Update on Hatchery Research and Use of State Funds to improve Larval Performance at Whiskey Creek Shellfish Hatchery

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In early 2008, the problems experienced at Whiskey Creek Shellfish Hatchery were thought to be attributable to the presence of *vibrio tubiashii*, a common marine bacteria that was found in unusually high concentrations in Netarts Bay during 2006 and 2007. Much of the early research and systems development at the hatchery were therefore targeted at reducing the concentration of *vibrio* and its toxins present in incoming seawater. However, further research in 2008 and 2009 has revealed that although *vibrio tubiashii* is a significant stress factor for shellfish larvae, it is not the underlying cause of the catastrophic mortality events that have brought production at Whiskey Creek to a standstill. Therefore, beginning in July 2008, the emphasis of research in the hatchery shifted toward understanding the fundamental changes in seawater chemistry that are influencing marine life in our coastal waters. The results of this research and our progress toward developing a solution will be detailed in the following sections.

Impacts of upwelling on shellfish larvae

In July 2008, a huge mortality event resulted in the loss of several billion bivalve larvae at Whiskey Creek, and brought production to a halt. While devastating for the hatchery, this event led us to perhaps our only clear revelation in 2008- *upwelling of nutrient rich, deep ocean water onto the continental shelf results in a sharp decline in the performance of bivalve larvae.*

When persistent winds blow from the North (for 24-48 hours or more), surface water near the coast is pushed offshore, and is replaced by cold, deep water masses. This upwelled water is characterized by very low pH (measured as low as 7.55-7.6 in 2008 and 2009). When this acidified water is observed in the hatchery, mass mortality events have followed in 24-48 hours. In these very extreme conditions, larvae of all sizes and species (Pacific oysters, Kumamoto oysters, Manila clams, and Mediterranean mussels) are dramatically affected. Although production was slow throughout the 2008 season, the sudden, catastrophic mortality events observed in July were clearly associated with this strong upwelling event.

An isolated example from this year can perhaps illustrate our sense of how upwelling/downwelling events are affecting the growth of shellfish larvae in our hatchery. To preface this, we should mention that larval production in the early months of the 2009 season has been quite good, with small larvae growing at normal rates for the first time since mid 2007. However, in mid-April 2009, winds began to blow from the north, and were sufficiently strong and sustained to produce an early upwelling event, which brought acidified seawater (pH=7.55) into Netarts Bay. Although this event was brief, significant mortality was observed over the next two weeks, particularly among small oyster larvae (both Pacific and Kumamoto).

A number of pH measurements were recorded during this following this recent upwelling event, and can provide some important insights into understanding Netarts bay. The following table shows the minimum daily pH measurements (recorded early each morning) on several days at the end of April.

4/21/2009	4/23/2009	4/24/2009	4/28/09	4/29/09
7.58	7.67	7.70	7.73	7.92

Admittedly, trying to determine trends in pH data is somewhat suspect- pH is affected by biology, and using it as a marker of upwelled water masses is therefore questionable. However, the data shown above gives a good general sense of the changes that follow an upwelling event. The minimum daily pH rises slowly in the 7-10 days after North winds relaxed, which suggests that upwelled water is slowly mixing with surrounding seawater on the continental shelf, or that biology (algae, etc) is slowly raising the pH by consuming excess CO_2 in the upwelled water. Which explanation, or combination of the two, makes the most sense is a question for the oceanographic community- however, the point for our purposes is plain: even though the upwelling has relaxed, the 'bad' properties of the seawater in Netarts Bay do not immediately vanish. During the entire period described in the table, slow growth and mortality of small larvae continued to plague the hatchery.

In fact, slow growth of small larvae continued through the first week of May, when minimum pH values continued to drop to 7.9-8.0 each day. Starting on May 1, winds began to blow from the South, and some fairly large storms passed through during the first week of the month, with strong sustained winds and gusts as high as 50 MPH. These winds presumably started a strong downwelling event and pushed the remaining 'bad' water offshore to reset the system. Following this storm, an immediate change was observed in the hatchery, and the growth of small larvae has been normal ever since.

This fairly isolated event greatly improves our confidence that upwelling of deeper ocean water is directly linked to poor performance of larvae in the hatchery. Unlike 2008, when poor performance throughout the season clouded our observations, production in 2009 was quite good both before and after this brief cycle of upwelling and downwelling, which provided a clear signal that larval mortality was directly related to this incoming water mass.

