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Final Report

Feasibility of a recirculating aquaculture system for early larval culture  
of *Crassostrea virginica*

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Priority Area III. – 1.  
Aquaculture/Mariculture: Improve the efficiency of  
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FID:

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Other Participants:

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Oyster Aquaculture Training Program

## Introduction

Oyster aquaculture in Virginia is a rapidly developing industry with greater than 75 million oysters planted in 2010, this compared to just 6 million in 2005 (Murray and Hudson 2011). Necessary to support this rapid development, among other things, is a large and consistent supply of larval and seed oysters, from which oyster growers start their crops. Providing this product to the aquaculture industry are hatcheries.

Hatcheries represent the tip of the pyramid in oyster aquaculture. Only a few hatcheries with a handful of workers support a much larger industry of many growout operations collectively employing hundreds of workers. Hatcheries offer advantages over collecting and rearing wild seed in that hatchery seed can be produced from disease resistant broodstock, made to be sterile (increasing its survival, growth rate, and meat yield) and are generally of higher quality and uniformity. These hatchery tricks offer to the grower important advantages that have helped to make their operations successful, driving the rapid development of the industry.

While hatcheries have been critical in the rapid development of the industry, their malfunction also has the potential to jeopardize it. Oyster hatcheries are notoriously inconsistent and are seemingly becoming more so as integral periods of good water quality, essential for predictability, become less frequent. Hatcheries rely heavily on good ambient water quality with varying levels of nominal filtration to produce oyster larvae and seed. Poor water quality, leading to inconsistency in production, can lead to seed and larvae shortages, which would have negative impacts on a budding industry. For example, as growers are just starting to develop large markets to move the vast amount of product being produced in Virginia, a shortage could lead to losses in those markets at a time when it is critical to demonstrate consistency.

Typical hatchery practices consist of collecting embryos from spawning adults and transferring them to large, lightly aerated tanks filled with filtered seawater. The seawater is sourced from a body of water adjacent to the hatchery. The resulting larvae are fed a diet of live micro-algae (also grown at the hatchery) on a daily basis with 100% water changes completed every other day. The quality of this water fluctuates throughout the season and, in some cases, even the day, making consistent production of larvae and subsequent seed production impossible. In the case of Oyster Seed Holdings (OSH), survival in the hatchery in 2011 from egg to ready-to-set larvae dropped from near 40% in March, April, and May to 5% or less for June-Sept. OSH also observed a similar trend in 2010. These fluctuations in hatchery efficiency due to ambient water quality severely limit overall production. The principal period of exceptional mortality is embryogenesis and early larval development.

Inconsistent larval production is not exclusive to Virginia or oysters for that matter, but is experienced by hatcheries all over the world producing various different species. One method

many hatcheries, especially fish and shrimp systems, employ to get around the inconsistencies of ambient water quality is the use of recirculating aquaculture systems (RAS). In short, these are systems that consist of a fixed volume of water pumped in a loop through the culture tanks then through a series of filtration steps -- essentially life support equipment -- before returning to the culture tanks. While these systems can be expensive to setup, the advantage of a properly operating one is a consistent source of culture water.

Very little research has been done on the use of RAS in shellfish culture. In fact, a literature search yielded no results for oyster culture in RAS. However, Dr. David Kuhn recently cultured adult oysters in RAS over an extended time period (publications are currently under review as part of standard academic protocols). Of the already published work regarding shellfish culture in RAS, one study by Merino et al. (2009) compared traditional shellfish larval culture of scallops *Argopecten purpuratus* (similar to standard methods described above) to that of RAS and found faster growth and shorter culture time, but lower survival in the RAS.

It is my belief that the use of RAS in shellfish culture, particularly for a system dedicated to the completion of embryogenesis and early larval development, could significantly increase the consistency and overall production capacity of a hatchery in a time of increasingly inconsistent ambient water quality. Given the lack of research in this area, especially on oysters, this would be a pioneering study with potentially wide impact in shellfish culture should it help to stabilize the rearing of young oyster larvae.

The purpose of this project is to determine the feasibility of early larval culture in RAS through the design, construction, and evaluation of multiple small scale RAS designs for early larval culture (zero to six days post-hatch) of *C. virginica* relative to standard larval rearing practices

### **Location and system design**

All experiments were conducted at Oyster Seed Holdings (OSH), a commercial oyster hatchery located on Gwynn's Island in Mathews County, VA. The entire study lasted approximately sixteen weeks, beginning on May 17 and ending on September 5, 2012.

This study used two systems: a recirculating aquaculture system (RAS) and a standard operating procedure system (SOP). Both systems consisted of three 200 L round, flat-bottom tanks (approximately 5% scale of OSH commercial gear). Water from each tank traveled through an outflow tube to a Pro Clear Aquatic Systems sump (Jacksonville, FL) located below the tank. In the RAS system water passed through several filtration devices located in the sump

before being returned to the tank via a sump pump. To retain larvae in these two systems, banjo screens were used. The banjo screen is a circular piece of PVC with Nitex screen glued to both sides. The screen can be different sizes depending on the size of the larvae. For the RAS and SOP systems, banjo screens with 20 and 48  $\mu\text{m}$  mesh was used, providing 14% and 31% open area, respectively. Three RAS designs were tested for this study, with each design requiring the addition of one or more filtration devices. All tanks were initially stocked with between three to six million eggs, depending on availability.

In RAS Design 1, once water exited the culture tank it passed through approximately 11 L of biological media (bio balls specifically) housed in the sump. From there water was pumped back into the tank at approximately 12 L/min, allowing the water in the tank to be completely replaced four times an hour. Water recirculated continuously, beginning when eggs were introduced to the tank, and larvae were fed once a day.

In RAS Design 2, once water exited the tank it passed through the bio ball filter and then a Marineland Marine Pro Series In-sump protein skimmer Model 100 (Blacksburg, VA) located in the sump. Water passed through a Laguna UV Sterilizer/Clarifier light (Mansfield, MA) on its return trip to the tank at approximately 12 L/min. This allowed water in the tank to be completely replaced four times every hour. Tanks remained static until larvae were a day old. During the static period and during normal work hours water recirculation was re-routed so that it did not recirculate through the culture tank, but still recirculated through the filtration devices. Water recirculated in this manner for approximately six to seven hours so that larval feed would not be removed from the water by filtration. Overnight the system received seventeen to eighteen hours of recirculation.

In RAS Design 3 a mechanical filter, activated carbon filter, and Kent Marine phosphate sponge filter media (Franklin, WI) were added to the system. The order of filtration became: activated carbon and phosphate sponge filter media, mechanical filter, bio ball filter, protein skimmer, and UV light. Tanks remained static until larvae were a day old. Water was allowed to recirculate through the culture tank and filtration gear for approximately six to seven hours during the day, with feeding occurring at the end of this period. At night water recirculation was re-routed to eliminate the culture tank but still permit water to recirculate through the filtration devices.

In RAS Design 4 the phosphate sponge filter media was removed, making the order of filtration: activated carbon, mechanical filter, bio ball filter, protein skimmer, and UV light. Tanks remained static until larvae were a day old. Water was allowed to recirculate through the culture tank and filtration gear for approximately six to seven hours during the day, with feeding occurring at the end of this period. At night water recirculation was re-routed to eliminate the culture tank but still permit water to recirculate through the filtration devices.

The control, or SOP system, consisted of three 200 L round, flat-bottomed tanks identical to those used in the RAS system. The SOP tanks had water recirculation, but without any filtration devices. Instead, water was collected in a 20 L bucket before returning to the tank at a flow rate of 12 L/min. At this flow rate, water in the tank was completely replaced four times

every hour. The SOP tanks followed the same feeding and recirculation schedule as the RAS tanks. Aeration was provided by an air stone located at the bottom of each tank.

## **Sampling**

### *Larvae*

Both RAS and SOP tanks were drained when larvae were two, four, and six days old. To ensure that the same water was used in the RAS tanks throughout the experiment, these tanks were drained into a separate 200 L holding tank with a 20 µm screen over top to collect the larvae. Larvae were rinsed into a beaker for counting and the tank was scrubbed with a sponge before transferring the water in the holding tank back into its original RAS tank. The SOP tanks were drained in a similar manner, with larvae collected on a 20 µm mesh screen, but the water was not saved. Instead, after each draining SOP tanks were filled with new seawater from the OSH head tank.

Larvae were counted under a microscope to determine survival and observations were made on their appearance, activity level, and cleanliness of the water. Larvae were then returned to their respective tanks.

### *Water Quality*

Twelve water quality parameters were measured: alkalinity, pH, temperature, dissolved oxygen, calcium, carbon dioxide, ammonia, nitrite, nitrate, phosphorus, potassium, and silica. Silica was added as a water quality parameter mid-way through the study. Ammonia, nitrite, nitrate, phosphorus, potassium, and silica were measured using a HACH DR 2700 Spectrophotometer (Loveland, CO). Alkalinity and calcium were measured using a digital titrator. Carbon dioxide was measured using a HACH carbon dioxide test kit (Loveland, CO). A HACH SensIon 156 Portable Multiparameter Meter (Loveland, CO) was used to measure dissolved oxygen. Temperature was recorded using a mercury thermometer and pH was measured with a Fisher Scientific accumet excel XL15 pH meter (Pittsburgh, PA).

The parameters that were measured daily from the RAS and SOP tanks were pH, temperature, dissolved oxygen, ammonia, nitrite, and nitrate. The remaining parameters were measured twice during the six days larvae spent in either the RAS or SOP systems – on the day eggs were added to the tanks and on one drain day, either day 2 or day 4. Additionally, the twelve water quality parameters were measured twice a week in ambient water and water from the OSH head tank.

## **Maintaining the system**

Daily maintenance of the RAS and SOP systems required cleaning the protein skimmers and mechanical filters, if present, as well as the banjo screens. Three times a week, after the RAS and SOP tanks had been drained, a sponge was used to scrub each tank. Tanks were then rinsed with freshwater before being refilled.

After each experiment the tubing used for water recirculation and aeration was disassembled and soaked in a mild bleach solution to remove any bacteria. The tubing was

rinsed with a thiosulfate solution to neutralize any remaining bleach and rinsed thoroughly with freshwater before the system was reassembled.

If water in the RAS system was replaced with new water from the head tank, the tubes, aeration lines, and filters were cleaned as described above. Additionally, the bio ball filter and sub pump were also cleaned, the former with a freshwater rinse, the later by scrubbing with a sponge before rinsing with freshwater.

### **Static vs. recirculation experiment**

In addition to the four experimental designs already described, a fifth experiment was conducted to test whether recirculation affected survival of larvae from the egg stage to day two. Two 200 L tanks were equipped with water recirculation but no filtration – the same set-up as the SOP system. Two 200 L tanks had no water recirculation and remained static for the entire experiment. Tanks were initially stocked with between three to six million eggs, depending on availability. Once eggs were added, recirculation began in the two tanks with this capability. The other two tanks remained static. After two days the tanks were drained and larvae counted to determine survival as described above. This experiment led to the change in protocol for RAS Designs 2 – 4 in regards to when recirculation was begun in the tanks after egg addition.

### **Sterilized water experiment**

Mid-way through RAS Design 1 a sudden drop in survival was observed, causing the quality of the water to be called into question. Since the only method of filtration used in RAS Design 1 was a bio ball filter, it was suspected that a contaminant had entered the water which the bio ball filter was unable to remove. The RAS Design 1 experiment was put on hold so another experiment, using sterilized water, could be conducted.

For the sterilized water experiment, the RAS tanks were filled with water from the OSH head tank and sterilized with 200 mL of bleach. Thirty minutes were allowed to pass before the bleached water was neutralized with 300 mL of thiosulfate solution. A separate 400 L tank was also filled with water from the OSH head tank and sterilized with 400 mL of bleach to be used as a reserve. Thirty minutes were allowed to pass before the bleached water was neutralized with 300 mL of thiosulfate solution. If the RAS tanks required more water to maintain the water level in the sump it was drawn from the 400 L reserve tank. The SOP tanks were filled with water from the OSH head tank. The SOP tanks were drained on days 2, 4, and 6 and the RAS tanks on days 2 and 6. Survival counts were taken on these days. The RAS water was saved in a separate container as previously described.

### **Calculating adjusted values**

Each of the twelve water quality parameters measured during this experiment existed on their own numerical scale. For example, temperature could range from 20 – 30°C yet nitrite might be between 0 – 0.01 mg/L. The large difference in these values made displaying all of the water quality data on the same graph difficult as the larger values would dwarf the smaller ones.

As a solution, the water quality values were either multiplied or divided by factors of ten so that they fell within the same numerical range. For some values no adjustment was needed, as was the case for pH, dissolved oxygen, carbon dioxide, nitrate, and silica. The values for alkalinity and temperature were divided by ten. The values for calcium and potassium were divided by 100. The values for ammonia, nitrite, and phosphorus were multiplied by 10.

## Results

### *RAS Design 1: Bio ball filtration with constant recirculation*

Four experiments using this design were conducted over an approximately four week period. The first experiment (round 1) ended on day 5 with an average survival of 0.25% in the RAS tanks and 23.6% in the SOP tanks (Fig. 2). In the second experiment (round 2), larvae in the RAS tanks died by day 2 so round 2 ended on this day. Average survival for round 2 was 0% in the RAS tanks and 1.4% in the SOP tanks (Fig. 3). For the third and fourth experiments (round 3 and round 4, respectively) larvae survived until day 6 and were able to be transferred to a flow-through system. Average survival for round 3 was 17.1% in the RAS tanks and 31.1% in the SOP tanks (Fig. 4). Round 3 had the highest survival to day 2 and day 6 out of all the other RAS rounds, regardless of the filtration components used. In round 4, average survival was 1.2% in both the RAS and SOP tanks (Fig. 5).

