FINAL REPORT

NEIWPCC Job Code:	0348-008
Project Code:	S-2022-018
Contractor:	Cornell Cooperative Extension of Suffolk County (CCE)
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Project Period:	December 2022 – June 2025
Date Submitted:	November 2025
Date Approved:	March 2026

Nutrient Bioextraction: Refinement of Atlantic Ribbed Mussel (*Geukensia demissa*) Aquaculture Methods

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This is a United States Environmental Protection Agency (U.S. EPA) funded project.

This project has been funded wholly or in part by the United States Environmental Protection Agency (under agreement LI-00A00688) to NEIWPCC in partnership with the Long Island Sound Partnership (LISP) and the New York State Department of Environmental Conservation (NYS DEC). The contents of this document do not necessarily reflect the views and policies of NEIWPCC, LISP, NYS DEC, or the US EPA, nor does mention of trade names, commercial products, or causes constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

Nitrogen pollution leads to critical impairments within the Long Island Sound, including eutrophication, hypoxia, and increased occurrences of harmful algal blooms (HABs). Although reduction targets for wastewater treatment plants have been met, nitrogen from non-point sources and legacy pollutants remain a significant contributor to water quality impairments within the Long Island Sound. Poor water quality and frequent or widespread HABs can have negative effects on natural ecosystems, shellfish restoration projects, and the cultivated species upon which the shellfish aquaculture industry relies.

Shellfish aquaculture has the potential to reduce nitrogen and bacterial concentrations in impaired waterbodies through ingestion and incorporation into tissue as the shellfish grow. When the shellfish are harvested, the nitrogen incorporated into their tissue is effectively removed from the ecosystem, a process known as bioextraction. Until recently, the Atlantic ribbed mussel (*Geukensia demissa*) has been under-utilized for this purpose, despite offering unique advantages over other species. Previous studies have demonstrated that the ribbed mussel has the potential to reduce nitrogen and bacterial concentrations in impaired water bodies and increase water clarity by filter feeding a wide range of particle sizes, including phytoplankton, bacteria, and suspended solids. A resilient species that can grow in highly impacted environments and under a broad range of temperatures and salinities, the ribbed mussel is an ideal species for wastewater remediation and water quality improvement projects in impaired water bodies, including those affected by bacterial inputs such as combined sewer overflows. Additionally, the ribbed mussel is also considered to be a non-edible species, enabling its deployment into impaired waterbodies, which are closed to shellfish harvest, and often the most in need of nitrogen and bacterial remediation.

Streamlining cultivation of ribbed mussels is a critical part of scaling up such remediation and restoration efforts, but there has been limited effort placed on developing reliable methods in the hatchery. Methods that can provide ribbed mussels for bioextraction and restoration projects are necessary, not only to maintain their natural populations in the wild, but also to protect salt marsh habitat that can be undermined and impacted when ribbed mussels are harvested and transplanted for such project purposes. Hatchery production of ribbed mussels is still in the early stages of development, and additional testing and refinement of methods are needed to ensure consistent and efficient approaches for the spawning and rearing of larvae and juveniles.

This project was conducted between November 2022 and June 2025 with the purpose of refining foundational hatchery culturing practices for ribbed mussels to establish a more efficient and replicable process for consistently producing large numbers of ribbed mussels in a hatchery setting. This project primarily sought to compare the traditional shellfish spawning method of thermal cycling in shallow tables (‘table spawn’) with the “bin-silo” method developed by Rutgers University to refine the aquaculture methods used by hatcheries. This comparison of the two spawning methods will contribute critical data and increase the base of knowledge needed for large-scale ribbed mussel production. The outcomes of this project may not only impact the potential for use of ribbed mussels in bioextraction operations, but also influence other large-scale uses, including restoration, living shorelines, and pathogen removal efforts associated with combined sewer overflows or treatment plants.

Adult ribbed mussels were collected from three different locations across north and south shores of Long Island, New York: (1) Gold Star Battalion Beach in Huntington Harbor, (2) Flax

Pond in Setauket, and (3) Bergen Basin in Jamacia Bay. Each batch of mussels went through conditioning periods of 7-10 weeks, and spawning trials using both the thermal cycling in a shallow table ('table spawns') and bin-silo methods commenced in March 2023. Throughout the 10 bin-silo spawning trials conducted between 2023 and 2025, this method provided repeated success to induce spawning across three years. In contrast, ribbed mussels did not release gametes during any of seven table spawning events. We also tested alternative table-spawning thermal profiles, including one designed to mimic the bin-silo with an extended cooling period, but this too failed to induce spawning. Of the bin-silo method trials that yielded successful spawning, the most successful larval production occurred with the re-conditioned (i.e. back-conditioned) wild-collected ribbed mussels from Flax Pond. In this trial, the mussels were initially conditioned for 8 weeks and spawned in the bin-silo, producing approximately 1 million larvae. The mussels were then re-conditioned for an additional 10 weeks, where they produced 8.9 million larvae in a second bin-silo 're-spawn' in May 2025. This suggests there was either a sub-optimal diet and/or conditioning duration, or an endogenous physiological component that was reflective of the natural seasonal *in-situ* ripening process. Additional quantitative research is suggested to further refine the broodstock conditioning phase for optimal gamete production. Finally, ribbed mussels from all three locations of the study across Long Island successfully spawned in bin-silo trials, confirming the broad applicability of this method.

The larval rearing success in this study was also highly variable, with most of the batches experiencing very high mortality rates. Our best successes with larval survival and setting occurred in the first year of the project and decreased during all subsequent years. This was attributed to mechanical difficulties with the running seawater system at the Flax Pond Laboratory, poor water quality and/or poor genetic quality. Regardless of these difficulties, these results confirm that bottlenecks in larval production and larval and juvenile survival remain, and that additional research is necessary to improve these hatchery methods.

This study demonstrates that the bin-silo method provides more consistent success than the table thermal cycling approach, making it the preferred technique for future larval production of ribbed mussels. These findings highlight the need for continued aquaculture research and refinement of protocols to establish reliable hatchery practices for large-scale ribbed mussel production, supporting nutrient bioextraction and restoration initiatives.

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1. INTRODUCTION

Controlling nitrogen pollution is a top priority within Long Island Sound (LIS). Although reduction targets for wastewater treatment plants have been met, nitrogen inputs from non-point sources and legacy pollutants are still significant contributors to water quality impairments within the Sound (LIS Partnership 2016). These impairments can include eutrophication, hypoxic “dead-zones”- areas of low oxygen, and increased occurrences of harmful algal blooms (HABs) (LIS Partnership 2015; Anderson and Taylor 2001). Poor water quality and frequent or widespread HABs negatively impact habitats like eelgrass beds that provide vital ecosystem functions, restoration efforts, such as the Long Island Shellfish Restoration Project, and commercially important cultivated species essential to the shellfish aquaculture industry like oysters and clams (Mizuta & Wikfors 2020; Gallagher 2024; Zhan et al. 2023).

Shellfish aquaculture has the potential to improve water quality by reducing nitrogen and bacterial concentrations in impaired waterbodies through ingestion and incorporation into tissue as the shellfish grow. When the shellfish are harvested, the nitrogen incorporated into their tissue is effectively removed from the system, a process known as bioextraction. Shellfish provide other environmental co-benefits, such as filtering water as they feed to remove nutrient-rich organic and inorganic matter from the water column, which improves water clarity for habitats like eelgrass beds, and sequestering carbon (CT Department of Agriculture 2025).

The Atlantic Ribbed Mussel, *Geukensia demissa,* has the potential to reduce nitrogen concentrations in impaired water bodies and increase water clarity by filter feeding suspended particles (e.g., phytoplankton, bacteria, and suspended solids) and removing them from the water column, making the species a strong candidate for shellfish restoration and nutrient bioextraction efforts (Jordan and Valiela 1982; Wright et al. 1982; Kreeger and Gatenby 2007; Kreeger et al. 2008; Kreeger et al. 2011; Galimany et al. 2013; Galimany et al. 2017; Moody and Kreeger 2020; Yap 2025). The species provides efficient filtration and digestion of a wide range of particle sizes, including fecal coliform bacteria (Wright et al. 1982; Riisgard 1988; Udelson et al. 2019), and appears to be a resilient species that can grow in highly impacted environments and under a range of temperatures and salinities (Bertness and Grozholz 1985; Franz 2001; Galimany et al. 2013; NYSDEC 2025). These advantages make the ribbed mussel an ideal candidate for wastewater remediation and water quality improvement projects, especially in impaired water bodies affected by combined sewage overflow (CSO) events, other forms of bacterial inputs to the marine environment, and excess nitrogen input.

In addition to the nutrient and bacterial removal ecosystem services offered by ribbed mussels, they are also an important species for salt marsh restoration projects. Ribbed mussels live partially embedded in marsh sediment in aggregations of individuals held together by their byssal threads, which bind to the roots of marsh plants and sediment to form dense aggregations (Moody and Kreeger 2020; Bertness and Grozholz 1985; Franz 2001). These aggregations can help mitigate coastal erosion and enhance climate change resilience by trapping wetland sediments, stabilizing salt marshes, and preventing them from washing away due to wave energy and storms, lending a protective benefit to coastal shorelines (Bertness and Grozholz 1985; Kreeger et al 2011).

Previously, ribbed mussels have been used in nutrient bioextraction studies because they can potentially be grown in areas closed for other shellfish harvest, as they are considered to be a non-edible species in New York. As the New York State Department of Environmental Conservation (NYSDEC) indicates, ribbed mussel meat is rubbery, tough to chew, and can also possess organic bacteria, which gives it a slightly metallic taste (NYSDEC 2025). Additionally, at low tide the mussels tightly close their shells, trapping waste products which can be toxic if eaten by humans (NYSDEC 2025).

While ribbed mussels have no current commercial market, ongoing work with the Nutrient Bioextraction Initiative and financial support from the Long Island Sound Partnership (LISP or LIS Partnership), formerly the Long Island Sound Study (LISS), are seeking to establish a potential commercial use for the species. There has also been recent interest in larger scale uses for ribbed mussels, including restoration, living shoreline projects, and for pathogen removal in urban estuaries (e.g., the Jamaica Bay Long Term Control Plan, AECOM USA, Inc. 2018). These expanding applications have highlighted the urgent need to develop hatchery-based spawning techniques, as large-scale restoration and bioextraction projects cannot rely on wild mussel populations alone, for both practical and ecological reasons. For example, removal of ribbed mussels from their habitat can be destructive not only to the individual animals, by tearing their byssal threads when separating them from the marsh, but also destructive to the surrounding salt marsh and coastal habitats which depend on the mussels' filtration and shoreline stabilization services. For this reason, it is essential that future large-scale projects involving ribbed mussels use aquaculture-sourced animals rather than wild-collected.

This project took place from November 2022 to July 2025, and followed a structured timeline involving broodstock collection, culturing of algae, hatchery conditioning, spawning trials, larval rearing, and grow-out using FLUPSY systems (Table A). Broodstock were collected from three different locations across north and south shores of Long Island, New York: Huntington Harbor, Flax Pond (Setauket), and Jamaica Bay, and conditioned at Stony Brook University's Flax Pond Marine Laboratory (Figures 1A-C). The bin-silo and table spawning methods (described below) were both applied across multiple trials. Cornell Cooperative Extension (CCE) of Suffolk County served as a key partner, providing hatchery management, data collection, and system maintenance.

The purpose of this pilot project was to reexamine and refine hatchery culturing practices for the non-commercial ribbed mussel species, and to develop a baseline for assessing the effectiveness of various hatchery spawning methods for ribbed mussel. The results of this project provide critical information to an emerging sector of the industry that is looking to culture ribbed mussel, potentially on a commercial scale.

This work was built off a previous project, *Utilizing Ribbed Mussel Aquaculture to Improve Water Quality in Long Island Sound (S-2022-003),* which included rearing *Geukensia demissa* using the Rutgers University "bin-silo" method (Landau 2014). The bin-silo method was compared to another commonly used hatchery shellfish spawning technique, thermal cycling ('table spawning'). This project sought to establish a more streamlined and replicable process for consistently producing large numbers of ribbed mussels in a hatchery setting, and for eventual commercial scaling of production for bioextraction operations within the Long Island Sound, as well as for other large-scale uses, including restoration projects, living shorelines, and pathogen removal associated with combined sewer overflows or treatment plants. This project is in alignment with the Nutrient Bioextraction Initiative, and supports the future implementation of programs such as, but not limited to: Long Island Sound Partnership's Comprehensive Conservation and Management Plan (LISP 2025) and NYSDEC's Long Island Watershed Program's Action Agenda, formerly the Long Island Nitrogen Action Plan (NYSDEC 2025).

2. TASKS COMPLETED

The following tasks were completed during this project:

Quality Assurance Project Plan and Approval

Version 1 -- This task included the development and finalization of project objectives, experimental design, and sampling methodology with all participants. This task was completed and approved in June 2023, prior to the collection of any data.

Version 1.1 – This task included an update to the QAPP to account for a repeatability study to test the modified bin-silo method with temperature changes. The final QAPP was signed and approved in January 2025.

Algae Production & Ribbed Mussel Conditioning

Adult broodstock of Atlantic ribbed mussels (*Geukensia demissa)* were collected from three locations across Long Island, NY (Figures 1A-C) for culturing in a hatchery, where they were conditioned, fed a combined microalgae diet, and used as broodstock for spawning.

Ribbed Mussel Spawns

The ribbed mussels were tested (2023-2025) in experimental trials using two different spawning methods; the table spawn and the bin-silo methods. A total of 7 table spawning trials were run over the duration of this project, and a total of 10 trials were attempted using the bin-silo spawning method over the duration of this project.

Ribbed Mussel Grow-Out

Once the larval ribbed mussels set and sufficiently grew to an average size of approximately 3-5 mm, juvenile mussels were placed into mesh grow out bags and deployed into a FLUPSY (Floating Upweller System) located at Gold Star Battalion Beach. Ribbed mussel growth was subsequently monitored over a two-year time period.

3. METHODOLOGY

RIBBED MUSSEL BROODSTOCK COLLECTION

From November 2022 to April 2025, wild adult Atlantic ribbed mussels were collected from three locations across Long Island, New York to be used as broodstock: Gold Star Battalion Beach in Huntington; Flax Pond Marine Laboratory in Setauket; and Bergen Basin in Jamacia Bay (Figures 1A-C). Once collected from the wild, mussels began conditioning in early January. While Jamaica Bay is located on the southern shore of Long Island, Huntington Harbor and Flax Pond are situated along the north shore, within the embayments of the Long Island Sound (LIS). These two north shore locations exhibit similar environmental parameters found in other north shore LIS embayments (Table B), so the ribbed mussels used in the study were representative of the typical spawning observed along the north shore of Long Island. As evidence of this, the Unified Water Study's LIS water quality data from 2018 was utilized to parameterize the temperature and salinity data collected at 3 representative stations along a west-east gradient in the LIS for both New York and Connecticut. Sampling for this water quality study occurred from

May-October for the following embayments in NY: Hempstead Harbor, Huntington Harbor, and Port Jefferson Harbor, and for CT: Stamford Harbor, Darian Harbor and Cover Harbor (Table B). The averages and standard deviations of temperature and salinities for these embayments exhibited relatively low variability (Table B).

Ribbed mussels have a geographic range from Nova Scotia, Canada to the Gulf of Mexico and are known for their tolerances to extreme changes in heat and salinity. As eurythermal and euryhaline animals, ribbed mussels can tolerate thermal conditions ranging from a lower limit of -22 °C to an upper limit of at least 56°C, and a salinity range of 5 ppt to 70 ppt (Wells 1961; Lent 1969). Therefore, the variation between the embayments in Long Island Sound is relatively small compared to what ribbed mussels can tolerate, and it is believed that Huntington and Flax Pond collected ribbed mussels (a more central embayment of the LIS) are fairly representative of other geographic locations within the Long Island Sound where ribbed mussels are typically found.