Related research- ocean acidification and potential effects on shellfish larvae

The presence of acidified seawater on the Oregon continental shelf has been well documented by recent oceanographic studies. Feely et al (2008) recorded upwelling events in July 2007, and recorded water masses acidic enough to be considered corrosive to shellfish. Shellfish build their shells from calcium carbonate (CaCO3), which has several different crystal forms. Many shellfish, as well as most corals in the ocean, form their shells from a type of calcium carbonate called aragonite, which is fairly easy to dissolve. Feely's measurements showed upwelled water that was corrosive enough to dissolve aragonite and literally eat away at the shells of many animals on the continental shelf.

It turns out that adult oysters form their shells from a type of calcium carbonate called calcite, which is much more difficult to dissolve, and they therefore are somewhat more protected than other animals. Adult mussels, for example, form their shells from aragonite. Recent studies have shown that under slightly acidified conditions, the growth of adult mussels is dramatically slowed, while the reduction in growth of adult oysters is only slightly reduced (Gazeau, et al 2007). Although this was a lab study, recent studies have shown that ocean acidification may be beginning to damage coral and shellfish in their natural environment (Langdon, et al (2003) and Cutlip, K (2008)).

Oyster *larvae*, however, are a different story altogether. A surprising amount of research has been conducted to determine what makes up the shell of oyster larvae (Weiss, et al 2002, Lee et al 2004, etc), and shows that the shells of oyster larvae are composed of aragonite, which makes them very susceptible to the acidified water observed on the Oregon continental shelf in 2007. Moreover, these studies also conclude that the shells of small oyster larvae (1-3 days old) are formed partially from amorphous calcium carbonate (ACC), which is EXTREMELY easy to dissolve. In fact, animals have to expend a lot of energy to form ACC even in normal conditions.

Adult Oyster Shell	Calcite	Harder to dissolve
Adult Mussel Shell, Older Oyster Larvae	Aragonite	Easier to dissolve
Young Oyster Larvae	Amorphous Calcium Carbonate (ACC)	Really easy to dissolve

This research was very enlightening, and seems to explain many of the problems we have observed in the hatchery. Most of the ongoing problems experienced in 2007 and 2008 involved the growth of young larvae. In many cases, once they reached the umboe stage (typically after 7-10 days), larvae had a much easier time growing to setting size. In even slightly acidified seawater, young larvae need to expend a great deal of energy to form shell, since it is composed partially of ACC. Under these same conditions, older larvae would have an easier time forming aragonite shell, but their overall growth should also be somewhat slower than in normal conditions.

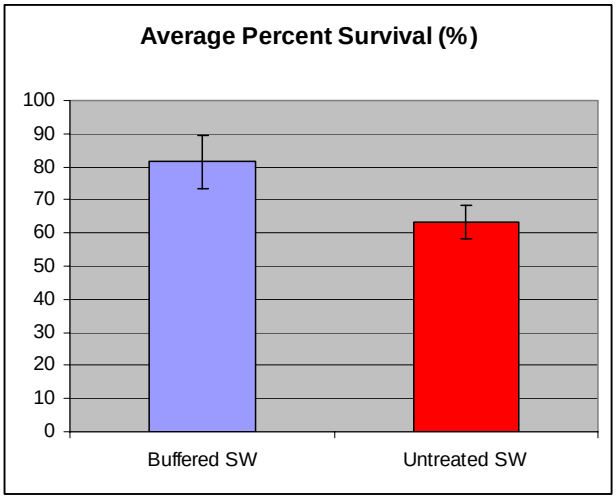
The experiment of Gazeau et al (2007) mentioned above supports this idea- in their example, we're considering the top two forms of calcium carbonate shown in the table above, calcite and aragonite. Even in only slightly acidified conditions, their study showed that the growth rate of animals was reduced. The growth of adult oysters (which form their shell from calcite) slowed slightly in acidified water, but not as much as adult mussels (which form their shells from aragonite). It seems plausible to apply this reasoning to growth of larvae in the hatchery. In the case of oyster larvae, we are now considering the bottom two carbonate forms in the table above. Under slightly acidified conditions, older larvae, which form their shells of aragonite, should show a slight reduction in growth. Younger larvae, however, which form their shells (partially) from ACC, would experience a much greater reduction in growth under the same conditions. Current research is directly investigating the effects of acidified water on oyster larvae, and supports the idea that young larvae are most at risk (Kurihara et al, 2007 and unpublished work currently under investigation by Whitman Miller of the Smithsonian Research Institute- referenced in Cutlip, K (2008)).

