VIRGINIA FISHERY RESOURCE GRANT PROGRAM

FRG 2021-02 Final Report

Title: Enhancing commercial bivalve hatchery operations through utilization of a recirculating larvae culture system

Project Investigator: Mike Congrove, Oyster Seed Holdings, Inc

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Signature of Principal Investigator Date

Introduction

Around the world, bivalve shellfish hatcheries are at the mercy of ambient water quality, which in some cases, inhibits production consistency or even results in complete production failure. These periods of poor production may be increasing, and are related to a whole host of surface water quality problems including, but not limited to, harmful algal blooms, upland runoff, pathogenic bacterial species, high rainfall events, and acidified water. Furthermore, often these failures go unexplained and unpredicted making preventing them in the future or adjusting filtration difficult. Additionally, the cost of waterfront property to set up and acquire ambient water for use is inhibitory to many startups as working waterfronts diminish. The development and use of a recirculating biological filter to effectively treat and reuse production water provides control and independence from changes in ambient water conditions that can stabilize production, and potential independence from an operational necessity to occupy expensive waterfront real estate.

Recirculating treatment systems for aquaculture (RAS) have integrated various filtration components to process waste and stabilize rearing water for aquatic organisms with minimal ambient water exchange, allowing operations to occur independent of adjacent ambient water sources (Malone 2013). RAS has been used to culture and maintain many different kinds of organisms in various production stages in aquaculture operations. The technology has been well established in many higher valued commercial finfish operations. However, RAS use for filter feeding and planktonic organisms has not been widely implemented, due mainly to cost and unknown risks of adapting new approaches.

The marine rotifer *Brachionous spp.,* an important live feed for marine finfish larvae, has been traditionally produced with static batch culture methods (Hoff and Snell 1987). However, in the last few decades, several foundational studies have established initial system designs (Dhert et al. 2001; Suantika et al. 2000, 2003) eventually leading to the development of a commercial RAS, transitioning many finfish hatcheries to a RAS production method for rotifer culture (Bentley et al. 2008; Pentair Aquatic Ecosystems. Apopka, FL, USA). Two of the project support participants in this proposed project team (Bentley and Schwarz) have worked extensively with these systems.

Historically, RAS has been underutilized within the commercial bivalve industry but in recent years, research and development has been increasing addressing implementation of RAS in bivalve aquaculture systems. Pruder (1975) demonstrated the technical feasibility of a closed RAS for the entire production cycle of *Mercenaria mercenaria* and *C. virginica*. Three large scale system configurations were described solving several technical constraints, but at this initial technical stage economic feasibility was not achieved. Other recent approaches have

targeted individual production phases utilizing RAS technology as a tool to solve specific problems. RAS technology has been most widely used in commercial bivalve aquaculture for depuration as well as wet storage systems. Dr. Schwarz, Co-PI on this proposal was instrumental in helping design one of the largest such commercial bivalve wet storage RAS systems in the U.S., specifically on the Eastern Shore of Virginia at Cherrystone Aquafarms. As the bivalve industry has expanded, prevention of zoonotics has become a critical factor, and commercial utilization of RAS depuration systems has allowed for purging of animals prior to sale. These systems increase product quality, are independent of ambient waters. and allow for the use of disinfectants, such as iodophors (Richards 1988).

The early larval period is considered the most sensitive phase of production and where the majority of losses associated with water quality problems occur. Commercial bivalve hatcheries have largely transitioned from natural microalgae to semi-closed algal culture systems for larval feed (Guillard 1975), but larval culture is still dominated by a sequential batch feeding and drawdown practice. The development of RAS for bivalve larvae production has attracted recent attention with several examples of successful trials at near commercial scale with multiple bivalve species (Asmani et al. 2016, Congrove 2012, Magesen and Jacobsen 2012, Merino et al. 2009 and Qiu et al. 2017).