### *RAS Design 2: Bio ball filter, protein skimmer, and UV light with overnight recirculation*

Four experiments using this design were conducted over an approximately three week period. In the first, second, and fourth experiments (round 7, round 8, and round 10, respectively) larvae were reared to day 6. The third experiment (round 9) was closed on day 2 due to insufficient numbers of larvae caused by high mortality. Survival to day 6 in round 7 averaged 1.4% in the RAS tanks and 0.34% in the SOP tanks (Fig. 6). Survival to day 6 in round 8 averaged 1.7% in the RAS tanks and 2.1% in the SOP tanks (Fig. 7). Survival to day 2 in round 9 averaged 0.28% in the RAS tanks and 0.62% in the SOP tanks (Fig. 8). In round 10 survival to day 6 averaged 3.13% in the RAS tanks and 17.1% in the SOP tanks (Fig. 9).

### *RAS Design 3: Mechanical, charcoal, phosphorus, and bio ball filters, with protein skimmer and UV light with daytime recirculation*

Five experiments using this design were conducted over an approximately three week period. In all rounds except round 14 the experiment was closed on day 2 due to high mortality. Average survival to day 2 in the first experiment (round 11) was 0% in the RAS tanks and 27.6% in the SOP tanks (Fig. 10). In the second experiment (round 12) average survival to day 2 was 0.47% in RAS tanks and 15.5% in SOP tanks (Fig. 11). In the third experiment (round 13) average survival to day 2 was 1.1% in RAS tanks and 0.79% in SOP tanks (Fig. 12). In the fourth experiment (round 14) larvae in the RAS tanks were discarded due to high mortality on day 2 and larvae from the SOP tanks were reared in the RAS tanks to determine if survival was

affected by the RAS environment. Survival to day 2 averaged 1% in the RAS tanks and 25.6% in the SOP tanks. The larvae from the SOP tanks experienced a 50% decline in survival from day 2 to day 6 when they were reared in the RAS system (Fig. 13). The fifth experiment (round 15) had 0.44% survival in the RAS tanks and 3.8% survival in the SOP tanks to day 2 (Fig. 14).

#### *RAS Design 4: Mechanical, charcoal, and bio ball filters, with protein skimmer and UV light with daytime recirculation*

Two experiments using this design were conducted over a one week period. Both experiments were closed on day 2 due to high mortality. In the first experiment (round 16) survival to day 2 averaged 0.03% in RAS tanks and 0% in SOP tanks (Fig. 15). In the second experiment (round 17) survival to day 2 averaged 0.02% in RAS tanks and 7.5% in SOP tanks (Fig. 16).

#### *Static versus recirculation experiment*

Given the low survival in the first RAS design, the original experimental plan was deviated from to determine if exposing eggs to constant water recirculation interfered with the hatch rate. The comparison between rearing eggs in a static versus a flow-through environment was repeated twice over approximately one week. In the first experiment for this design (round 5) larvae were reared in the system until day 2. Average survival for round 5 was 16.3% in tanks where eggs were exposed to constant water flow and 15.3% in static tanks (Fig. 17). In the second experiment (round 6) larvae were reared in the system for six days to determine if an extended time period resulted in a greater difference in survival. In round 6 average survival to day 2 was 42% in tanks that exposed eggs to constant water flow and 57% in static tanks (Fig. 17). Average survival to day 6 was 11.3% in the tanks with constant flow and 28.6% in static tanks. While Round 6 had the highest survival of larvae compared to the other rounds, it should be noted that no filtration components were used in the static versus recirculation experiments, so larvae were not reared under RAS conditions.

#### *Water Quality*

Twelve water quality parameters were measured: alkalinity, pH, temperature, dissolved oxygen, calcium, carbon dioxide, ammonia, nitrite, nitrate, phosphorus, potassium, and silica. These parameters were measured in the RAS and SOP tanks during the experiments described above as well as in the head tank water used by the OSH hatchery and the ambient water.

Alkalinity was similar in the head tank and ambient water, ranging from 71 – 93 mg/L CaCO<sub>3</sub> and from 71 – 94 mg/L CaCO<sub>3</sub> in ambient water during the course of the summer. Average alkalinity in the RAS tanks was similar to that of the head tank and ambient water, ranging between 71 – 89 mg/L CaCO<sub>3</sub> (Fig. 18). The pH was also similar between the head tank and ambient water, though lower overall in the head tank, ranging from 7.53 – 8.15 compared to 7.63 – 8.36 in ambient water. Average pH in the RAS tanks was slightly higher than the head tank and ambient water, ranging from 7.95 – 8.26 (Fig. 19). Temperature was similar for the head tank, ambient water, and RAS tanks, ranging from 23.9 – 30°C in the head tank, from 23.5



– 30.5°C in ambient water, and from 23.9 – 30.8°C in the RAS tanks (Fig. 20). Dissolved oxygen tended to be higher in the RAS tanks, ranging from 6.66 – 8.21 mg/L. Dissolved oxygen in the head tank ranged from 2.13 – 8.23 mg/L and from 4.18 – 8.31 mg/L in ambient water (Fig. 21). Calcium ranged from 462 – 599 mg/L CaCO<sub>3</sub> in the head tank, from 444 – 637 mg/L CaCO<sub>3</sub> in ambient water, and from 442 – 626 mg/L CaCO<sub>3</sub> in RAS tanks (Fig. 22). Carbon dioxide ranged from 8 – 100 mg/L in the head tank, from 6 – 220 mg/L in ambient water, and from 5 – 102 mg/L in RAS tanks (Fig. 23). Ammonia ranged from 0.03 - 0.63 mg/L in the head tank and from 0.11 – 0.47 mg/L in ambient water. In RAS tanks ammonia concentration averaged between 0.06 – 0.26 mg/L (Fig. 24). Nitrite was similar for the head tank and ambient water, ranging from 0 - 0.2 mg/L in the head tank and from 0 - 0.1 mg/L in ambient water. In the RAS tanks nitrite had a higher concentration ranging from 0.002 – 1.09 mg/L (Fig. 25). Nitrate was similar for the two water sources, ranging from 0.1 – 0.5 mg/L in the head tank and from 0 – 0.6 mg/L in ambient water, but had a higher range in the RAS tanks, from 0.3 – 2.2 mg/L (Fig. 26). Phosphorus ranged from 0.02 – 3.7 mg/L in the head tank and from 0 – 2.37 mg/L in ambient water. In the RAS tanks phosphorus averaged between 0.07 – 1.21 mg/L (Fig. 27). Potassium concentrations were similar for all three water sources, ranging from 150 – 350 mg/L in the head tank, from 170 – 310 mg/L in ambient water, and from 180 – 325 mg/L in RAS tanks (Fig. 28). The measurement of silica did not begin until the end of July. For the head tank, silica ranged from 0.7 – 4.3 mg/L and from 0 – 4.9 mg/L in ambient water. In RAS tanks silica ranged between 11 – 4.1 mg/L (Fig. 29).

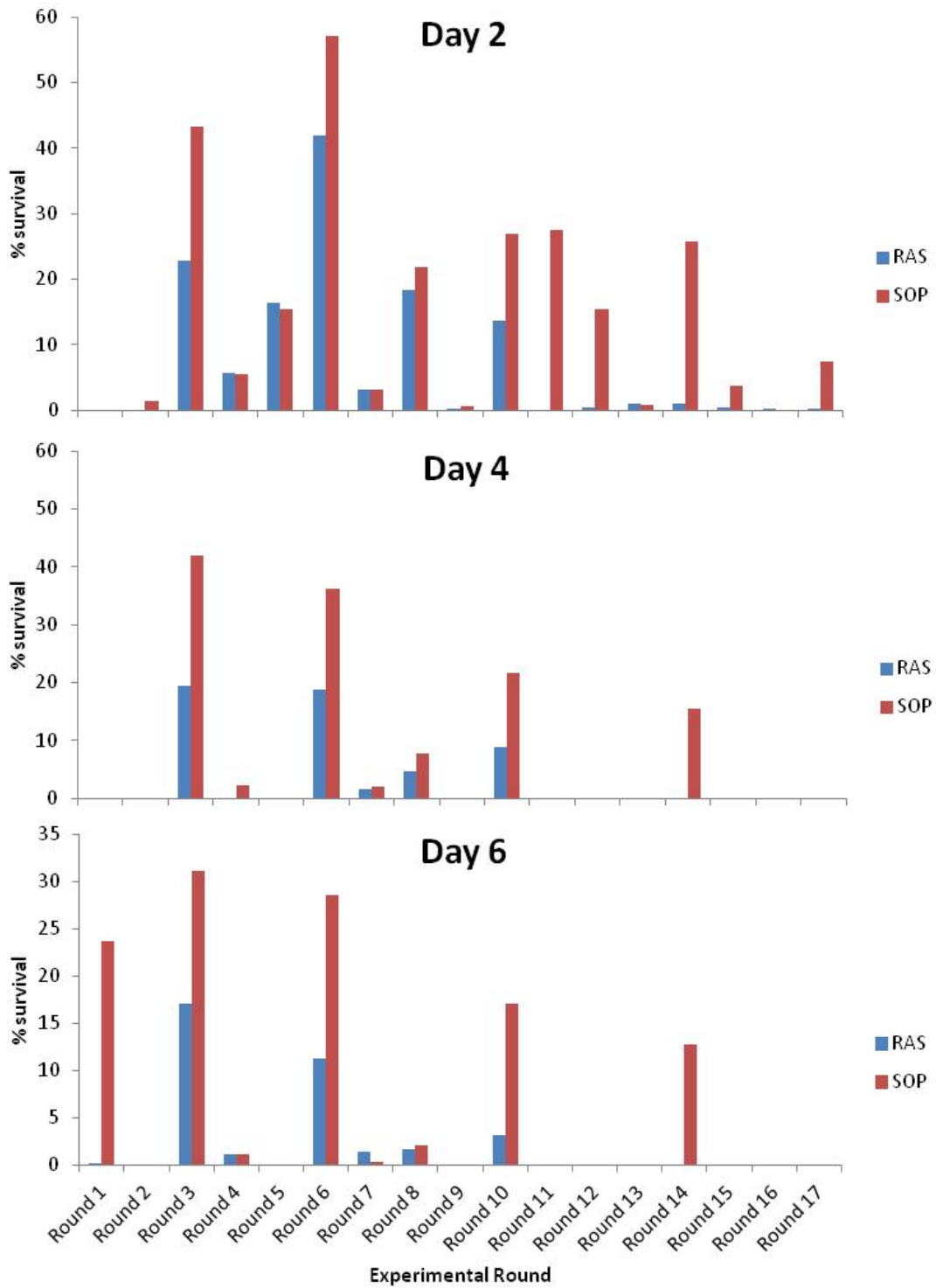


Figure 1. Cumulative survival at day 2, 4 and 6 for all experimental rounds completed.

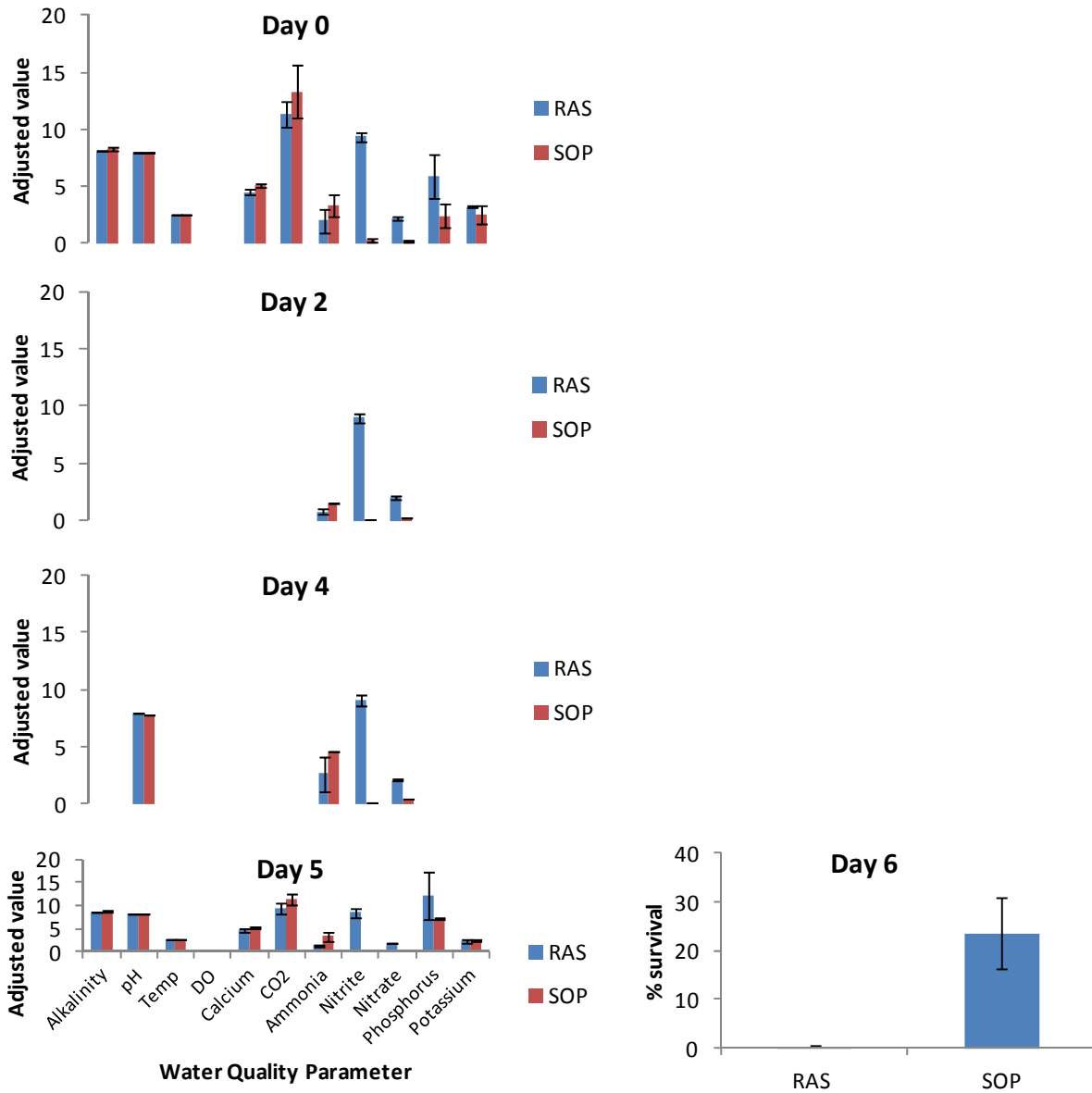


Figure 2. The water quality parameters measured in Round 1 for six days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 6 is given for both systems. Error bars =  $\pm 1$  standard deviation.  $n=3$

