Final Report

Water Chemistry Studies for Oyster Hatcheries in the Chesapeake Bay

April 3, 2013

Prepared By:
Virginia Marine Resources Commission
And Virginia Tech

Grant Year 2010

NOAA Grant #NA10NOS4190205
Task 2.02

This project was funded by the Virginia Coastal Zone Management Program at the Department of Environmental Quality through Grant #NA10NOS4190205, Task 2.02 of the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, under the Coastal Zone Management Act of 1972, as amended.

Product 1: Provide wet-Lab Equipment for Six Hatcheries

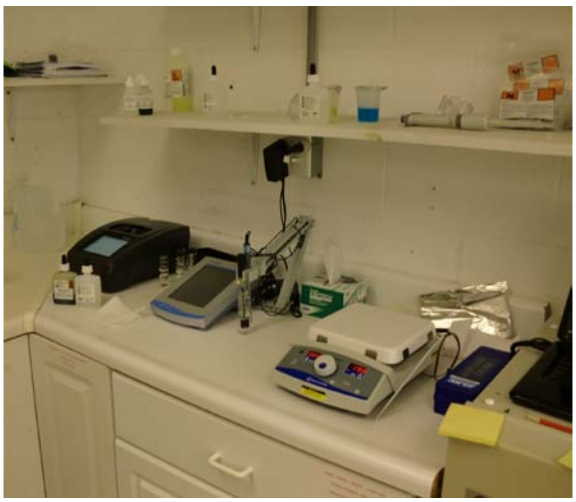
Oyster production in Virginia has increased more than 10 fold over the past ten years. Much of this increase has been the growth of hatchery based aquaculture and private investment on leased ground. All shellfish hatcheries in Virginia are privately owned, and the future of the modern industry rests on their successful production of eyed larvae and seed oysters. In 2010 and especially 2011, there were unexplained production problems at all of the hatcheries throughout the State. Water quality issues were suspected, but there were no standards for water quality monitoring among hatcheries, and there was no way to link production difficulties with changes in water quality. After several meetings between hatchery managers and academia, Dr. David Kuhn from Virginia Tech and Jim Wesson from MRC developed a standardized program to begin monitoring various water quality components at six Virginia hatcheries. Secretary of Natural Resources, Doug Domenech provided funds to begin monitoring water quality, and analyzing the collected data. The Virginia Coastal Zone Management Program provided funds for standardized onsite laboratory equipment for each hatchery. Significant effort and in-kind match has been undertaken by each hatchery. Water chemistry parameters monitored include alkalinity, ammonia, calcium, carbon dioxide, iron, nitrate, nitrite, pH, phosphorus, potassium, salinity, silicate, and temperature. These parameters were monitored several times a week at various locations in the hatchery when they were producing larvae. It was anticipated that carbonate chemistry issues resulting from ocean acidification could be involved with larvae production issues, but in 2012 that did not appear to be the case. Water quality in 2012 was much better than in 2010 and 2011, most likely because of the dryer weather conditions. Oyster larvae production was very good at most hatcheries until after July. Larval production declined in August, and a high level of ammonia was the only abnormal water quality parameter observed.

All of the hatcheries will continue with the water chemistry monitoring in 2013. Water quality data is now routinely shared between the six hatcheries, Virginia Tech, and MRC. Meteorological conditions are very different this winter and spring from those observed in 2012. Rainfall has been higher and temperatures colder in 2013, and one hatchery has already had issues with elevated potassium. Monitoring is the first step in recognizing issues, and may suggest possible adaptations that can be implemented in the shellfish hatcheries—even though in many cases there will be no explanation for the water quality changes that are being observed.

Besides the water chemistry parameters that were monitored by each hatchery, other potential problems with water quality were also examined. Intake and hatchery tank water samples were routinely sent from each hatchery to Dr. David Kuhn's lab for microbial analysis. Included in the analysis are aerobic plate counts, total coliforms, fecal coliforms, *Escherichia coli*, and pathogenic bacteria in the Vibrionaceae family. The general population trend of these bacteria increased with warmer waters. Surprisingly, very low levels of coliforms were observed throughout the 2012 growing season. Many bacteria species were identified and quantified in the Vibrionaceae family including: *Aeromonas hydrophila*, *Vibrio alginolyticus*, *V. cholerae*, *V. fluvialis*, *V. parahaemolyticus*, *V. vulnificus*, *Pasteurella multocida*, *Pasteurella* spp., *Pasteurella pneumotropica*, *Photobacterium damselae*, and *Shewanella* spp. Bacteria from the Vibrionaceae family can release toxins that are harmful to shellfish larvae.

There are hundreds of pollutants that could be in the Chesapeake Bay that could be toxic to shellfish larvae. It would be cost prohibitive to analyze for all of these parameters in the water at each hatchery. Fortunately, the majority of these pollutants can be absorbed by charcoal in a carbon filter. Charcoal was collected from a carbon filter that had been filtering Chesapeake Bay water for several weeks at one of the hatcheries. This charcoal was subjected to a priority pollutant scan. The results of the charcoal analysis indicated that it was relatively clean. The most important pollutant that was identified was bromoform. Bromoform is very toxic to aquatic life and can be released by some algae species in the Chesapeake Bay water. For this reason, bromoform and other VOCs have been selected as an additional parameter to monitor in the water at all six of the shellfish hatcheries.

This is a very new industry, and many other water quality issues, both biological and chemical will be continue to be monitored by the industry and Dr. Kuhn. Below are photos of the monitoring equipment in the various labs.