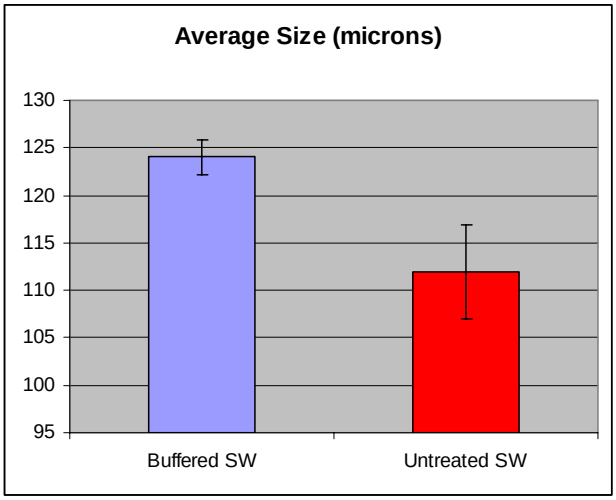
This line of reasoning also seems to explain the massive mortality events observed during periods of strong upwelling. During these periods, when the pH drops to 7.6 or lower, seawater is directly corrosive to aragonite, and both young and old larvae will experience a great deal of stress. In these periods, larvae throughout the hatchery may feel the direct effects of acidification, or simply expend so much energy building shell that they are weakened, and can more easily succumb to normal stressors like bacteria blooms in larval tanks.

Hatchery experiments- Fall 2008

In late summer 2008, experiments began in the hatchery to test the effects of low pH on oyster larvae. Conveniently, the ocean provided all the low pH water necessary for these experiments, which acted as our low pH control. Carbonate buffers were used to artificially raise the pH in some tanks, and early results were quite promising. The graphs on the following page show the results of a buffering experiment conducted in small (10 L) buckets- untreated seawater in these experiments ranged from 7.7-7.8 during the 9 day experimental period, and treated seawater was buffered to an approximately constant pH of 8.3. A marked increase in both larval survival and growth can be seen in the buffered seawater tanks.

pH Bioassay- Day 9 (August 15, 2008)





In the hatchery itself, early attempts to buffer seawater in large tanks (6000-20000 gallons) also met with some success, and the performance of older larvae (especially mussels and clams) was dramatically improved, especially during conditions when incoming seawater was extremely low (minimum pH of 7.6). However, the results in large tanks were less impressive with younger larvae, and in general, buffering alone did not solve all of our problems. As is becoming the trend in this battle, however, our failure to fix the hatchery did lead to an important further insight.

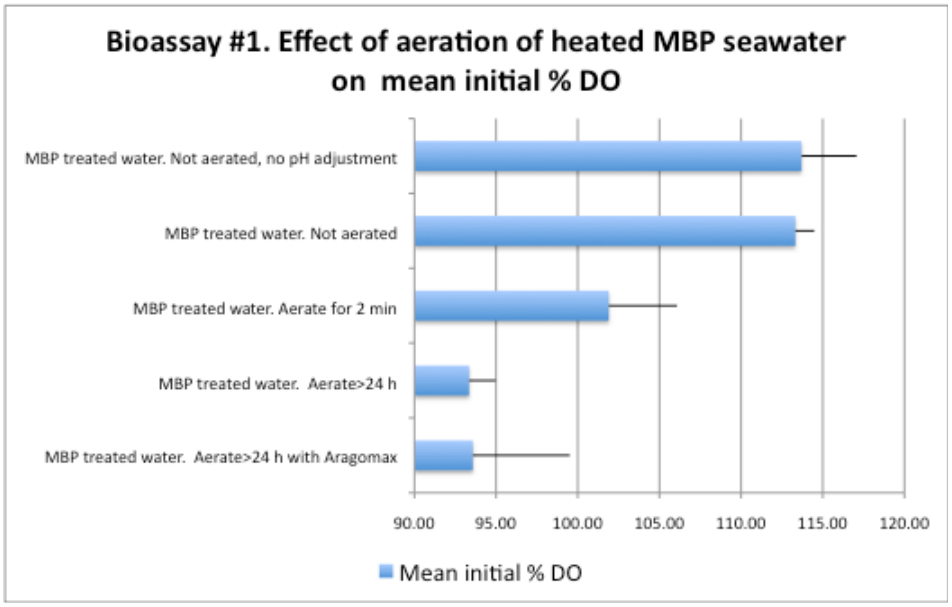
From these concurrent experiments, it became clear that what happens in a 10L bucket is quite different than what happens in a 6000 gallon tank. Whatever changes take place in a small container of buffered seawater appear to happen very quickly, while the same processes appear to be much slower in a large volume of water. Seawater chemistry involves a very complex set of interacting reactions- it is likely that although the 'quick fix' of adding buffers to a large volume of water improves conditions to a degree, buffering alone cannot address the underlying problems which force this system out of balance.

