**Data description**

These data were collected as part of a collaborative effort between ORD and the Smithsonian Environmental Research Center (SERC) to develop a standardized, longitudinal biodiversity monitoring program for the Great Lakes, with the specific aim of detecting introduction of non-native species. The Great Lakes Invasives Sentinel Sites Network (GLISSNet) is intended to provide data to managers on the rate of non-native species introduction and to provide early warning on potentially damaging invasive species. It is intended to address EPA’s statutory requirements to mitigate risks of new introductions to the Great Lakes under the auspices of the 2018 Vessel Incidental Discharge Act.

Samples were collected from Duluth/Superior Harbor in Lake Superior in 2021 using multiple sampling gears to obtain a broad representation of biodiversity present at sentinel sites. To sample benthic organisms we used Hester-Dendy samplers as well as oyster crates. Samplers were deployed through the summer and retrieved in the fall for processing. For Hester-Dendy’s, biomass was scraped from individual plates and preserved in a DMSO Salt Solution (10% DMSO, 8% EDTA, saturated with NaCl; Also called DNE Solution, referencing the three main components of the solution). Oyster crates, once retrieved, were soaked for 4 hours and then the soak water was filtered for environmental DNA through 0.45 um filters. Filters were stored at -20⁰ C until DNA extraction. To sample zooplankton we pumped water through a modified trash pump (Honda GX160 non-rammer engine) and filtered through a specially designed plankton net (80µm mesh) that floats horizontally on the water due to the shallow conditions of the collection sites. Zooplankton samples were preserved for metagenetic analysis in DMSO Salt Solution.

Filters and biomass from Hester-Dendy and zooplankton pump samples were processed for DNA using QIAGEN PowerSoil extraction kits as per manufacturer’s protocol. DNA metabarcoding was conducted targeting the COI locus using previously published amplification primers. Library preparation and sequencing was conducted using dual indexing 2x300 paired-end Illumina MiSeq sequencing according to standard protocols. All data are stored as raw sequence files in .fastq format at "C:\Users\jdarling\OneDrive - Environmental Protection Agency (EPA)\Documents - Vessel Biomonitoring\General\Sample Analysis\GLISSNet 2021 data."