Jamaica Bay Long-Term Control Plan

Ribbed Mussel (*Geukensia demissa*) Project

Phase III - Mesocosm Research Procedures and Protocols

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Table of Contents

List of Figures

1 Introduction

The New York City Department of Environmental Protection (DEP) proposes to mitigate the influence of pathogens derived from combined sewer overflow (CSO) events by installing a community of ribbed mussels (*Geukensia demissa*) in two Jamaica Bay tributaries. This approach could be adopted in various waterbodies, however, the two areas this project will be targeting are Bergen and Thurston Basins. DEP has contracted with Hazen and Sawyer and Cornell Cooperative Extension to conduct a literature review and an array of experiments with ribbed mussels to determine the feasibility of the project goal. The project has been broken up into several phases beginning with a literature review (Phase I), followed by laboratory-based trials (Phase II) that will scale up to mesocosm simulations (Phase III) and then field trials (Phase IV):

- Phase I – Literature Review and Preliminary Microcosm Experiments
- Phase II – Microcosm Experiments (laboratory bench-top experiments)
- Phase III – Mesocosm Experiment (scaled physical model)
- Phase IV – In-situ Pilot Study (deployment in Bergen)

The purpose of this report is to document the procedures and protocols of the mesocosm (scaled down tank with flow through) experiments. **Section 2** describes the pre-experiment protocols for collecting seawater, maintaining the algae systems and Ribbed Mussels. **Section 3** describes the specific procedures and protocols for initiating the experiment, sample collection schedule and methodology. Section 4 describes the various experimental levels for the mesocosm experiment related to the density of ribbed mussels deployment and the bacterial loads introduced in a stepped manner to mimic their varying levels during a CSO event. Two parallel tanks were configured and each experiment was run as three replicates with a paired control. **Figure 1-1** shows the installation which includes a header tank, a collection tank and a controlled bacterial input tank. **Figure 1-2** shows the bacterial feed tank with the feed pump.

Figure 1-1: Experimental Tank Setup showing the Main tank the collector tank and the header tank (in the back)

Figure 1-2: Bacterial input tank and feed pump

2 Pre-Experiment Protocols

2.1 Ribbed Mussel Maintenance

- Water is pumped to a 600gal holding tank and is used to supply the algae system and for refilling the Ribbed Mussel (RM) holding tanks after the tanks are cleaned.
- RM were held in static tanks as shown in **Figure 2-1**, with circulating seawater that is maintained at 24°C and 25ppt (±1ppt)
- RM were fed a combination of algae paste and fresh cultivated algae of various species (**Figure 2-2**).
- Holding tanks were drained, cleaned and refilled Mon, Weds, Fri each week.