On each collection date, 90 to 120 mussels were collected haphazardly (without a systematic or structured approach) from the shoreline, targeting animals that were loose or attached to rocks on the shoreline rather than removing them from the marsh bank, in an effort to minimize damage to the marsh ecosystem. These locations were chosen for wild collection due to proximity to Cornell Cooperative Extension facilities as well as ribbed mussel abundance. They were collected at low tide while their shells were closed, then brought to the hatchery at Gold Star Beach in May of 2023 (Figure 1A). They were rinsed, scrubbed, cleaned, and placed in running seawater tanks until they were ready to be brought to the Flax Pond facility later that same day. In spring 2024, and winter 2025, more ribbed mussels were collected, as needed, to be used as broodstock from Flax Pond in East Setauket and Bergen Basin in Jamaica Bay. All animals were brought to the hatchery in Flax Pond Marine Laboratory in Stony Brook, NY. To ensure consistency and cleanliness with collection methods used in 2023, they were also rinsed with freshwater, gently scrubbed by brush, rinsed with saltwater, and placed in broodstock holding tanks which were held at the ambient water temperature, generally ranging between 7°C and 10°C.

ALGAE CULTIVATION

To produce a consistent microalgae diet to feed ribbed mussel broodstock and spawned larvae, several species of algae were cultured at Flax Pond Marine Laboratory in a use-specific algae culturing room using standard methods (Helm et al. 2004). This included the cultivation of stock cultures *Pavlova lutheri*, *Tisochrysis lutea* (*T-ISO), Isochrysis galbana (C-ISO), Chaetoceros* sp., *Thalassiosira Weissflogii*, and *Tetraselmis* sp. (Figure 2A). These were scaled up to 500mL Erlenmyer flasks, then further scaled to 2L flasks and 15L containers once an adequate algal cell density developed (Figure 2A). Algal cultures were then introduced to the continuous culture SeaCAPS system, where they were periodically fed to broodstock and larvae via overhead transfer lines (Figures 2B). Cell densities were determined using a hemocytometer as part of the daily feedings.

Algae production expanded during the spring 2025 spawning season to keep up with feeding demand for broodstock and larvae. Eight 250L Kalwall tubes were incorporated into the algae system, which houses 16 individual 500L bags of algae (Figures 2B and 2C). Kalwall tubes are batch culture systems where algae can be raised quickly, but are drawn down without replenishment (i.e. in batches, Figure 2C). These served as a viable feeding option for larvae,

providing an algae source separate from the SeaCAPS system, helping to keep up with feeding demand. Algae was continuously cultured at the Flax Pond facility to ensure an ongoing supply (Figure 2A).

Ribbed Mussel Aquaculture

Ribbed Mussel Broodstock Conditioning

In preparation for the culturing and spawning process, the ribbed mussels were conditioned for 7-10 weeks using the following protocol. Approximately 15-20 adult mussels were grouped and placed into one small plastic planter pot (13cm x 15cm), with 3 to 6 pots in each conditioning tank (Figure 3). This allowed for efficient tank cleanings without tearing or damaging the byssal threads, which can stress the animals. These pots were placed into static tanks and initially held at ambient water temperature to match the water temperature from where they were collected (Figure 3). Water temperature was increased slowly over the first 1-2 weeks until it reached a temperature of 20°C, which was maintained until they were used in the spawning trials. During this time, cultured algae was provided to each conditioning tank *ad-libitum* to the mussels on a daily basis. Algae clearing was monitored through visual assessments periodically throughout the day. Although feeding was *ad-libitum*, we aimed for approximately 4.0% of dry tissue weight daily feeding ration, as recommended by Rutgers University (S. Tower, pers. comm., January 2023). Cultivated algal species *Pavlova lutheri*, *T-ISO, C-ISO, Chaetoceros* sp., *Thalassiosira Weissflogii*, and *Tetraselmis* sp. were simultaneously added from the SeaCAPS continuous culturing system (Figure 2B). The broodstock tanks were cleaned regularly on Monday, Wednesday, and Friday of each week by draining the water, rinsing the plastic pots, cleaning the tanks with oxalic acid, and adding new seawater. The broodstock were returned to the water once it reached the appropriate temperature.

Ribbed Mussel Spawns

The ribbed mussels were tested in experimental trials (2023-2025) using two different spawning methods; the table spawn and the bin-silo methods. The table spawn method is a more traditional approach to spawning shellfish in a hatchery. The use of thermal cycling in table spawning has particular appeal in commercial hatcheries, as this method allows for easier identification of individual animals that are actively spawning, which leads to better sperm to egg fertilization control, genetic control, cleanliness, and potential scheduling efficiency for hatchery staff. The bin-silo method, originally developed at Rutgers University's Aquaculture Innovation Center (Landau 2014), utilizes both "controlled thermal shock" and "overnight incubation" to trigger spawning in bivalve broodstock, and without sacrificing animals.

Water Quality Measures

All shellfish tanks received filtered seawater (1µm) in a large holding (head) tank with recirculating filters and UV sterilization. To improve conditions for ribbed mussel (*Geukensia demissa)* larval production in 2025, the seawater filtration system was enhanced with a five-stage pretreatment and a five-stage post-treatment (filter sizes: 10µm, 5µm, 1µm, 0.5µm, 0.2µm) prior to entering the head tank. Seawater for bin-silos received additional filtration through a 1µm filter bag. Water quality parameters in the broodstock tanks were maintained at a pH of 8.0, salinity of 28 ppt, and temperature of 20°C. The bin-silo tanks were filled at 32°C and were allowed to slowly cool overnight to induce spawning. Seawater quality parameters

remained stable throughout the monitoring period, with pH values averaging 8.0 and salinity consistently measuring at 28 ppt.

Thermal Cycling Spawning Method

The thermal cycling spawning method involved the use of shallow, open-top tanks for the trials (Figures 4A and 4B). Forty (n= 40) broodstock animals were placed into a shallow table tank (length 90 cm x width 60 cm x depth 10 cm) initially filled with filtered seawater at 15°C. Multiple submersible aquarium heaters were used to gradually increase the water temperature approximately 1°C every 5-10 minutes over the course of 60-90 minutes. The target temperature for the first thermal cycle was 30°C. Once the target temperature was reached and maintained for a short period (~30 minutes), the water was drained, and the tank was immediately refilled with a new volume of 15°C seawater. This process was repeated 3-4 times, with each cycle involving a gradual temperature increase to a slightly greater endpoint than the previous one as follows: (1) 15°C to 30°C, (2) 15°C to 32°C, (3) 15°C to 34°C (Figures 5A-5G). Each heating phase lasted approximately 60 to 90 minutes. We further experimented with different thermal profiles to see if they could induce spawning (Figures 5A-5G). We also tested alternative table-spawning thermal profiles, including one designed to mimic the bin-silo method with an extended cooling period (see: bin-silo spawning method section below).

Throughout the process, broodstock were intermittently fed small amounts of live algae, such as *Chaetoceros* sp. and *T-ISO,* to simulate a natural phytoplankton bloom that can be used to stimulate spawning for other commercial shellfish species. Observations were made throughout the cycle for signs of gamete release. OBJ If no spawning had occurred after the second thermal cycling temperature was reached, one or two animals were selected at random and sacrificed to extract their gametes directly. Animals were shucked and either eggs or sperm were collected and diluted with seawater. The gamete solution was slowly added to the spawning tank to serve as a pheromonal stimulus and induce spawning (Helm et al. 2004). Typically, table spawn trials ran for 3-4 days, 2 weeks apart, for a total of 7 attempts. This table spawning method was used primarily in the spring 2023 spawning trials (Table C) and focus was subsequently placed on the bin-silo methodology to determine its repeatability.

Bin-Silo Spawning Method

The bin-silo system (Landau 2014) consists of a 19L cylindrical mesh-bottom silo that is inserted into a 200L bin (Figures 6A-6D). The silo is suspended using a PVC pipe across the opening of the bin, and water is circulated using an airlift to create a downwelling effect in the tank (Figures 6A-6D). Prior to a spawning trial, the adult broodstock were cleaned by rinsing with freshwater, gently scrubbed with a soft-bristle brush to remove contaminants, and then placed back into the planter pots. The mussels also had their shells oriented vertically, with the outer edge facing upward and hinge ligament resting at the bottom, allowing for unobstructed gaping (Figure 6B). Staff were instructed to avoid damaging or detaching byssal threads, as this stress has been observed to disrupt spawning activity. Three replicate bin-silo tanks were each stocked with three planter pots, each containing 15-20 conditioned ribbed mussels (n=~45-60 ribbed mussels per bin-silo; Figure 6B). Prior to adding the broodstock, each bin was filled with 1µm filtered seawater and heated to 32°C using multiple submersible aquarium heaters. Once the target temperature was reached, the heaters were removed and ribbed mussels were added to the bin-silo. To promote valve gaping behavior, *Chaetoceros* and *T-ISO* were added to the barrels. The system was left undisturbed overnight, allowing the temperature to decline gradually from

32°C to ambient temperature. The following morning, the contents of the bin were drained through a stacked sieve system consisting of a 28µm and 100µm mesh sieve. The 100µm mesh sieve was added to remove any contaminants, while the 28µm sieve collected any eggs or early-stage larvae that may be present. This was done to improve collection efficiency and purity. Through qualitative visual observations, this appeared to have a positive impact on sample cleanliness. Since the bin-silo method is a more gradual thermal cooling process, it is re-run over successive days (3-5 days) with the same broodstock (planter pots) and therefore, each 3-5 day period was considered a single spawning 'trial,' repeated 1 to 2 weeks apart depending on conditioning status (Table C). On average, ribbed mussels would be submerged for 15-20 hours, and a total of 10 trials were attempted using the bin-silo spawning method over the duration of this project.

Ribbed Mussel Spawning Monitoring

This project was an observational study comparing different spawning methods that took place in a hatchery setting, so spawning success and larval production were monitored under two hatchery spawning methods, within-facility monitoring of tank conditions (temperature and salinity) to ensure that they met the conditioning and thermal cycling methods. Spawning success was measured as a discrete dichotomous variable with the possible outcomes of using these spawning methods either "success" or "failure" to remove ambiguity and reduce external variables.

Larval animals were grown in 350L conical shaped tanks up until the settlement stage (Figure 6E). Once set, they were housed on floating downwellers in 2023 (Figure 6F). It was found that this design was ineffective at holding the animals due to their ability to crawl over the side walls, so improvements were made to the downweller design in 2024 to create a standing downweller with an airlift that pushed water over the juvenile mussels and preventing them from escaping over the side walls (Figure 6G).

Monitoring of ribbed mussels was conducted by Cornell Cooperative Extension of Suffolk County staff throughout the project period. The ribbed mussels were monitored throughout the conditioning process to ensure that temperatures were appropriate and held constant at 20°C, especially as sudden large changes in temperature may trigger spawning at inappropriate times. Salinity remained at 28 ppt, which is consistent with the typical ambient water from where the mussels were collected. Broodstock mussels were fed and cleaned according to established hatchery protocols (Helm et al. 2004). Assessments of spawning success from the trials were also monitored and measured. During spawning trials, larvae were measured for size using sieves and counted by pipetting 1mL samples onto a Sedgewick-Rafter slide at 40x magnification, counting total number of larvae from a uniform solution on each plate (Figure 7). The sample was collected in a 10L bucket, suspended and mixed by plunging the contents with a graduated cylinder for homogenization, and then collected by pipette. Three samples were counted (triplicate), and the average was determined to account for the 10L (eg. 45 animals per 1mL * 10,000mL per 10L = 450,000 animals in 10L) to provide an average count and estimates of variability. However, if one of the counts fell far outside of the 3 measures (>3 standard deviations), an additional measure was taken (or more, if needed) to determine which values are most representative of the densities.

Ribbed Mussel Sampling Schedule

Sampling to assess spawning success (described above) was dependent on the timing of spawning trials and therefore occurred at the end of each spawning trial. Larvae were counted when the conicals were drained down for cleaning, which typically occurred on Monday, Wednesday and Friday of each week. The larval phase usually lasts 10-14 days, and up to 3 million larvae were cultured in a single 350L conical. Larvae were fed *Pavlova* sp. beginning at 1.0 L on Day 1, with daily feedings increased by 0.5 L over the rearing period, with algal cell concentrations averaging 3 million cells per mL. To meet nutritional requirements, a mixed diet of *Pavlova* sp. with either *T-ISO* or *C-ISO* was also provided. These strains were selected for their small cell size, which is suitable for larval consumption and common among other commercial shellfish species due to their higher nutritional value (Helm et al. 2004). No counting was performed during the downweller phase as the larvae transition from a planktonic phase and settle to the bottom of the bucket, hence rendering volumetric counts inaccurate.

Additional Novel Pilot Test of Hall Effect Sensors

To further supplement knowledge on the physiological behavior of ribbed mussels during a spawning event, a pilot study was conducted during the spawning trials. This pilot study was not included in the QAPP, so data collected from this additional study is considered for screening purposes only. This pilot study utilized an arbitrarily selected group of ribbed mussels (n=15) from the 2025 bin-silo trials in an effort to monitor valve gaping behavior evidenced through hall effect valve gape sensors, and was carried out by Stony Brook University School of Marine and Atmospheric Sciences (SoMAS) graduate student, Bryanna Porter-Pompey, and Dr. Nils Volkenborn (Porter-Pompey 2025; Appendix 2). Valve gape sensors operating on a hall effect were deployed on 15 ribbed mussels divided into the 3 test groups (n=5 each, Group A= Flax Pond, Groups B and C = Bergen Basin) during each spawning trial to offer insights into possible unique physiological patterns related to the feeding and spawning events of ribbed mussels. Valve gape sensors are a relatively modern tool used to record various metrics related to the valve gape of bivalves and have been used in studies assessing the effects of a suite of environmental parameters on their feeding activity and physiological behavior at a high resolution (Porter-Pompey 2025).

The valve gaping sensors utilized in this study are powered by an Arduino logger connected to a power source. Each Arduino logger is capable of connecting up to 8 sensors, however only 5 ports were employed on each of the 3 Arduino loggers used here (Appendix 2, Methods). The sensor is attached to the right shell (top) closest to the shell margin across from the hinge to best detect opening and closing. The magnets are attached to the left shell directly opposite the sensor (Appendix 2, Image 1). Sensors and magnets are attached to shell using Loctite Super Glue. The Arduino logger records the raw signal on a 16GB SD card at a frequency of 6Hz either until the SD card is full or until the observation period has ended. Temporal variability of the raw signal was processed in an analysis script in RStudio which produces valve gaping behavior metrics of interest (i.e. percent of time open hr^{-1} and partial closure frequency), as displayed in Figure 8.

4. QUALITY ASSURANCE TASKS COMPLETED

PROJECT MANAGEMENT

Quality assurance measures were implemented throughout the duration of this project. Each project partner ensured all involved staff members were trained on proper protocols, sampling methods, laboratory protocols, and data analysis. The most current copy of the approved Quality Assurance Project Plan was distributed to all project partners in PDF format. The Quality Assurance Project Plan was maintained by the NYS DEC Project Lead in electronic format throughout the length of the project. All data was recorded in appropriate data sheets, and instrument calibration forms completed and retained. The QAPP was amended to update staff changes in 2025 and include an additional task to conduct a repeatability study to determine the success of spawning methods using a modified bin-silo method. All data for this project is being stored on the Cornell Cooperative Extension, NYS DEC, and NEIWPCC servers.

Data Generation and Acquisition

All portions of this study were conducted at Stony Brook University's Flax Pond Marine Laboratory, and collection, cleaning, and trial preparation of ribbed mussels were handled by trained Cornell Cooperative Extension staff members who followed the QAPP protocols. Animals were separated and labeled by collection date and location on their corresponding holding trays and conditioning tanks to track and reference each batch.

Water quality was initially maintained through a seawater filtration system to eliminate any potential harmful or invasive marine organisms. Additionally, adult Atlantic ribbed mussels were scrubbed with a brush and briefly dipped in fresh water to eradicate any protists, plankton or other flora and fauna attached to shells and byssal threads. Between each trial, the barrels were emptied and sanitized thoroughly with oxalic acid.

