CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Syma Ebbin syma.ebbin@uconn.edu, Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Nancy Balcom at (860) 405-9107.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Name of Submitter: J. Evan Ward

Date of Report submission: October 21, 2024

Project #: ____ LI 00A00284____ Check one: [ ] Progress Report [X] Final report

Duration (dates) of entire project, including extensions: From [3/1/2021] to [8/31/2024].

Project Title or Topic: Establishing robust bioindicators of microplastics in Long Island Sound: Implications for reliable estimates of concentration, distribution, and impacts

Principal Investigator(s) and Affiliation(s):

1. J. Evan Ward, University of Connecticut

2. Sandra E. Shumway, University of Connecticut

A. <u>COLLABORATORS AND PARTNERS</u> *(List any additional organizations or partners involved in the project.)*

Norm Bloom and Son, Norwalk, CT
Michael Gilman, Branford, CT
Jim Markow, Noank Oyster Cooperative, Mystic, CT

B. PROJECT GOALS AND OBJECTIVES:

Objective 1: In the laboratory, identify one or more suspension-feeding bioindicators that indiscriminately ingest all suspended MP to which they are exposed.

Objective 2: In the field, determine if the concentrations and types of microplastics in the gut of several species of suspension feeders are representative of those in the surrounding environment.

C. LISS CCMP IMPLEMENTATION ACTIONS: (*List the top 3 primary CCMP Implementation Actions that this project addresses. LISS CCMP Implementation Actions can be found at https://longislandsoundstudy.net/2021/01/ccmp-implementation-actions-supplemental-documents/)*

HW-26: Determine whether monitoring of surrogate species, including those of high conservation priority, is a cost-effective way to track habitat restoration.

SC-9: Raise awareness through various media formats about the Sound's water quality conditions that could impact human health.

D. PROGRESS: (*Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

Research was planned out and QAPP was written. QAPP was approved and signed.
Objective 1:

Particle selection assays were conducted using multiple microplastic types, sizes, and shapes. The tunicate, *Molgula manhattensis* and the slippersnail, *Crepidula fornicata* were offered aged polyester microfibers of different lengths (65, 500, 1000 µm), nylon microfibers of different lengths (70, 500, 1000 µm), nylon (70 µm length) and polyester (65 µm length) microfibers, or polyethylene (26 µm diameter) and polystyrene (31 µm diameter) microspheres during a 2-h exposure (n=10 for each set of exposures and each species). Based upon feces and pseudofeces collected during and after exposure to microplastics, it was shown that slippersnails and sea grapes both exhibited size-based rejection of nylon fibers, rejecting longer fibers at higher proportions. Slippersnails retained more fibers in the gut than tunicates. Polymer type did not influence ingestion of fibers or spheres. Sea grapes were the most indiscriminate feeders when compared with slippersnails, oysters, and mussels, but were able to egest MP just as quickly. Although sea grapes rejected proportionally fewer MP than slippersnails, neither species will make an ideal bioindicator because they do not ingest all plastic particles they encounter, they egest the particles quickly, and do not accumulate MP in their tissues.

This work has been summarized with reported results in chapter 6 of Kayla Mladinich Poole's dissertation (included as an attachment with report). Further, Ward and Mladinich Poole conducted a series of endoscopy experiments where slippersnails were offered nylon or polyester fibers of different lengths (one length at a time) and 500-µm diameter polystyrene spheres to qualitatively observe the handling of individual particles. The summary video will be included with the progress report and shows the animals rejecting longer fiber lengths and both consuming and rejecting 500-µm polystyrene spheres. The videos emphasize the effect size has on particle handling in slippersnails and provide novel observations of particle handling in slippersnails; the snails reject larger proportions of the longer fibers and larger beads than smaller particles.

Objective 2:

Oysters, water, and sediment have been collected from all three stations (eastern, western, and central Long Island Sound) for 2 years. Slippersnails and sea grapes have not been found consistently at all three locations, therefore, another summer of sampling is necessary to

sample water and snails or sea grapes around the original sampling locations for comparison. All water samples have been filtered and are in the process of being analyzed. Initial water samples from the western (N=3) and central Long Island Sound (N=3) locations reveal low microplastic concentrations with 0-4 microplastics found in any one of the 10-L water samples. Similarly, initial results show that slippersnails (N=3) and sea grapes (N=3) from the central sound location have low concentrations of microplastics in their guts with 0-2 MP/individual. Low microplastic concentrations have been observed repeatedly in oyster-gut samples taken by our group across Long Island Sound on and off aquaculture farms (0-8 MP/ individual; Mladinich et al., 2023). More field samples are being processed, but issues with the FTIR microscope have also led to delays with processing times.