Initial work on *C. virginica* larval RAS production showed poor results with only 6 of the 17 conducted trials resulting in survival beyond 6 days post fertilization (Congrove 2012). When system components and operation protocols are compared to other studies (Merino et al. 2009 and Magnesen and Jacobsen 2012), large differences can be seen with the water recirculation rate and system design. Both Merino et al. (2009) and Magnesen and Jacobsen (2012) operated at tank exchange rates of 25%/h and 4%/h, respectively. While Congrove (2012) reported much higher tank exchange rates, 400%/h, during intermittent periods. This high exchange rate was identified in the conclusion as a likely cause of the poor performance. System components may also have been undersized when compared to Merino et al. (2009) and Magnesen and Jacobsen (2012). More recent studies have been conducted with a small-scale *C. gigas* RAS system in 5 L tanks (Asmani et al. 2016) and with a larger scale *C. angulata* RAS system in 450 L culture tanks (Qiu et al. 2016). Both studies reported high survival in the RAS systems (85% and 80%, from D-larvae), demonstrating suitability of RAS for the culture of larval *Crassostrea spp.*

Preliminary work to date

In the 2019 season, the project participants operated small scale batch RAS systems for oyster larvae culture at the Virginia Tech AREC facility and the Virginia Institute of Marine Science Eastern Shore Lab. Limited larvae runs were completed at both locations with positive preliminary findings, but funding and resources limited the ability to run multiple larvae cycles.

In 2020, one of these RAS systems was setup at the OSH hatchery (Figure 1), again, with no external funding, and were successful in running 3 complete larval groups through the system from spawn to competent larvae. Two of the three larval groups were completed using batch larvae culture methods, while the third was completed using continuous flow larvae culture methods. In all trials overall survival (from egg to eyed larvae) was similar in RAS and standard larvae culture methods. In trial 1, overall survival in RAS was 18% compared to 16% in standard larvae culture. In trial 2, overall survival in RAS was 15% compared to 14% in standard larvae culture. In trial 3, overall survival was substantially lower, but similar between RAS and standard larvae cultures at 2% for both. The reason for the lower survival rate in trial 3 remains unresolved, indicating the need for closer examination of water quality issues.

These preliminary trials demonstrate proof of concept, at least at very small scale. Further work is needed to scale up the components to ensure feasibility at larger scales as well as to accommodate higher density, continuous flow larvae culture systems.

Figure 1: Preliminary small-scale RAS at OSH in 2020. The bioreactor/filter is the black tank to the left.

Objectives

The primary purpose of this project is to evaluate the efficacy of a scalable commercial RAS larvae culture system. The following objectives will facilitate this evaluation:

- Objective 1: Build as designed, a RAS larvae culture system capable of producing 10M eyed larvae per week.

- Objective 2: Establish appropriate prokaryotic and eukaryotic bioreactor biofilm populations to facilitate the necessary biological filtration necessary in a RAS.

- Objective 3: Integrate the RAS larvae culture system into OSH larvae production operations so there are respective cohorts of each spawn in standard larvae culture and RAS larvae culture. Record performance of larvae in standard culture and RAS culture for each cohort

- Objective 4: Characterize the bacterial and eukaryote populations in the respective bio- reactors.

Methods

Objective 1

Based on RAS larvae culture experiment conducted by the team in 2019 and 2020, a RAS larvae culture system has been designed by the team with a production capacity of 10M eyed larvae per week. A process flow diagram, Figure 2, is provide below to describe the components required and how they will interact in the overall production system. The OSH crew will build, commission, and operate this system with assistance from the team as needed.

Objective 2

In conjunction with project partners at VA Tech and VIMS, OSH staff will utilize “starter” cultures of bioreactor media preconditioned for ammonia oxidizers from the VIMS ESL lab to inoculate RAS bioreactors. After inoculation, OSH staff will be responsible for scaling up the reactor populations using heterotrophic biofilm conditioning protocols developed at VIMS ESL. This is a critical step to establishing an effective bioreactor. OSH will only proceed with larvae production after confirmation by VA Tech and VIMS ESL staff that the reactors are ready and capable of processing water to support larval culture.