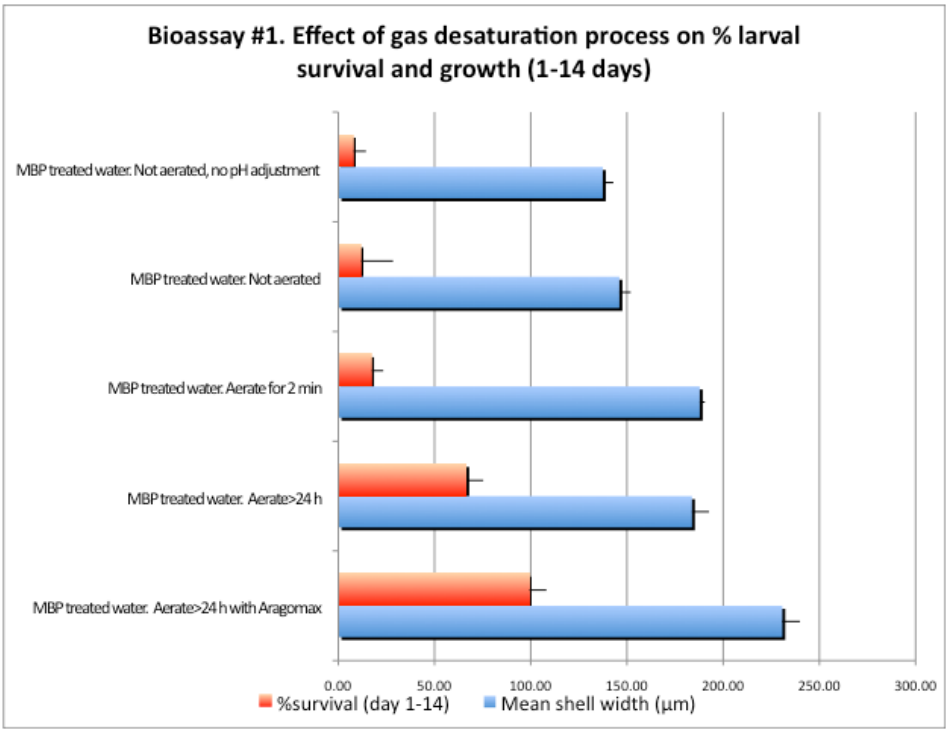
Feely et al (2007) states that the real culprit in this caper is atmospheric CO_2, derived from human inputs. In their opinion, high CO_2 in the air has increased the pCO_2 (partial pressure of CO_2) in seawater, which is ultimately forcing down the pH of the ocean. In addition to human influences, the pCO_2 of seawater can be influenced by biology- respiration of animals in seawater can greatly increase the pCO_2 of seawater in coastal areas. It is likely that biology in our bay, especially during the dead zone events of the past several years, contributed to high pCO_2 along our coast, and has helped to push this system further out of balance.

Unlike most gases, CO_2 is fairly difficult to remove from seawater, even with vigorous aeration. As a result, it can take several hours to significantly reduce CO_2 concentrations in a large volume of seawater. This idea may help to explain the differences observed in small containers of buffered seawater, where the high surface to volume ratio could allow CO_2 to escape quickly.

Degassing experiments

Unfortunately, until quite recently (this week, actually) we haven't had the tools available to assess pCO_2 concentrations directly. However, Chris Langdon at MBP has conducted some relevant work using O_2 saturation as a proxy for the saturation of other gases in seawater. In addition to elevated pCO_2, Chris' work also considers the direct effects of other gases such as nitrogen and methane, which can be directly toxic to shellfish larvae in supersaturated conditions. Some of Chris' work with degassing is summarized in the graphs below and shows a dramatic increase in the survival and growth of larvae after vigorous aeration and storage for a 24 hour period.





Whether the changes produced by degassing are related to high nitrogen, methane, or increased pCO_2, it is clear that some measurable changes take place when tanks of seawater are vigorously aerated. It is also clear that these changes can result in improved performance of larvae.

Based on these results, we began to measure O_2 saturation in tanks at Whiskey Creek. The following table shows $\%O_2$ measurements from several locations in the hatchery. The probe used to collect these measurements may be slightly inaccurate, but the measurements are reliable and repeatable. Therefore, the relative differences between readings can be used to gain valuable insights about gas saturation.