Hatchery equipment was inspected prior to use (initially) for cleanliness and any needed repairs or adjustments (Kemp et al. 2006; Hadley et al. 1997). Equipment (i.e. tanks, heaters, and chillers) were cleaned with oxalic acid (as needed) and rinsed with ambient water prior to use. Tanks were cleaned with oxalic acid during drain-downs every Monday, Wednesday, and Friday throughout this project. A more complete breakdown is provided below.

Calibration of all instruments was performed prior to each use and in accordance with the manufacturer's instructions. Instrument and equipment calibration frequency was dependent on the equipment manufacturer's recommendations. A sampling event is defined as an instance of measuring the environmental parameters. Sampling occurred within 5-15 minutes after the calibration was complete. Calibration records included: date, time, name of the individual doing the calibration, and the calibration results. No calibration failures or drift were observed in this study. There were no issues identified regarding calibrated instruments used, and no data that did not meet the manufacturer-developed acceptance criteria. Calibration logbooks were retained as part of the data for this project and will be kept for a minimum of five years.

Diligent training of hatchery staff ensured measurement accuracy and consistency in observational techniques for this project. One trained observer worked on this project, and measurements were taken using straight-forward measures and objective techniques, such as counting individual mussels. Standard Operating Procedures (SOPs) are training documents in the QAPP.

Records and raw data including handwritten notes, data sheets, analysis logs, and results of instrument calibrations have been scanned into electronic format, with raw data entered into an Excel database. Computer-entered data was cross-referenced with field or hatchery data and sample analysis results to confirm accuracy.

The overarching goal of this study was to determine how to replicate spawns using viable and repeatable processes. Broodstock with a shell length of 70-90mm were collected from the three locations (Huntington Harbor, Flax Pond, and Jamaica Bay) and conditioned by being maintained at the same water temperatures and fed a similar diet of microalgae. Conditioning of the various batches was staggered with animals for the first spawn starting 2-3 weeks prior to the next batch.

The comparability of the data collected was assured by treating all broodstock mussels identically during the conditioning period, and only differing treatment of the mussels during the spawning trials. Conditioned mussels were randomly selected and assigned to the different spawning trials, and spawning success was determined using the same methods at the end of each of the trials.

All of the planned number of trials were run according to a pre-established schedule. Because this work was conducted within a hatchery, there were only minimal changes to the schedule, as the mussels were not reliant on external environmental conditions such as weather.

Special Training

Each project partner ensured their staff were trained on the proper protocol and sampling methods and retained documentation of this information. These records were scanned by the Project Lead, held in both electronic and paper form, and will be held on the NYS DEC server, and in the NYS DEC Region 1 Office, respectively, for a period of at least 5 years. The Project Lead ensured that all individuals involved with the project received and were familiar with the QAPP to ensure proper adherence to the procedures outlined within. At CCE, the hatchery manager was responsible for assuring training requirements were satisfied, including use of the Hatchery Data Sheet and the SOP utilized for algae culturing.

Assessment and Oversight

The NYS DEC Project Lead thoroughly briefed contract partner staff before and after beginning their respective implementation tasks to identify any emerging/unanticipated problems. This was done through virtual meetings; identification of potential problems was also done through email correspondence and phone calls. Corrective actions or significant changes to the project were reported to the NYS DEC Project Lead, NEIWPCC QA Program Manager designee, NEIWPCC Project Manager, and the EPA Project Officer, and also reported in quarterly reports to the EPA. A problem encountered in this project was that QAPP approval was delayed until June 2023, so environmental data collected prior to that is considered for screening purposes only. It should also be noted that the additional pilot study using Hall Effect Sensors (see Methodology section) was conducted as a supplemental study and not included in the QAPP, so data collected from this additional study is considered for screening purposes only. No issues were encountered that required a suspension of work or any corrective actions.

There was an audit ordered by NEIWPCC in the summer of 2023 to assess conformance and compliance to the Quality Assurance Project Plan in accordance with the NEIWPCC Quality

Management Plan. This field assessment occurred during the monitoring and maintenance of juvenile cultured ribbed mussels at the FLUPSY (Floating Upweller System) in Huntington Harbor. One minor note pointed out by the Aquaculture Coordinator for this project was that there was a calibration table included in the QAPP version 1 that was not referenced during this study because it was included erroneously in this project's QAPP and therefore not relevant to this work. This was an issue with the writing of the QAPP and not the implementation of the QAPP and therefore is not a quality assurance concern for this project. No compliance issues were found, and no corrective actions were necessary in any aspects of the field or hatchery project components. The NYS DEC Project Lead prepared quarterly progress reports during the course of the study until January 2025 when the QAPP was amended for the contract project partner (Cornell Cooperative Extension of Suffolk County) to prepare quarterly reports. Quarterly progress reports from the project partner were submitted to the NYS DEC Project Lead, NYS DEC Project Manager, and the NEIWPCC Project Manager, and included the current status of ongoing work, accomplishments, and problems encountered.

5. RESULTS

From 2023 to 2025, all algae culturing, broodstock rearing, and a series of 13 spawning trials were successfully executed at Flax Pond Marine Laboratory. Once conditioned for at least 7 weeks, ribbed mussels from the various sites were placed into either a table or bin-silo for spawning trials.

The table thermal cycling spawns yielded no successful spawns (0%), regardless of which thermal profile was tested, including a gradual cooling to mimic a bin-silo thermal profile (Table C, Figures 5A-5G). For instance, we used the more conventional temperature profile that our hatchery typically uses to induce Northern quahog (*Mercenaria mercenaria*, commonly known as hard clam) spawning. This approach involved a temperature increase over 1.5-2 hours, then a brief cooling period of 30 minutes, tank drain-down and refilling, followed by another warming period over 1.5-2 hours (Figures 5A-5G). When there was no evidence of spawning successes, we sacrificed several of the conditioned ribbed mussels for inducing spawning through chemical cues, but this also failed to induce gamete release (Helm et al. 2004). In addition to gamete release, these sacrificed animals are qualitatively examined to confirm gamete development. We experimented with more rapid temperature increases over a 1 hour period, as well as letting mussels sit at the target temperature for longer periods of time, as much as 45 minutes (Figure 5G); which also did not yield any spawning. We further attempted a more gradual thermal cycling approach that attempted to mimic the bin-silo approach where we increased the temperature to the target value over 2 hours, with an extended cooling period of 6 hours (Figure 5E). This method also did not lead to a spawn.

In contrast to the table spawning method, the bin-silo method yielded repeatable spawning successes throughout all three years of the study, with a success rate of 60% (See Table C for total larval counts across these trials). The highest single-trial count in 2023 was 3,529,500 larvae. Throughout the 2025 spawning season, high larval counts were measured for several trials, with a peak count of 8,817,998 larvae in a single trial. This was observed with Flax Pond ribbed mussels that were initially conditioned for 8 weeks and spawned (produced ~ 1,000,000 larvae), and then re-conditioned for an additional 10 weeks and re-spawned, which produced the peak count of ~8.9 million larvae in May 2025. This was the most successful spawn observed overall, that occurred from reconditioned mussels over two successive days (Day 1= 3.2 million larvae, and Day 2= 5.6 million larvae).

Overall, the larval rearing success in this study was also highly variable within and between years, with most of the batches experiencing very high mortality rates. Of all the successful spawning trials resulting from the bin-silo method, the highest survivorship of larvae was in 2023, and limited to no survivorship in 2024 and 2025. In general, our best successes with larval survival and setting occurred in the first year of the project and all subsequent years decreased. This was attributed to mechanical difficulties with the running seawater system at the Flax Pond Laboratory, poor water quality, and/or poor genetic quality. In addition, the larvae that successfully metamorphosed were transferred to modified downwellers (Figure 6F), and when large enough were moved to the FLUPSY units at the Gold Star growout facility at Huntington for use in the NEIWPCC LIS-S-2022-003 bioextraction project (Yap 2025; Figure 9). The average annual growth observed on the juvenile mussels over a two-year period was 8.48 mm year (Figure 10).

Based on the raw voltage signals of all animals examined from the hall effect sensors used in the novel pilot test, there were clear behavioral patterns present, as characterized by extended periods of valve opening events, short and relatively quick partial valve closures within opening events, and periods of extended near-closures within opening events (Appendix 2, Figure 1). Within test groups which spawned twice (groups B and C), common gaping behavior was observed within individuals during spawning events, but not shared with every mussel attached to sensors in the respective group (Appendix 2, Figures 2 and 3).

All mussels in which valve gaping data was collected (n = 15) were open for a majority of the spawning period (>50% time open hr^{-1}). In Group A (n=5), mussels were open for a longer period during the spawning period than before or after the spawning period (Appendix 2, Figure 1). Mussels examined in Group A exhibited an average percent (±standard error, SE) of time open $mussel^{-1}$ hr^{-1} of 76.0 (±1.7) pre-spawn (02/20/2025 12:00–02/26/2025 12:00), 91.1 (± 2.8) percent of time open $mussel^{-1}$ hr^{-1} during the spawning period (02/27/2025 13:30–02/28/2025 09:30), and 82.6(±1.5) percent of time open $mussel^{-1}$ hr^{-1} during the post-spawn period (03/01/2025 10:30–03/06/2025 09:30).

Contrasting to Group A, mussels in Group B were typically open for less time during the spawning periods than during the pre-spawn and post-spawn periods (Appendix 2, Figure 2). Mussels examined in Group B (n=5) exhibited an average (±SE) percent of time open $mussel^{-1}$ hr^{-1} of 84.9 (±1.4) pre-spawn (02/18/2025 13:30 – 02/24/2025 12:30), 74.8 (±3.8) percent of time open $mussel^{-1}$ hr^{-1} during spawn #1 (02/25/2025 14:40—02/26/2025 08:40), 70.7 (±4.8) percent of time open $mussel^{-1}$ hr^{-1} during spawn #2 (02/27/2025 14:00—02/28/2025 09:00), and 81.4 (±1.1) percent of time open $mussel^{-1}$ hr^{-1} post-spawn (03/01/2025 10:00—03/07/2025 09:00).

In contrast to both Group A and Group B, mussels in Group C did not appear to have a substantial difference in the duration of valve opening events pre-spawn, during spawn attempts, and post-spawn (Appendix 2, Figure 3). Mussels examined in Group C (n = 5) exhibited an average (±SE) percent of time open $mussel^{-1}$ hr^{-1} of 78.6 (±1.8) during the pre-spawn period (02/21/2025 12:00—02/26/2025 08:00), 71.8 (±4.2) during spawn attempt 1 (02/26/2025 12:50—02/27/2025 10:00), 74.6 (±3.6) during spawn attempt 2 (02/27/2025 13:00—02/28/2025 09:30), and 71.5 (±1.7) during the post-spawn period (02/28/2025 13:30—03/04/2025 17:00).

Further examination into the partial closures (minute valve pulses where the valve is not fully open and not fully closed) may be necessary to understand precise behaviors which may be linked to spawning. Some mussels exhibited gaping behavior during spawning events not seen in other mussels within the same group, as described in Appendix 2. It should also be noted that

this hall effect sensors pilot study data is considered for screening purposes only, as it was not included in the QAPP.

6. CONCLUSIONS & DISCUSSION

Considerable variability existed in both spawning response and larval survivorship between the bin-silo and table spawn methods. Ultimately, the bin-silo method demonstrated potential as a viable approach for hatchery-based production of ribbed mussels (*Geukensia demissa*), and these findings serve as a foundation for future refinement of mussel cultivation and bioextraction techniques.

The bin-silo method consistently yielded positive spawning results within and between years, while in contrast, the table spawn had no successful spawns. The repeatability of the bin-silo approach is essential for any large-scale hatchery production of ribbed mussels. The bin-silo method is also more practical and efficient compared to the table spawn method, as it does not require constant monitoring and supervision by staff throughout the spawning process. Table spawns, however, require hours of constant supervision, monitoring manipulations, and can even extend in duration if no animals spawn during the expected period. Additionally, thermal cycling with table spawns did not yield any spawns by the ribbed mussels in this study regardless of the type of temperature profile used. In 2023, a table spawn was conducted to mimic a bin-silo spawn with a single peak temperature. However, this did not successfully yield larvae. The ribbed mussels from all three locations, Huntington Harbor, Flax Pond, and Jamaica Bay, successfully spawned in bin-silo trials, further confirming the broader regional applicability of this method. For these reasons, the bin-silo method was deemed more efficient and was thus repeated throughout the duration of this project, while the table spawning method was not.

Despite the overall success of the bin-silo method, there were also occasional challenges, including a higher than expected larval mortality observed during the first week of rearing. Throughout the project, some zooplankton and other potential predators were observed in water samples, potentially leading to larval predation and high mortality. In an attempt to reduce larval mortality, ribbed mussel broodstock were scrubbed with brushes and dipped in freshwater before placement in bin-silos to prevent any predators or contaminants from entering the system (Aji 2011). To further refine our drain-downs, a 100µm mesh sieve was added to prevent additional contaminants from settling with early-stage larvae (Figure 5D). However, these attempts did not significantly improve survivorship, leading us to speculate other potential causes of mortality, described below.

It is also important to note that Flax Pond Laboratory's seawater system was disabled in winter of 2024 and remained on a backup system, which complicated storage and water distribution throughout the facility (the system was restored and functioning again in late summer 2025). This may have adversely affected the survival of larvae during this time period as well. Therefore, while the bin-silo method of spawning yielded higher larval counts, this may not have provided the ideal environment for larval development, especially as we continued to transition to a highly purified filtration system to try and resolve these issues (2024 & 2025). Additionally, the modest egg output compared with that of other bivalves suggests that something is likely limiting the broodstock ripening during the conditioning phase. Suboptimal algal diets, absence of essential microbial supplements, insufficient conditioning periods, or a combination of these factors may underlie the observed outcomes.

The project also revealed some general insights regarding conditioning and spawning of ribbed mussels. For instance, broodstock were successfully conditioned on a mixed-algal diet that

supported gonad development and delivered repeatable spawns in bin-silo systems when water was heated to 32°C and allowed to cool gradually overnight. This suggests that a controlled and gradual thermal fluctuation may support both acclimation and spawning activity. However, greater larval production typically seemed to occur during the middle of a bin-silo trial rather than right at the initial start date. We also observed that the highest bin-silo spawning success was observed with Flax Pond ribbed mussels that were initially conditioned for 8 weeks and spawned (produced ~ 1,000,000 larvae), and then re-conditioned for an additional 10 weeks and respawned, which produced ~ 8.9 million larvae in May 2025. This suggests either a sub-optimal diet and/or conditioning duration, or an endogenous physiological component that was reflective of the natural *in-situ* ripening process, may be the underlying cause(s), thus requiring additional research. Finally, the possibility of spawning success based on lunar cycle was observed in 2025, since success tended to coincide with new and full moons. Though qualitative, and based on a very limited number of trials, it may be of interest to schedule future spawns around the moon phase. For instance, other species of shellfish, such as the Green Mussel, *Perna viridis*, and the Pacific Oyster, *Crassostrea gigas,* have been shown to synchronize spawning based on lunar cycles, often timing spawns to the highest tides of the new and full moons. This increases the dispersal and mixing of their gametes (i.e., increasing fertilization success), as well as increasing predictability for population-wide spawning synchrony, optimizing the probability of spawning success and genetic diversity (Baldevieso et al. 2021; Payton and Tran 2019).

While some larval rearing was carried through to post-set, very few survived past this life stage, likely due to chronic challenges in water availability, water quality, and potentially attributed to lack of chemical cues in the hatchery at this time. The metamorphosis stage, undergone by marine invertebrates as they grow from pelagic larvae into juveniles, is an energetically intensive and stressful process, a factor which may have also hindered ribbed mussel survivorship past the larval stage (Shilling et al. 1996; Videla et al. 1998; García-Esquivel et al. 2001). Another factor to consider is the role of potential bin-silo restrictions, as it is unclear how many males or females spawn during the bin-silo trials. If only a few individual ribbed mussels spawn, this could lead to a genetic bottleneck and poor survivorship of larvae as a result.