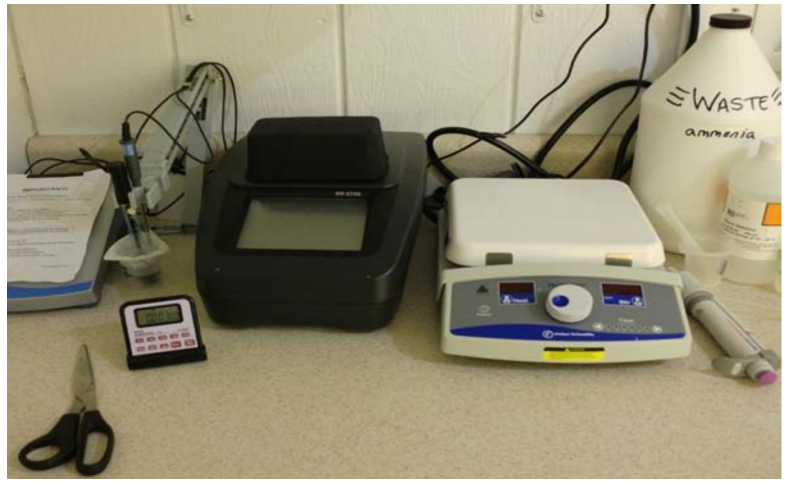
Cherrystone Oyster Hatchery
Northampton County

KCB Oyster Hatchery
Northumberland County

Middle Peninsula Oyster Hatchery
North River, Mathews County

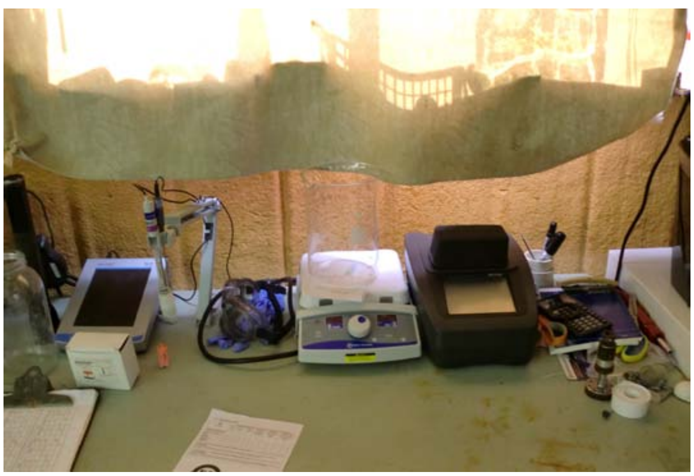
Oyster Seed Holdings Hatchery

Gwynn Island, Mathews County



Oyster Seed Holdings Hatchery
Gwynn Island, Mathews County

Walker Brothers Hatchery
Willis Wharf, Accomack County



Tom's Cove Hatchery
Assateaugue Channel, Accomack County

Product 2: Facilitate Data Transfer between Hatcheries, VMRC, and Virginia Tech

All data from each hatchery has been tabulated. Each hatchery has been assigned a letter to maintain anonymity. This data has been provided to the hatcheries upon request. The data set is relatively new, and all hatcheries will continue to provide water chemistry results throughout 2013. An example of an excel spreadsheet has been included.

Data Analysis by Virginia Tech:
Oysters and clams are an essential part of Virginia's near-shore ecosystems, and a vital part of life in our coastal communities. A healthy oyster industry means a strong economy in Eastern Virginia, jobs for processors and oystermen, and a healthier Chesapeake Bay. This plan is assisting the struggling oyster industry by identifying and mitigating bottlenecks that occur at oyster and clam hatcheries, the major source for replenishing oyster stocks in Virginia waters.

Background

Over the last 7 years, Virginia's oyster aquaculture industry has developed rapidly, with the number of hatcheries increasing from one in 2005 to eight in 2010. This allowed for an increase in oysters planted from approximately 6 million in 2005 to nearly 77 million in 2010. Oyster hatcheries have become the backbone of the private oyster industry, providing jobs at the hatcheries, in grow-out operations, processing operations, and other areas.

Virginia shellfish hatcheries have recently experienced significant production difficulties. In the 2010 and 2011 seasons, between June and August, production of oyster larvae was significantly lower than previously reported. While some improvement was noted during the month of September, hatchery production was still limited, and was not considered satisfactory. This issue is occurring at alarming rates among many of the shellfish hatcheries located in Virginia. For example, production at Oyster Seed Holdings Hatchery decreased from 100 million larvae per week in late May 2011 to less than 10 million larvae per week in mid-June 2011 and remained at that level for several months. Similar issues were observed during the same period of time at many of the other hatcheries. In some cases, zero production was observed over a several week period.

For oysters, during the unsuccessful periods, young spat are exhibiting an empty gut (between one and six days post-hatch) and/or significant deformities, resulting in extremely high mortality rates. Water quality testing protocols at these hatcheries were often basic (e.g. dissolved oxygen, pH, and temperature) to non-existent. Analysis of trends in these parameters yields no clues as to what could be happening to affect oyster larvae so dramatically. Without comprehensive water quality monitoring and a strategic plan to explore the problem, it has been be very difficult for the industry to discover a solution. Potential solutions could include implementation of a specialized filtration system, balancing water chemistry through supplementation using salts or other chemicals, or implementation of biosecurity plans. Water quality in surface waters changes over time due to temperature, precipitation, and anthropogenic activity. Moreover, algae and many pathogenic bacteria proliferate during the warmer summer months. It is likely that a chemical constituent or biological agent is causing the detrimental effect on hatchery production. Accordingly, implementation of a long-term water quality monitoring plan was implemented in 2012 to track water chemistry and test for the presence of biological agents in the source water and system water at oyster hatcheries.

Collaborations

Six industry partners are serving as research sites including Cherrystone Aqua-Farms (Cheriton, VA), KCB Oyster Holdings (Lottsburg, VA), JC Walker Brothers (Willis Wharf, VA), Oyster Seed Holdings (Grimstead,VA), Tom's Cove Aquafarms (Chincoteague, VA), and Ward Oyster (Mathews, VA). These hatcheries are well distributed around the coastal waters of Virginia from near river outlets within the Chesapeake Bay to the Atlantic seaside, thereby representing a wide range of water conditions. On this effort, Dr. David Kuhn is serving as the principle investigator in collaboration with Dr. Jim Wesson and the six industry partners. Karen Hudson (Virginia Institute for Marine Science - VIMS) has been helpful in bringing in partners to support this project including but not limited to Thomas Murray (VIMS, economic analysis), Mark Luckenbach (VIMS, water quality plan advisor), Dr. Dwight Gledhill (NOAA, carbonate chemistry expert), and Brad Warren (Sustainable Fisheries Partnership, ocean acidification expert).