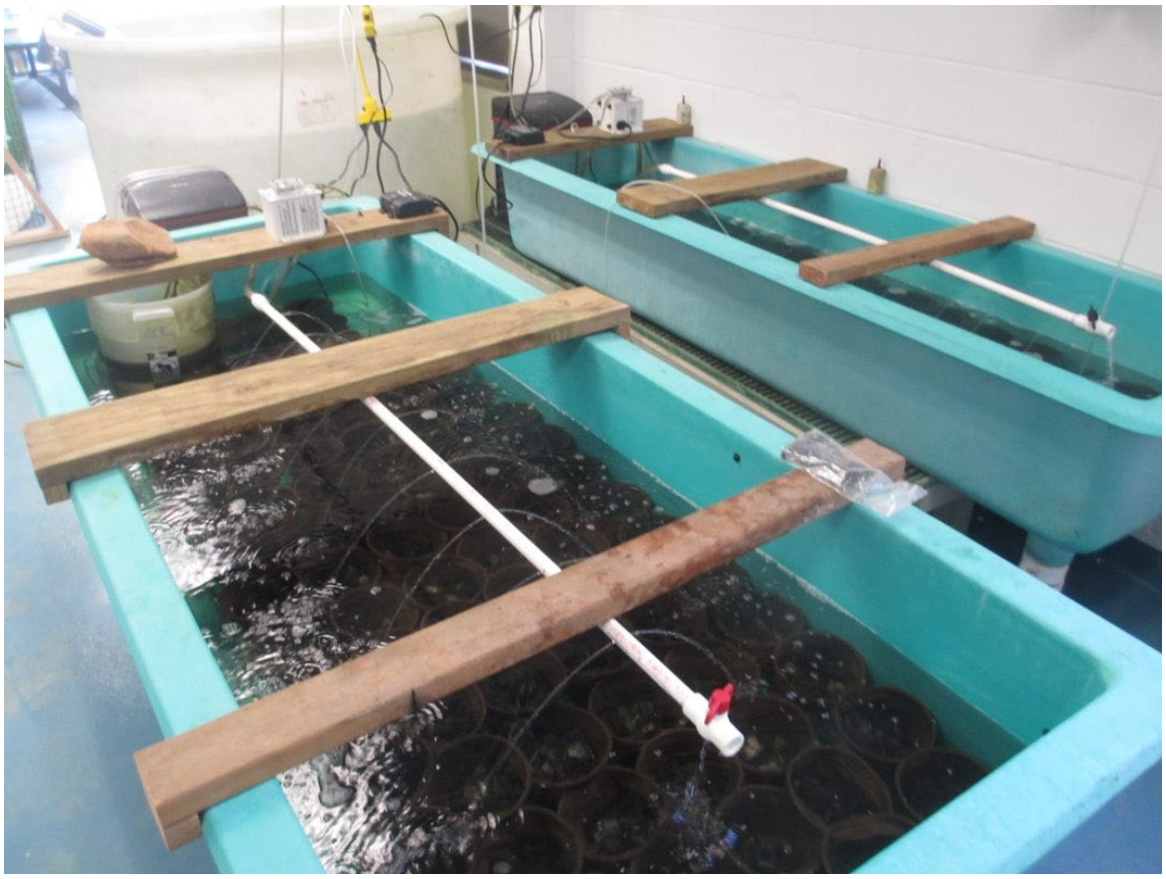

Figure 2-1: Ribbed Mussel holding tank with circulating seawater

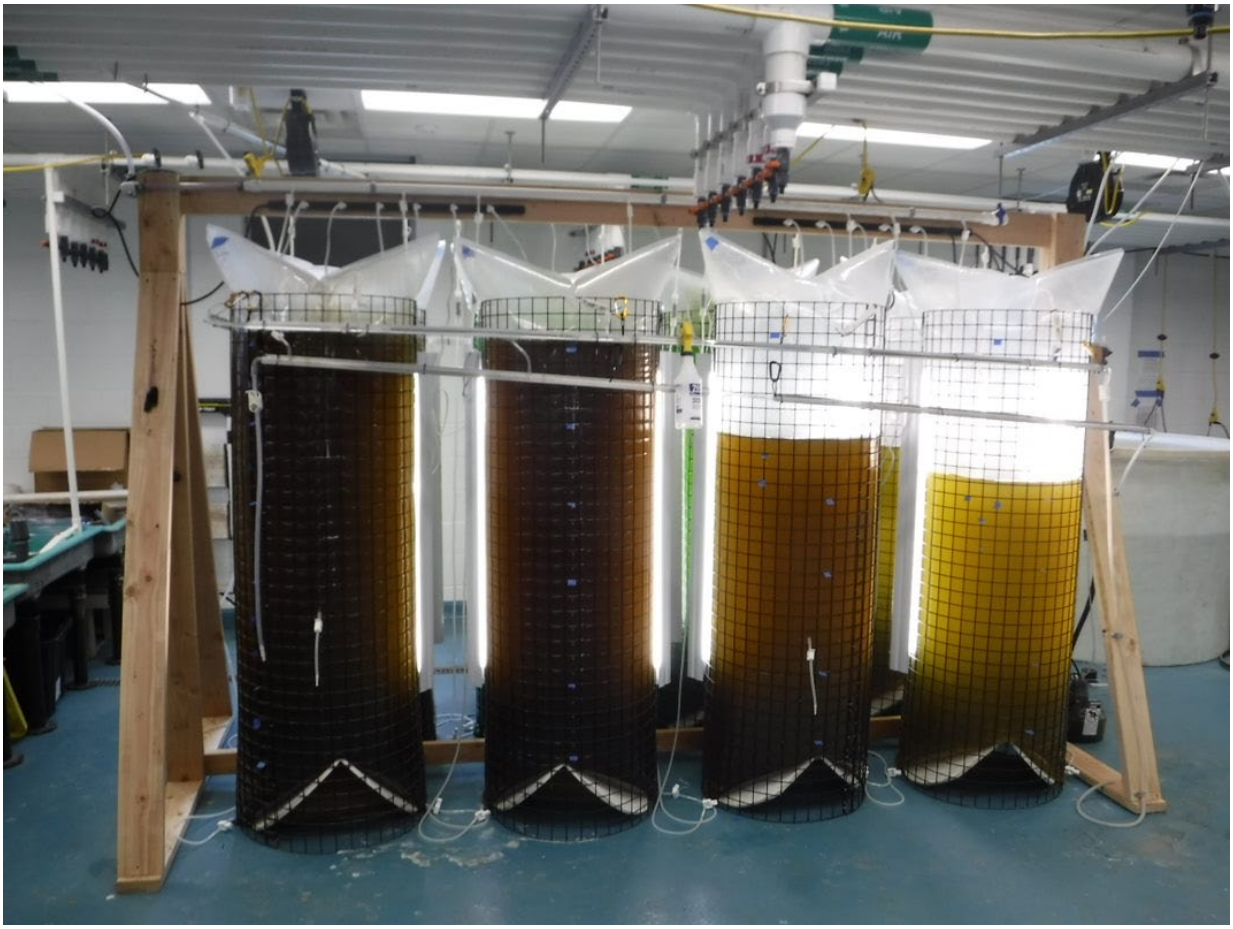

Figure 2-2: Algae growing tanks for feeding Ribbed Mussels

2.2 Water Collection

- Seawater is pumped from Flax Pond and held in 800gal reservoir tanks (**Figure 2-3**)
- Water is filtered first through a 50µm bag and then through 1µm bags before entering the reservoir tanks.
    - The last mesocosm trials were done without filtering the water
- Water is pumped from the reservoir tanks into the two 1000L experimental head tanks.
    - Salinity is adjusted to 25ppt (±0.25)
    - Water is heated to 24°C for 24hrs prior to the start of the experiment.

Figure 2-3: Seawater holding tanks for the Mesocosm experiments

3 Experimental Procedures and Protocols

- At the start of the experiment, bacteria was added to the bacteria head tank as well as added directly into the head and experimental tanks.
- Bacteria was added to 1.6L of seawater, swirled in a 2L flask and distributed into the head tank (600mL), the front of the experimental tank (600mL) and the back of the experimental tank (400mL).
    - The purpose of this was to evenly distribute the bacteria solution throughout the experimental system.
    - After the bacteria was added, the system was given 20mins to homogenize before taking any samples.
- In parallel, bacteria was continuously dripped into each head tank as described above.
- The experiment ran for 4 hours with samples taken every 20 mins from the front (influent) and back (effluent) of the tank.
    - Samples were taken before the bacteria was added to serve as blanks.
- 500µl samples were taken from the left, right and center of tank from both the influent (**Figure 3-1**) and effluent (**Figure 3-2**) ends of the experimental tank. The samples were capped and placed in a cooler on ice before being taken to be analyzed via flow cytometry. The 3 samples were averaged together to calculate clearance.
- Additional samples (50mL) were taken and analyzed using standard IDEXX Enterolert techniques used for measuring Enterococci .
    - These were taken at the start as a blank, time zero (20mins after bacteria added), then at each hour for a total of 10 samples. The blank and time zero samples were only taken from the influent whereas the rest were taken at both ends of the tank.
- RM were suspended in mesh bags inside the experimental tanks (**Figure 3-3** and **Figure 3-4**). Any dead RM observed in the bags were removed and replaced with new ones. After each experimental level was complete, all RM were removed and restocked with new animals.