To improve spawning survivorship and scale-up production, the primary challenge remains achieving larval survival in conical tanks and progressing individuals to the setting stage. During the 2025 season, no ribbed mussels reached the setting stage, which resulted in 100% mortality. Despite decreasing hatchery contamination through filters finer than 1µm and eliminating predators or competitors, it is possible that the water may have been overly ‘polished’ or sanitized to support larval settlement. This is based on wild ribbed mussels, which naturally anchor in sediment with abundant bacteria and nutrients. The removal of small, bacteria-sized particles is essential to their survival at this stage (Moody and Kreeger 2020; Galimany et al. 2013). Therefore, it is possible that our increased filtration and UV sterilization process removed essential microbes, seston, and cues required for larval survival and for successful setting (Anderson and Padilla 2024). For example, ribbed mussel larvae may need ecological cues, such as presence of *Spartina alterniflora* (saltmarsh cordgrass), as they have a mutualistic relationship in natural wetlands (Anderson and Padilla 2024; Whaley and Alldred 2023). More investigation is needed to confirm this, as the cues may differ from those that trigger clam and oyster mass spawns.

To achieve potential success with the table spawning method, it may prove beneficial to conduct successive trials throughout the week similar to bin-silo spawns. However, this would reduce the

efficiency of this spawning method since greater efforts are required to monitor and manipulate the thermal cycling over multiple days. The bin-silo method does not require such 'active' monitoring, but does reduce genetic controls.Future valve gaping behavioral studies could potentially help increase confidence in identifying species-specific spawning behavior and estimating the number of actively spawning ribbed mussels in the bin silo, where video recording sensor-attached ribbed mussels can visually indicate which mussels spawned. Although general behavioral patterns were observed through the valve gape sensors before, during, and after spawning, we could not determine whether certain behaviors (i.e., extended partial closures or metrics, such as the frequency of quick and short partial closures and the percent of time open hr^{-1}) are directly linked with active spawning. The aforementioned description of the percent of time open hr^{-1} metric in groups A, B, and C exemplifies the need to identify which specific individuals were spawners in order to exclude non-spawning individuals from behavioral analysis during presumed spawning. Thus, it is suggested that ribbed mussels attached to hall effect sensors in the novel pilot study, are video recorded so that spawning individuals are identified and the exact time of spawning is recorded. With this, distinct patterns associated with spawning may be able to be identified and assessed within other individuals. This could present itself as any of the observed gaping behaviors listed previously. The application of valve gaping hall effect sensors may also provide a non-destructive method to detect spawning patterns in wild populations and identify periods when ribbed mussels are in reproductive readiness. Anticipating spawning events could allow for strategic deployment of larval settlement collectors, augmenting and assisting hatchery production. In addition, it's noted that this Hall Effect Sensors study was not included in the QAPP, so these conclusions are considered for screening purposes only.

Overall, this study has provided the baseline information necessary to assess the effectiveness of various hatchery-based spawning methods for *Geukensia demissa*, contributing to the critical data need and building the base of knowledge about how ribbed mussel production may be scaled for large-scale restoration, bioextraction, and other research projects and industry needs. Ribbed mussels hold great potential for nutrient bioextraction, which can be deployed on a larger scale in local waters, providing new employment and research opportunities, as well as offering significant benefits to the ecosystem and coastal communities through improved water quality, shoreline stability, and coastal resilience.

Future projects may focus on independently studying the effects of adjusting variables such as water temperature at each stage of the process, the length of the conditioning period, the quality of algal food, and the seasonal or synchronized endogenous timing of spawning. Once each variable is independently examined, additional studies may focus on increasing yield or speed of production. Future research could also include pairing hatchery-reared adults with *in-situ*–conditioned controls, quantifying the role of associated novel algae and bacteria in gametogenesis, exploring wild spat collection methods as supplementation to hatchery production, and investigating alternative nutritional regimes aimed at scaling fecundity. Once improvements to the conditioning methods for broodstock are determined, research should then focus on larval rearing and settlement to increase survival at these stages as well. Addressing these gaps will transform the promising technical advances reported here into a reliable method for achieving hatchery spawning and juvenile supply for future restoration and bioextraction projects.

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8. APPENDICES

Appendix 1. Tables and figures for information collected during the study and additional data that informed the study.

Table A. Project Timeline. Different cohorts of spawning ribbed mussels were managed and denoted in 2025 with the following letters: B, C, 2A, 2B

Timeframe	Task	Location	Notes
Nov 2022-Mar 2023	Broodstock collection and initial conditioning begins	Huntington Harbor and Gold Star Beach Hatchery	Approx. 40-60 loose mussels collected to avoid marsh disturbance
Mar-April 2023	Initial table spawn and bin-silo spawning trials	Gold Star Beach Hatchery	2 table trials and 2 bin-silo trials; first successful spawn on April 6
April-May 2023	Continued bin-silo spawning and larval rearing	Gold Star Beach Hatchery	Over 3.5 million larvae collected in one trial
April-Nov 2023	Dock infrastructure installation and water quality monitoring	Huntington Harbor	Temperature loggers and periodic field sampling conducted
June-Oct 2023	Larval rearing, post-set transfer to FLUPSY, and grow out monitoring	Gold Star Beach Hatchery	Growth monitoring bi-monthly through fall
Jan-Mar 2024	Broodstock collection/conditioning for spring/fall trials	Bergen Basin, Jamacia Bay	Mussels held at Flax Pond Marine Laboratory
April-Sep 2024	Spring/fall spawning trials using table and bin-silo methods	Flax Pond Marine Laboratory	Bin-silo spawns unsuccessful
Oct-Dec 2024	Analysis of 2024 trial failures, preparation for 2025 trials	Flax Pond Marine Laboratory	Conditioning protocols adjusted
Jan-Mar 2025	Intensive bin-silo spawning trials from Flax A and Bergen B/C groups	Flax Pond Marine Laboratory	Improved success; over 1 million larvae produced across trials
Mar-Apr 2025	Continued Bergen B/C spawning, with mixed results	Flax Pond Marine Laboratory	One trial yielded 56,000 larvae
May 2025	Flax A respawn group bin-silo trials	Flax Pond Marine Laboratory	Most successful single-day spawn: >5.5 million larvae
June 2025	Final bin-silo spawning attempts using Bergen 2A & 2B groups	Flax Pond Marine Laboratory	No successful spawns from 3 final trials
June-Sep 2025	Continued larval rearing and post-set grow-out in FLUPSY	Gold Star Beach Hatchery	End of hatchery rearing cycle; continued growth monitoring and size-sorting

Table B. Unified Water Study's Long Island Sound (LIS) water quality data (2018). Average temperature and salinity were collected along a west-east gradient in the Long Island Sound for both New York and Connecticut. Variability between sites was quite low. This data was used to help inform ambient temperature and salinity ranges across the Long Island Sound for the purposes of this study.

Location	Avg Temp	SD Temp	Avg Salinity	SD Salinty
Stamford Harbor, CT	19.82	4.19	25.03	1.44
Cover Harbor, CT	18.94	3.77	25.48	1.88
Darian River (Harbor), CT	19.06	3.69	24.88	3.19
Port Jefferson Harbor, NY	20.35	4.31	26.45	0.77
Hempstead Harbor, NY	21.39	2.84	25.35	0.99
Huntington Harbor, NY	19.93	3.73	25.53	0.75

Table C. Total spawn and larval counts of ribbed mussel (*Geukensia demissa*) from spring 2023 to spring 2025 with controlled conditioning of broodstock. Total larval counts include all spawns that occurred for a given cohort of ribbed mussels. Broodstock were fed a constant diet of microalgae and held at 20°C in holding tanks. Dashes (-) indicate either a very low number of gametes released, or no spawning was detectable, and therefore considered unsuccessful.

Season & Year	Spawn Method	Total # of Trials	Ribbed Mussel Group	Conditioning Period	Total Larvae at First Count
Spring 2023	Bin-Silo	2	Gold Star	8 weeks	3,529,500
Spring 2023	Table	7	Gold Star	10 weeks	-
Fall 2024	Bin-Silo	1	Bergen	8 weeks	-
Spring 2025	Bin-Silo	1	Flax A	8 weeks	1,051,000
Spring 2025	Bin-Silo	1	Bergen B	8 weeks	554,000
Spring 2025	Bin-Silo	1	Bergen C	7 weeks	56,000
Spring 2025	Bin-Silo	1	Flax A (respawn)	10 weeks	8,817,998

Spring 2025	Bin-Silo	3	Bergen 2A & 2B	10 weeks	-

Figure 1A. Aerial image of Gold Star Battalion Beach in Huntington, NY. The red box indicates the area where adult ribbed mussels (*Geukensia demissa*) were collected.

Figure 1B. Aerial image of Flax Pond. The red box indicates the area where adult ribbed mussels (*Geukensia demissa*) were collected. The Flax Pond Marine Laboratory is also labeled, serving as the base for research activities.

Figure 1C. Aerial image of Bergen Basin near JFK International Airport. The red box indicates the area where adult ribbed mussels (*Geukensia demissa*) were collected.

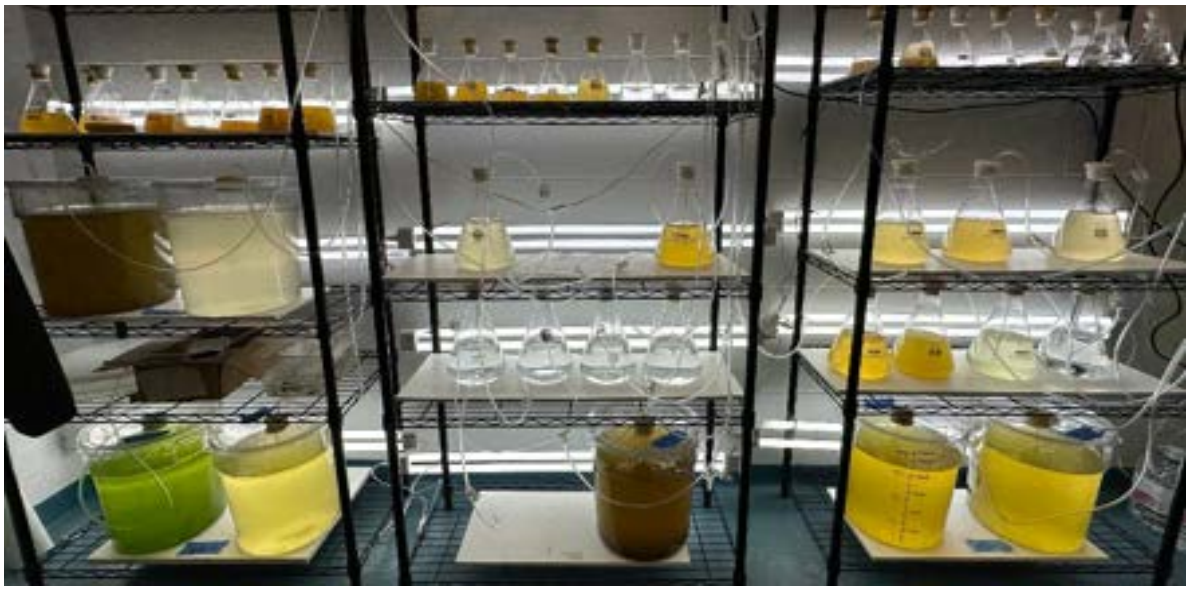

Figure 2A. Algae stock cultures at Flax Pond Marine Laboratory. A combined microalgae diet of *Pavlova lutherie*, *Tisochrysis lutea (T-ISO), Isochrysis galbana (C-ISO), Chaetoceros* sp., *Thalassiosira Weissflogii*, and *Tetraselmis* sp. was used for feeding.

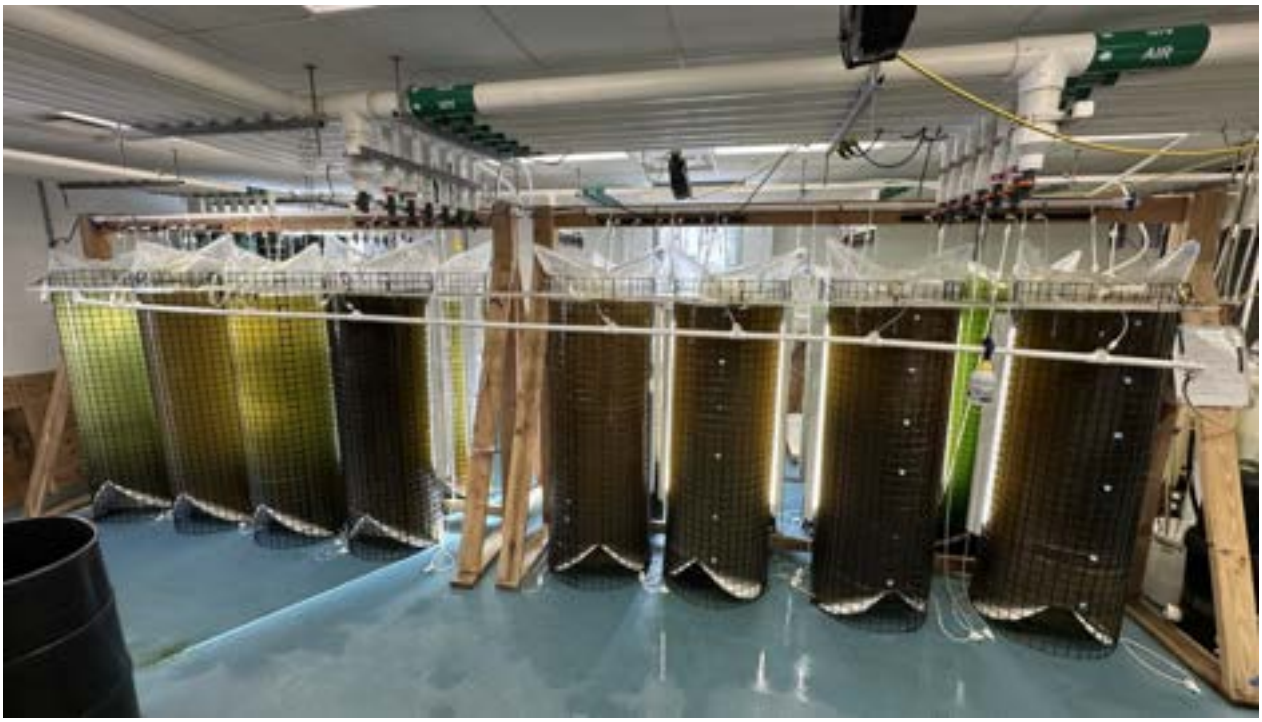

Figure 2B. SeaCAPS algae system. Sixteen 500L bags of algae were constantly maintained and fed to ribbed mussel broodstock and larvae. As treated seawater continuously drips into each bag, all overflow algae spills into a PVC line which deposits into a single container. Once reaching a certain level, this algae is periodically pumped into broodstock and larval tanks through overhead transfer lines.

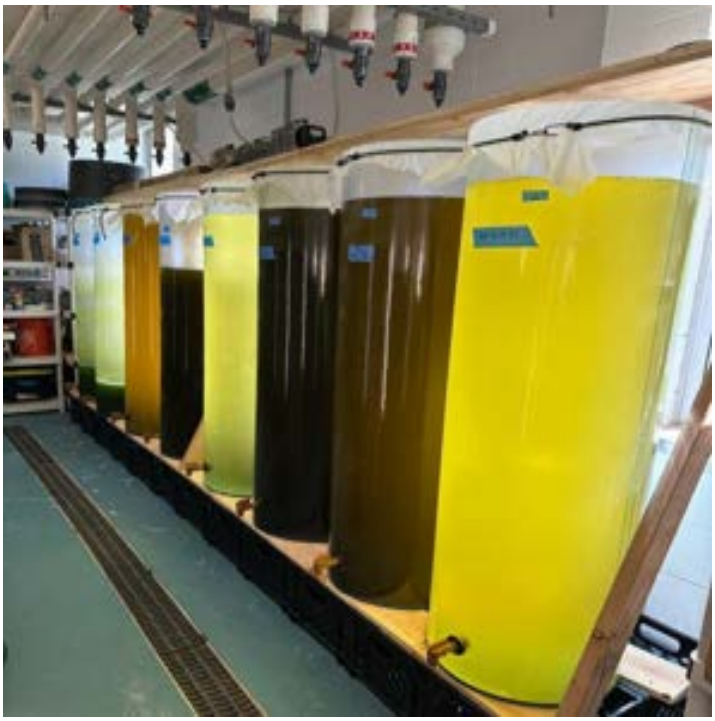

Figure 2C. Kalwall tubes used to grow algae. During the spring 2025 season, eight 250L Kalwall tubes were incorporated into our algae production system to expand overall algae output. This provided a slightly faster way to bring up algae cultures, helping to keep up with feeding demand independent from the SeaCAPS system.