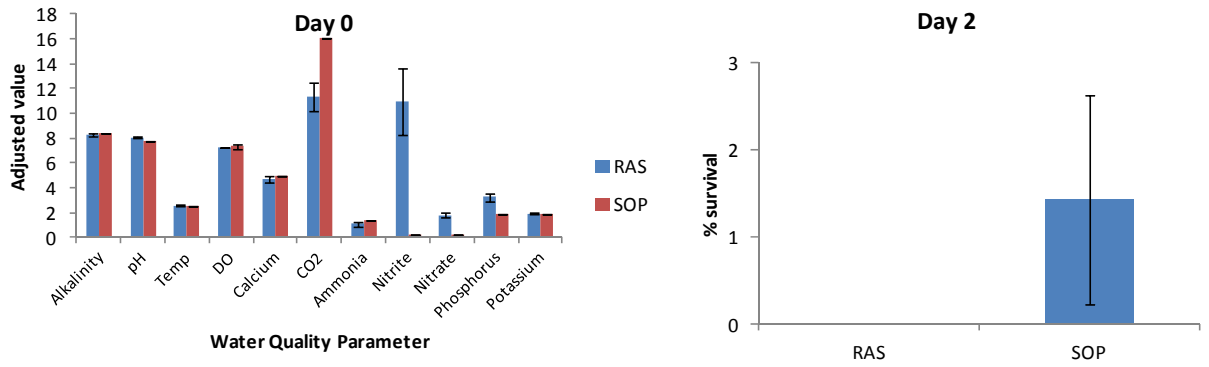


Figure 3. The water quality parameters measured in Round 2 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation.  $n=3$

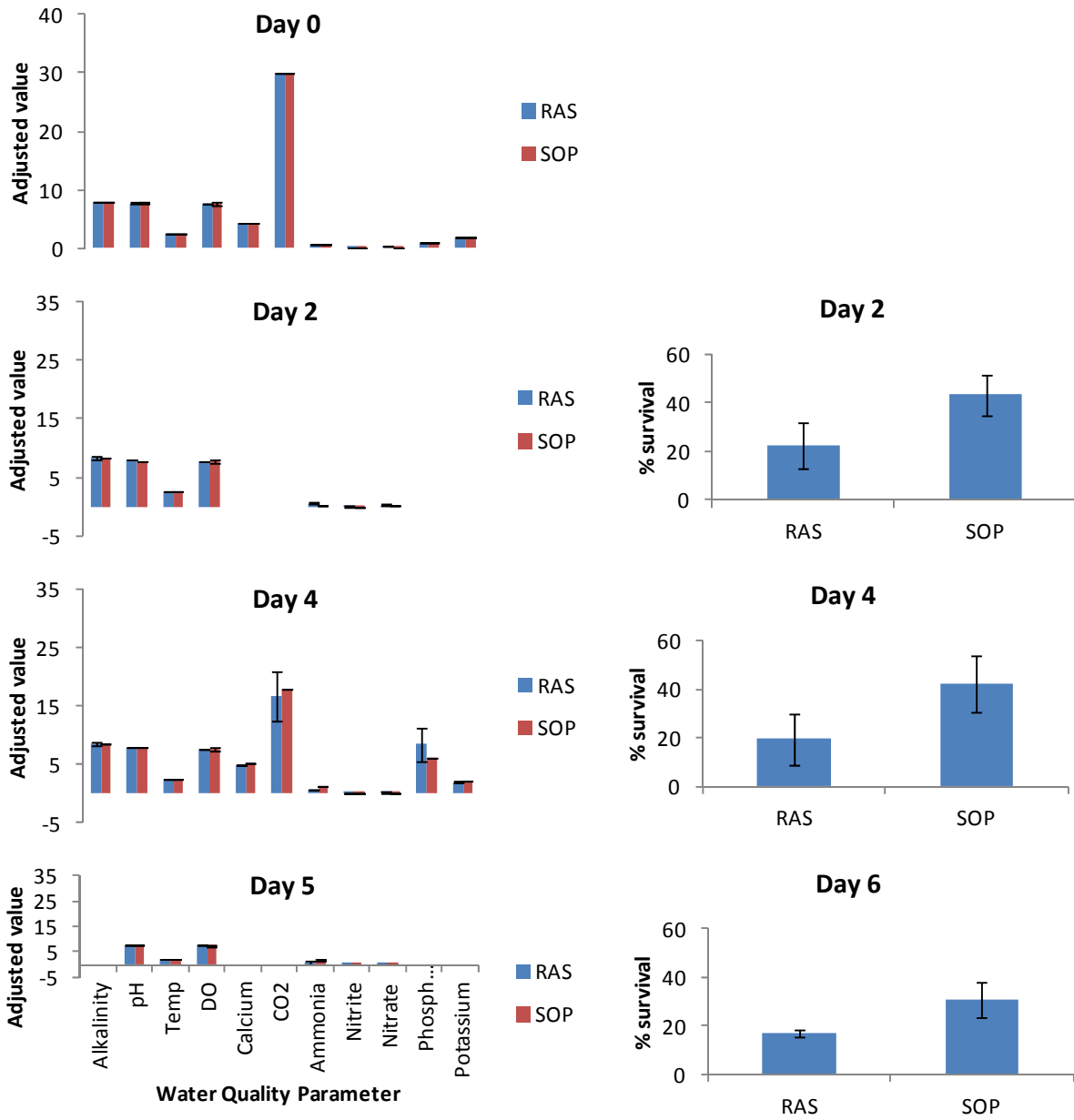


Figure 4. The water quality parameters measured in Round 3 for six days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to days 2, 4, and 6 is given for both systems (right hand column). Error bars =  $\pm 1$  standard deviation. n=3

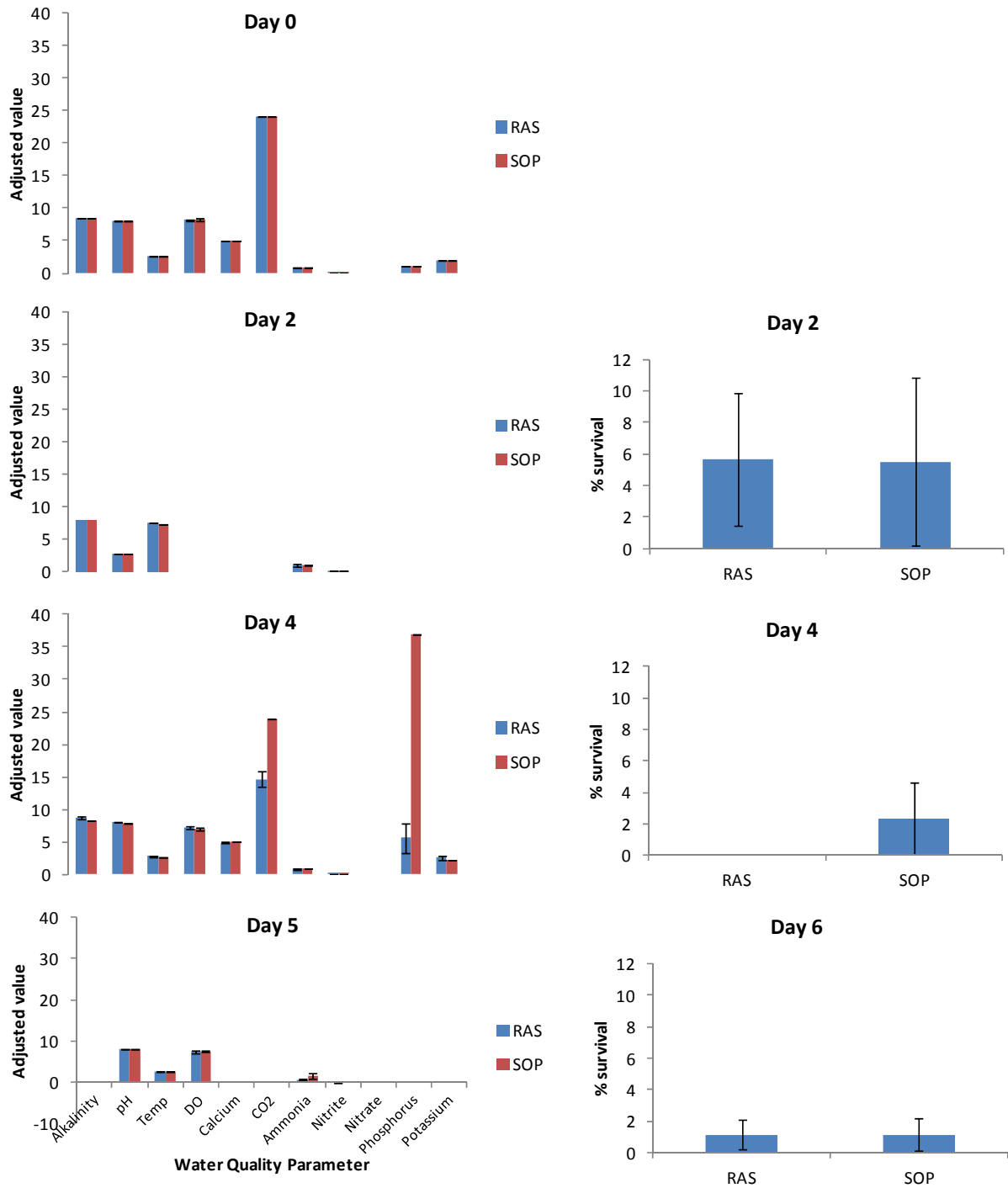


Figure 5. The water quality parameters measured in Round 4 for six days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to days 2, 4, and 6 is given for both systems (right hand column). Error bars =  $\pm 1$  standard deviation. n=3

