D-net
15	Oconee River	30-May-22	N 32°30.064' W 082°52.481'	59.13	24.8	6.46	0.066	0.03	6.95	D-net

RESULTS

Nearly 500 individual specimens were collected and 31 species in the classes Branchiopoda, Gastropoda, Insecta, Malacostraca, and Ostracoda were identified (*Table II*). Five of the thirty-one identified species are newly documented in Georgia, representing possible range extensions (*Cypridopsis vidua* (O.F. Müller, 1776); *Eurycerus microdontus* Frey, 1978; *Gyrinus pleuralis* Fall, 1922; *Simocephalus serrulatus* (Koch, 1841), and *Trichocorixa borealis* Sailer, 1948. The 3D images were uploaded to the Georgia College Aquatic Sciences Center Webpage under the Aquatic Macroinvertebrate Gallery (https://www.gcsu.edu/gcsu-asc-aquatic-macroinvertebrates-gallery).

Forty-five sequences of mitochondrial COI DNA and 28 sequences of nuclear 18s rDNA were amplified and uploaded to GenBank (*Table II*). Uncorrected pair-wise nucleotide distances between sequences from multiple specimens of one species were between 0.000-0.214 (COI) and 0.000-0.006 (18S), confirming the accuracy of our morphological identification. Amphipods identified as *Hyalella spinicauda* Soucek and Lazo-Wasem, 2015 were collected from Lake Oconee (sample 2), Lake Sinclair (sample 3), and the lower Oconee River (sample 15). DNA distances between amphipod sequences from Lake Oconee and Lake Sinclair were 0.000-0.014 (COI) and 0.000-0.004 (18S), but the distances in sequences between the Lakes and the lower Oconee River were 0.226-0.237 (COI) and 0.195-0.202 (18S).

Table II. List of species identified (based on classification in World Register of Marine Species); the collecting site value corresponds with the collecting site value in *Table I*; * represents superorder.

Class	Order	Family	Species	Collecting Site	Genbank 18S	Accession Numbers COI
Branchiopoda	Anomopoda	Bosminidae	*Bosmina longirostris* O.F. Müller, 1776	1		
		Chydoridae	*Leydigia acanthocercoides* (Fischer, 1854)	6		OQ918572
			Oxyurella brevicaudis Michael & Frey, 1983	8		
		Daphniidae	*Daphnia lumholtzi* Sars, 1885	1	OQ924624, OQ924625	OQ918569, OQ918570
		Daphniidae	*Simocephalus serrulatus* (Koch, 1841)	3		OQ918576
		Eurycercidae	*Eurycercus microdontus* Frey, 1978	8		
	Ctenopoda	Sididae	*Sida crystallina* O.F. Müller. 1776	2, 6, 8		OQ918573, OQ918574, OQ918575
		Sididae	*Latona setifera* (O.F. Müller. 1776)	8		OQ918571
Gastropoda	Hygrophila*	Lymnaeidae	*Pseudosuccinea columella (Say, 1817)*	7		OQ918577
		Physidae	*Physella acuta* (Draparnaud, 1805)	3, 13, 7		OQ918580, OQ918581
	Littorinimorpha	Cochliopidae	*Littoridinops tenuipes* (Couper, 1844)	13		OQ918605
Hexapoda	Coleoptera	Gyrinidae	*Dineutus serrulatus serrulatus* Leconte, 1868	14		OQ918582, OQ918583
			Gyrinus plureuralis Fall, 1922	14		OQ918584, OQ918585, OQ918586
	Hemiptera	Corixidae	*Trichocorixa borealis* Sailer, 1948	15		
		Gerridae	*Metrobates hesperius* Uhler, 1871	6		OQ918587
		Veliidae	*Mesovelia mulsanti* White, 1879	3		
			Rhagovelia obesa Uhler, 1871	5		OQ918592, OQ918593
	Odonata	Aeshnidae	*Boyeria vinosa Say, 1839*	4		OQ918591
	Diptera	Chironomidae	*Chironomus ochreatus* (Townes, 1945)	3, 7, 8, 15		OQ918594
Malacostraca	Amphipoda	Caprellidae	*Paracaprella tenuis* Mayer, 1903	11	OQ924642, OQ924643, OQ924644	OQ918602, OQ918603, OQ918604
		Corophiidae	*Monocorophium tuberculatum* (Shoemaker, 1934)	11	OQ924635, OQ924636, OQ924637, OQ924638	