E. PROJECT PUBLICATIONS, PRODUCTS, PRESENTATIONS AND PATENTS: *(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles, reports, and abstracts not previously provided.)*

Journal Articles (List URLs):

Mladinich, K., Holohan, B. A., Shumway, S. E., & Ward, J. E. (2023). The relationship between microplastics in eastern oysters (*Crassostrea virginica*) and surrounding environmental compartments in Long Island Sound. *Mar Environ Res, 189*, 106040. https://doi.org/10.1016/j.marenvres.2023.106040

Shumway, S. E., Mladinich, K., Blaschik, N., Holohan, B. A., & Ward, J. E. (2023). A critical assessment of microplastics in molluscan shellfish with recommendations for experimental protocols, animal husbandry, publication, and future research. *Rev Fish Sci Aquac*, DOI: 10.1080/23308249.2023.2216301.

Conference Papers:

Proceedings or book chapters:

Mladinich, K., Shumway, S.E., Vondolia, G. K., & Ward, J. E. Plastics in the Sea: An Overview. In **Plastics in the Sea: Occurrence and Impacts**; Shumway, S. E. and J. E. Ward (eds.), Elsevier Science Publishers. In Press.

Ward, J.E., K. Mladinich, N. Blaschik, B.A. Holohan, & Shumway, S.E. Interactions between microplastics and particle-feeding bivalve molluscs: implications for trophic transfer and toxicological effects. In **Plastics in the Sea: Occurrence and Impacts**; Shumway, S. E. and J. E. Ward (eds.), Elsevier Science Publishers. In Press.

Web sites, Software, etc.:

Technical Reports/Other Publications:

Other Products (including popular articles):

Publications planned / in progress:

Chapter 6 of Mladinich Poole's dissertation will be combined with the endoscopy work and a set of endoscopy videos for sea grapes or another tunicate species for a publication in the next year.

Patents: *(List those awarded or pending as a result of this project.)*

Presentations and Posters: *(Include name and date of the conference or meeting, whether it was a talk or poster, if it was invited, and who the presenter was.*)

Mladinich Poole, K. (August 2023). Selective Ingestion of Microplastics by Suspension-Feeding Invertebrates: Investigations into Sources, Fate, and Concentrations in Coastal Environments. Dissertation Defense.

Ward, J.E., K. Mladinich Poole, B.A. Holohan & S.E. Shumway (September 2023). Know Your Bivalve: Understanding Feeding Processes is Critical to Interpreting Results from Microplastic Research. Molluscs workshop, Cambridge, UK. Talk

Mladinich Poole, K. (November 2023). Establishing Robust Bioindicators of Microplastics in Long Island Sound: Implications for Reliable Estimates of Concentration Distribution and Impacts. Long Island Sound Study STAC Meeting.

Kayla M. Poole, Bridget A. Holohan, Sandra E. Shumway, J. Evan Ward (March 2024). Investigating suspension-feeding invertebrates as bioindicators of microplastics. National Shellfisheries Assoc. Annual meeting, Charlotte, NC. Talk

Ward, J. E. (November 2023). Seminar on plastics and microplastics. Americas Boating Club (formerly the Power Squadron) of Greenwich CT. Talk

Ward, J.E., K. Mladinich Poole, B.A. Holohan & S.E. Shumway (May 2024) Investigating suspension-feeding invertebrates as bioindicators of microplastics. Long Island Sound Conference, Port Jefferson, NY. Talk

F. FUNDS LEVERAGED: *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*
None

G. STUDENTS: ***(****Document the number and type of students supported by this project.)*
*Note: **"Supported"** means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. "**New**" students are those who **have not** worked on this project previously. "**Continuing**" students are those who **have** worked on this project previously. If a student volunteered time on this project, please use section G, below.*

Total number of **new*** K-12 students who worked with you: 0
Total number of **new** undergraduates who worked with you: 0
Total number of **new** Masters degree candidates who worked with you: 0
Total number of **new** Ph.D. candidates who worked with you: 0