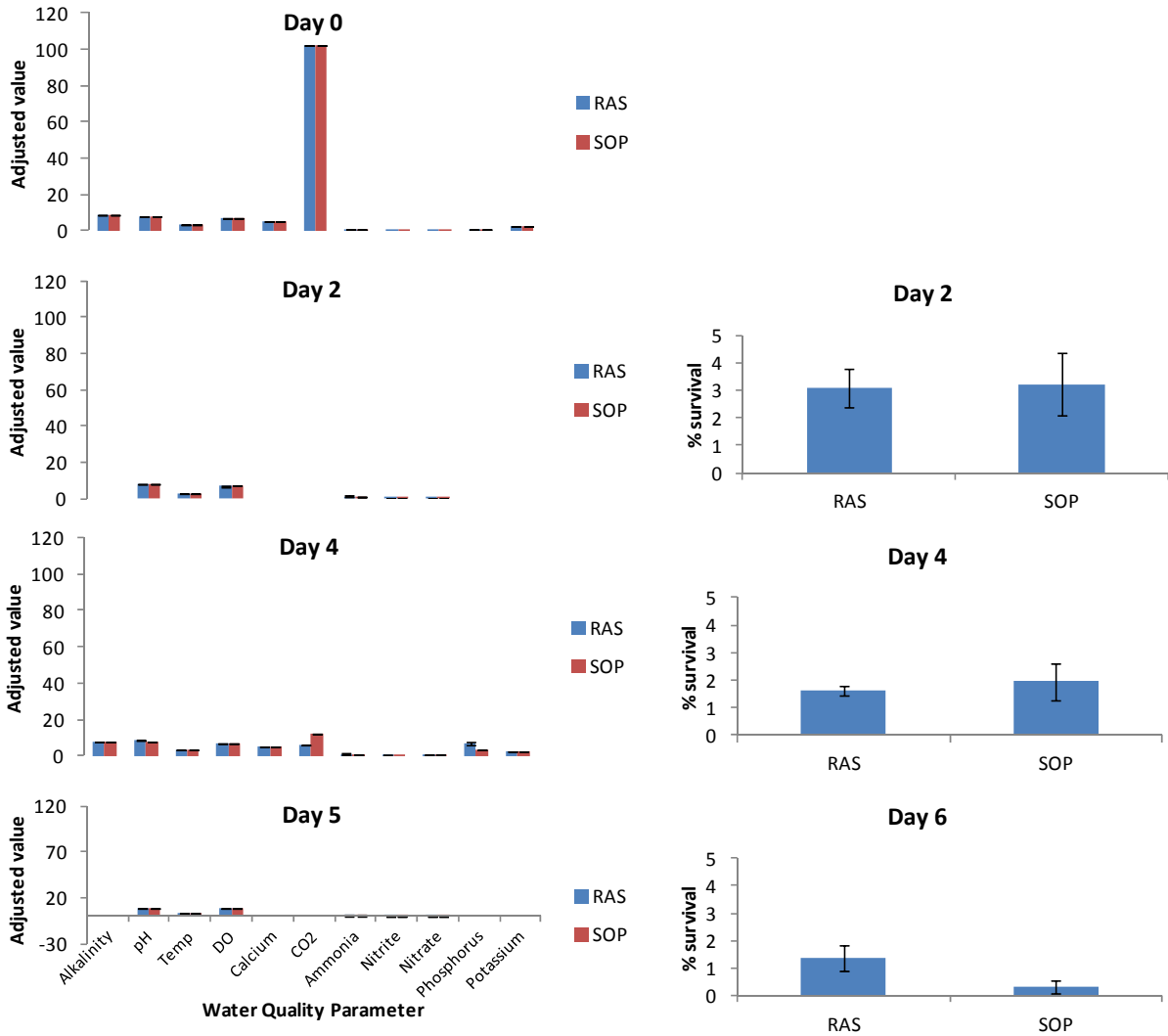


Figure 6. The water quality parameters measured in Round 7 for six days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to days 2, 4, and 6 is given for both systems (right hand column). Error bars =  $\pm 1$  standard deviation.  $n=3$

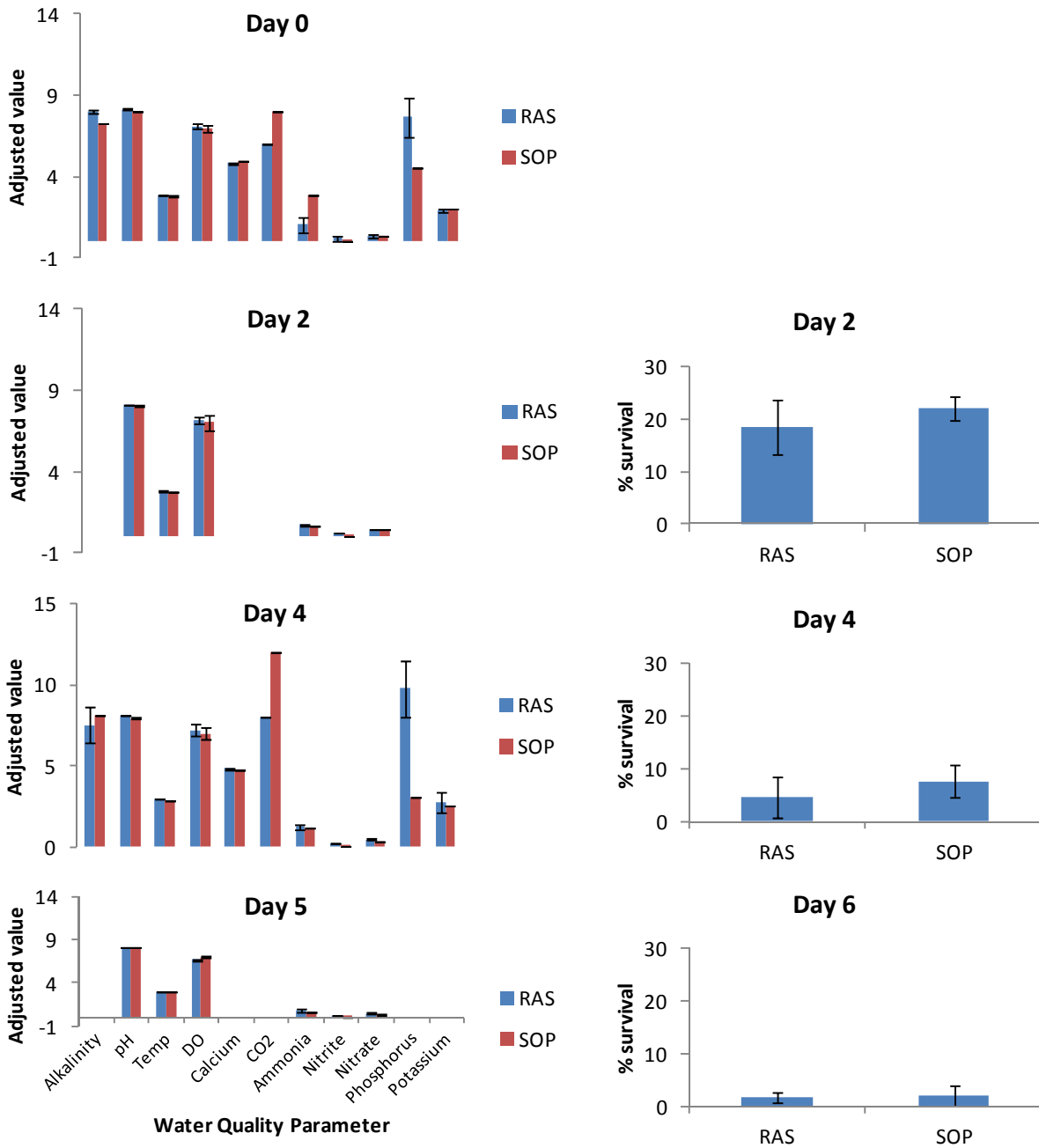


Figure 7. The water quality parameters measured in Round 8 for six days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to days 2, 4, and 6 is given for both systems (right hand column). Error bars =  $\pm 1$  standard deviation.  $n=3$



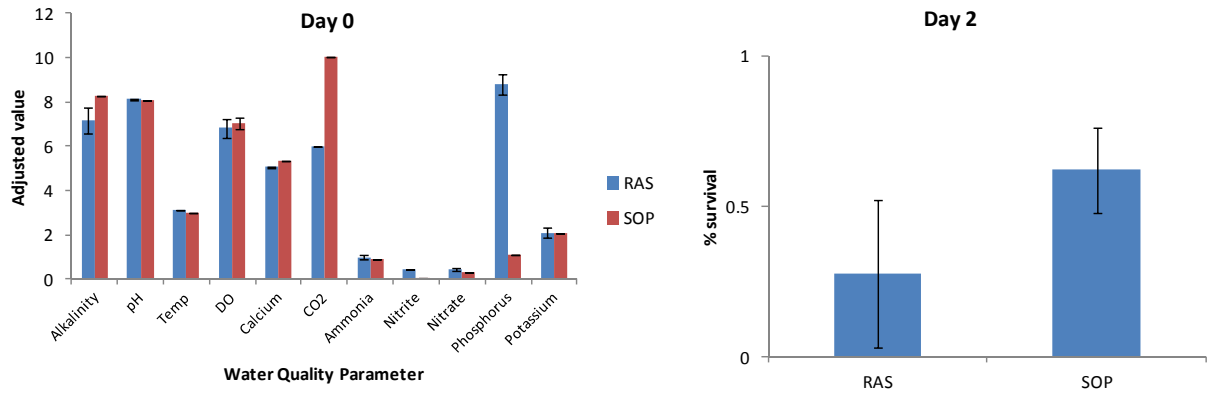


Figure 8. The water quality parameters measured in Round 9 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation.  $n=3$

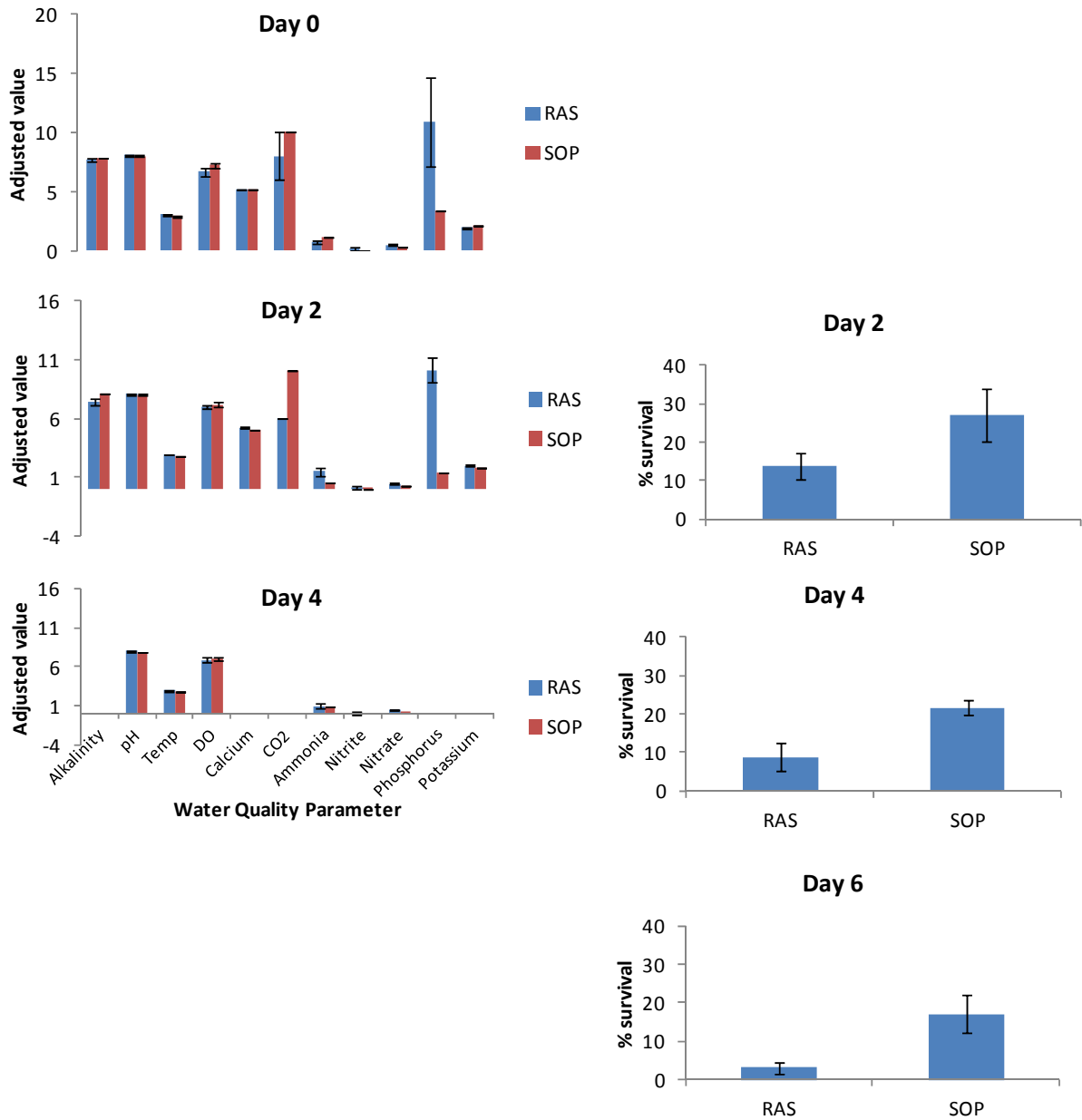


Figure 9. The water quality parameters measured in Round 10 for four days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to days 2, 4, and 6 is given for both systems (right hand column). Error bars =  $\pm 1$  standard deviation.  $n=3$

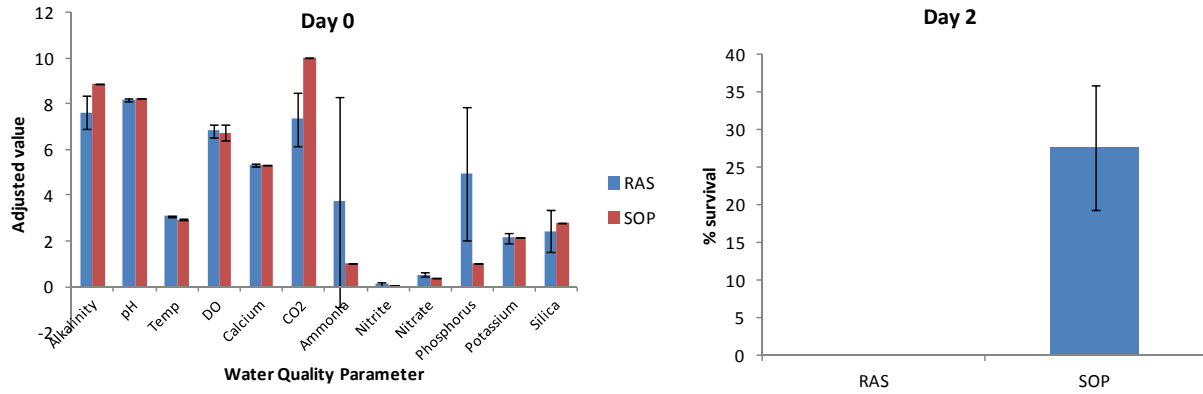


Figure 10. The water quality parameters measured in Round 11 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation. n=3