		Crangonyctidae	*Crangonyx* cf. *serratus* (Embody, 1911)		OQ924626, OQ924627, OQ924628	
		Gammaridae	*Gammarus tigrinus* Sexton, 1939	3	OQ924645, OQ924646, OQ924647	OQ918607, OQ918608
		Haustoriidae	*Parahaustorius longimerus* Bousfield, 1965	13		OQ918597, OQ918598, OQ918599, OQ918600
		Hyalellidae	*Hyalella spinicauda* Soucek & Lazo-Wasem, 2015	1	OQ924629, OQ924630, OQ924631, OQ924651	OQ918595, OQ918596, OQ918612
		Melitidae	*Melita nitida* Smith, 1873	3, 7, 8, 15	OQ924639, OQ924640, OQ924641	
		Pleustidae	*Incisocalliope aestuarius* (Watling & Maurer, 1973)	11	OQ924632, OQ924633, OQ924634	OQ918601
	Decapoda	Albuneidae	*Albunea paretii* Guérin-Méneville, 1853	11		
		Palaemonidae	*Palaemon paludosus* (Gibbes, 1850)	9	OQ924648, OQ924649, OQ924650	OQ918578, OQ918579
	Ispopoda	Asellidae	*Lirceus fontinalis* Rafinesque, 1820	14		OQ918606
Ostracoda	Podocopida	Cyprididae	*Cypridopsis vidua* O.F. Müller. 1776	13		OQ918589
				3, 7		

DISCUSSION

While Georgia is recognized for its aquatic biodiversity, many of the macroinvertebrates that inhabit its rivers, streams, and coastlines are not well-documented. This research documented and identified 31 species of aquatic macroinvertebrates in central Georgia. This project also resulted in the creation of a freely available biodiversity database that can be expanded upon by other studies involving the Georgia College & State University Aquatic Sciences Center. The species identifications, 3D images on the website, and the addition of molecular sequence data provide a baseline for future studies involving aquatic macroinvertebrates. These baseline data will aid both graduate and undergraduate students that intend to study aquatic environments, particularly in selecting locations based on presence of particular invertebrate species. The addition of molecular sequence data for 31 species that were not previously represented in GenBank will also allow the use of eDNA more comprehensively.

Macroinvertebrates that were sequenced in this study could be compared to morphologically identical macroinvertebrates sampled from different areas to detect cryptic speciation. Upon investigation of the *Hyalella azteca* (Saussure, 1858) complex, one species was split into several different species, including *H. spinicauda*. White (2011) and White et al. (2019) documented intraspecific sequence distances for amphipods as 0-0.023 for 18S rDNA and 0.2101-0.2143 for COI mtDNA. Interspecific distances were documented as 0.090-0.295 for 18S rDNA and 0.248-0.327 for COI mtDNA (White, 2011; White et al. 2019). *Hyallela spinicauda*, collected from Lakes Oconee and Sinclair were morphologically identical to amphipods collected from the lower Oconee River. However, the high DNA distances suggest that cryptic speciation may be occurring due to the separation of these habitats by the Sinclair Dam, which causes a major shift in habitat and water flow. Despite the variation in the DNA sequences, only one specimen from the lower Oconee was sequenced and this result needs further investigation in order to confirm whether or not cryptic speciation is occurring.

In total, five of the 31 identified species had not been previously documented from Georgia, representing a possible range extension. *Cypridopsis vidua* was previously documented in South Carolina (Ferguson 1958). *Eurycerus microdontus, S. serrulatus,* and *T. sexcincta* were all previously documented in Florida (Layne 1979; State of Florida Department of Environmental Regulation 1983; Epler and Denson 2017). *Gyrinus pleuralis* was previously documented from Colorado to California, and north to Alberta (Oygur and Wolfe 1991). Many of these organisms have a cosmopolitan distribution and were likely just not previously identified in Georgia. *Daphnia lumholtzi* was the only organism found to be exotic and potentially invasive to Georgia (Havel et al. 1995).

Taxa from sensitive groups were collected from Lake Oconee, the Ocmulgee River, Lake Sinclair, Sapelo Island, the Ogeechee River, and the Ohoopee River, suggesting that these are oxygen rich environments. An oxygen rich environment is typically important for the survival and success of aquatic life and can also serve as an indicator of quality water conditions. However, our measured oxygen levels at Lake Sinclair and Sapelo

Island were relatively low. It is likely that the species collected are not sensitive taxa, even though other species in the order may be sensitive taxa. This highlights the importance of identifying organisms to species level. Additional and more thorough sampling of the collection sites might allow the use of properly identified aquatic macroinvertebrates in indicating the health of the streams, lakes, and rivers from which they were collected. While this project cannot serve as an indicator of the biological diversity in Georgia, as the sampling time and techniques were limited and differed between sampling sites, it provides a framework that can be used to document and monitor diversity, and additionally serves as a snapshot of the aquatic macroinvertebrate biological diversity.