Total number of **continuing**** K-12 students who worked with you: 0
Total number of **continuing** undergraduates who worked with you: 0
Total number of **continuing** Masters degree candidates who worked with you: 0
Total number of **continuing** Ph.D. candidates who worked with you: 1

Total number of volunteer hours: 0

*(Note: *__New__ students are those who have not worked on this project previously. **__Continuing__ students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

Student Name: Kayla Mladinich Poole
Degree Sought: Ph.D.
Thesis or Dissertation Title: Selective ingestion of microplastics by suspension-feeding invertebrates: Investigations into sources, fate, and concentrations in coastal environments
Date of thesis completion: December 1st 2023
Date of graduation: December 2023

H. VOLUNTEER HOURS:

(List the number of hours provided to the project by volunteers, i.e., individuals who were not compensated in any way or for whom involvement is not part of their paid occupation. This could be students or citizens. What was their contribution?)

I. PICTORIAL: Please provide high resolution images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study. Attach images as separate files (do not embed). Include links to websites associated with the research project. Please include proper photo credits and a caption with date, location, names of people, and activity. These images are useful to document your project in future CTSG publications, websites and presentations.

J. HONORS AND AWARDS: *(List any honors or awards received during the reporting period, for anyone working on the project. This can be for best paper or poster, university awards, etc.*) Specify:

a) Name of person or group receiving recognition:
b) Name of award or honor:
c) Group or individual bestowing the award or honor:
d) What it was for:
e) Date:

K. DATA MANAGEMENT PLANS: Proposals funded in 2014-2016 and later cycles are required to have a data management plan in place. All environmental data and information collected and/or created must be made visible, accessible, and independently understandable to general users, free of charge or at minimal cost, in a timely manner (typically no later than two years after the data are collected or created). This is a reminder that your CTSG funded research data needs to be archived and accessible as outlined in the data management plan you submitted with your proposal. If there have been any modifications,

adjustments, or new information available regarding the location, timing, type, formatting and metadata standards, content, sharing, stewardship, archiving, accessibility, publication or security of the data produced please elaborate here.

FOR FINAL DEVELOPMENT AND RESEARCH GRANT REPORTS, PLEASE COMPLETE THIS SECTION:

L. PROJECT OUTCOMES AND IMPACTS

RELEVANCE OF PROJECT: *(Describe briefly the issue/problem / identified need(s) that led to this work.)*

As the amount of bulk plastic increases in near-shore waters, so too does the contamination of estuarine organisms by their microscopic by-products. This research addressed several important questions regarding MPs (MP) in Long Island Sound (LIS), and addressed the Clean Waters and Healthy Watersheds, Thriving Habitats and Abundant Wildlife, and Sustainable and Resilient Communities (specifically Marine Debris-Monitoring/Research) objectives of the RFP. Our work was the first to examine multiple suspension feeders for potential use as a robust bioindicator of environmental MP, which is critically needed for an efficient and standardized method of monitoring plastic-particle abundance in marine systems.

RESPONSE: *(Describe briefly what key elements were undertaken to address the issue, problem or need, and who is/are the target audience(s) for the work.)*

The work provided both field observations and laboratory experiments to probe different invertebrate suspension feeders as bioindicators of MP. In the field, the work examined the concentrations and types of microplastics in the gut of the slipper-snail *Crepidula fornicata*, the solitary tunicate, *Mogula manhattensis*, and the eastern oyster *Crassostrea virginica*. Additionally, samples were taken from water and sediment in the area around the animals to determine environmental load. In the laboratory, the research identified the sizes, shapes (microspheres & microfibers), and polymer types (polystyrene, polyethylene, nylon, polyester) that were most readily ingested vs rejected in pseudofeces. The goal was to identify the animal that ingested the widest range of MP, indiscriminately, and thus was the best bioindicators of environmental MP.

A well-suited bioindicator species for MP pollution would be a critical tool for monitoring MP pollution in the LIS. Understanding the MP concentrations and polymers that dominate the LIS can be used to inform management directly on the degree of MP and potential sources to regulate. End users of the results of this study would include state and federal managers, NGOs (e.g., Save the Sound, The Nature Conservancy), and the aquaculture industry. Quantifying the load and distribution of MP in LIS is an important first step in addressing the concerns of these groups. Additionally, both species have congeners that are members of near-shore environments worldwide and, therefore, will serve as good model species for other regions.