Progress Summary - 2012 Efforts

The goal of this project is to implement a rigorous water quality monitoring program to track water chemistry and test for the presence of biological agents in the source water and system water at each hatchery. From this data we will identify potential water quality and/or biological agents that could be causing the larval development issues at these hatcheries based on correlations with hatchery success rates. Once an issue is identified, a solution can be implemented. In 2012, the activities outlined below are currently underway.

Overall, production of shellfish larvae was not impaired in 2012 compared to the high level of impairment observed in 2010 and 2011. This is good news for production but makes correlating water quality with larvae production success rates difficult. It is speculated that water quality in the Chesapeake Bay was not as poor as the previous two years because rain events were not as severe in 2012. Major rain events create large volumes of runoff and consequently significant pollution loading and dilution of carbonate chemistry in the Chesapeake Bay area. It is anticipated within a year or two the Chesapeake Bay will revert to conditions similar to what was observed during 2010 and 2011.

Activity 1 - Water Chemistry

Each hatchery has been equipped with onsite laboratory equipment so they can conduct water quality analysis on a continuous basis. Significant efforts and in-kind matching have been undertaken by each hatchery. Water chemistry parameters monitored include alkalinity, ammonia, calcium, carbon dioxide, iron, nitrate, nitrite, pH, phosphorus, potassium, salinity, silicate, and temperature. Each site has been monitoring these parameters several times a week during much of the 2012 growing season. In the fall and winter of 2011-2012, Dr. Jim Wesson provided personnel to track some of these parameters with training and equipment provided by Dr. David Kuhn. Water quality parameters observed during the 2012 season have been generally more favorable compared to those experienced in the fall of 2011. At this juncture, it is speculated that ammonia could be a problem because it is extremely toxic to aquatic organisms. Even though levels have been low in 2012, concentrations in 2011 were alarmingly high. Lastly, many of these parameters feed into *Activity 2*.

Activity 2 - Carbonate Chemistry

Using data collected during *Activity 1 - Water Chemistry,* we can estimate the calcium carbonate saturation state, Ω, of the water, which is represented by the following equation:

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}^*}$$

Where $[Ca^{2+}]$ and $[CO_3^{2-}]$ are concentrations of ions in solution and K_{sp} is the solubility product at a given temperature, salinity, and pressure. Other water chemistry parameters, i.e. alkalinity and pH, can be measured to estimate the calcium carbonate saturation state. If the calcium carbonate saturation state is less than one, then calcium carbonate can readily dissolve. Conversely, if the calcium carbonate saturation state is greater than one, then calcium carbonate does not readily dissolve. Calcifying organisms (e.g. oysters and clams) typically live in conditions where the calcium carbonate saturation state is greater than one and thrive in conditions where it is much greater than one. Ocean acidification can contribute to lower calcium carbonate state values. In areas influenced by rivers and runoff, such as the Chesapeake Bay, the calcium carbonate saturation state can also be lowered by freshwater dilution. This phenomenon is particularly true during and after heavy rainfall events. In 2012, all of the hatcheries had calcium saturation state values greater than one. However, values were approaching or close to one, the minimum threshold, in many instances. We do not have ample data from the 2010 and 2011 seasons. It is reasonable to assume that calcium saturation state values were lower during this period due to significant rain events. This could occur again within a year or two depending on future weather.

Activity 3 - Microbial Analysis

Intake and hatchery tank water samples are routinely sent from each hatchery to Dr. David Kuhn's lab for microbial analysis. Included in the analysis are aerobic plate counts, total coliforms, fecal coliforms, *Escherichia coli*, and pathogenic bacteria in the Vibrionaceae family. The general population trend of these bacteria increased with warmer waters. Surprisingly, very low levels of coliforms have been observed throughout the 2012 growing season. This is further evidence that rain events have been minimal in 2012. Many bacteria species were identified and quantified in the Vibrionaceae family including: *Aeromonas hydrophila*, *Vibrio alginolyticus*, *V. cholerae*, *V. fluvialis*, *V. parahaemolyticus*, *V. vulnificus*, *Pasteurella multocida*, *Pasteurella* spp., *Pasteurella pneumotropica*, *Photobacterium damselae*, and *Shewanella* spp.

Bacteria from the Vibrionaceae family can release toxins that are harmful to shellfish larvae. Recent sampling events included microbial analysis of larvae and water. Results showed that the bacteria from the Vibrionaceae family were higher in the larvae compared to the water where they were cultured. Bacteria can be vertically transmitted from brood stock to eggs/sperm and consequently to larvae.

Activity 4 - Other Pollutants

There are hundreds of pollutants that could be in the Chesapeake Bay that could be toxic to shellfish larvae. It would be cost prohibitive to analyze for all of these parameters in the water at each hatchery especially because levels may vary from day-to-day. Fortunately, the majority of these pollutants can be absorbed by charcoal in a carbon filter. For this reason, charcoal was collected from a carbon filter that had been filtering Chesapeake Bay water for several weeks, thereby capturing all pollutants collected on the charcoal over a long period of time. This charcoal was subjected to a priority pollutant scan which is often used for Phase One assessment of samples when there is an unknown contaminant. Analysis includes hundreds of constituents inclusive of volatile organic carbons (VOC), semi-volatile carbons (sVOC), pesticides, herbicides, polychlorinated biphenyls (PCBs), heavy metals, cyanide, and phenols. The results of the charcoal analysis indicated that it was relatively clean. The most important pollutant that was identified was bromoform. Bromoform is very toxic to aquatic life and can be released by some algae species in the Chesapeake Bay water. For this reason, bromoform and other VOCs have been selected as an additional parameter to monitor in the water at all six of the shellfish hatcheries.