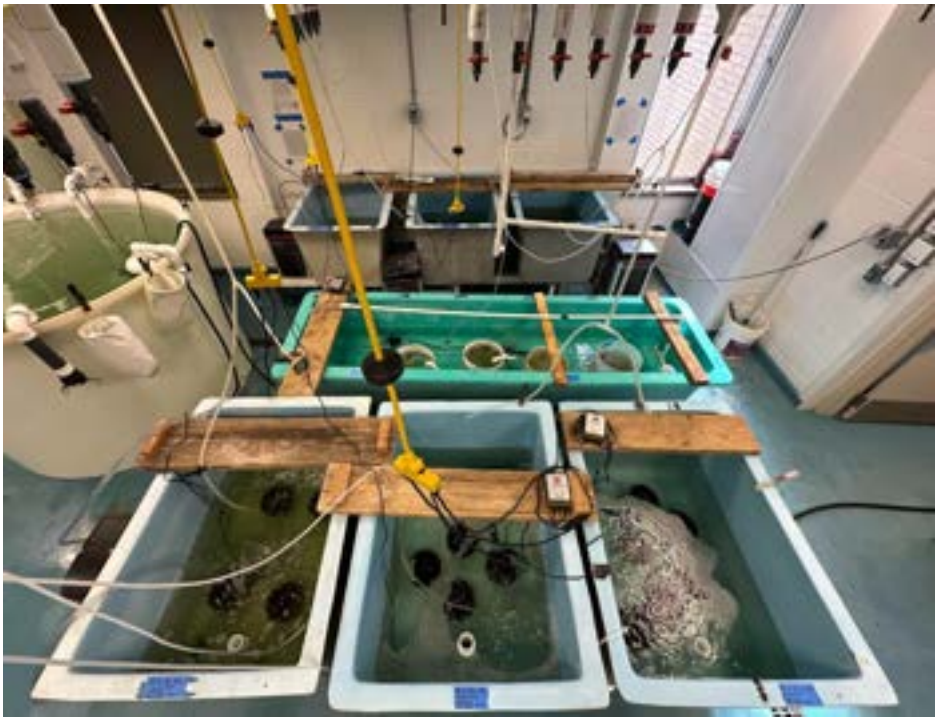

Figure 3. Broodstock conditioning tanks at the Flax Pond hatchery. Ribbed mussels were housed in planter pots held at 20°C and continuously fed a mixed microalgae diet. Air stones and pumps were used to circulate water.

Figure 4A. Ribbed mussel table spawn setup. Each spawn consisted of 40 animals. Seawater temperature manipulated by controlled heating system.

Figure 4B. Close-up view of ribbed mussel table spawn setup. Ribbed mussels were placed in a shallow tank filled with 15°C seawater and a small water circulator.

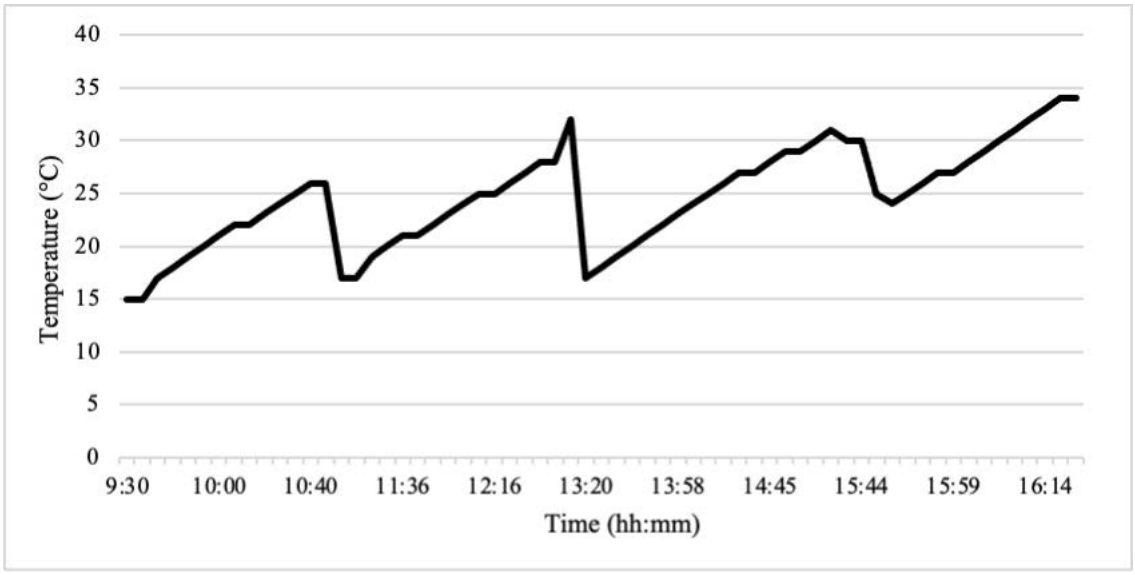

Figure 5A. Temperature (°C) profile over time (hh:mm, 24-hour) for table spawn #1. Two clear cycles with drain-downs at ~26°C and ~32°C; each followed by refill with 15°C seawater. Algae food added intermittently.

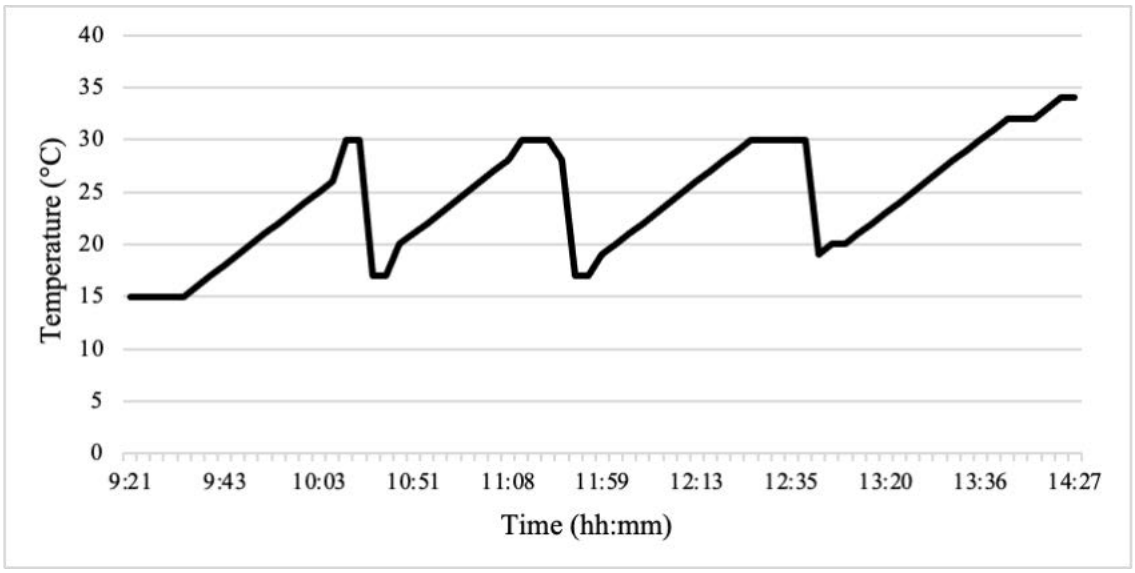

Figure 5B. Temperature (°C) profile over time (hh:mm, 24-hour) for table spawn #2. Cycling to ~30°C, drain-down, 15°C refill, then a second cycle with drain-down near ~28–30°C. Algae were added between cycles; and include sacrificial gametes after the second cycle to stimulate spawning.

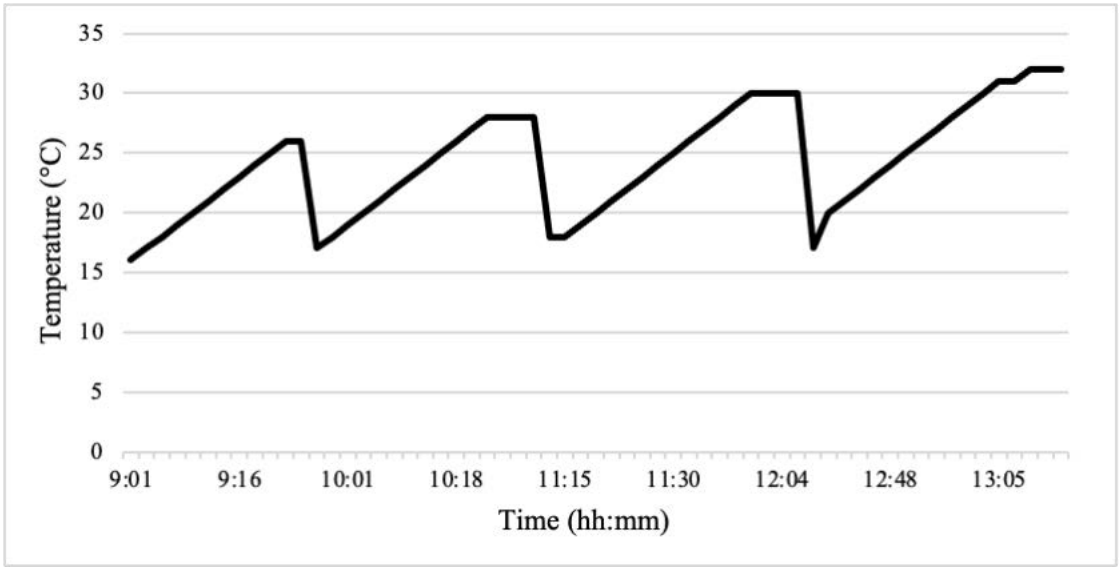

Figure 5C. Temperature (°C) profile over time (hh:mm, 24-hour) for table spawn #3. Three cycles with drain-downs at ~26°C, ~28°C, and ~30°C; each drop reflects 15°C seawater added after draining. Algae additions occurred intermittently, and sacrificial gametes were introduced to stimulate spawning after the second cycle.

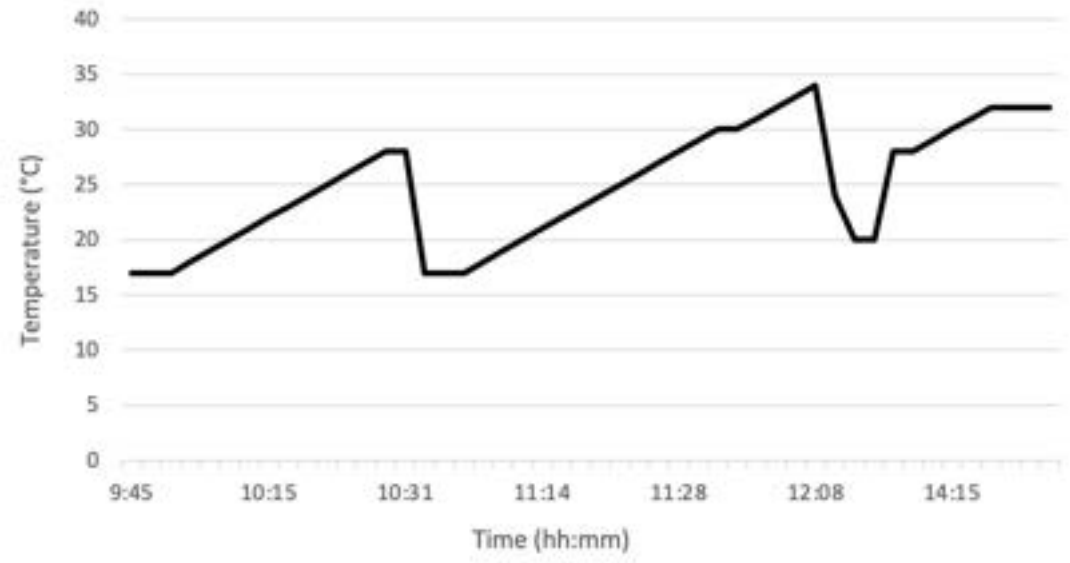

Figure 5D. Spawn #4 (table spawn). Temperature (°C) changes over time (hh:mm). Progressive heating into the low-30s with brief cold-water additions, then drain-down near ~28°C and refill with 15°C seawater. Algae additions occurred intermittently; after the second cycle, sacrificial gametes were introduced to stimulate spawning.

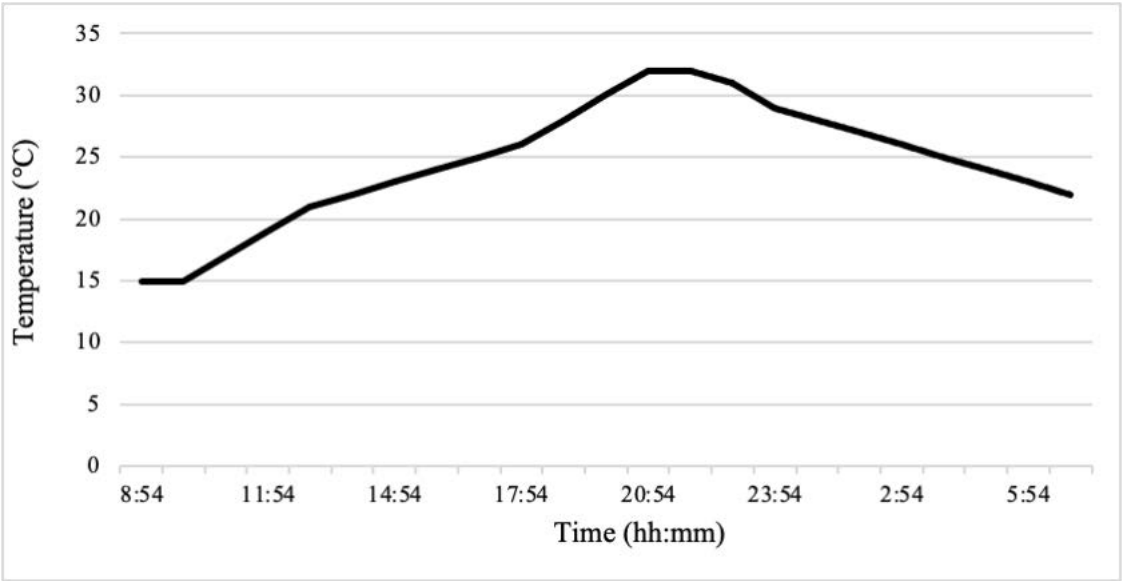

Figure 5E. Spawn #5 (table spawn). Temperature (°C) profile over time (hh:mm). Long, stepped warm-up from 15°C with algae additions early; no drain-down captured in this series. Heaters were later unplugged, leading to gradual cooling. This table spawn was conducted to mimic a bin-silo spawn, where one peak temperature is reached and cooled off overnight.