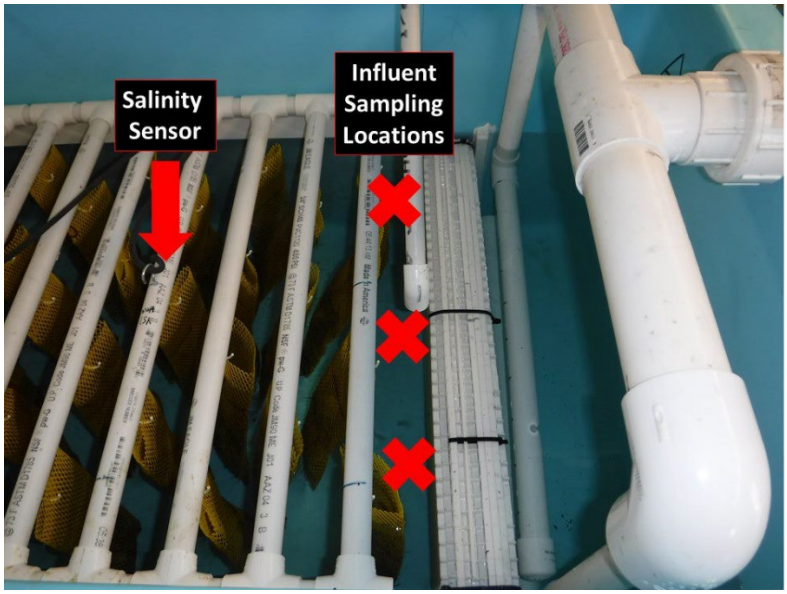

Figure 3-1: Influent sampling locations and salinity sensor

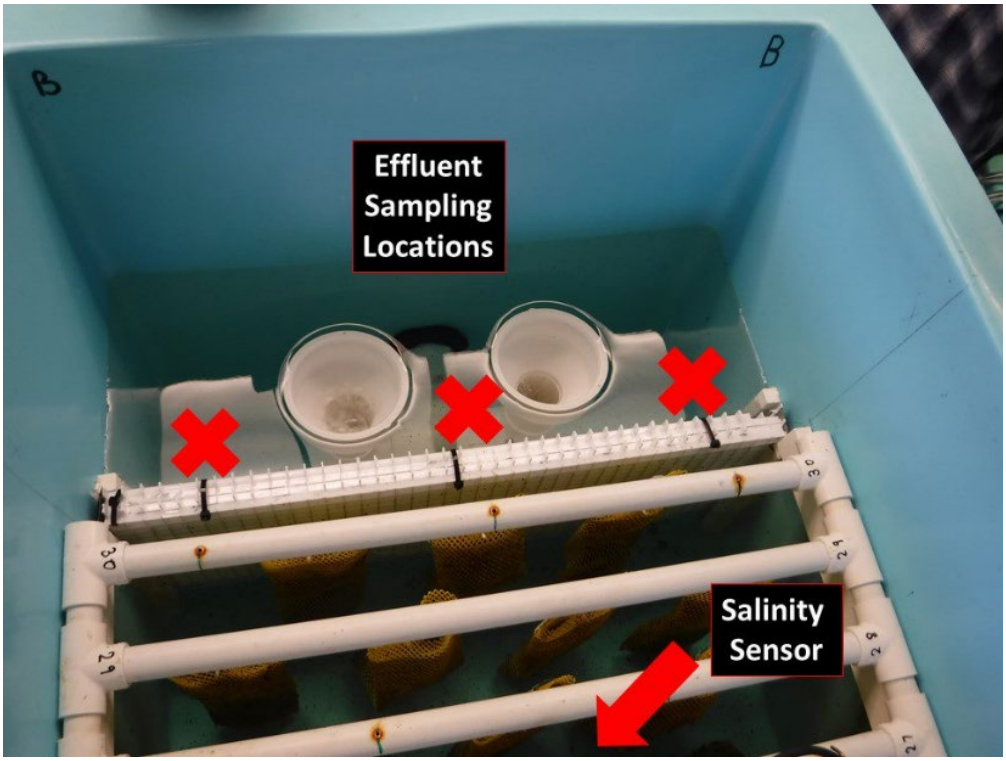

Figure 3-2: Effluent sampling locations

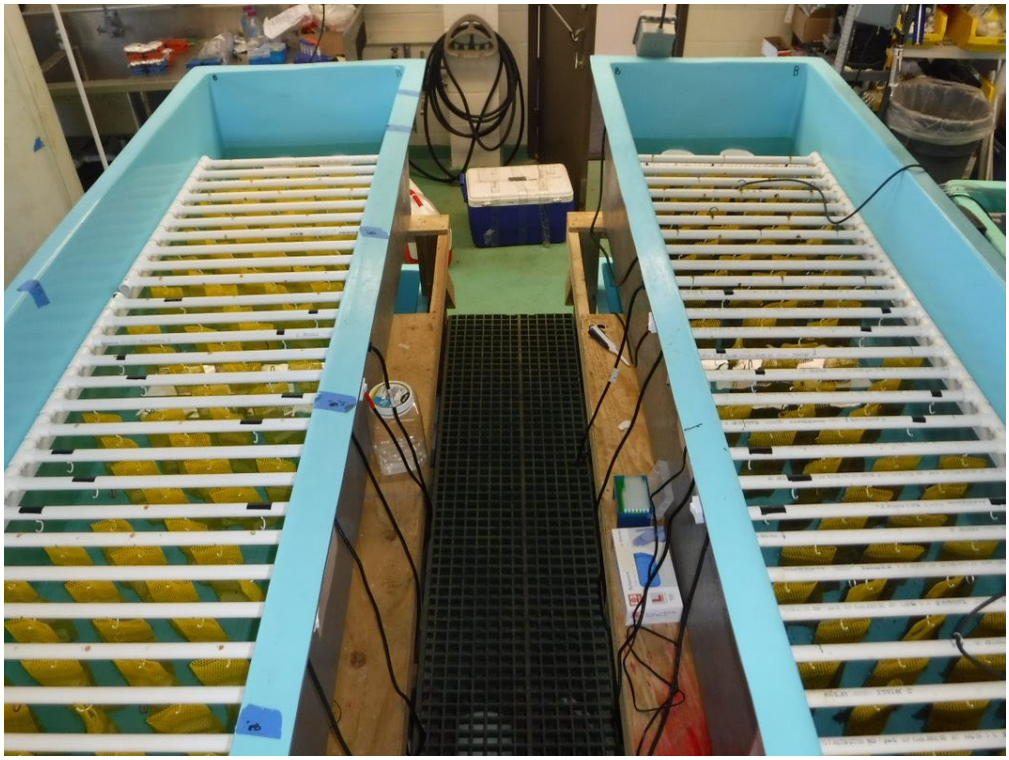

Figure 3-3: Ribbed Mussels suspended in mesh bags arranged in 4:3:4:3 configuration

Figure 3-4: Close-Up of Ribbed mussels suspended in mesh bags

4 Experimental Levels Tested per Trial

Ribbed Mussel Density: Two levels of Ribbed Mussel were tested as described below. Three replicates were conducted with a paired control at each level.

- **Medium Density**: uniform distribution of 105 bags for a total of 720 RM with continuous bacteria input with concentration of 10,000 *E. faecalis* cells per mL. Alternating rows of 4 and 3 bags, each containing 6 to 7 RM.
- **Max Density:** uniform distribution of 105 bags for a total of 1440 RM with continuous bacteria input with concentration of 10,000 *E. faecalis* cells per mL. Alternating rows of 4 and 3 bags, each containing 13 to 14 RM.

Bacteria pulse and Freshwater input: Four trials were evaluated ranging from continuous bacteria input in pulses and steps to mimic the increase and decay bacterial loads during a CSO event as described below. Each of the four trials were conducted as three replicates with paired controls

- **Bacteria Pulse:** uniform distribution of 105 bags for a total of 720 RM with continuous bacteria input. Alternating rows of 4 and 3 bags, each containing 6 to 7 RM. The experiment started with 1,000 *E. faecalis* cells per mL. At the 1-hour mark, an additional dose of bacteria was added to increase the concentration load to 10,000 *E. faecalis* cells per mL, which was maintained for 30 min before all bacterial input was stopped.