Objective 3

OSH staff will operate and maintain the RAS larvae culture system. OSH staff will spawn new larvae cultures weekly in the system to maintain a commercial-like rate of production. OSH staff will provide all husbandry and operation of the system once spawning begins. This will include conducting mass spawns, the majority of which will go into standard OSH larvae production (SOP), but a portion (approximately 40M embryos form each spawn) will be put into RAS larvae culture.

Larvae performance metrics to be collected on both standard larvae culture and RAS larvae culture for every cohort including drop interval survival, grade split proportions, gut density, larvae activity, overall survival form egg to eyed. For at least 3 cohorts, setting rate will be estimated for respective standard and RAS larvae treatments.

Objective 4:

Samples of the bioreactor media will be collected periodically for activity measures, DNA extraction for microbial community analysis of prokaryotes and eukaryotes, and visual identification of eukaryotes. Periodic microscopic examination of eukaryotes in the system may be used as indicators of system performance similar to the practice followed in waste water treatment plants, and PIs will develop a photographic guide that will allow operators to make such assessments. Activity measures will be based on respiration rates for heterotrophic and autotrophic activity (ammonia oxidizers) by multichannel oxygen meter. Basic water quality measures will be analyzed periodically to track ammonia, nitrate + nitrite, pH, and alkalinity.