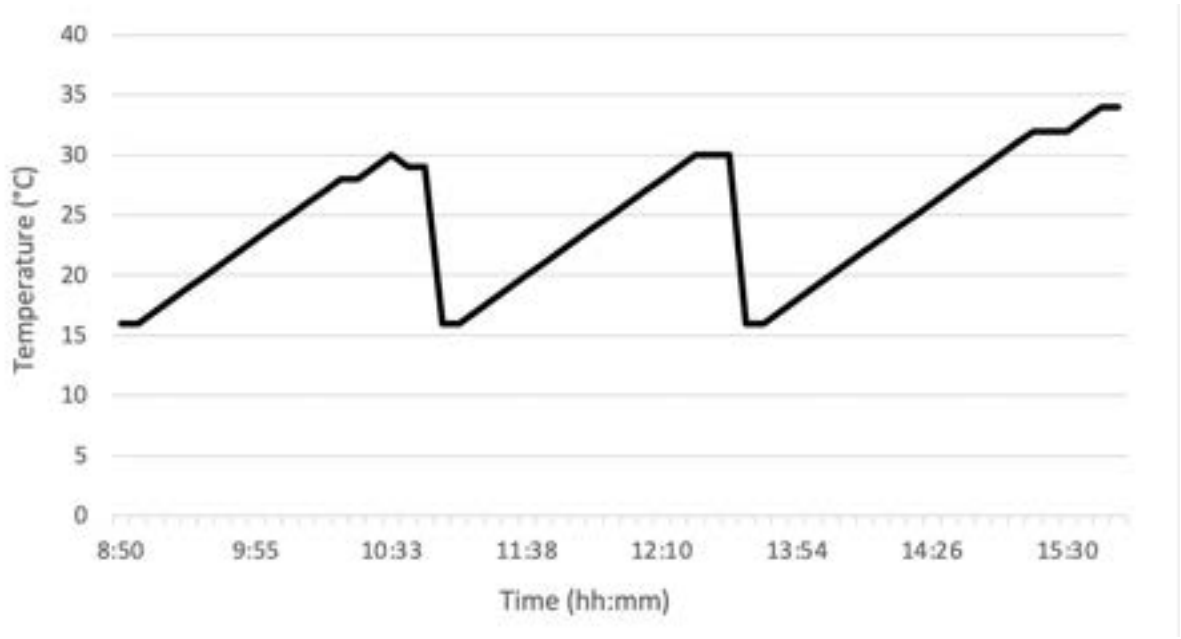

Figure 5F. Spawn #6 (table spawn). Temperature (°C) profile over time (hh:mm). Multiple cycles with drain-downs around ~30°C and ~34°C (plus an early maintenance drain near ~15°C); each abrupt decrease follows refill with 15°C seawater. Algae were added intermittently; later cycles stepped to higher plateau temperatures.

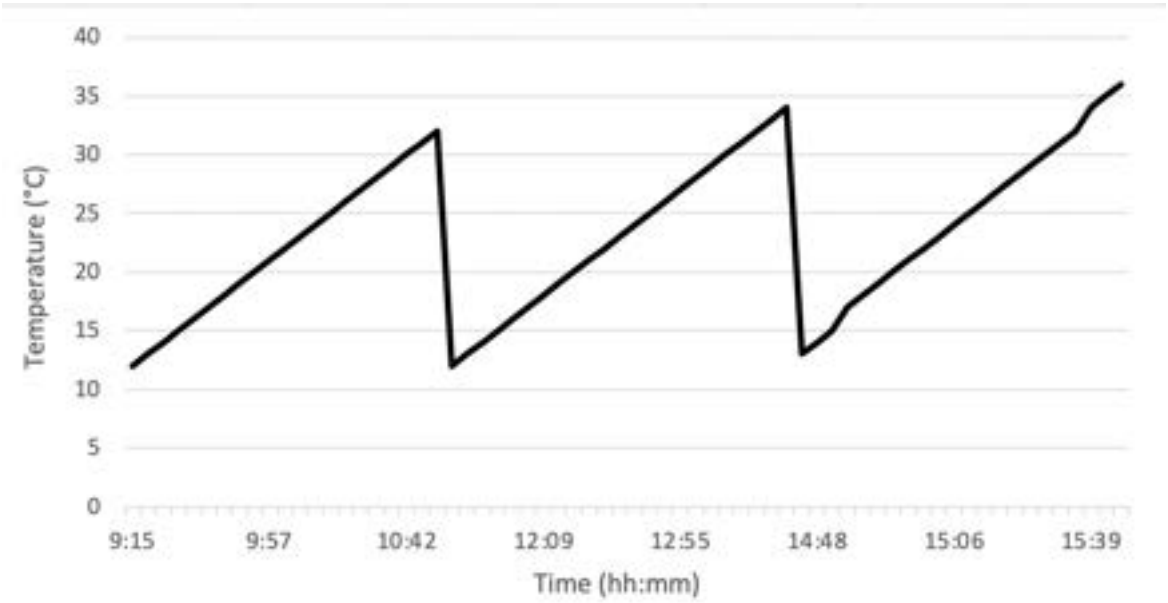

Figure 5G. Spawn #7 (table spawn). Temperature (°C) profile over time (hh:mm). Progressive warm-up with holds at ~34°C and ~36°C (second and third thresholds). Algae additions occurred intermittently.

Figure 6A. Bin-silo barrel setup. Each trial consisted of three replicate barrels that contained a downweller airline to recirculate water and introduce air into the barrel for the duration of the trial. At the end of each trial, all barrels were drained down on a 100µm and 28µm mesh sieve.

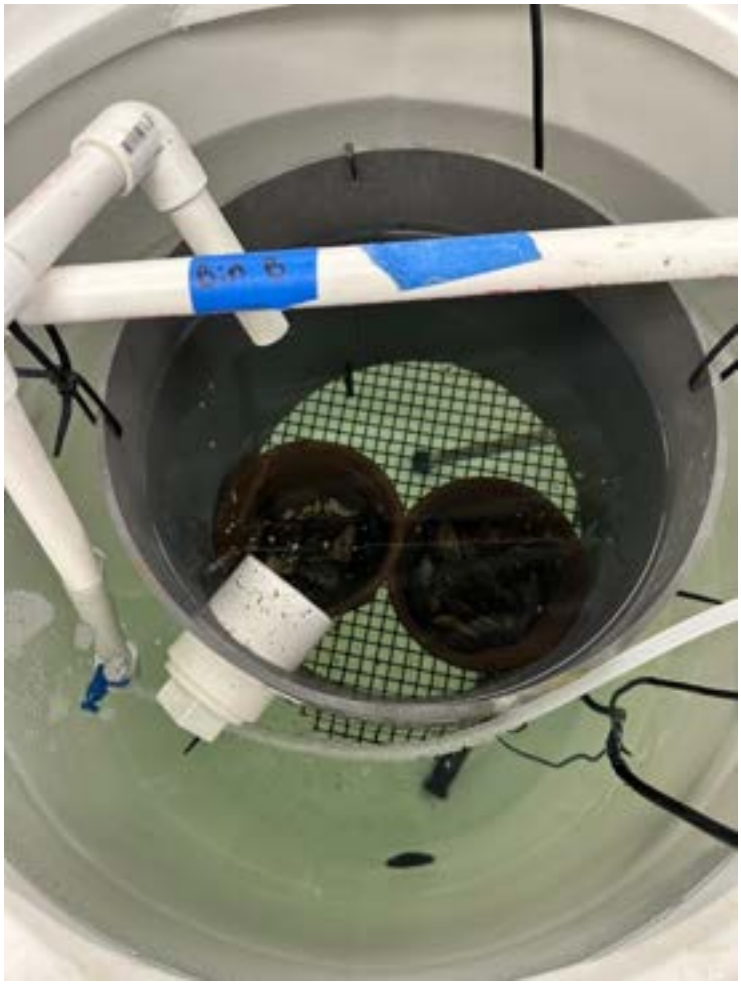

Figure 6B. Ribbed mussel bin-silo setup. 15-20 Ribbed mussels are grouped together in planting pots (13cm x 15cm) and placed into a suspended downweller hanging over a 200L bin of 32°C seawater. A PVC airline pipe constantly pumps water on top of the mussels, while also circulating any potential sperm or eggs that release.

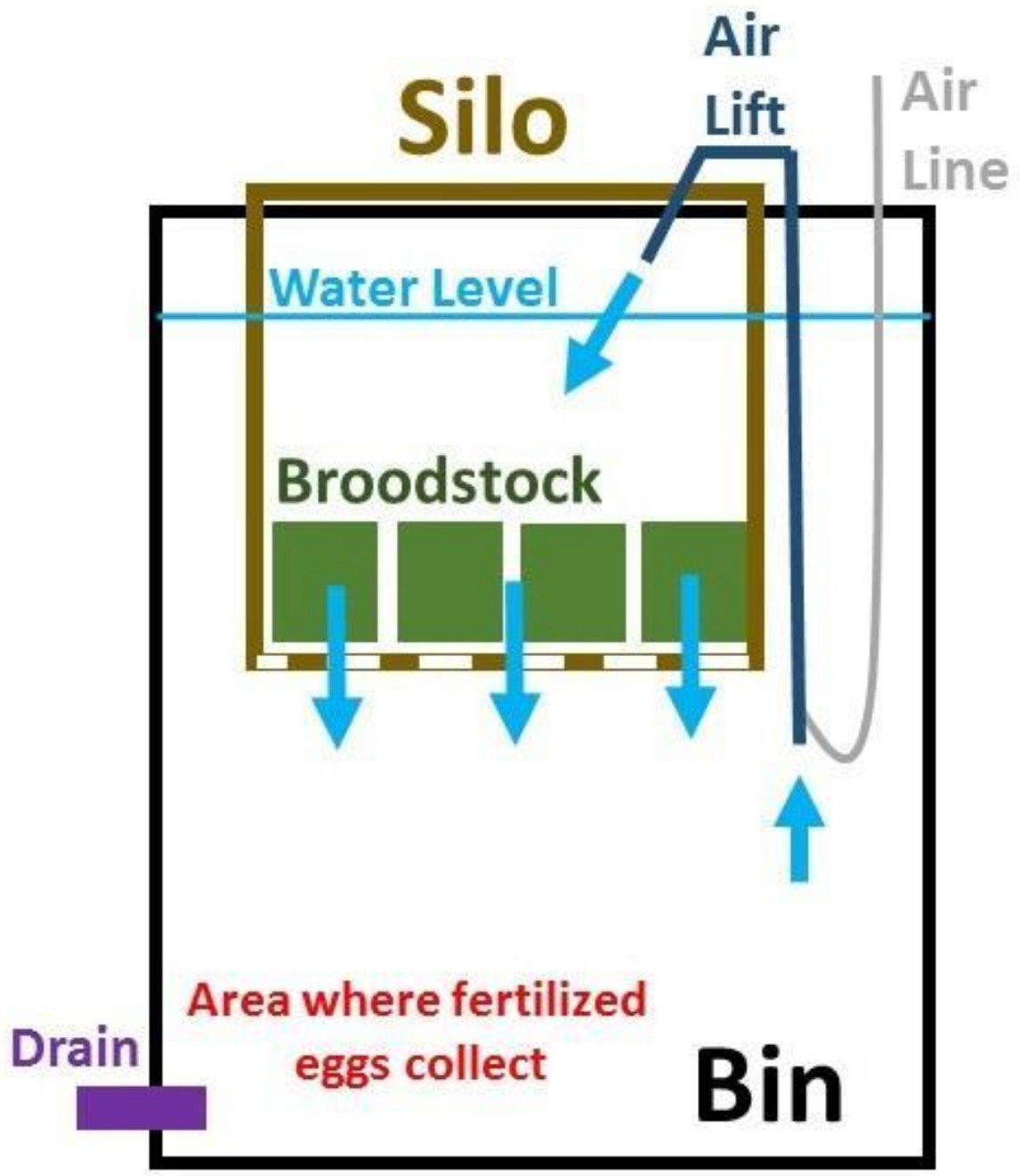

Figure 6C. A cross-sectional diagram of a bin-silo setup used for spawning ribbed mussels. Blue arrows indicate direction of water flow throughout bin-silo set-up.

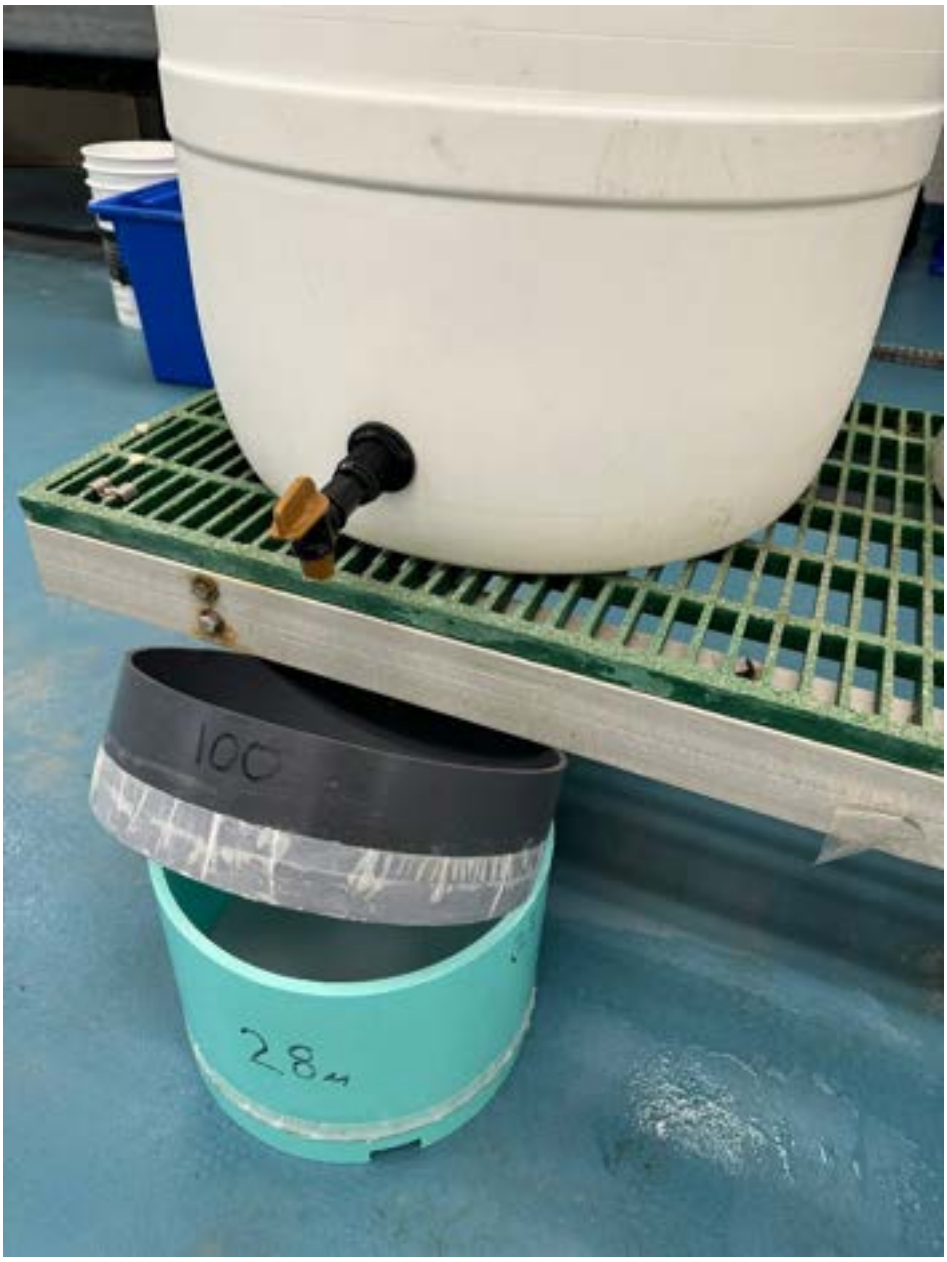

Figure 6D. Bin-silo drain-down setup. After running a trial, each replicate barrel was drained down onto a 100µm and 28µm mesh sieve. All contents sitting on the 28µm sieve were promptly rinsed into a clean bucket and examined under a microscope for potential larval collection.

Figure 6E. 350L conical tanks used to hold larvae. Drain-downs occurred on Monday, Wednesday, and Friday to replace the tanks with new water and remove waste.