- **Bacteria Stepped Feed & Freshwater input**: uniform distribution of 105 bags for a total of 720 RM with continuous bacteria input. Alternating rows of 4 and 3 bags, each containing 6 to 7 RM. Bacteria started at 1,000 MPN/ml, increased at 1 hour to 10,000 MPN/mL, decreased at hour 2 to 5,000 MPN/mL and decreased at hour 3 to 1,000 MPN/mL. A slug of freshwater (100L) was also added to the influent at 1 hour (**Figure 4-1**).
- **Spatial Arrangement Variation with Bacteria Step Feed & Freshwater Input:** Total of 720 RM used in unequal distribution. Front third of tank contained 35 bags with alternating rows of 4 and 3 bags, each containing 6 to 7 RM. Middle third of tank contained 24 bags with alternating rows of 4 and 3 bags, each containing 6 to 7 RM. The rear third of the tank contained 35 bags with alternating rows of 4 and 3 bags, each containing 9 RM. Bacteria started at 1,000 MPN/ml, increased at 1 hour to 10,000 MPN/mL, decreased at hour 2 to 5,000 MPN/mL and decreased at hour 3 to 1,000 MPN/mL. A slug of freshwater (100L) was also added to the influent at 1 hour.
- **Unfiltered seawater, Spatial Arrangement Variation with Bacteria Step Feed & Freshwater Input:** Total of 720 RM used in unequal distribution with unfiltered seawater. Front third of tank contained 35 bags with alternating rows of 4 and 3 bags, each containing 6 to 7 RM. Middle third of tank contained 24 bags with alternating rows of 4 and 3 bags, each containing 6 to 7 RM. The rear third of the tank contained 35 bags with alternating rows of 4 and 3 bags, each containing 9 RM. Bacteria started at 1,000 MPN/ml, increased at 1 hour to 10,000 MPN/mL, decreased at hour 2 to 5,000 MPN/mL and decreased at hour 3 to 1,000 MPN/mL. A slug of freshwater (100L) was also added to the influent at 1 hour.