Figure 2: Process flow diagram for RAS larvae culture system

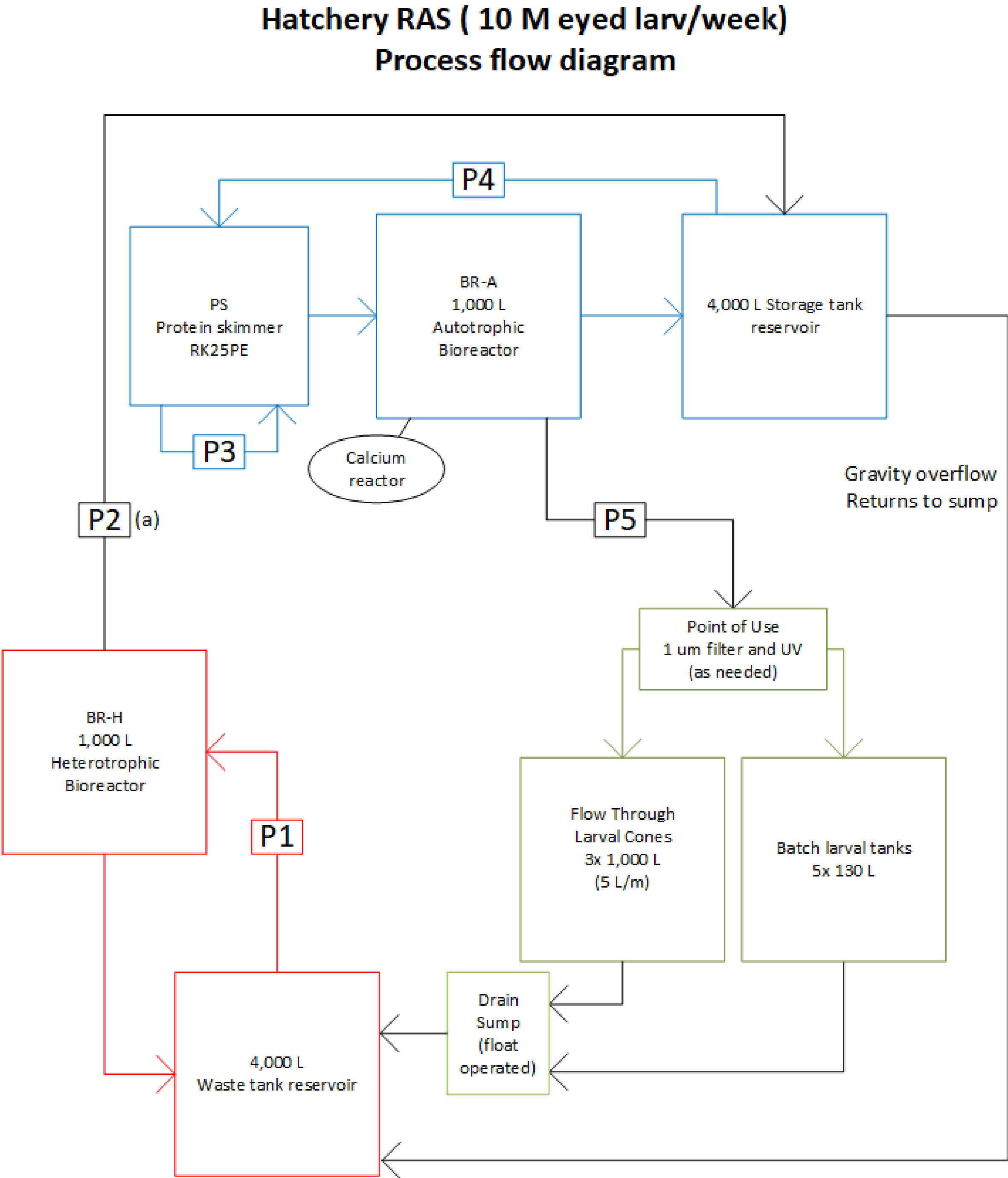


Figure 3: Photo of complete pilot scale system.

Results

The pilot-scale RAS larvae culture system was constructed as designed (see figure 3.) and put into operation in mid May. The heterotrophic bioreactor system was put through a 2 wk "start-up" starting on April 21, 2021 as directed by Co-PI Snyder. A summary of the startup protocol used is included at the end of this report. Results of bacteria and microflagellate counts from are reported in Future 4 below. Initial fill and dose with 100 mg L^{-1} organics resulted in a rise in bacterial numbers peaking in 24 hours, followed by increase in microflagellates grazing on bacteria at 48-54 hours (4-4.5 days). Second dose of 50 mg L^{-1} organics resulted in another bacteria peak at 8 days followed by a decrease in bacteria and microflagellates in the planktonic phase, even with an additional 10 mg L^{-1} organic dose. This indicated the microbial biomass in the system shifting to the biofilm phase from plankton phase as desired. complete drain and refills at 12 and 14 days, completes the planktonic to biofilm shift and prepared the system for use.

Figure 4: Bacteria and Microflagellate counts form heterotrophic bio-reactor start-up.