Ultimately, the documentation of aquatic macroinvertebrate diversity is crucial for monitoring the health of the environment. The presence and morphological characteristics of the organisms can provide details about the environment from which they were identified and indicate environmental change. Aquatic macroinvertebrates provide an important food source for most animals in aquatic systems. Without these organisms, many systems would not be able to sustain the ecosystem services they provide today; in turn, impacting human use of these ecological systems. Additionally, the use of genetic analysis to inform morphological identifications is a valuable tool not only to confirm identifications but to discern between cryptic species.

ACKNOWLEDGMENTS

Funding was provided by Georgia College & State University's Aquatic Sciences Center Summer Fellowship. Georgia College & State University's Department of Biological and Environmental Sciences provided support in the form of space and equipment. Finally, a special thank you to Heidi Mead for instructing the authors on the use of the imaging system.

REFERENCES

Anderson, N.H. and Sedell J.R. 1979. Detritus processing by macroinvertebrates in stream ecosystems. Annual Review of Entomology, 24, 351–377. https://doi.org/10.1146/annurev.en.24.010179.002031.

State of Florida Department of Environmental Regulation. 1983. Bioassays of Picketville Road Landfill Leachate Jacksonville, Duval County, Florida. Report submitted to the State of Florida Department of Environmental Regulation, Jacksonville, FL. 133 pp.

Bouchard, R.W. 2004. Guide to Aquatic Invertebrates of the Upper Midwest. http://www.aquaticinsects.org/index.html Water Resources Center, University of Minnesota: St. Paul, MN, USA, 1–215.

Bousfield, E.L. 1965. Haustoriidae of New England (Crustacea,Amphipoda). Proceedings of the United States National Museum, 117, 159–239

Bright, E. 2013. Aquatic Insects of Michigan. http://www.aquaticinsects.org/index.html.

Bruno, M.C., J.W. Reid, and S.A. Perry. 2005. A List and Identification Key for the Freshwater, Free-Living Copepods of Florida (U.S.A.). Journal of Crustacean Biology, 25(3), 384–400. https://doi.org/10.1651/C-2538.

Couper, S. 1844. in S.S. Haldeman,editor. A monograph of the freshwater univalve Mollusca of the United States, including notices of species in other parts of North America. 7. J. Dobson: Philadelphia, 1–32.

Dillon, R.T. 2019. The Freshwater Gastropods of Georgia. The Freshwater Gastropods of North America. https://www.fwgna.org/FWGNC/dkey.html

Draparnaud, J.P.R. 1805. Histoire naturelle des mollusques terrestres et fluviatiles de la France. Levrault & Schoell: Paris, 164 pp.

Embody, G.C. 1911. A new fresh-water amphipod from VirgIa with some notes on its biology. Proceedings of the United States National Museum, 38, 299–305.

Epler, J.H. 2001. Identification Manual for the Larval *Chironomidae* (*Diptera*) of North and South Carolina. Report submitted to the North Carolina Department of Environment and Natural Resources: Raleigh, NC. 53 pp.

Epler, J.H. 2006. Identification Manual for the Aquatic and Semi-Aquatic Heteroptera of Florida. Report submitted to the Florida Department of Environmental Protection: Tallahassee, FL. 195 pp.

Epler, J.H. and D.R. Denson. 2017. New Records of Corixidae and Micronectidae (Insecta: Heteroptera: Corixoidea) from Florida, with a Checklist of All Species Known from the State. Entomological News, 126(5), 410–414. https://doi.org/10.3157/021.126.0510.

Fall, H.C. 1922. The North American Species of Gyrinus (Coleoptera). Transactions of the American Entomological Society, 47(4), 269–306. https://www.jstor.org/stable/25077050.

Ferguson, E. 1958. Freshwater Ostracods From South Carolina. The American Midland Naturalist, 59(1), 111–119. https://doi.org/10.2307/2422379.

Fischer, S. 1854. Über die in der Umgebung von St.-Petersburg vorkommenden Crustaceen aus der Ordnung der Brachiopoden und Entomostraceen. Mémoires de l'Académie impériale des sciences de St. Pétersbourg, 7, 1–14.

Frey, D.G. 1978. A new species of Eurycercus (Cladocera, Chydoridae) from the Southern United States. Tulane Studies in Zoology and Botany, 20(1–2), 1–25.