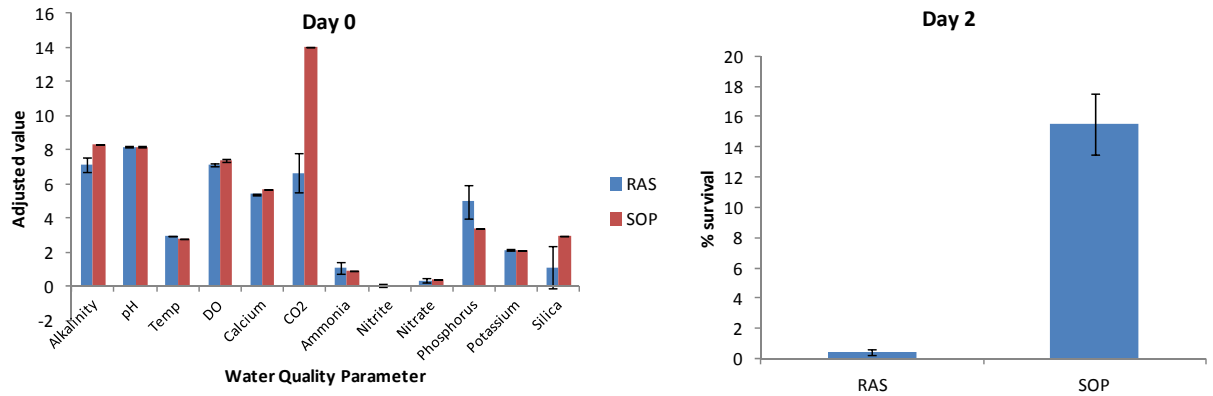


Figure 11. The water quality parameters measured in Round 12 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation. n=3

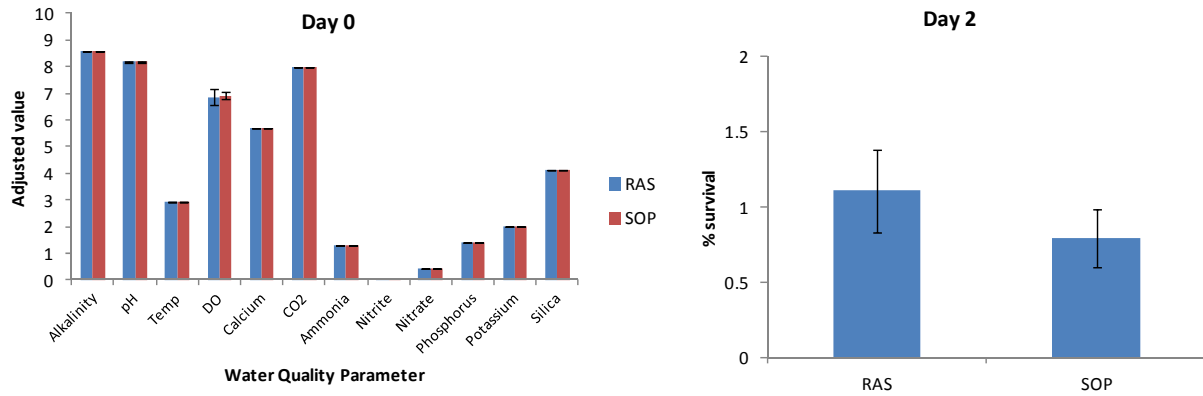


Figure 12. The water quality parameters measured in Round 13 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation. n=3

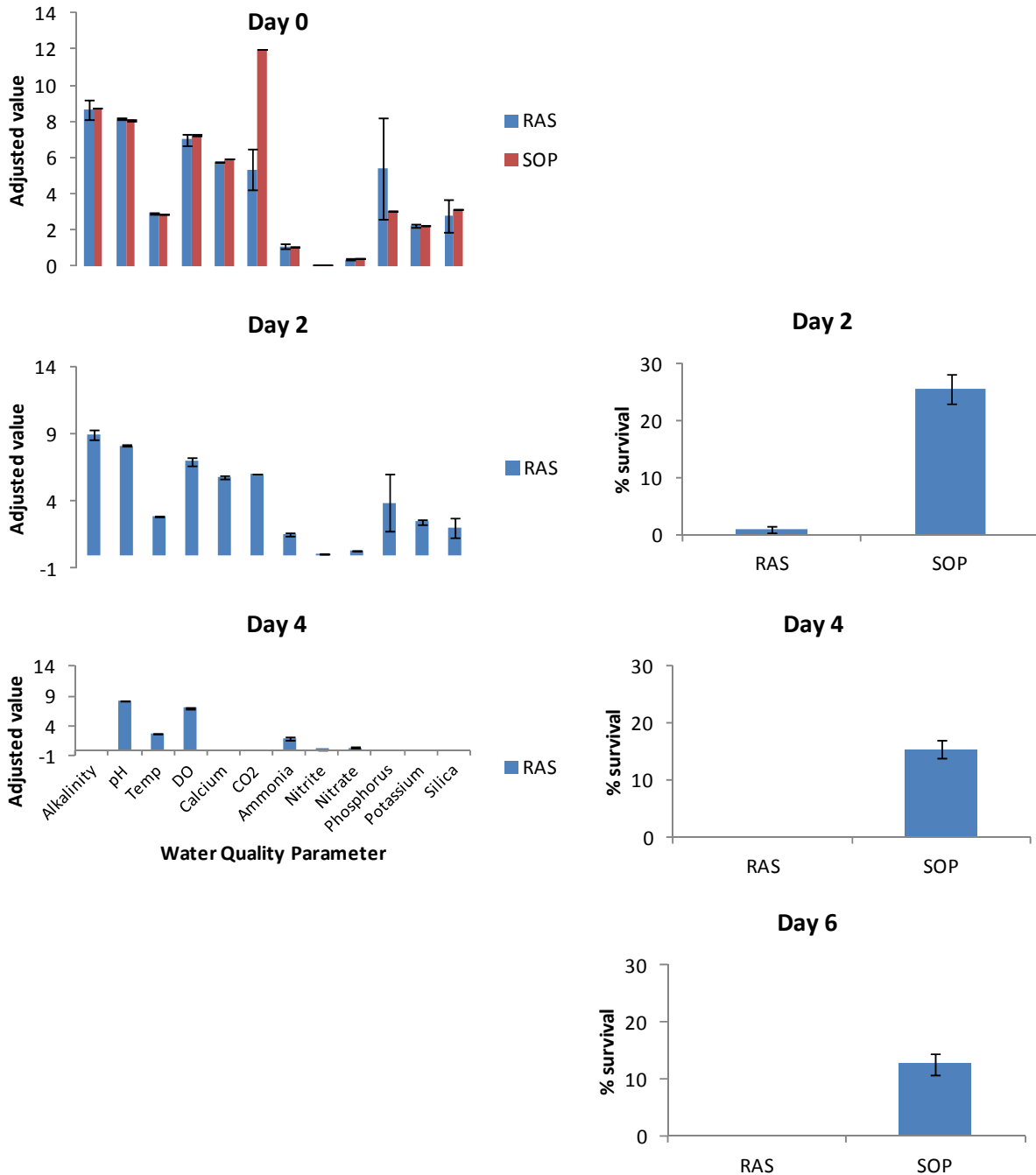


Figure 13. The water quality parameters measured in Round 14 for four days of larval culture in the RAS (blue bars) and SOP (red bars) systems. After day 2 survival in the RAS tanks was low enough to warrant discarding the larvae from those cultures. Survival in the SOP tanks was high, so larvae from the SOP tanks were reared in the RAS system. Water quality parameters are displayed as adjusted values. Survival to day 2 is given for both systems. Survival for SOP larvae reared in the RAS system is labeled as SOP in the graph for days 4 and 6 (right hand column). Error bars =  $\pm 1$  standard deviation.  $n=3$

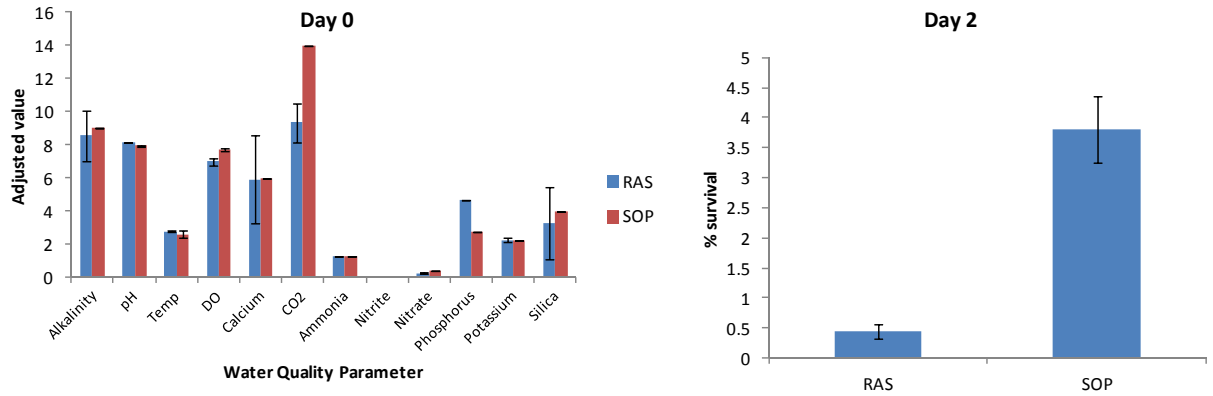


Figure 14. The water quality parameters measured in Round 15 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation.  $n=3$

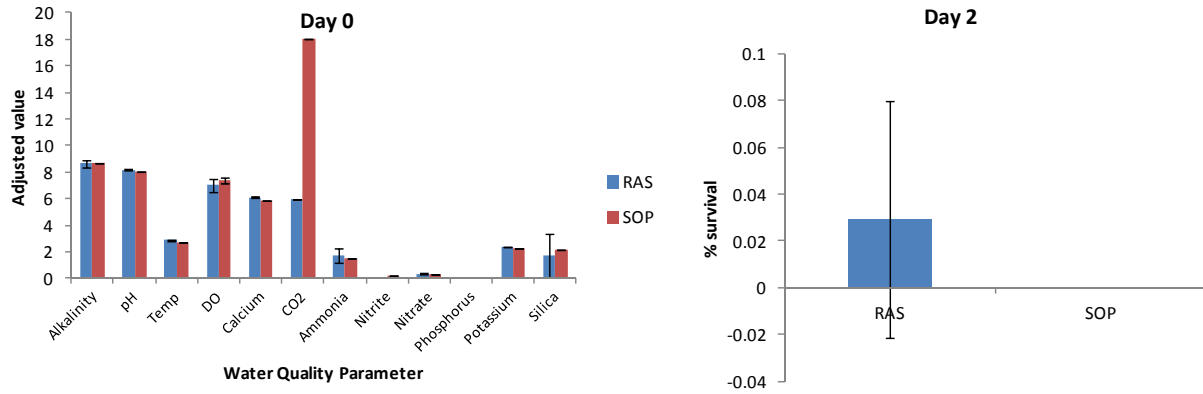


Figure 15. The water quality parameters measured in Round 16 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation. n=3



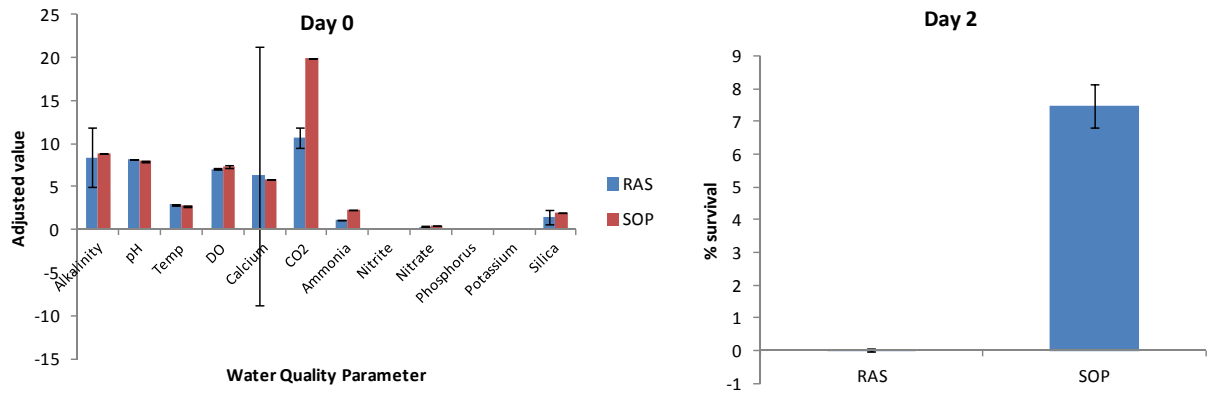


Figure 16. The water quality parameters measured in Round 17 for two days of larval culture in the RAS (blue bars) and SOP (red bars) systems. Water quality parameters are displayed as adjusted values. Survival to Day 2 was considered insufficient to continue the experimental round and the cultures were closed. Error bars =  $\pm 1$  standard deviation. n=3