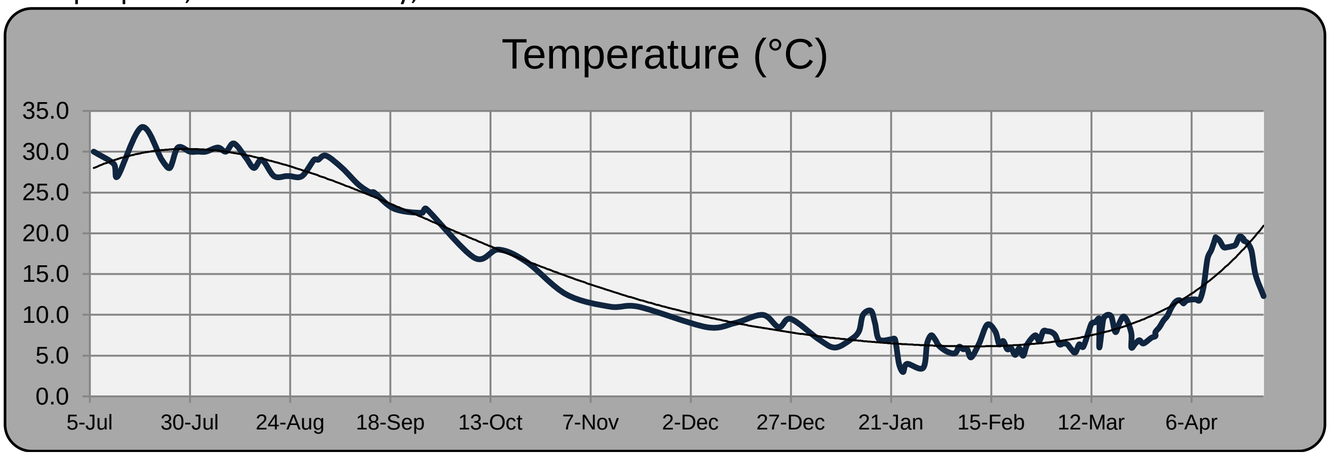
Activity 5 - Filter Unit Processes and Carbonate Chemistry Manipulation

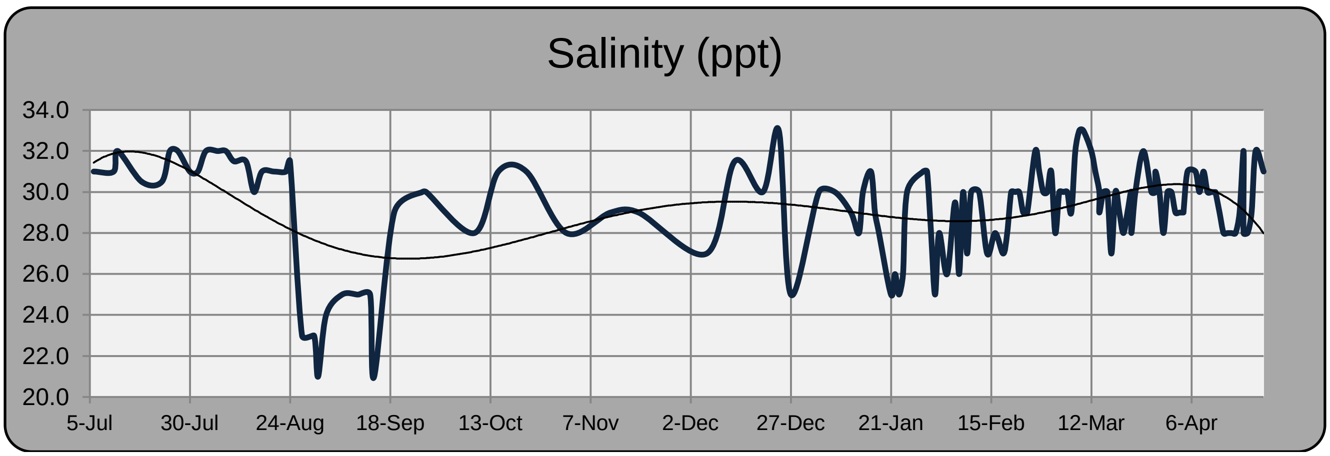
Potential solutions to the issues identified in *Activities 1-4* could include the installation and use of a specialized filtration system, balancing water chemistry through supplementation using salts or other chemicals, or implementation of biosecurity plans. All three of these areas have been attempted in some form at some of the shellfish hatcheries. Some examples include: bicarbonate and calcium oxide have been supplemented to larvae culture water to increase the carbonate saturation state described under *Activity 2*; bacterial biosecurity plans have been reviewed and assistance has been provided to reduce bacterial loadings in the larvae culture systems, an issue that was described under *Activity 3*; and, carbon filters have been installed to remove priority pollutants described in *Activity 4*. The evaluation of the effectiveness of the measures taken above was offset by the relatively successful season that the hatcheries experienced in 2012.