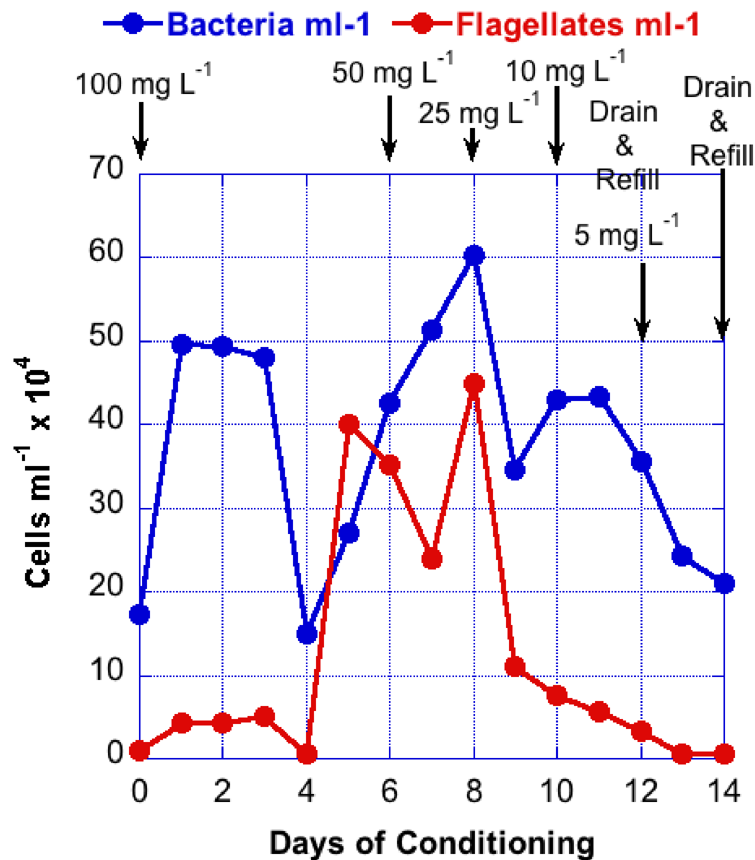
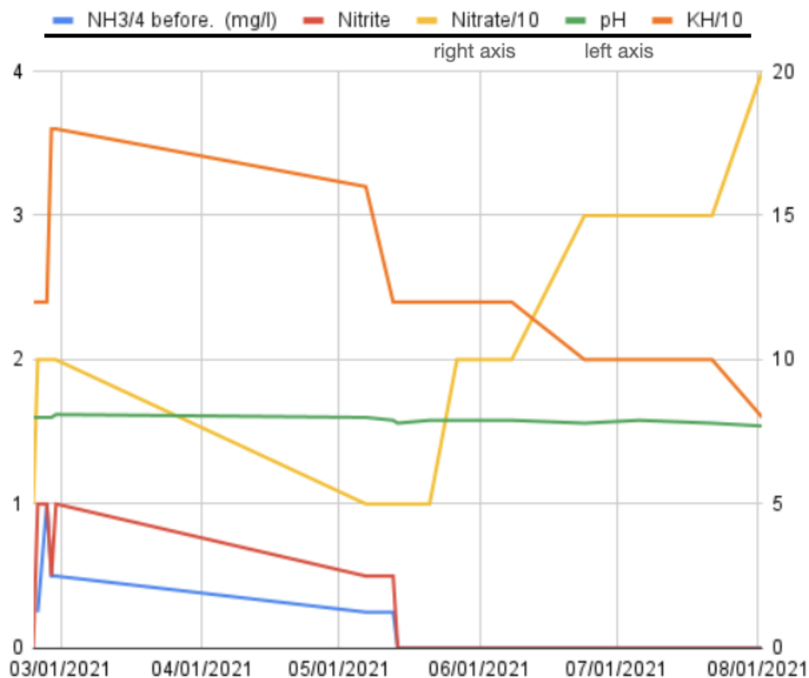


Figure 5: Ammonia, nitrate, nitrite, pH and alkalinity of autotrophic reactor during culture period.



Nutrient and carbonate chemistry parameters monitored are reported above in figure 5. Generally, these parameters were within acceptable range, but note that pH and alkalinity drop over time. There was a failure of the calcium reactor during the project period which was mean to control these two parameters.

A total of 15 larvae cultures were attempted with the first spawn started on 5/13/21 and the last spawn started on 8/19/21. For each RAS larvae culture attempted, a SOP analog was also attempted as a relative control on RAS performance. Each spawning event was conducted via a strip spawn using working-water from the respective systems. Embryos destined for the RAS system were created by suspending stripped eggs in seawater from the RAS system and completing fertilization in RAS water. Embryos destined for the SOP system were created by suspending stripped eggs in seawater from the SOP system and completing fertilization in RAS water.

Of the 15 cultures attempted in the RAS system, 8 resulted in viable pediveliger larvae. The quantity of larvae produced ranged from 0.1-3.9 million larvae. The percentage of pediveliger larvae produced from each completed culture ranged from 0.3-7.8% with an average of 4.8%. Hatch rate (survival of larvae from eggs to 2 day old larvae) ranged from 14-71% with an average of 42%. Of the 15 SOP cultures attempted, 10 resulted in viable pediveliger larvae. the percentage of pediveliger larvae produced from each completed culture ranged from 0.5-13.8% with an average of 4.3%. Hatch rate ranged from 13-35% with an average of 26%.