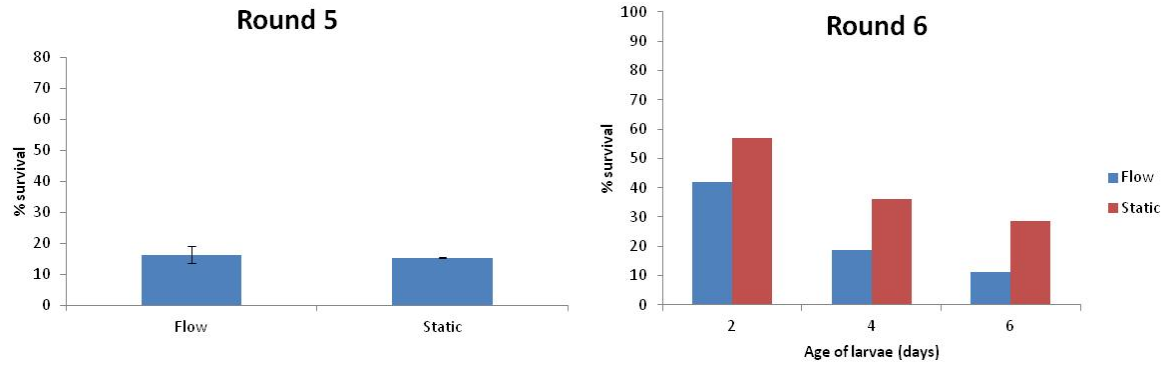


Figure 17. Survival for larvae exposed to continuous water recirculation from the time of fertilization (flow) and for larvae exposed to no water recirculation during their time in culture (static). Error bars =  $\pm 1$  standard deviation.  $n=2$

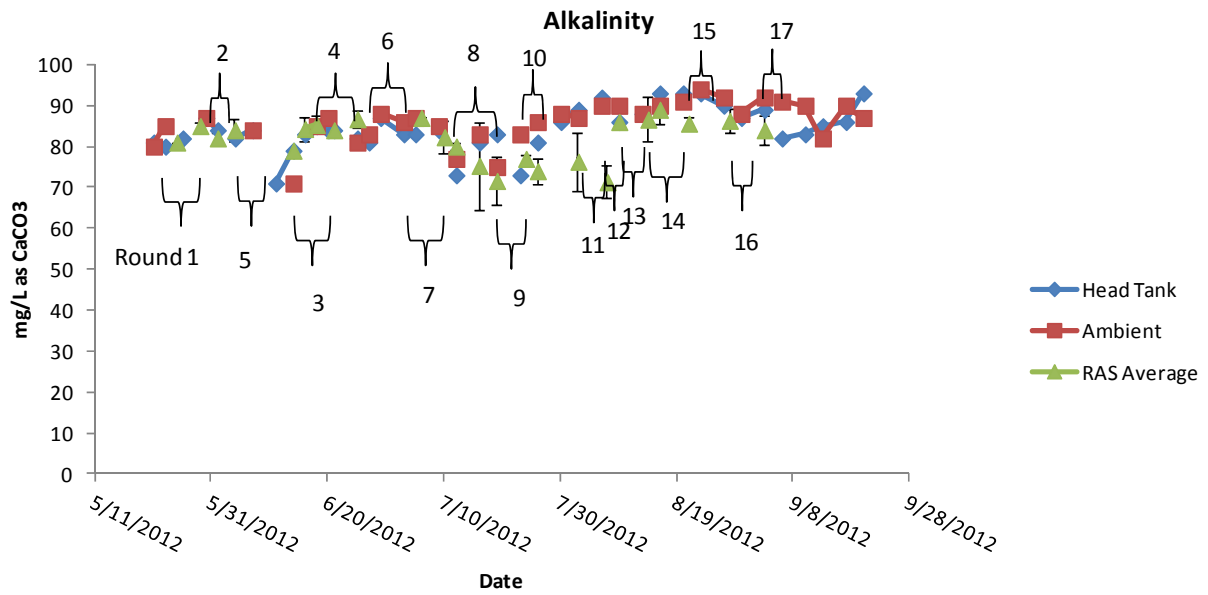


Figure 18. The alkalinity measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average alkalinity for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

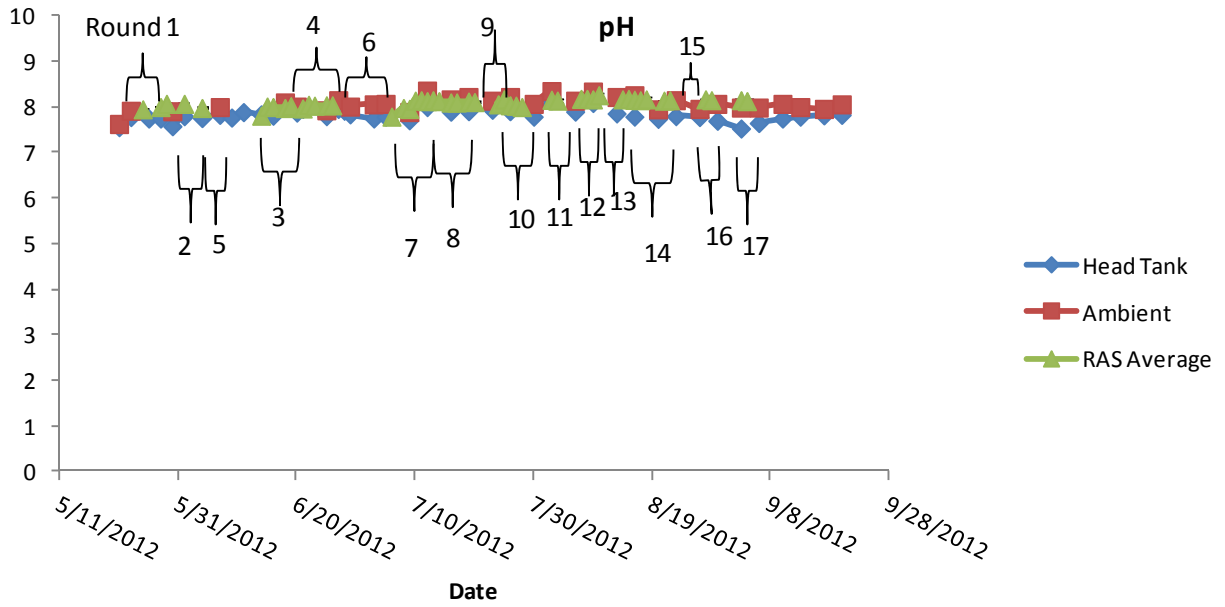


Figure 19. The pH measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average pH for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer.

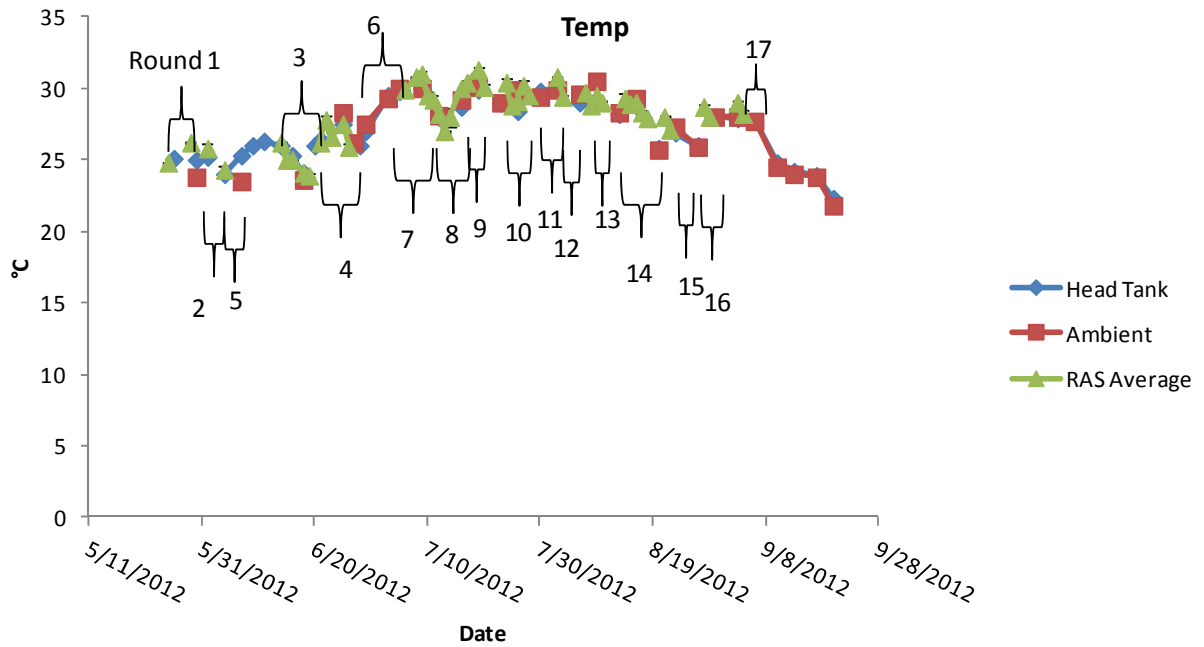


Figure 20. The temperature measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average temperature for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

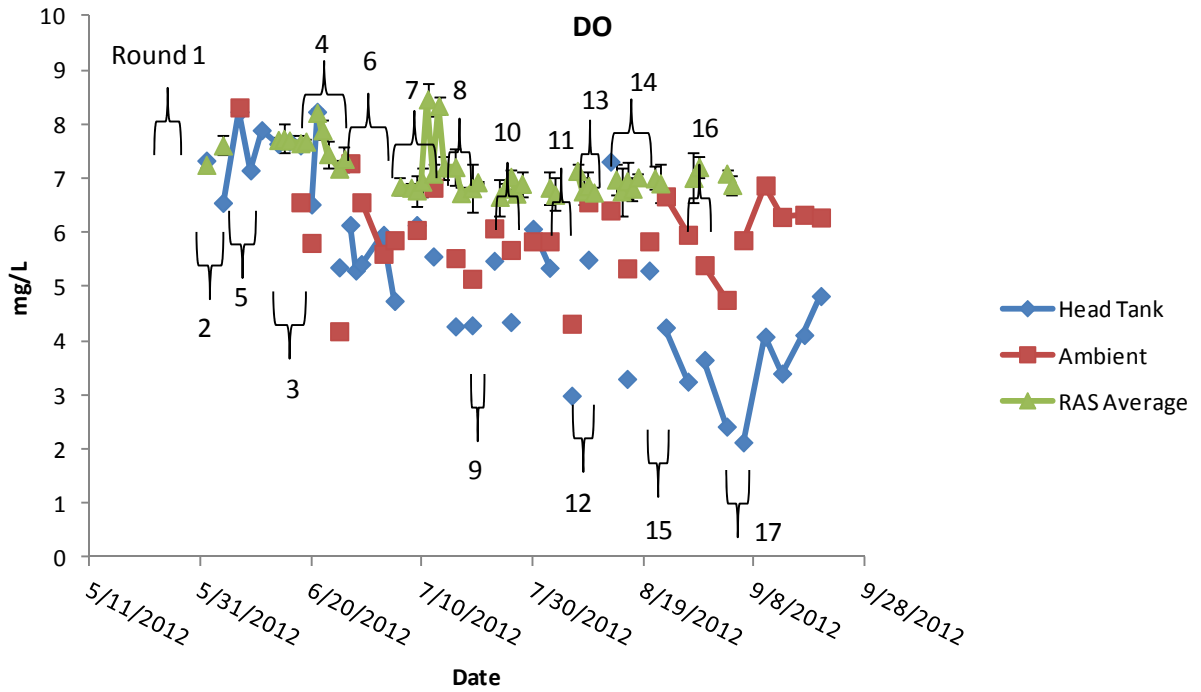


Figure 21. The dissolved oxygen measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average dissolved oxygen concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

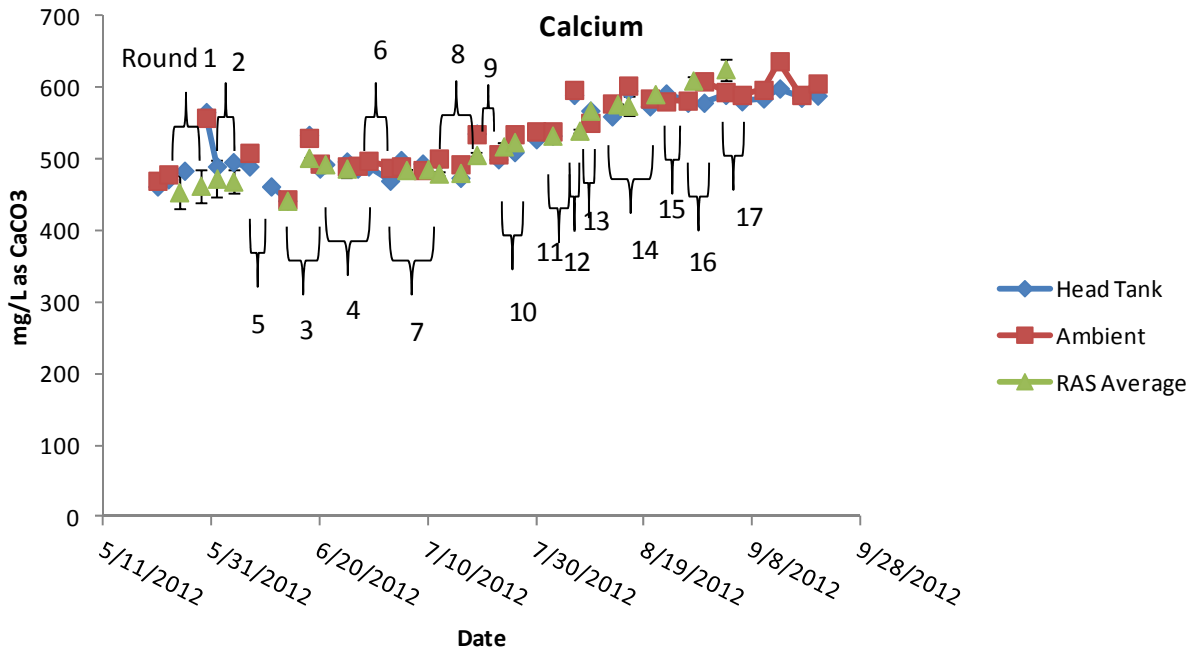


Figure 22. The calcium measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average calcium concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