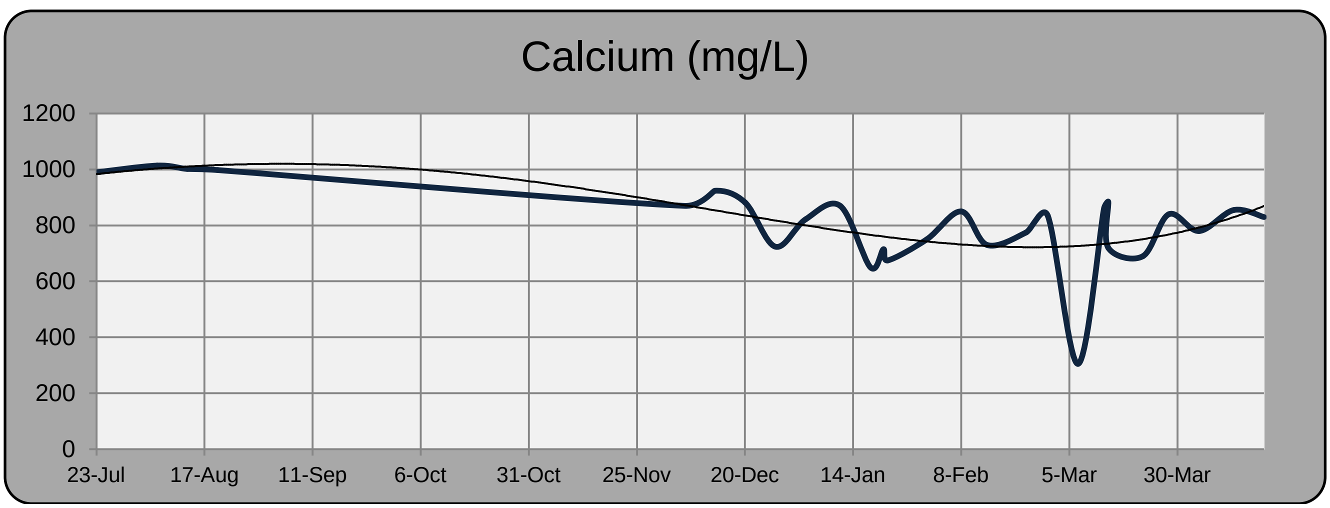
Conclusion

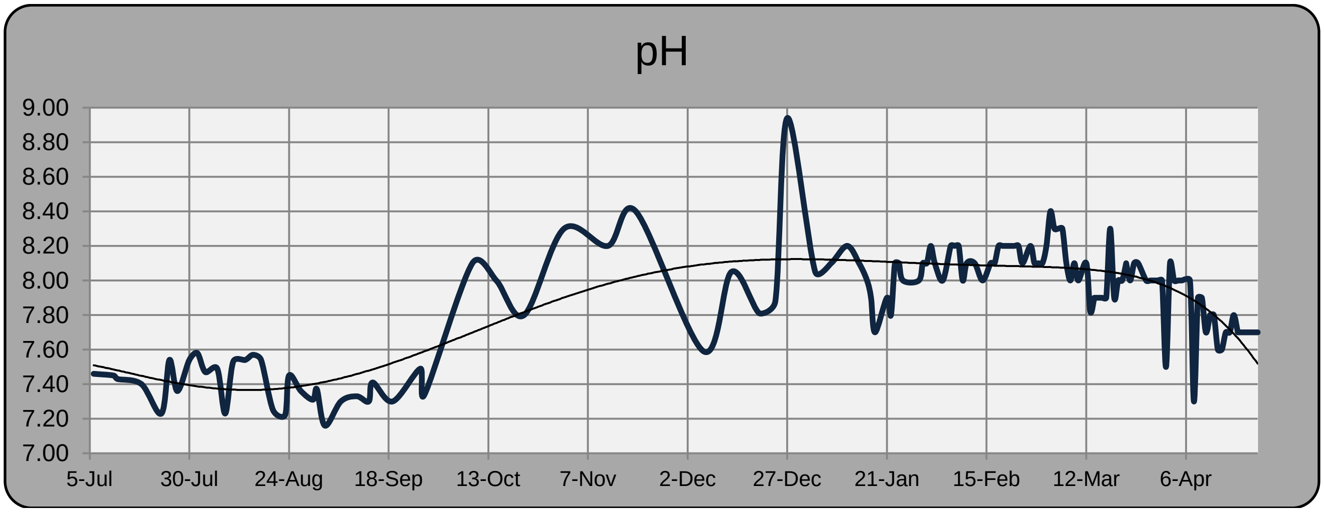
The 2012 larvae production season was relatively successful which is likely a result of no significant rain events that would have otherwise contributed to a high level of pollution and dilution of water resulting in reduced calcium carbonate saturation state values. Increased pollution would have also contributed to an increase in bacterial and algal (consequently higher levels of bromoform) activity in the Chesapeake Bay. Since the 2012 season was relatively successful, it was difficult to correlate potential issues and mitigation strategies. It is anticipated that within the next couple of years, rain events will be significant again and will result, once again, in poor larvae production rates similar to that were observed during the 2010 and 2011 seasons. Moreover, other factors may contribute to poor larval production rates. The data collected during the 2012 season has been very helpful in that potential issues have been identified. Before the 2012 season, no water quality data was collected and potential issues were based 100% on speculation.

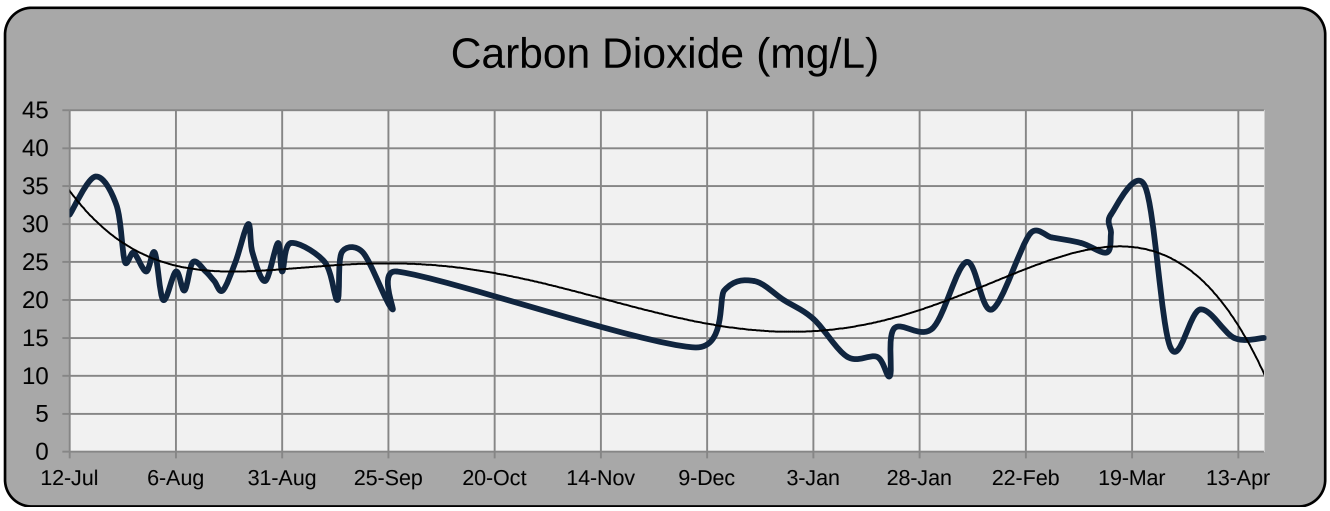
Example plots, water chemistry, from actual data collected at one of the shellfish hatcheries:

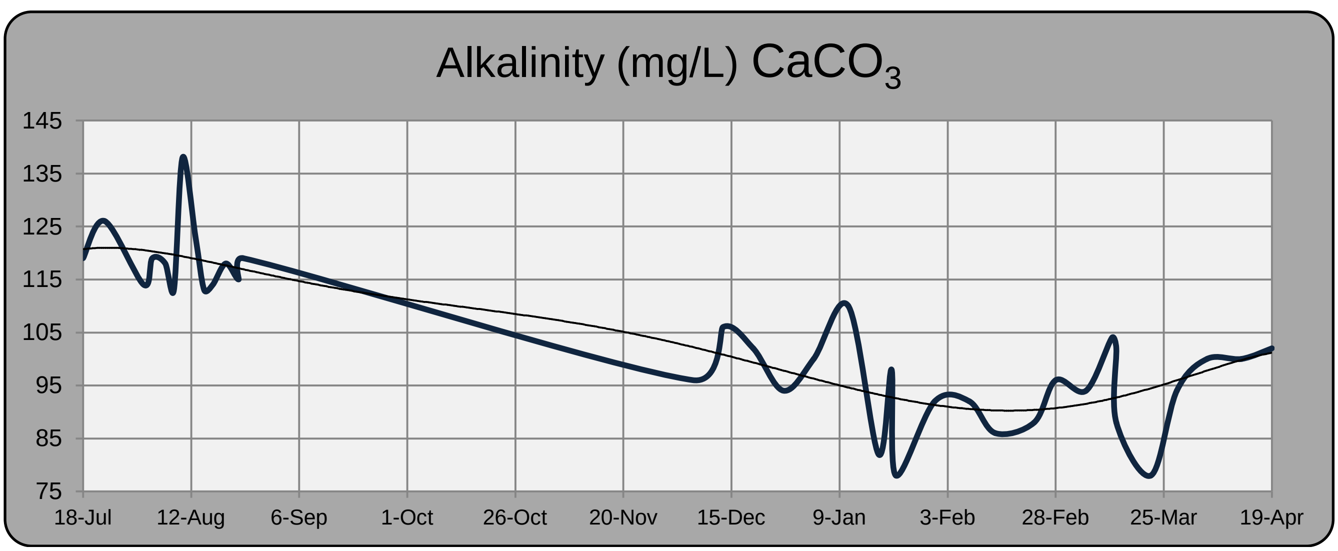


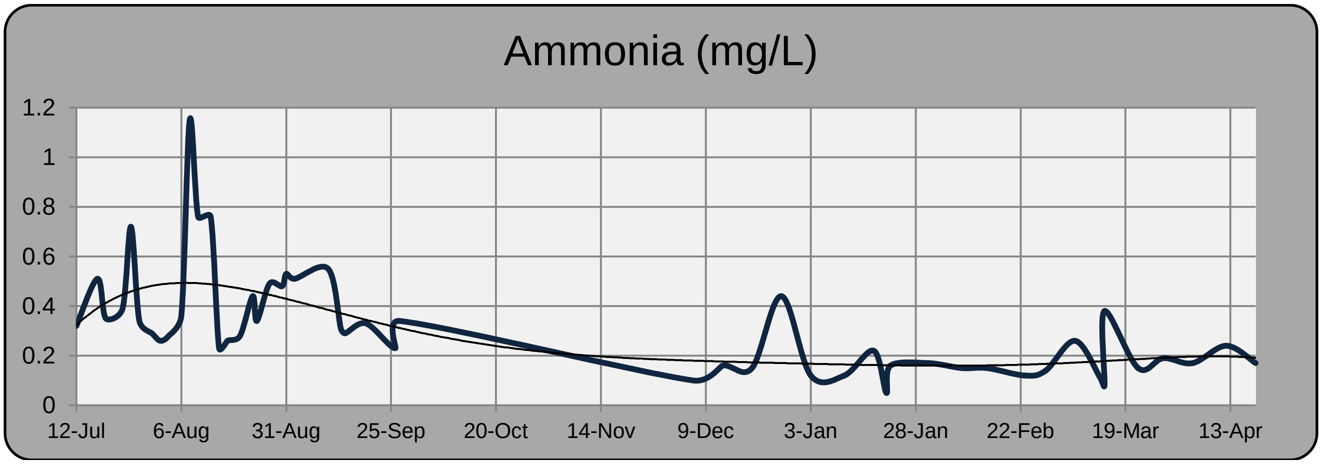


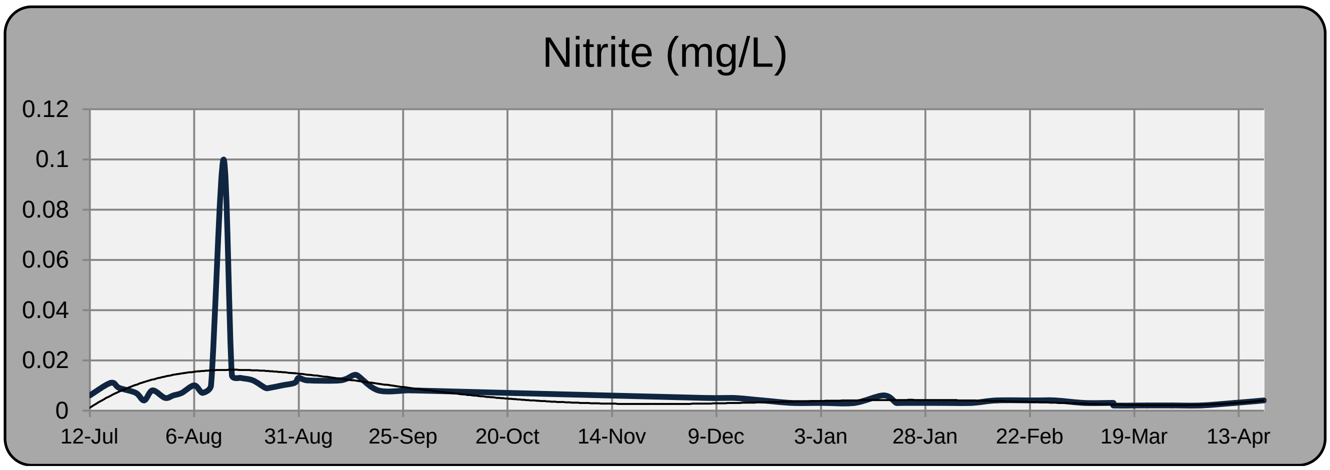


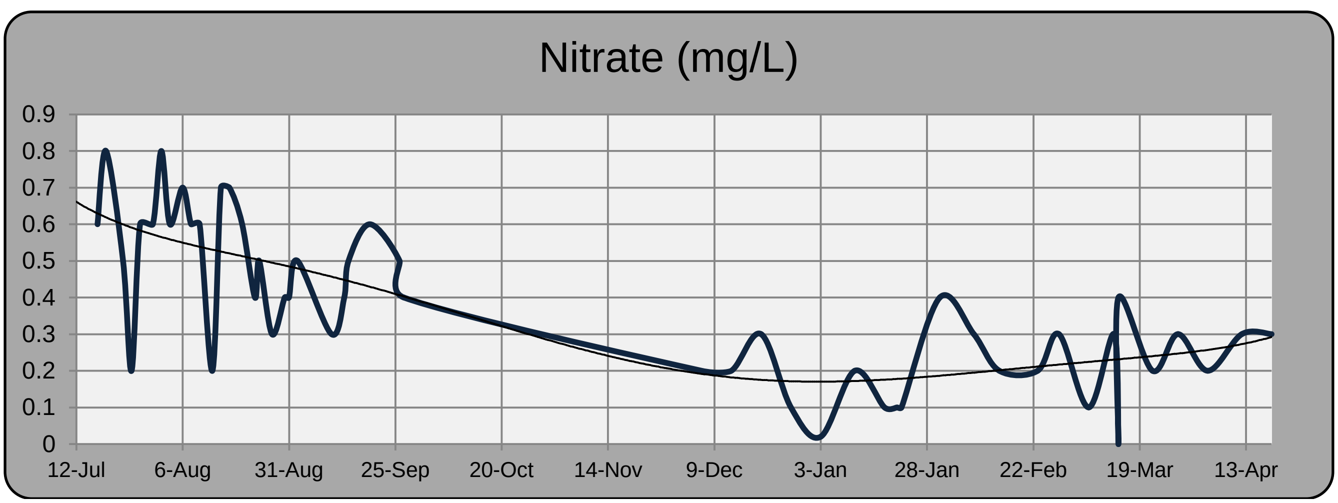

pH
9.00
8.80
8.60
8.40
8.20
8.00
7.80
7.60
7.40
7.20
7.00
5-Jul
30-Jul
24-Aug
18-Sep
13-Oct
7-Nov
2-Dec
27-Dec
21-Jan
15-Feb
12-Mar
6-Apr


Carbon Dioxide (mg/L)
45
40
35
30
25
20
15
10
5
0
12-Jul
6-Aug
31-Aug
25-Sep
20-Oct
14-Nov
9-Dec
3-Jan
28-Jan
22-Feb
19-Mar
13-Apr


Alkalinity (mg/L) $CaCO_3$
145
135
125
115
105
95
85
75
18-Jul
12-Aug
6-Sep
1-Oct
26-Oct
20-Nov
15-Dec