Figure 6: Hatch rate (percent survival to day 2) for the RAS culture in blue and the SOP culture in green for each culture attempted. Organized by start date of spawn.

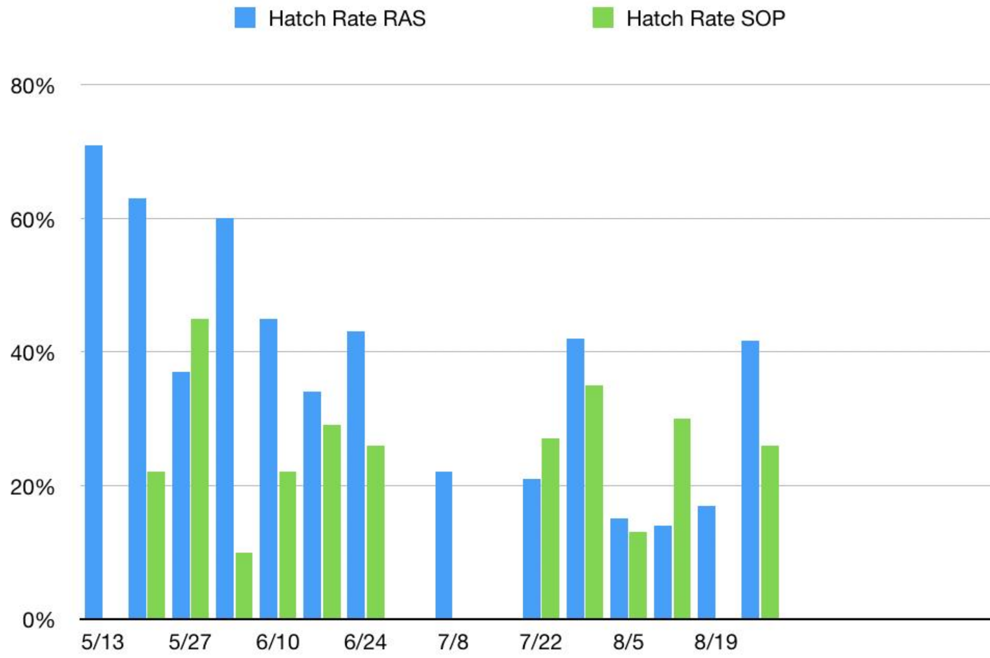
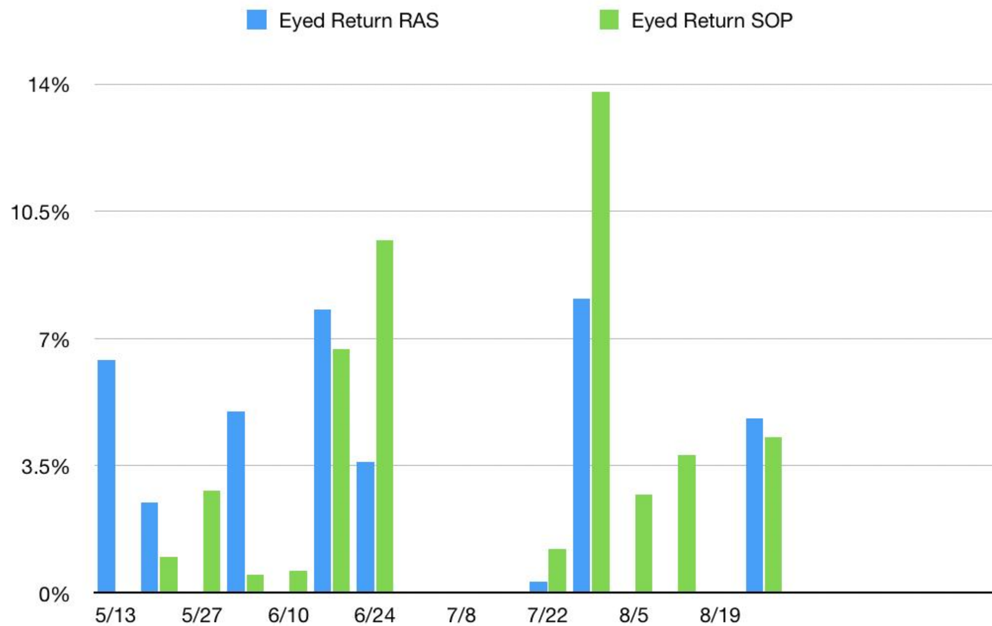