Figure 6F. Ribbed Mussel downweller tank set up for post-set ribbed mussels. A PVC water circulator and airline constantly pumped water on top of the mussels. This design proved ineffective at holding the animals due to their ability to crawl over the side walls.

Figure 6G. Modified ribbed mussel downweller tank set up for post-set ribbed mussels. A PVC airline pipe constantly pumped water on top of the mussels. This downweller was designed to prevent mussels from escaping over the side walls.

Figure 7. Ribbed mussel larvae on a 1 mm Sedgewick rafter slide counting grid. Each larvae seen here is approximately 30μm in size.

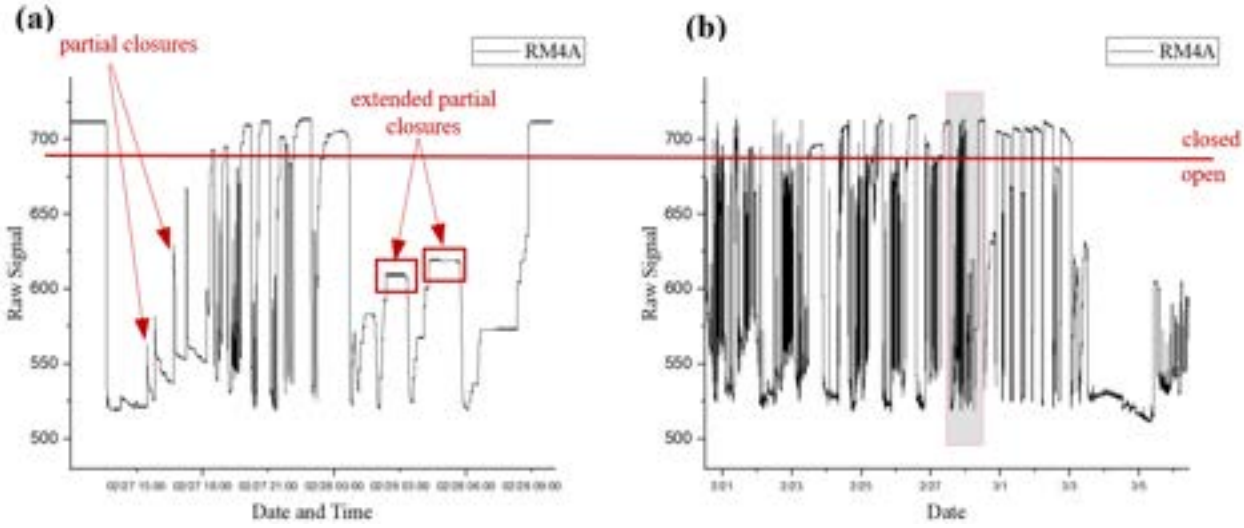

Figure 8. Raw voltage signal of the valve gaping behavior of an individual ribbed mussel in group A (RM4A). The leftmost figure (a) displays the gaping behavior during the induced spawning period occurring 02/27/2025 13:30–02/28/2025 09:30. On the y-axis is the unitless raw signal and on the x-axis is the date and time of the observation in MM/dd HH:mm format. This figure also demonstrates commonly seen gaping behavior portrayed as partial closures and extended partial closures. The highlighted partial closures are characterized as relatively short gaping pulses where the mussel is closing but does not proceed to a full closed position. The extended partial closures are a prolonged variation of the partial closures where the mussel

remains in a partially closed position for a relatively extended period of time. The rightmost figure (b) displays the gaping behavior of the same individual over an extended period of time: pre-spawn (2/20/2025 12:00–02/26/2025 12:00), during the spawning period, and post-spawn (03/01/2025 10:30—03/06/2025 09:30). The y-axis displays the raw signal of the gaping behavior of the mussel, and the x-axis displays the date of observation in M/dd format. This is a continuous record during the time frame. The spawning period shown in (a) is highlighted within a red box in (b). A red line extending across (a) and (b) is a closure threshold where a raw signal above this threshold implies the mussel is fully closed and a raw signal below this threshold implies the mussel is open to some degree. Partial closures fall below this threshold.

Figure 9. Ribbed mussel Floating Upweller System (FLUPSY) setup used in a bioextraction pilot study. Juvenile ribbed mussels were held in mesh grow-out bags and a milk crate within a FLUPSY trough.

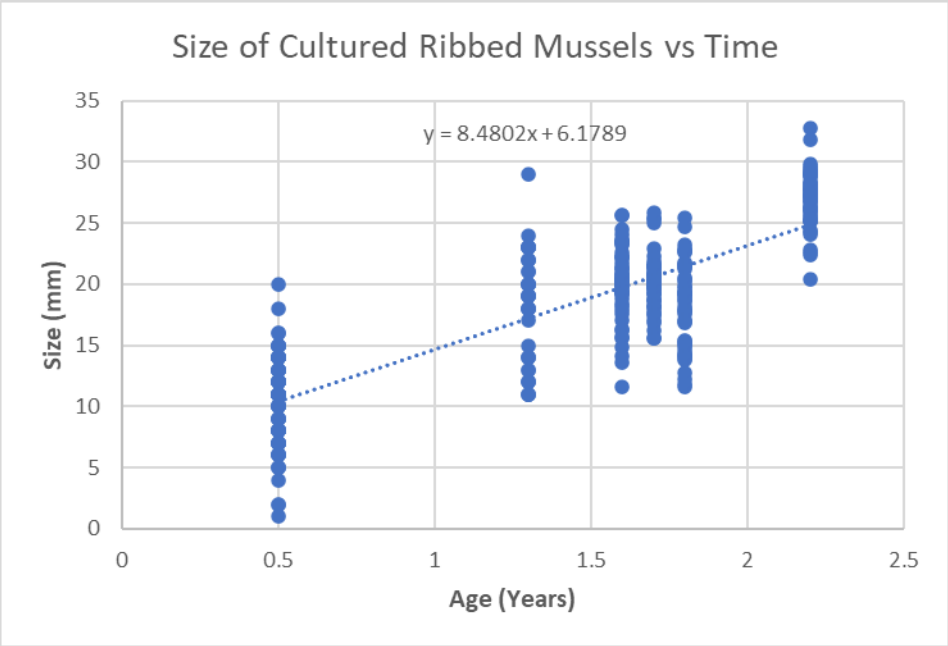

Figure 10. Change in size (shell length, mm) of cultured ribbed mussels that were grown for approximately 2 years at the Huntington grow-out site in a FLUPSY unit. While there was considerable size variation within each sampling interval, a linear regression model (blue line) yielded an average growth rate of 8.48 mm per year.

Appendix 2. Detecting Ribbed Mussel, *Geukensia demissa,* spawning through hall effect valve gaping sensors

Report on the Observations Conducted During February and March 2025

Prepared by: Bryanna Porter-Pompey

July 2025

1. Background

1.1 Valve Gaping Sensors

Various iterations of valve gaping sensors have been used on bivalves to assess their feeding activity and behavior in response to environmental conditions in recent decades. In 2003, Tran et al. used their variation of a valve gaping sensor on the Asiatic clam, *Corbicula fluminea*, in an effort to detect behavioral responses to the contaminant cadmium. It was discovered that valve closure duration and frequency may be linked to the concentration of contaminants as well as the duration of exposure to the tested contaminants. The study also assessed the potential effect of the sensor on the animal thereby influencing the collected data and found that the non-invasive sensor does not pose a significant effect on gaping behavior. In 2007, Frank et al. described their novel valve gaping sensor used to finely measure valve gape in the Eastern Oyster, *Crassostrea virginica*, and continued to describe the use of their sensor in conjunction with water clearance rate assays. The authors exhibited that valve gape alone is an imperfect proxy for clearance rate, yet remains a crucial indicator when utilized in conjunction with other physiological measurements. In 2016, Porter and Breitburg used their variation of a valve gaping sensor on *C. virginica* to detect behavioral responses to normoxic, hypoxic, and superoxic dissolved oxygen concentrations. It was found that extended valve closure periods during severe hypoxic events significantly contrasts the closure duration of oysters during normoxic events in which oysters were closed for a comparatively shorter duration. In 2017, valve gaping sensors were deployed on the ocean quahog, *Arctica islandica*, from February 2014 to September 2015 to characterize their variable behavior to seasonal changes in environmental conditions (Ballesta-Artero et al. 2017). Valve gaping behavior examined on ribbed mussels is a novel endeavor, even more so during spawning events in which there is no known published work on this topic to date.

1.2 Ribbed Mussel Spawning

In an attempt to fill the knowledge gaps associated with the spawning of the ribbed mussel, *Geukensia demissa*, hall effect sensors, a variation of the valve gaping sensor technique are used in observations occurring from February through March 2025 in which there were multiple spawning events. The collected data attempts to characterize potential patterns in the valve gaping behavior pre-spawning, during spawning, and post-spawning of mussels observed.

2. Materials and Methods

2.1 Organism Acquisition

Ribbed mussels were sourced from two different locations contrasting in environmental conditions- Flax Pond in Setauket and Bergen Basin in Jamacia Bay. The animals used in observations were divided into three different groups based on location of collection. The mussels in group A were collected from Flax Pond and the mussels in group B and group C were collected from Bergen Basin. Each group received daily maintenance and supply of food.

2.2 Hall effect sensors and Arduino loggers

Non-invasive hall effect valve-gaping sensors were employed on a total of 15 ribbed mussels to gather valve gaping data which may later be quantitively assessed. These sensors operate on a hall effect which, when connected to a power source, generates a voltage difference across a conductor or semiconductor. When a hall effect sensor's proximity to a magnet changes, it alters the voltage signal, which is recorded on a connected Arduino logger with 4 measurements every second or 232 measurements per minute. The sensors are attached to the outside of the mussel's left shell valve in a region closest to the opening of the valve (Images 1-2.). The circuit is completed, and gaping detectable, when a magnet is placed on the direct opposite right shell valve of the animal resulting in the highest raw signal. Typically, two magnets are suitable to maintain a high signal, however additional magnets may be used to improve signal quality of weak signals. When signals collected over a period of time are graphed, it is inferred that the highest signal on the graph indicates the animal is fully closed. In contrast, the lowest signal indicates the animal is open to an extent, or the magnet and sensor have gained distance from each other, contributing to the lower signal.

One board may attach up to 8 animals although only 5 per group were used among 3 Arduino boards (n = 15) in these initial observations. Three of the examined mussels were placed in a single plastic planter in such a way that the animals were fixed upright so that the magnets remain untouching. The remaining two mussels were placed in another planter in a similar fashion. Sensors and magnets were attached to the animals using Loctite, a quick drying adhesive. Mussels attached to hall effect sensors in all three groups formed byssal threads in the days following sensor attachment.

At the conclusion of observations, gaping data was acquired from the Arduino loggers on a 16GB SD card and uploaded to the computer application, Origin, for viewing. Raw data was analyzed using an R-script in RStudio version 3.3.1, then quantitatively assessed.

Images 1-2. From left to right. Image 1 displays a group of 3 ribbed mussels freshly attached to hall effect sensors and magnets. Mussels are held in an upright position in a planter through the attached hall effect sensor having been zip tied to a slit in the planter. Mussels are placed in such a way that the magnets do not interfere with each other. Image 2 displays an apparently acclimated mussel with extended byssal threads and a partially open valve.

3. Results

1.2 Spawning Trials

A total of four spawning attempts were conducted during the observational period. Spawning events were captured during attempts 2, 3, and 4 through the observation of gametes. A spawning event was noted in group B during attempt 2 conducted on February 25 at 14:10 until February 26 at 08:35. Group C was the only group in which a spawning event occurred during attempt 3 conducted on February 26 at 12:50 until February 27 at 09:15. During the fourth attempt, which occurred from February 27 at 13:30 until February 28 at 09:05, all groups A, B, and C showed signs of a spawning event having occurred.

2.2 Valve Gaping Patterns

During the spawning periods in Groups A, B, and C, all mussels observed displayed clear gaping patterns captured by the hall effect sensors. These patterns appear as extended periods of openings in which partial and relatively quick valve claps are visible (Figure 1a; Figure 1c), periods of extended full closures (Figure 2a; Figure 2d), and extended periods of openings with relatively minimal activity (Figure 1c). In groups B and C, gaping patterns between the two spawning periods appear similar within the same mussel, however varies between different mussels in the same group (Figures 2a,b; Figures 3a,b). For example, Figure 2a displays a period of closure lasting about 3 hours, followed by an extended opening event with several brief valve claps, or partial closures, within that opening event. The opening event lasting nearly 9 hours is then followed by another closure event of about 3 hours. This mussel displayed a similar pattern during spawn 2. In the same group, the individual in Figure 2d displays contrasting behavior characterized as several periods of extended full closures with relatively brief openings, each lasting about 20 minutes. This individual displayed a similar valve gaping pattern can during the second spawning period. In group C (Figure 3), RM3C (Figures 3a,b,c) displayed similar gaping behavior during the first and second spawning periods in which it has two distinct closure events, each lasting 5-7 hours, separated by two distinct opening events, each lasting 3-5 hours. Within each opening event, activity may be seen through multiple partial closures, or valve claps. In the same group, RM4C contrasts RM3C through its gaping behavior in both spawning periods. The mussel begins each period with a series of closure events each lasting for 30-40 minutes. After these closure events, the animal follows these repetitive closure events with an extended opening event lasting for more than 10 hours with several spikes of partial closures within these opening events. Throughout mussels in group A (Figure1), although all animals observed displayed opening events lasting majorly throughout the entire spawning period, many mussels displayed apparently variable gaping behavior with some more active than others as evidenced through partial closures and percent of time open hr^{-1} as well as brief periods of full closures as in RM4A (Figure 1e).

Analysis of valve gaping behavior was performed on individuals examined in groups A and B. The percent of time open metric was calculated by an R-script for each hour of the respective observation periods. By averaging these values across individuals in either respective treatment (n = 5±SE), the percent of time open $mussel^{-1}$ hr^{-1} metric is calculated. Amongst all examined mussels in group A,

individuals exhibited an average percent of time open mussel^{-1} hr^{-1} of 76.0±1.7 pre-spawn (02/20/2025 12:00–02/26/2025 12:00), 91.1±2.8 percent of time open mussel^{-1} hr^{-1} during the spawning period (02/27/2025 13:30–02/28/2025 09:30), and 82.6±1.5 percent of time open mussel^{-1} hr^{-1} during the post-spawn period (03/01/2025 10:30—03/06/2025 09:30). In group A, mussels were more commonly open during the spawning period than before or after the spawning period. Mussels examined in group B exhibited an average (n = 5±SE) percent of time open mussel^{-1} hr^{-1} of 84.9±1.4 pre-spawn (02/18/2025 13:30–02/24/2025 12:30), 74.8±3.8 during spawn 1 (02/25/2025 14:40—02/26/2025 08:40), 70.7±4.8 percent of time open mussel^{-1} hr^{-1} during spawn 2 (02/27/2025 14:00—02/28/2025 09:00), and 81.4±1.1 percent of time open mussel^{-1} hr^{-1} post-spawn (03/01/2025 10:00—03/07/2025 09:00). Contrasting group A, mussels in group B were commonly open for less time during the spawning periods than during the pre-spawn and post-spawn periods. Mussels examined in Group C (n = 5) exhibited an average (±SE) percent of time open mussel^{-1} hr^{-1} of 78.6 (±1.8) during the pre-spawn period (02/21/2025 12:00—02/26/2025 08:00), 71.8 (±4.2) during spawn attempt 1 (02/26/2025 12:50—02/27/2025 10:00), 74.6 (±3.6) during spawn attempt 2 (02/27/2025 13:00—02/28/2025 09:30), and 71.5 (±1.7) during the post-spawn period (02/28/2025 13:30—03/04/2025 17:00).