9-Jan
3-Feb
28-Feb
25-Mar
19-Apr


Ammonia (mg/L)
1.2
1
0.8
0.6
0.4
0.2
0
12-Jul
6-Aug
31-Aug
25-Sep
20-Oct
14-Nov
9-Dec
3-Jan
28-Jan
22-Feb
19-Mar
13-Apr


Nitrite (mg/L)
0.12
0.1
0.08
0.06
0.04
0.02
0
12-Jul
6-Aug
31-Aug
25-Sep
20-Oct
14-Nov
9-Dec
3-Jan
28-Jan
22-Feb
19-Mar
13-Apr


Nitrate (mg/L)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
12-Jul
6-Aug
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25-Sep
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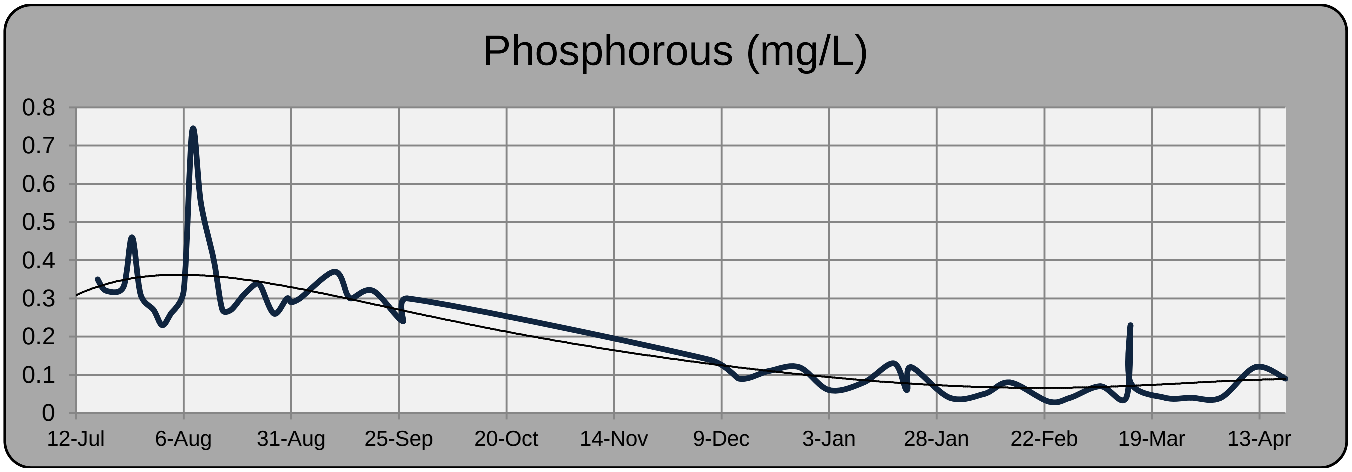
22-Feb
19-Mar
13-Apr