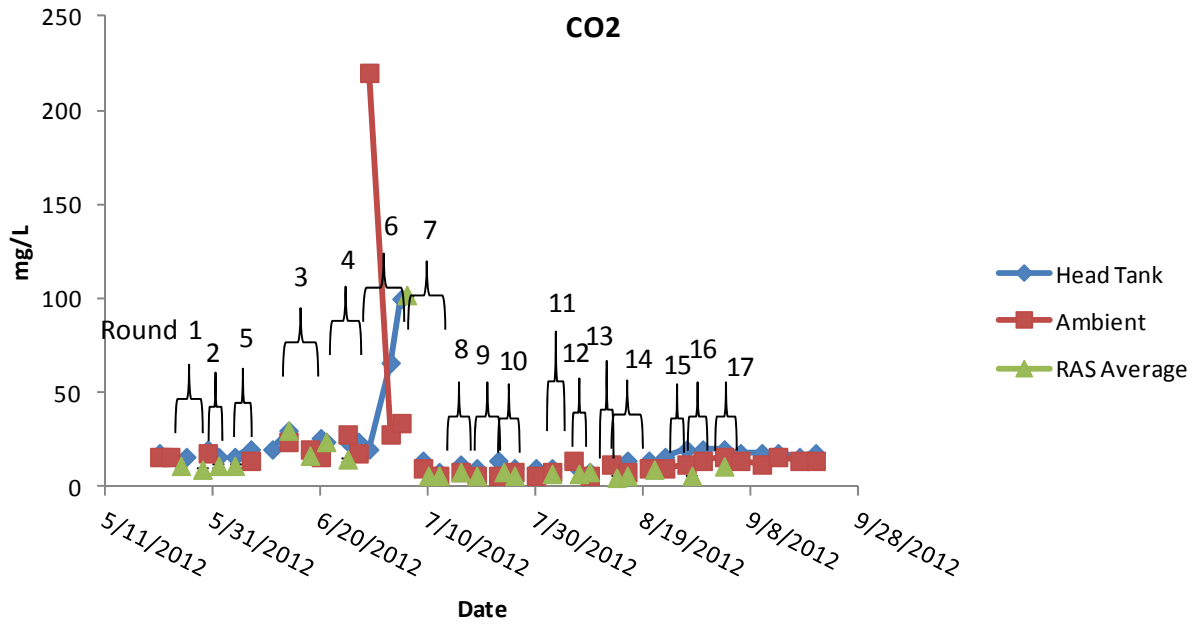


Figure 23. The carbon dioxide measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average carbon dioxide concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$



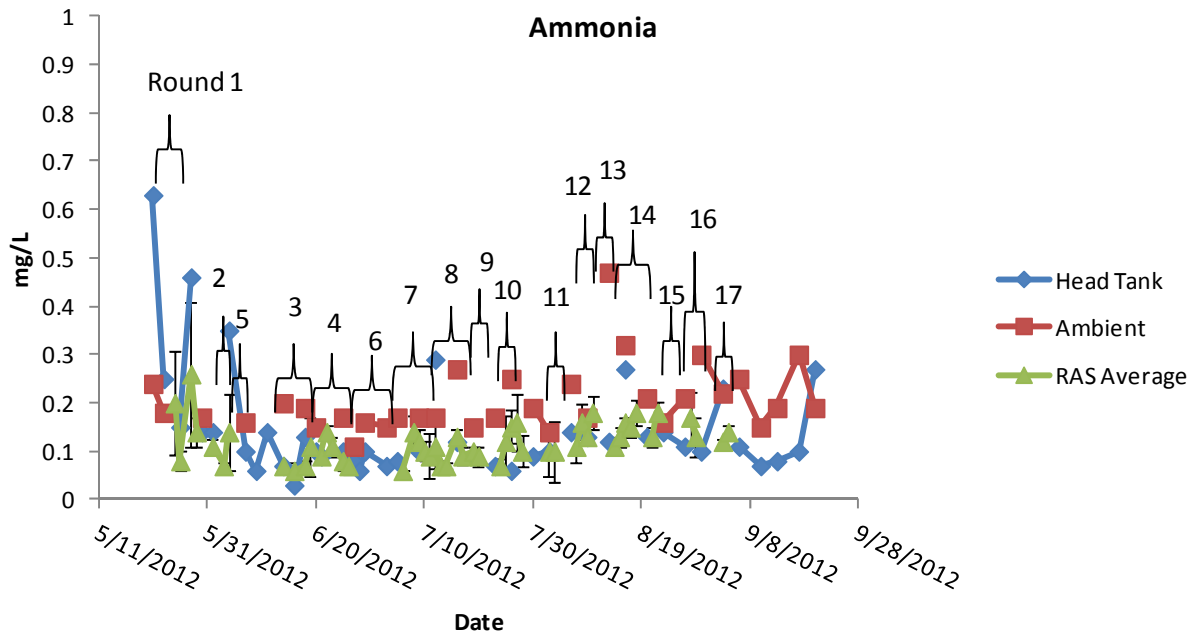


Figure 24. The ammonia measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average ammonia concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

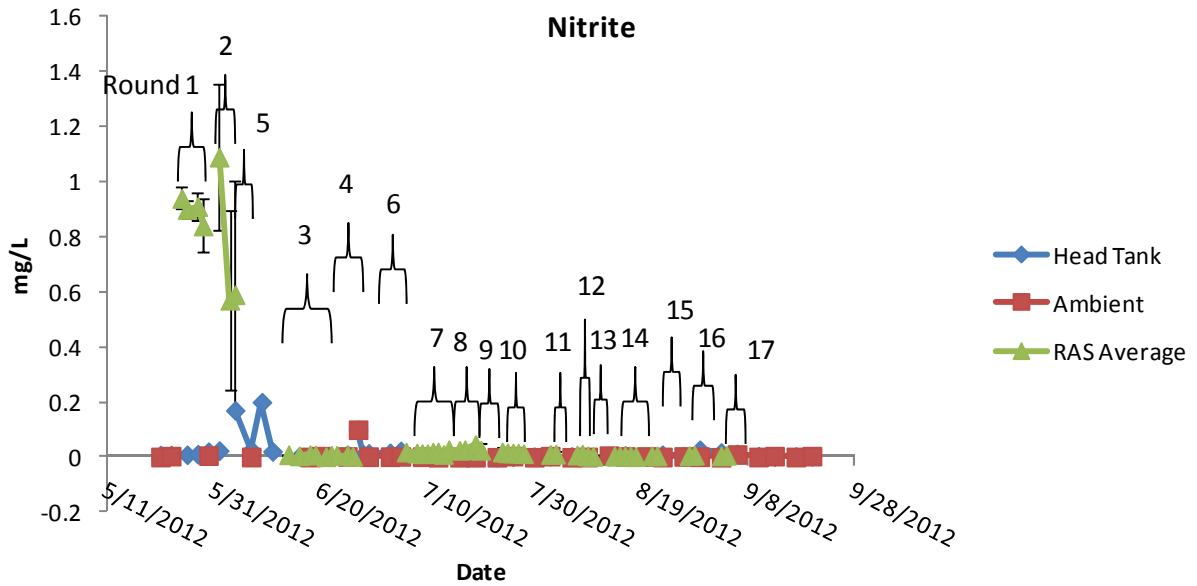


Figure 25. The nitrite measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average nitrite concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

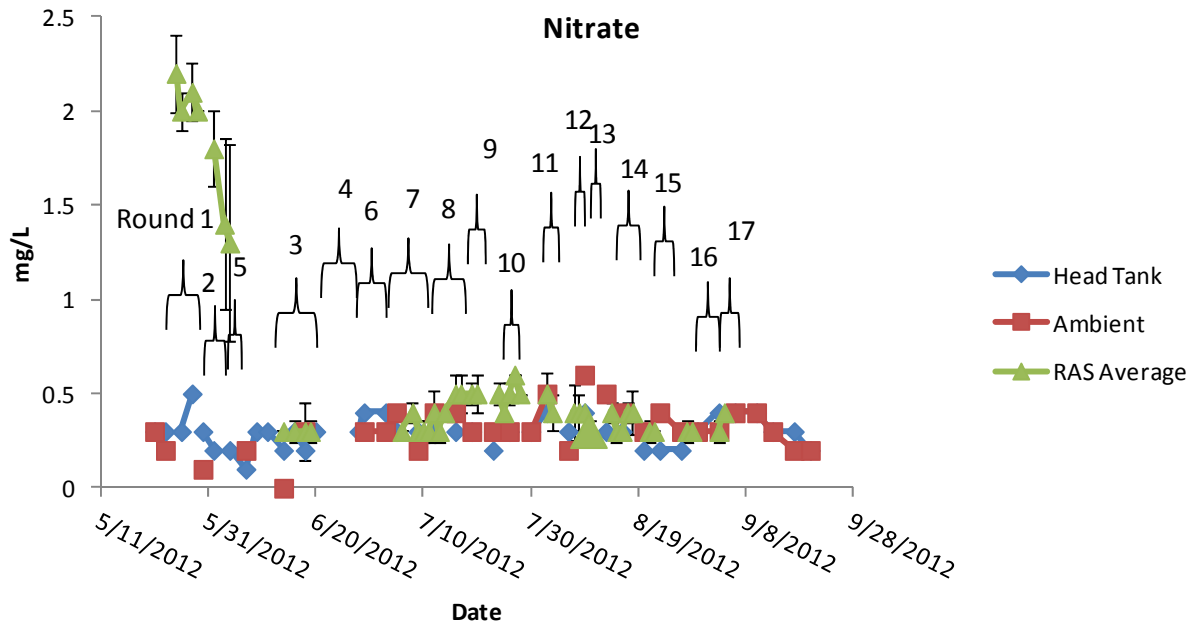


Figure 26. The nitrate measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average nitrate concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

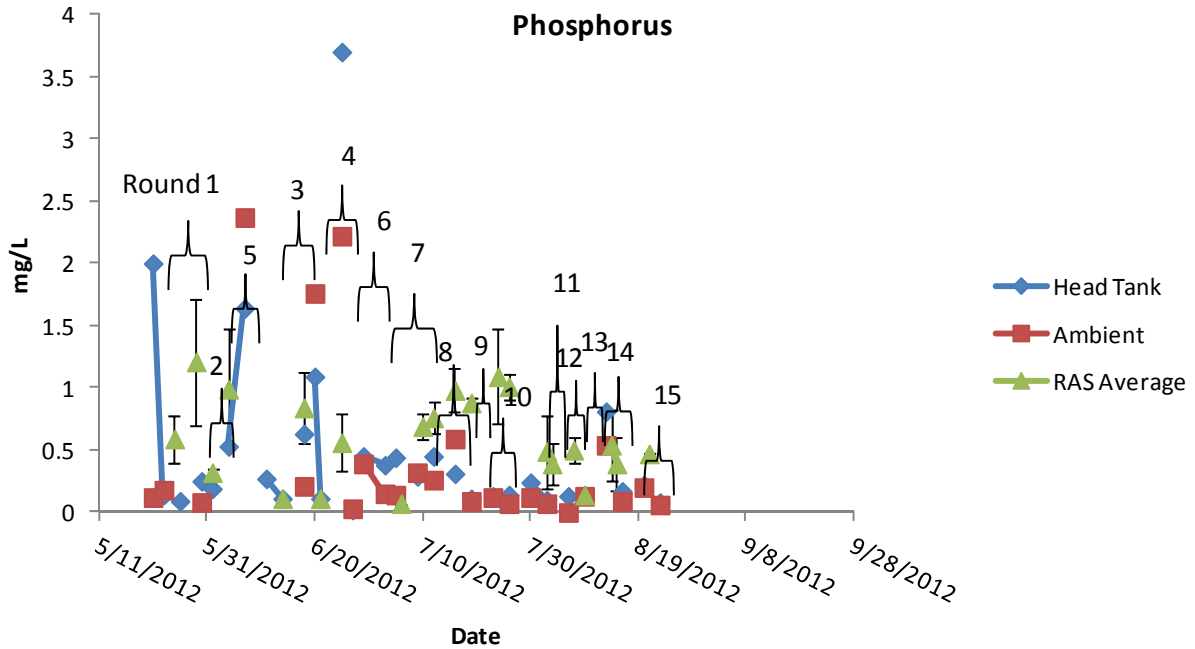


Figure 27. The phosphorus measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average phosphorus concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