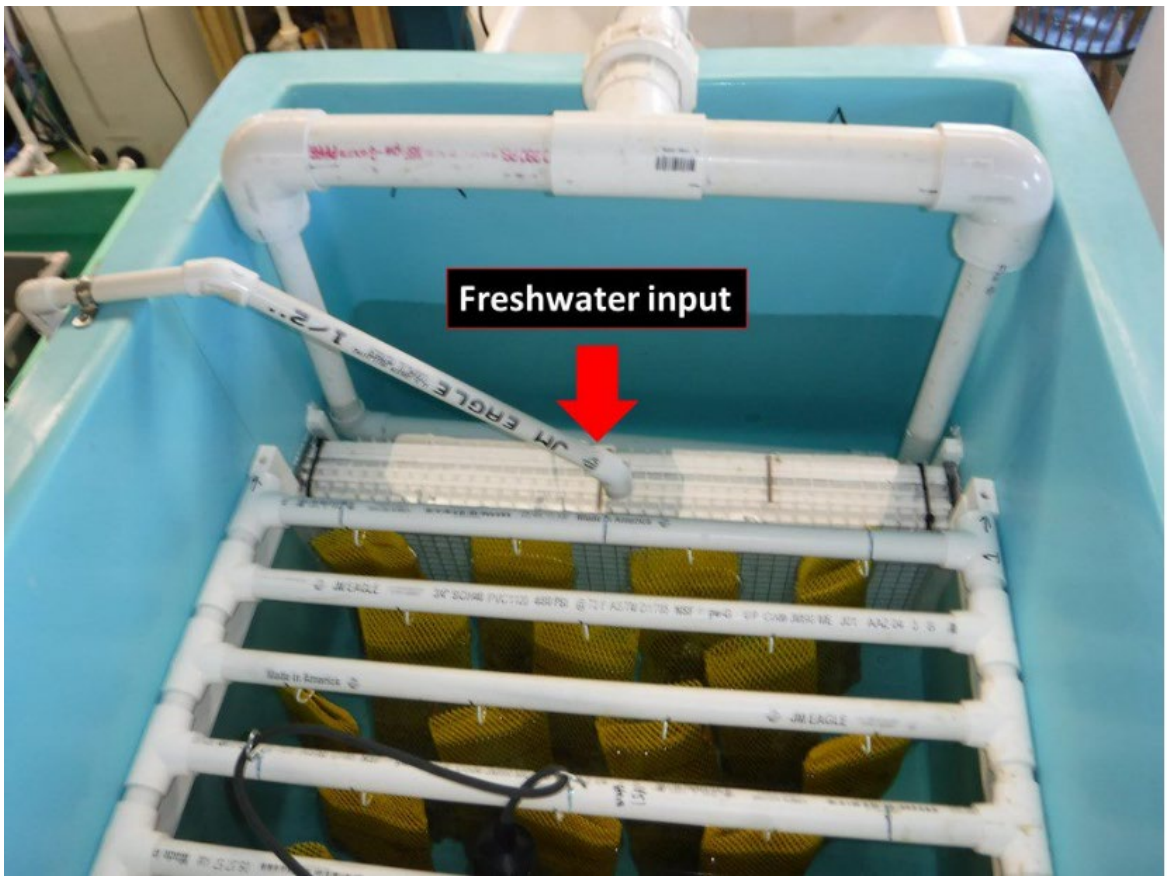

Figure 4-1: Freshwater input at the head-end of the tank

Figure 4-2: Experimental tank set-up with the header tanks shown in the foreground