4. Discussion

4.1 Observed Valve Gaping Patterns

Although there was clear spawning having occurred within all groups during their respective attempts, as indicated by the presence of gametes, it was not clear exactly which mussels were the spawning individuals and which specific mussels did not spawn based on valve gaping data alone. This being so, it is unclear whether valve gaping data depicted through hall effect sensors is distinct spawning behavior or typical feeding or respiration behavior because spawning individuals were visually indistinguishable from non-spawning individuals by the time gametes were discovered at the conclusion of a spawning attempt. As a whole, those mussels examined all exhibited general valve gaping behaviors in the form of brief partial closures, extended partial closures, full closures, and extended opening events. Several of those individuals examined in groups B and C displayed a similar pattern of behaviors across both spawning periods, however sometimes varied across individuals within the same group. This variation may imply that among those mussels attached to valve gaping sensors, spawning may not have occurred in all individuals. The percent of time open hr^{-1} metric examined in group A was greatest during the spawning period when compared to the pre- and post-spawn periods. In contrast, the percent of time open hr^{-1} metric examined in group B was lowest during both spawning periods compared to the pre- and post-spawn periods. Broadly, the percent of time open hr^{-1} for mussels in group C was, on average, greatest pre-spawn and reduced during spawns 1 and 2. However, the average post-spawn attempt percent of time open mussel^{-1} hr^{-1} for those in this group was similar to that of the spawning attempt periods. This contrast in broad behavior during spawning events in groups A, B, and C further exemplifies the possibility of non-spawners being included in analysis. To date, no statistical analysis such as a one-way repeated measures ANOVA has been performed on the valve gaping behavior between observation periods.

4.2 Adjustments to Methods

In future work, it would be beneficial to create a video timelapse focusing on the group of mussels attached to sensors so that it would be properly identifiable as to which animal had released gametes during spawning attempts. This timelapse with a time stamp may also be used to identify the exact time of spawning so that quantifiable analysis may be done within exact spawning times in an effort to characterize and measure pre-spawn, spawning, and post-spawn behavior through the high-resolution gaping data, and properly state whether the behavior significantly differentiates between each observation period.

Once the individuals and their exact spawning times are identified, a conducive measure can be made of the probable distinct spawning behavior in the laboratory. Once identified, it is anticipated that when applied in-situ, this method may be used to identify spawning events in the wild in combination with measured environmental parameters. Statistical analysis such as ANOVA with corresponding post-hoc tests would be applicable and necessary in analysis.

Possible continuation of work may include a linear regression analysis or Spearman's rank correlation test between applicable valve gaping metrics and the concentration of gametes observed. This work is completely novel and has the potential to fill a large knowledge gap pertaining to the minute physiological behavior of ribbed mussels both in-lab and *in situ* in relation to spawning behavior.

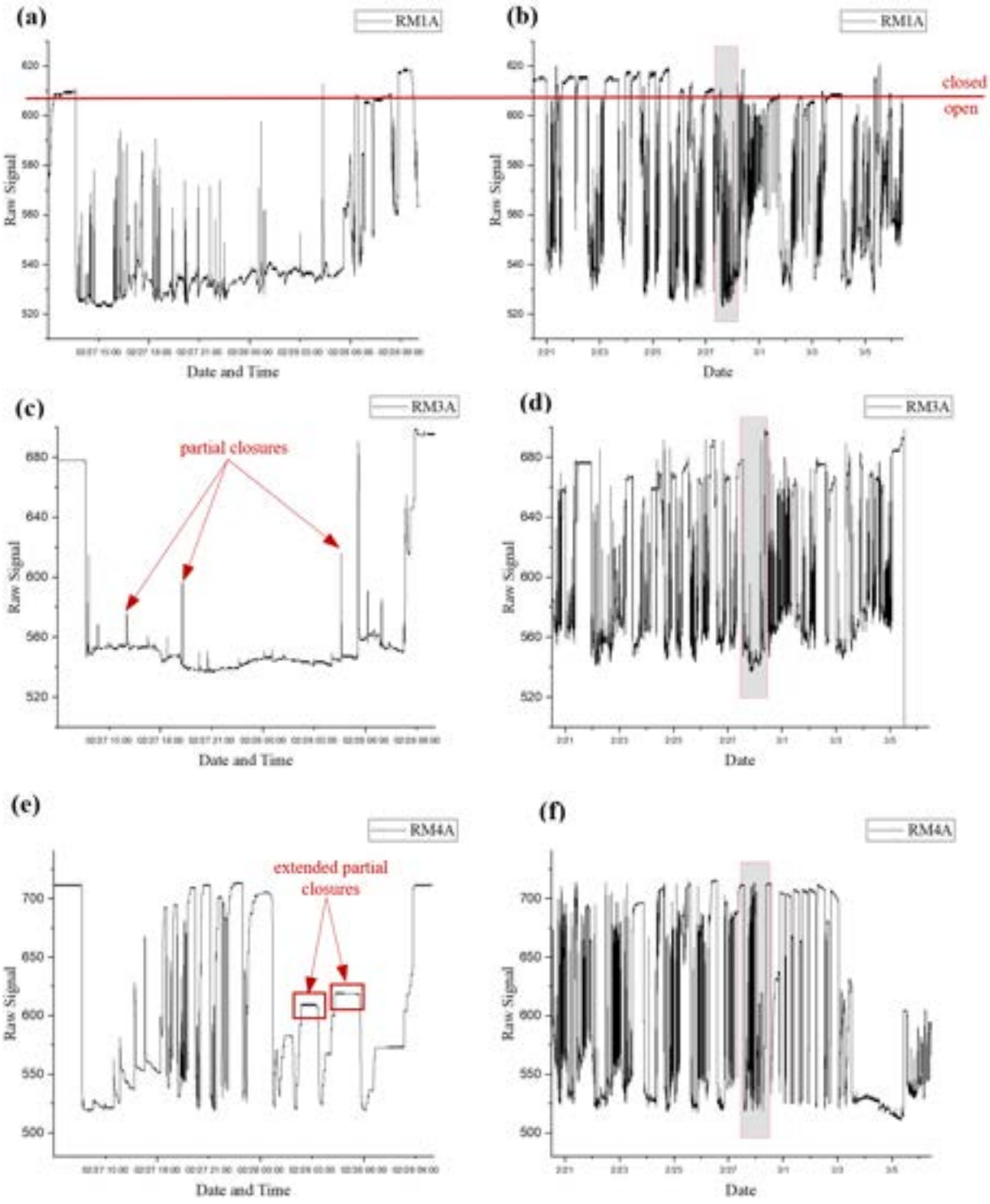

Figure 1a-f. Time-series of valve gaping observations of 3 individuals (RM1A, RM3A, RM4A) within group A. Figures in the leftmost column (a, c, e) depict the raw valve gaping signal of the 3 individuals during the induced spawning period of 02/27/2025 13:30—02/28/2025 09:05. The y-axis displays the raw signal gathered from the individuals and the x-axis displays the date and time of the observation period. Partial closures are present in all mussels examined but is exemplified in figure (c). The extended partial closures are exemplified in figure (e). The rightmost column (b, d, f) displays raw gaping behavior during

the full observation period including pre-spawn (02/20/2025 12:00—02/26/2025 12:00), the spawning period, and post-spawn (03/01/2025 10:30—03/06/2025 09:30). Figures within this column contain a shaded box highlighting the spawning period displayed in (a, c, e).

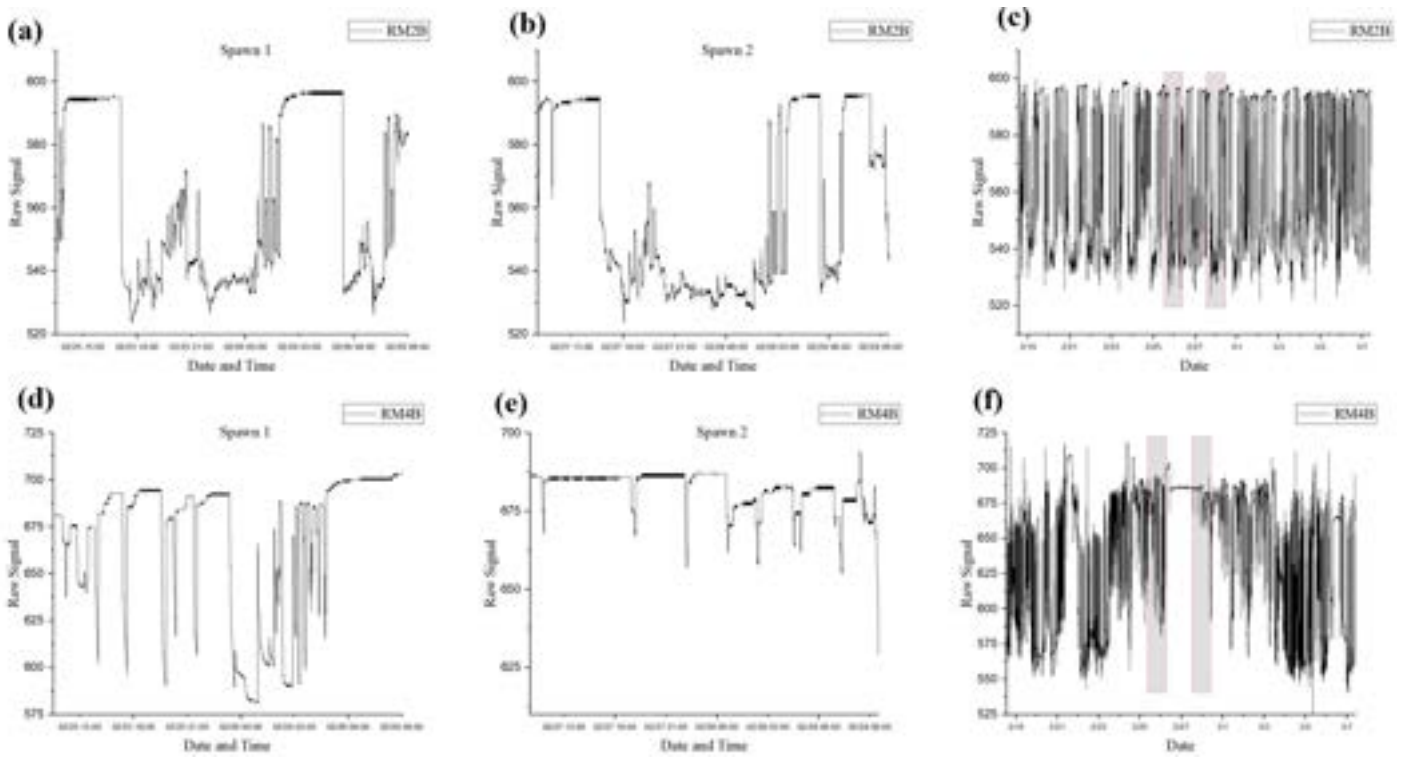

Figure 2a-f. Time-series of valve gaping behavior of 2 individuals (RM2B and RM4B) within group B. Figures in the leftmost column (a, d) depict the raw valve gaping signal of the 2 individuals during the first induced spawning period of 02/25/2025 14:40—02/26/2025 08:40. Figures in the middle column (b, e) depict the raw valve gaping signal of the 2 individuals during the second induced spawning period of 02/27/2025 14:00—02/28/2025 9:00. Figures in the rightmost column (c, f) depict the raw valve gaping behavior of the two individuals during the entirety of the observation periods including pre- (02/18/2025 13:30—02/24/2025 12:30) and post-spawn (03/01/2025 10:00—03/07/2025 09:00). The shadowed boxes within figures of this column serve to highlight the depicted spawning periods in the leftmost and middle columns. The y-axes display the raw signal gathered from the individuals and the x-axes display the date and/or time of the observation period.

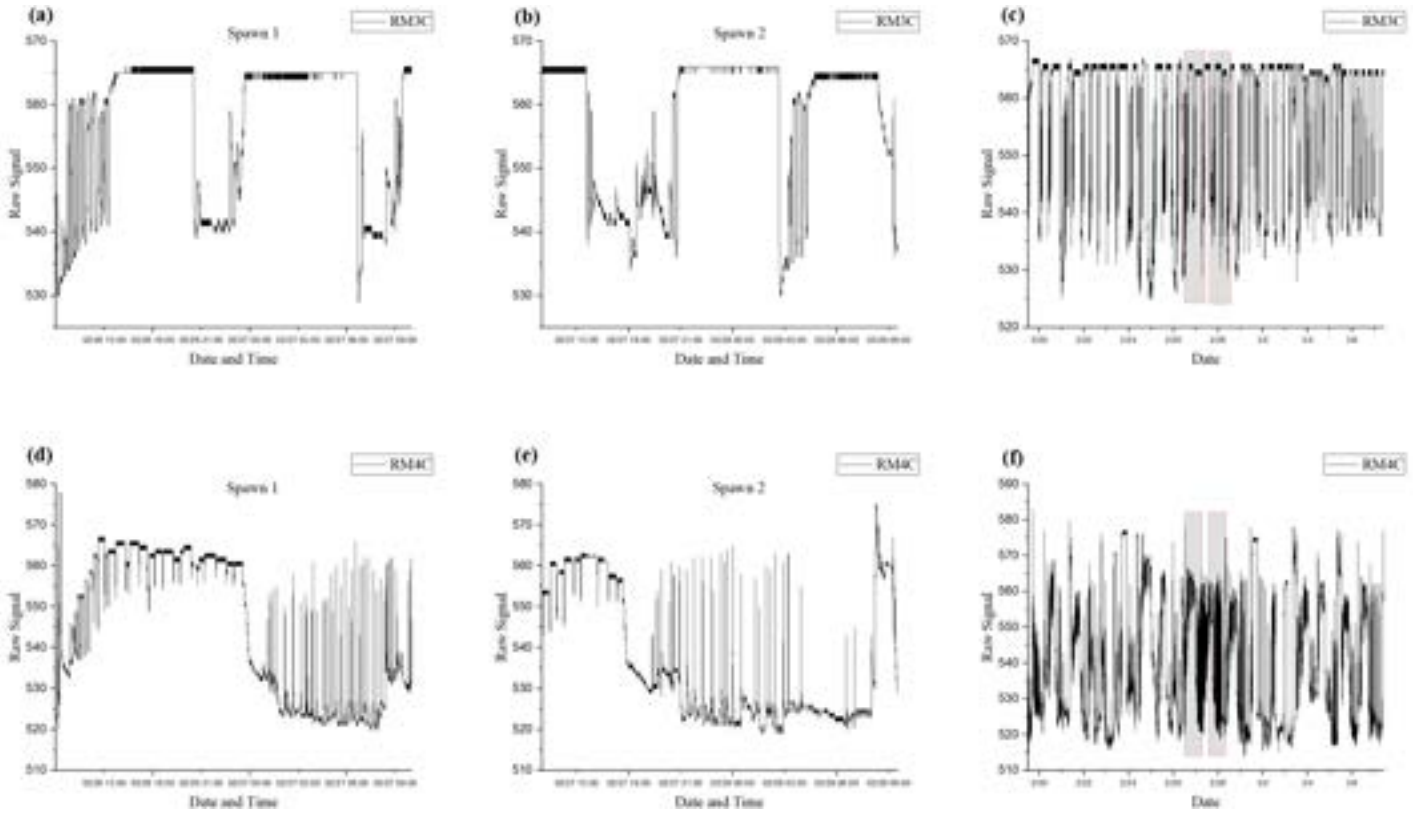

Figure 3a-f. Time-series of valve gaping behavior of 2 individuals (RM3C and RM4C) within group C. Figures in the leftmost column (a, d) depict the raw valve gaping signal of the 2 individuals during the first induced spawning period of 02/26/2025 12:50—02/27/2025 09:15. Figures in the middle column (b, e) depict the raw valve gaping signal of the 2 individuals during the second induced spawning period of 02/27/2025 14:00—02/28/2025 9:00. Figures in the rightmost column (c, f) depict the raw valve gaping behavior of the two individuals during the entirety of the observation periods including pre- (02/18/2025 13:30—02/24/2025 12:30) and post-spawn (03/01/2025 10:00—03/07/2025 09:00). The shadowed boxes within figures of this column serve to highlight the depicted spawning periods in the leftmost and middle columns. The y-axes display the raw signal gathered from the individuals and the x-axes display the date and/or time of the observation period.

5. References

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