Georgia Department of Natural Resources. 2023. Georgia's Aquatic Species Diversity. https://georgiawildlife.com/georgias-aquatic-species-diversity.

Gibbes, L.R. 1850. On the Carcinological Collections of the Cabinets of Natural History in the United States: With an Enumeration of the Species Contained Therein, and Descriptions of New Species. Proceedings of Third Meeting of American Association for Advancement of Science, 3, 165–201.

Gouy M., S. Guindon, and O. Gascuel. 2010. SeaView version 5.0.4 : a multiplatform graphical user interface for sequence alignment and phylogenetic tree building.

Molecular Biology and Evolution, 27(2), 221–224. https://doi.org/10.1093/molbev/msp259.

Guérin-Méneville, F.E. 1853. Mélanges et Nouvelles. Revue et Magasin de Zoolie Pure et Appliquée, 2(5), 45–48.

Gustafson, G.T. and K.B. Miller. 2015. The New World whirligig beetles of the genus *Dineutus* Macleay, 1825 (Coleoptera, Gyrinidae, Gyrininae, Dineutini). ZooKeys, 476, 1–135. https://doi.org/10.3897.8630.

Hall, T.A. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95–98.

Haney, J.F., Aliberti, M.A., E. Allan, D. Allard, D.J. Bauer, W. Beagen, S.R. Bradt, et al. 2013. An Image based Key to the Zooplankton of North America version 5.0. University of New Hampshire Center for Freshwater Biology. http://cfb.unh.edu/cfbkey/html/begin.html.

Havel, J.E., W.R. Mabee, and J.R. Jones. 1995. Invasion of the exotic cladoceran *Daphnia lumholtzi* into North American reservoirs. Canadian Journal of Fisheries and Aquatic Sciences, 52, 151–160. https://doi.org/10.1139/f95-015.

Karr, J.R. 1999. Defining and measuring river health. Blackwell Science Limited, Freshwater Biology 41, 221-234. 10.1046/j.1365-2427.1999.00427.x

Koch, C.L. 1841. Deutschlands Crustaceen, Myriapoden und Arachniden. Ein Beitrag zur deutschen Fauna. Volume 27. Regensberg: Herrich-Schäffer. 32 pp.

Layne, J.N. 1979. Natural Features of the Lake Annie Tract, Highlands County, Florida. A report submitted to Archbold Biological Station, Lake Placid, FL. 81 pp.

LeConte, J. 1868. The Gyrinidae of America, north of Mexico. Proceedings of the Academy of Natural Sciences of Philadelphia, 20, 365–373.

Mayer, P. 1903. Die Caprellidae der Siboga-Expedition. Siboga-Expeditie. Uitkomsten op zoologisch, botanisch, oceanographisch en geologisch gebied verzameld in Nederlandsch Oost-Indie 1899-1900 aan boord H.M. Siboga. 34, 160 pp.

Michael, R.G. and D.G. Frey. 1983. Assumed Amphi-Atlantic distribution of Oxyurella tenuicaudis (Cladocera, Chydoridae) denied by a new species from North America. Hydrobiologia, 106, 3–35.

Müller, O.F. 1776. Zoologiae Danicae prodromus, seu animalium Daniae et Norvegiae indigenarum characteres, nomina, et synonymy imprimis popularium. Hallageriis: Havniae. 282 pp.

Murray, K., D. Batzer, J. McHugh, and L. Roberson. 2018. Aquatic Insects of the Ogeechee River. A report submitted to the Ogeechee Riverkeeper, Savannah, Georgia. 40 pp.

Oygur, S. and G.W. Wolfe. 1991. Classification, distribution, and phylogeny of North American (north of Mexico) species of Gyrinus Müller (Coleoptera, Gyrinidae). Bulletin of the American Museum of Natural History, 207, 1–97. http://hdl.handle.net/2246/901.

Pilliod, D.S., C.S. Goldberg, M.B. Laramie and L.P. Waits. 2013. Application of environmental DNA for inventory and monitoring of aquatic species. U.S. Geological Survey Fact Sheet, 2012-3146, 4. https://pubs.usgs.gov/fs/2012/3146/ .

Rafinesque, C.S. 1820. Annals of Nature or Annual synopsis of new genera and species of animals, plants, etc., discovered in North America. First annual number. Thomas Smith: Lexington. 16 pp.

Sailer, R.I. 1948. The Genus *Trichocorixa* (Corixidae, Hemiptera). The University of Kansas Science Bulletin, 32, 289–407.

Sars, G.O. 1885. On some Australian Cladocera raised from dried mud. Forhandlinger i Videnskabs-Selskabet i Christiania, 8, 1–46.