Phosphorous (mg/L)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
12-Jul
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31-Aug
25-Sep
20-Oct
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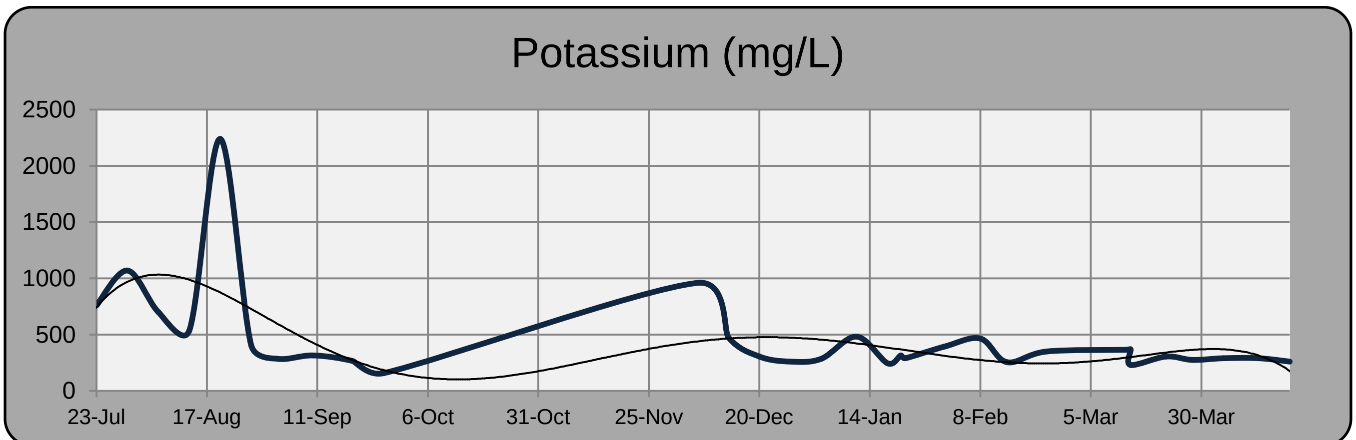
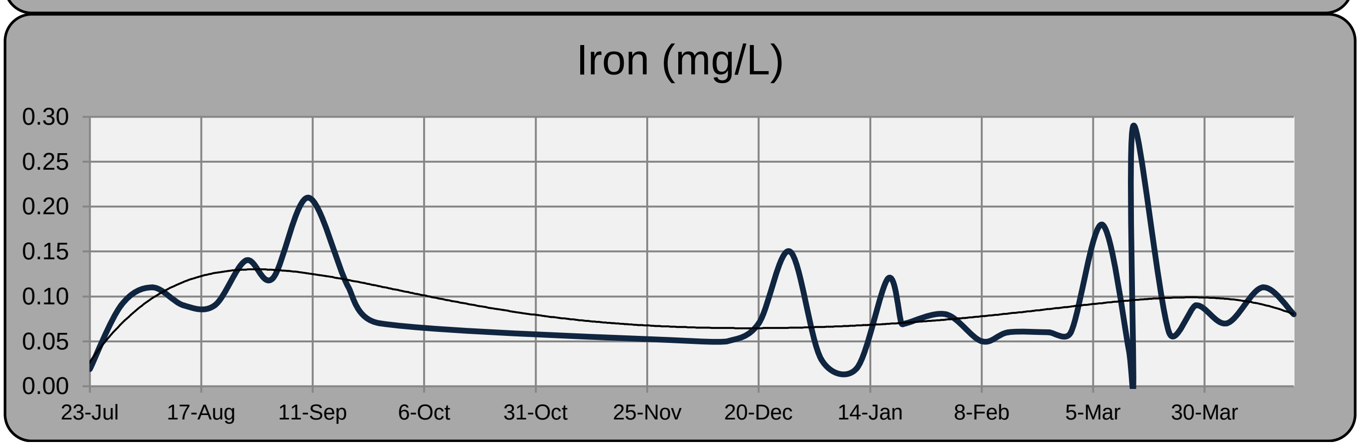
9-Dec
3-Jan
28-Jan
22-Feb
19-Mar
13-Apr


Potassium (mg/L)
2500
2000
1500
1000
500
0
23-Jul
17-Aug
11-Sep
6-Oct
31-Oct
25-Nov
20-Dec
14-Jan
8-Feb
5-Mar
30-Mar


Iron (mg/L)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
23-Jul
17-Aug
11-Sep
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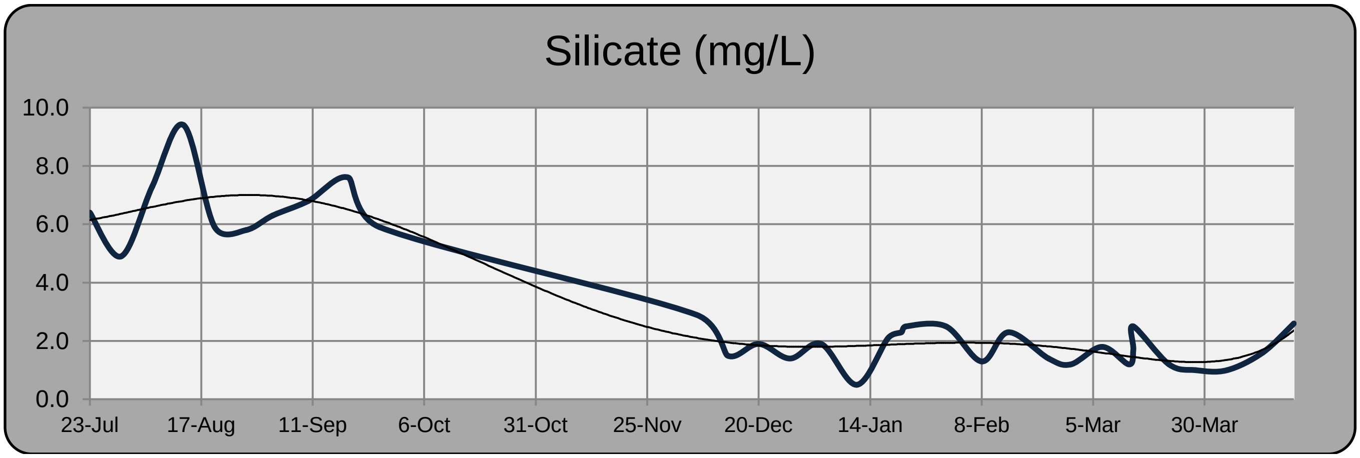
25-Nov
20-Dec
14-Jan
8-Feb
5-Mar
30-Mar


Silicate (mg/L)
10.0
8.0
6.0
4.0
2.0
0.0
23-Jul
17-Aug
11-Sep
6-Oct
31-Oct
25-Nov
20-Dec
14-Jan
8-Feb
5-Mar
30-Mar