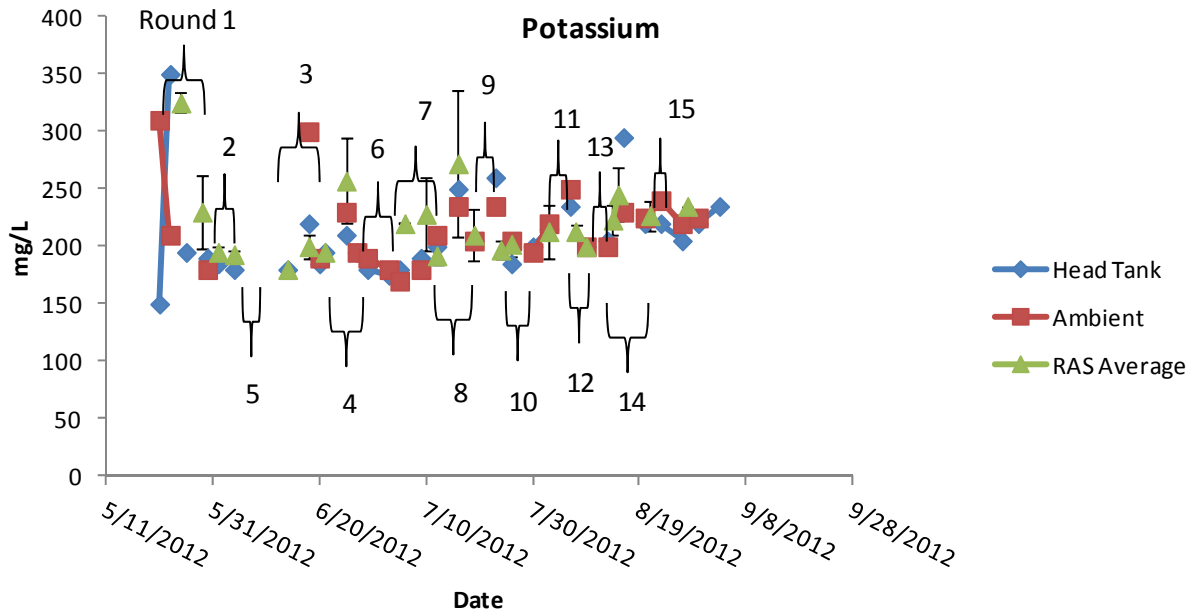


Figure 28. The potassium measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average potassium concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

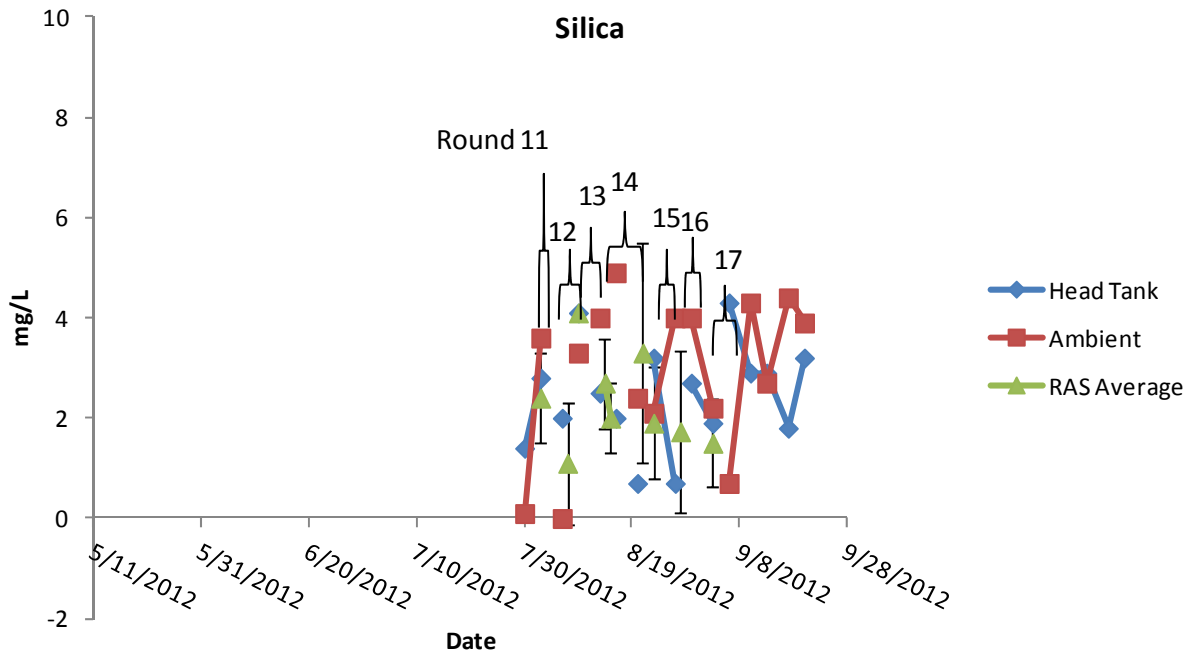


Figure 29. The silica measurements for the OSH head tank water (blue points) and ambient water (red points) during the course of the summer. The average silica concentration for the RAS tanks is also included (green points). The numbered brackets indicate when each experimental round was conducted during the summer. Error bars =  $\pm 1$  standard deviation.  $n=3$

## Discussion

The overall goal of these trials was to compare a standard method of culturing oyster larvae (referred to here as SOP (standard operating protocol) to an experimental recirculating aquaculture system or RAS. In the vast majority of trial runs the SOP performed better than the RAS (figure 1). It should be noted that larval survival in both SOP and RAS treatments was generally lower than is typically observed in commercial hatcheries operating on SOP and in particular survival of both RAS and SOP cultures were lower than analogous commercial cultures in the OSH facility.

One possible explanation for the generally low survival could be the small size of the culture systems. While the cultures were treated proportionally to commercial scale cultures at OSH with respect to density and diet, it is possible that these relationships are not linear and that at extreme ends of the spectrum, in this case the small end, protocols disproportionate to those in use at larger scales are necessary.

Another possible explanation has to do with water flow and is more critical of the potential of RAS system use for culturing oyster larvae. In both the SOP and RAS treatments water was recirculated through the culture tanks. This was done because it was a necessity for the RAS systems and determined to be too large an artifact not to subject the SOP treatments to as well (despite this not being part of standard larval rearing. In the case of the RAS systems, water left the culture tanks through an overflow and fell into a sump where it passed through multiple, and various filtration units before being pumped back into the culture tank. In order to prevent larvae from leaving the culture tank two sided screens (banjo screens) were placed inside the tank and connected to the overflow. The water circulation was mimicked in SOP systems with identical overflow and banjo apparatus allowing water to fall into a 5 gallon bucket, then be pumped with an identical pump back into the culture tank, passing through no filtration. After trial runs 1-4 suspicion that this water recirculation was inhibiting embryogenesis was confirmed in trial run 5-6 after which a change in operating protocol was made to allow embryogenesis to occur in the absence of water flow in both SOP and RAS systems. It is probable that this water flow, and in particular the vacuum that exists at the surface of the overflow, banjo screens, can decrease survival of early larvae as well. It is worth noting that OSH operates flow through culture with similar overflow apparatus for late larval culture (days 6 - end). OSH began using flow through culture for younger animals, but found that survival was better if they were cultured in static systems to day 6. Given this, the key to culturing oyster larvae in RAS may be in tailoring the hydrodynamics to be more satisfactory for young larvae.

The first four trial runs were performed using RAS design 1 which consisted of biological filtration only, with 22 plus hours of recirculation/filtration per day. Feeding was done during work hours, with continued recirculation as there was no filtration steps that would directly eliminate the feed. In trial one and two survival was virtually zero. This was likely due to the fact that the biological filters had not fully cycled yet and while maintaining ammonia at an

acceptable level had not yet controlled nitrite (figure 2 and 3). By run 3, nitrite levels were low and RAS survival was vastly improved though still not equal to SOP (figure 4). Water quality parameters were largely equivalent between RAS and SOP in run 3 with the only exception being higher phosphorus in the RAS. Run 4 had the most similar survival between the two treatments of the entire project, albeit low at around 2% by day 6 (figure 5). The equalizer here may have been a spike in CO<sub>2</sub> and phosphorus that was observed after the first SOP water change.

As a result of the generally low survival runs 5 and 6 consisted of a side experiment to investigate to what extent water recirculation may be hampering embryogenesis as defined by survival to day 2. Here duplicate treatments of SOP as outlined above and a normal SOP with no artificial recirculation was compared. While results from run 5 were similar, survival of both treatments were low possibly due to a poor spawn. In run 6 survival was better in the standard SOP (static) with no recirculation than in experimental SOP (flow) both cumulatively and in interval survival. With this information the decision was made to run both systems (RAS and SOP) static for the first 24 hours in subsequent runs to allow embryogenesis to take place in a lower energy environment without recirculation and water flow.

Having little success in runs 1-4 with the RAS system, runs 7-10 were completed using RAS design 2 where a protein skimmer and a UV sterilizer was added after the biological filtration. The protein skimmer was added generally to ensure that dissolved organics were being removed from the system and more specifically in an effort to reduce phosphorus levels which up to this point had been consistently higher in the RAS system. Important to note is that excess levels of phosphate can inhibit the precipitation of calcium carbonate and is therefore critical to control in a RAS system meant to support oyster larvae (Pokrovsky et al. 1993) The UV sterilizer was added as a result of observation of scavenging microbes in RAS water after subsequent cultures. It became apparent that failing larval cultures were compromising the water quality of the system and since there was an emphasis on minimizing water changes, UV sterilization was incorporated to manage this. A water change was completed prior to run 7 as it was after each change in RAS design. At the time of this water change was a spike in ambient CO<sub>2</sub> which is represented in the water quality readings for both treatments in run 7. This was the only exceptional parameter during run 7 and despite generally low survival between the two treatments, is the first and only run where the RAS system had higher cumulative survival after 6 days (figure 6). For the remainder of the design two runs, RAS survival remains low with the main difference in water quality profiles between the two treatments being phosphorus. Levels in RAS rose dramatically over these runs and led to another change in RAS design.

RAS design three added activated carbon and a phosphate adsorbing media in an attempt to reduce the phosphorus levels in the RAS systems. This change failed to lower the phosphate levels, and generally low survival was observed in the RAS systems for all runs of this design. It is possible that the phosphate media was having some negative affect on the larvae, though there were no other monitored water quality parameters that seem to be pushed out of balance through



the use of this media. Never the less, there could be some unrecorded or unknown toxic effect. It is also important to note that these trials occurred during the month of August during which time OSH was struggling with commercial larval culture. While there were not any exceptional water quality readings of those being monitored it is likely that some change in water quality, possibly bacterially related was generally causing poor larval performance.

While culture success was generally a bust for design three one positive observation came out of this design. In order to facilitate a longer effective feeding period, the recirculation schedule was reversed between RAS design two and three so that in three, the systems were only recirculated during work hours, therefore receiving approximately 6-7 hours of recirculation. This is in contrast to design two which received nearly three times that at 17-18 hours of recirculation. Despite this change however, there were no significant changes in the water quality profiles between the two designs suggesting that the metabolic load of young oyster larvae on a RAS is relatively low allowing for the possibility of lower flow systems which may ultimately be necessary for successful and consistent larval culture.

Presuming that the phosphate media could be negatively affecting the larvae, design four is identical to design three with the exception of the phosphate media being removed. While after the normal water change between system designs, recorded phosphorus was much lower, performance of the two cultures attempted, run 16 and 17 was very poor and therefore little information was gained. These were done very late in the season (mid-September) and often broodstock condition is in question at that time, this case being no exception.

It should be noted that in only one case were larvae transferred out of their respective RAS or SOP culture tanks and put into what was to be the second phase of larval culture , a flow-through culture system (FTS). This was because in all other cases, there were insufficient numbers of surviving larvae or larvae of insufficient health from RAS treatments to complete this work. In the one trial run that was transferred to FTS, RAS larvae survived poorly, presumed to be weak coming out of the RAS system.

## **Conclusion**

Consistent larval production in commercial oyster hatcheries is of paramount importance to maintain a strong oyster aquaculture industry. The least controllable in the calculus of consistent large scale oyster larval production is ambient water quality. To effectively remove ambient water quality from this equation through the use of recirculating aquaculture systems has the potential to revolutionize how hatcheries operate. However, at least in the forms tested here, RAS is not yet a viable option for culturing early oyster larvae.

While the RAS systems tested here do not present as viable alternatives for standard hatchery culture protocol, they did provide a lot of useful information and insight that can lead the path toward a viable system. Most important it would seem is the realization that metabolic demand on the system will be low (at least for early larval culture). Six hours of recirculation,

approximately 20-24 nominal exchanges per day were sufficient to maintain acceptable water quality levels. It is possible that the true minimum required recirculation period is even less. Given that one of the biggest problems with larval performance in these trials seemed to stem from the hydrodynamics associated with recirculation, the possibility to minimize the duration or intensity of flow (most importantly the latter) suggests a direction for continued investigation. Integral in future investigation to ensure that the precipitation of calcium carbonate is not being inhibited will be finding a way to control phosphate levels at which these RAS designs failed.

I want to thank Tom Murray and the Virginia Sea Grant Fisheries Resource Grant Program for their support and the opportunity to complete this investigation. I also want to thank Stephanie Marsich who was hired as the technician to complete the operation, maintenance, and data collection for this project and for her dedication and countless hours of water quality processing.

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