Saussure, H. 1858. Memoire sur divers crustaces nouveaux des Antilles et du Mexique. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève, 14, 417-496.

Say, T. 1817. Description of seven species of American fresh water and land shells, not noticed in the systems. Journal of the Academy of Natural Sciences of Philadelphia, 1, 13–18.

Say, T. 1839. Descriptions of new North American neuropterous insects and observations on some already described by (the late) Th. Say. Journal of the Academy of Natural Science of Philadelphia, 8, 9–46.

Sexton, E.W. 1939. On a new species of *Gammarus* (*G. tigrinus*) from Droitwich District. Journal of the Marine Biological Association UK, 23, 543–551.

Shoemaker, C.R. 1934. The amphipod genus *Corophium* on the east coast of America. Proceedings of the Biological Society of Washington, 47, 23–32.

Sinniger, F., J.D. Reimer, and J. Pawlowski. 2010. The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: description of two new genera. Marine Biodiversity, 40, 57–70. https://doi.org/10.1007/s12526-009-0034-3.

Smith, S.I. 1873. In: Verrill, A. E. Report on the invertebrate animals of Vineyard Sound and the adjacent waters, with an account of the physical characters of the region. Report of the United States Committee of Fish and Fisheries, 1871 and 1872. 295–747.

Soucek, D.J., E.A. Lazo-Wasem, K.M. Major, and C.A. Taylor. 2015. Description of two new species of *Hyalella* (Amphipoda: Hyalellidae) from Eastern North America with a revised key to North American members of the genus. Journal of Crustacean Biology, 35(6), 814–829. https://doi.org/10.1163/1937240x-00002373.

Taylor, S.J. 1991. Species List, Distribution and Keys to the *Gerromorpha* (*Insecta: Heteroptera*) of Illinois. A report submitted to the Illinois Department of Conservation, Springfield, IL. 34 pp.

Thorp, J.H. and A.P. Covich. 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press: Boston, MA. 911 pp.

Townes, H.K. 1945. The Nearctic species of Tendipedini [Diptera, Tendipedidae (= Chironomidae)]. American Midland Naturalist, 34, 1–206. http://dx.doi.org/10.2307/2421112.

Uherek, C.B. and F.B.P. Gouveia. 2014. Biological monitoring using macroinvertebrates as bioindicators of water quality of Maroaga Stream in the Maroaga Cave System, Presidente Figueiredo, Amazon, Brazil. International Journal of Ecology, 2014, 1–7. https://doi.org/10.1155/2014/308149.

Uhler, P.R. 1871. Notices of some Heteroptera in the collection of Dr. T. W. Harris. Proceedings of the Boston Society of Natural History, 14, 93–109.

Urban, L., Holzer, A., Baronas, J.J., Hall, M.B., Braeuninger-Weimer, P., Scherm, M.J., Kunz, D.J., Perera, S.N., Martin-Herranz, D.E., Tipper, E.T., Salter, S.J., M.R. Stammnitz. 2021. Freshwater monitoring by nanopore sequencing. eLife. https://doi.org/10.7554/eLife.61504.

Wallace, J.B. and J.R. Webster. 1996. The Role of Macroinvertebrates in Stream Ecosystem Function. Annual Review of Entomology, 41, 115–139. https://doi.org/10.1146/annurev.en.41.010196.000555.

Watling, L. and D. Maurer. 1973. A new euryhaline species of Parapleustes (Amphipoda) from the east coast of North America. Proceedings of the Biological Society of Washington, 86, 1–8.

White, F.B. 1879. List of the Heteroptera collected in the Amazons by Prof. J. W. H. Trail in the years 1878-1875, with descriptions of the new species. Transactions of the Entomological Society of London, 267–276.

White, K.N. 2011. Nuclear 18s rDNA as a species-level molecular marker for *Leucothoidae* (Amphipoda). Journal of Crustacean Biology, 31 (4), 710-716. https://doi.org/10.1651/11-3489.1

White, K.N., J. Reimer and J. Lorion. 2016. Preliminary analyses reveal strong genetic structure in populations of *Leucothoe vulgaris* (Crustacea: Amphipoda: Leucothoidae) from Okinawa, Japan. Systematics and Biodiversity, 14(1), 55–62. https://doi.org/10.1080/14772000.2015.1078856.

White, K.N., K. Duval, and K. Travis. 2019 Anthropogenic range extension of *Leucothoe eltoni* Thomas, 2015 (Crustacea: Amphipoda: Leucothoidae). Marine Biodiversity, 49, 1595–1693. https://doi.org/10.1007/s12526-018-0928-z