RESULTS: *(Summarize findings and significant in terms of the research and any related education or outreach component; cite benefits, applications, and uses stemming from this project, including those expected in the future. Include qualitative and quantitative results.)*

Major results of the study include the following: 1) samples of water and sediment in LIS have a relatively low number of MP (i.e., 0-4 MP/10L of water); 2) slipper snails, sea

squirts, and oysters in LIS have in their guts a relatively low number of MP (i.e., 0-2 MP/individual); 3) laboratory results suggest that of the three suspension feeders, sea squirts appear to be a non- selective particle feeder and could be a bioindicator candidate for MP in the LIS environment -although studies with additional species need to be carried out. Currently, sample processing continues, and additional samples will be analyzed and used in future publications. Numerous presentations have been made based on this research, and another talk will be given at the upcoming Aquaculture 2025 meeting in New Orleans (Kayla M. Poole, Bridget A. Holohan, Sandra E. Shumway, J. Evan Ward. Investigating suspension-feeding invertebrates as bioindicators of microplastics. Aquaculture 2025, New Orleans, LA)

Consider the following as they apply to your research and any related outreach/education.

- What new tools, technologies, methods or information services were developed from this work? Have any been adopted / implemented for use and by whom?

  The work used standard procedures to collect and maintain suspension feeders, and established methods and QA/QC protocols to analyze plastic particles. As such, no new tools, technologies or methods were developed.

- What are the environmental benefits of this work? Have policies been changed? How has conservation (of ecosystems, habitats or species) been improved?

Establishing a bioindicator species for LIS would be central to addressing marine debris ecosystem targets and providing a method for MP monitoring in the LIS. As the results of the study are novel, no policies have been changed at this point in time.

- What are the social payoffs of this work? Who has benefited from this work? Have attitudes / behaviors of target audience changed? Elaborate. Have policies been changed?

Microplastics are pervasive in some marine environments and have received considerable attention in the media and from the general public as well as state and federal managers. As such, results of this study provide valuable fact-based information on several issues: 1) the relatively low number of MP that reside in oysters as a result of the animals' ability to preferentially reject many types of MP; 2) the potential use of non-selective particle feeders as bioindicators of MP; and 3) the relatively low number of MP in the surrounding environment. Data such as these allay immediate fears regarding plastic pollution in LIS and allow the public to focus on long-term strategies to keep MP pollution low, such as reducing input of macroplastic, for future generations. End users who could benefit from the outcomes of this study include state and federal managers, NGOs (e.g., Save the Sound, The Nature Conservancy), and the aquaculture industry. With continued presentations of the work at public forums and scientific meetings, attitudes will potentially change, for example, by having stakeholders focus on macroplastic pollution which is one of the main sources of MP in the LIS.

- What are the economic implications / impacts of this work? (Where possible, please quantify.) Have new businesses been created /or existing businesses retained as a result of this research? Have new jobs been created or retained? Are new businesses or jobs anticipated?

The main goal of the funded research was to create new knowledge that could be used in the future for economic benefit. Certainly, the results of this study could benefit the bivalve aquaculture industry in LIS which could be impacted by the public's concern over MP contamination of species such as oysters.

J. Stakeholder Summary (This is an abstract of your research and findings written for a lay audience)

Microplastics are pervasive components of many marine environments. As such they have received considerable attention in the media and from concerned citizens who want to protect the marine environment and human health. Unfortunately, much of the information available is not only incorrect, it has often been exaggerated. To obtain a more informed idea of the actual environmental load of MP in LIS and some of its inhabitants, this study examined the concentration of plastic particles in water, sediment and three filter-feeding animals – specifically the slipper snail, a sea squirt and oyster. Overall, the results demonstrate that currently the concentration of MP in LIS is low (example: 0-4 MP in found in about 2 ½ gallons of water; 0-2 MP in individual animals). Laboratory work demonstrated that both oysters and slipper snails selectively reject many types of plastic particles prior to ingestion. Sea squirts also rejected MP but to a lesser extent than the other two filter feeders. Thus, sea squirts might be an animal that could be used as an indicator of MP in the environment – although studies with additional species need to be carried out. Finally, the outcomes of this study provide additional information which demonstrates that oysters in LIS contain few plastic particles and should reduce concerns of consumers regarding the presence of MP in shellfish. The result of this study will be shared through the scientific literature and outreach efforts with user communities.