Figure 7: Percentage of recovered pediveliger larvae from each attempted spawn. RAS cultures in blue, Sop cultures in green. Organized by start date of spawn



Conclusions/Recommendations

Generally, we were happy with the results obtained during the course of this project. While we did not achieve the designed target capacity of the system, we did produce some pediveliger larvae and so have proven that the concept is feasible, with the focus now on optimization. One acknowledges shortcoming in the project was the failure of the calcium reactor early on in the trials. This led to an absence of carbonate chemistry control and subsequent decreased pH and alkalinity. While we thought at the time, and had originally hypothesized that the low biomass of the larvae being cultured in the recirculated water would not be sufficient to reduce significantly the pH or alkalinity; whether form the larvae directly, or from the biofilms in the reactors, or most likely a combination of the two, we did observe a decrease in pH from 8.1 to 7.7 and a reduction of alkalinity form 180 to 80ppm which undoubtedly induced stress in larvae. This verifies that some method of carbonate chemistry control will indeed be necessary for RAS larvae culture.

Larval performance was measured using two metrics: hatch rate, and survival of eyed larvae. One of the more surprising results from the trial was that the hatch rate, (survival form day 0-2) was nearly always better in the RAS treatment with an average of 42% across all cultures compared to only 26% in SOP (Figure 6). It is not yet clear what mechanism might have caused this difference, and without monitoring of water parameters in SOP culture it is impossible to

compare the two water sources. It is our intention in future work to maintain water quality monitoring for both the RAS and SOP.

The period in which the trials were conducted, was sub-optimal in terms of SOP performance with hatch rates and survival to eyed larvae lower than normal. Under normal water quality conditions hatch rate and survival to eyed larvae for SOP cultures is around 45% and 20% respectively. During the trial period SOP hatch rate and survival to eyed larvae was 26% and 4.2%, substantially lower than normal. These numbers imply a "period of poor water quality" during the trial period. Despite this "poor water" the RAS system returned consistently good hatch rates and frankly is a good reason to maintain a RAS system even if no larvae are cultured past day 2 in it (assuming equal performance of RAS and SOP after). This is because the hatch rate represents a significant early loss of total larvae culture population size and any survival gains made here represent a proportionally larger savings.

Survival to eyed larvae was similar between the two treatments (Figure 7), and while initially this may seem like a good result, it is the intention that the RAS system perform better than SOP during a "poor water" period, therefore transforming what would be poor larvae culture performance in SOP to normal larvae culture performance in RAS. After all, the biggest potential gain in maintaining a RAS larvae culture system is the expectation of consistent larvae culture despite fluctuations in water quality.

DNA processing of media samples obtained to characterize the makeup of the biofilms has not yet been completed. Once available an addendum to this summary will be made.

We are fortunate to have two more seasons of work on this system supported by a Saltonstall-Kennedy Grant secured by Virginia Tech. We want to thank Virginia Sea Grant and the Virginia Fishery Resource Grant Program for supporting this important work and helping to pioneer in Virginia the use of RAS for larvae culture. Without the support of VASG we not only would have missed out on the important results reported here, but it is likely would not have also been supported by the S-K program. Results of this project were presented at the Virginia Aquaculture Conference in January, 2022 and the video recording of the talk made available on the Conference website (https://vaaquacultureconference.com/). Results of this and future work on the OSH RAS system will be published at the OSH website at https://www.oshoyster.com/.