Source	% O_2 (3/29)	$\%O_2$ (4/1)
Cold Seawater (12 degC)	105%	88.5%
Heated Seawater (25 deg C)	113%	98%
Heated Seawater in tank immediately after filling	94%	
Heated Seawater in tank after 1 hour	86%	
Heated Seawater in tank filled overnight	84%	
Treated Seawater from existing seawater treatment system (heated)	84%	84%

To begin a discussion of this data, we should state that whenever cold seawater is heated in a heat exchanger, the water *has* to become supersaturated. Cold water holds more dissolved gases than warm water, so rapid heating means that there will temporarily be too much gas in the heated water when it comes out of the pipe. With that said, there are some interesting points to be made about the measurements shown above.

The equilibrium $\%O_2$ in these measurements seems to be 84%. When a tank is filled slowly overnight and allowed to sit (with gentle aeration), it eventually settles in at 84% saturation and stays at 84% over the next 48 hours. Over the past year, we have observed improved performance of larvae in tanks filled

overnight, when compared to tanks filled during the day. The differences in $\%O_2$ seen above may help explain these differences in performance, and illustrate how residence time remains an important factor in adjusting seawater chemistry. If it takes over one hour to reduce O_2 concentrations to equilibrium in a large tank, then it should take significantly longer to drive off stubborn gases like CO_2.

The data above also shows that treated seawater (passed through the existing treatment system) seems to be effectively degassed, and exits the system at 84% saturation. Vigorous aeration is an integral component of the treatment system (by happy accident), since the treatment system contains both a protein skimmer (with a venturi air injector) and a fluidized bioreactor powered by a tremendous amount of aeration. In addition, the system is designed to maximize residence time in the tanks, which seems to be a critical component of the degassing process.

Developing a comprehensive approach to the 2009 growing season

Although our understanding of water quality problems is admittedly imperfect, our investigation into ocean acidification research and early experiments with buffering and degassing of upwelled water provided us with some valuable insights, and provided some direction for continued research in 2009. In short, our strategy was to take a comprehensive approach to the problem, and modify the existing treatment system to address any potential stressors that could arise in the 2009 season.

Reducing the prevalence of Vibrio tubiashii in the hatchery

It is important to note that although *vibrio tubiashii* is no longer the primary focus of our research efforts, it is a significant stressor for oyster larvae. It was therefore critical that we take steps to reduce bacterial contamination in the hatchery, both to improve the overall health of larvae and to remove an additional variable that could further complicate our continued research efforts. Therefore, a significant portion of the funds received from the state of Oregon was used to improve the cleanliness of the hatchery and limit the prevalence of vibrios in our seawater systems.

The first step in this process was simply to shut down completely and dry out the facility for a period of six weeks, to eliminate any lingering sources of contamination from the previous season. During this period, much of the plumbing throughout the hatchery was replaced, and systems were modified to improve the process of disinfecting seawater lines. All media in the external sand filters was also replaced. Finally, all algae stocks from 2008 were discarded and replaced with fresh cultures from outside sources. The rigorous maintenance conducted in the winter months allowed us to enter the 2009 with a clean slate, and minimize the effects of an additional stressor that could potentially confound our continued research efforts.

In my mind, continued work to better identify and understand vibrio outbreaks is a critical component of our continued research efforts. My own understanding of vibrio improved when I began to think of the high prevalence of vibrio in the bay a *symptom* of water quality problems, rather than the cause. *Vibrio tubiashii* is a fast-growing, opportunistic bacterium which primarily acts as part of the ocean's 'clean up crew'. Its role in the environment is to break down organic matter, and when there is a lot of decaying organic matter in the ocean, we can expect there to be a lot of vibrio around to clean up the mess. Therefore, it is hoped that vibrio research will continue in earnest, with the goal of understanding the environmental factors that contribute to vibrio blooms in our coastal waters. If we can better understand the chemical cues that produce vibrio outbreaks in coastal bays, we may better understand the underlying changes in seawater chemistry that contribute to larval mortality.

Increased use of selected broodstock

During the 2008 season, MBP selected broodstock was used for commercial spawns on a number of occasions. In several instances, we observed that MBP groups fared better than larvae spawned from 'wild' broodstock. Certainly, use of MBP broodstock was not a magic bullet- during periods of extremely poor water quality, MBP larvae also experienced significant mortality. Nevertheless, using selected broodstock seemed to confer a definite advantage to larvae, and increased use of MBP broodstock has become a primary goal for the 2009 season. A large number of MBP larvae have already been produced this season, and early reports from growers have been very positive.

In a concerted effort to identify resistant MBP families, Chris Langdon and Mark Camara have planned and carried out a commercial spawn at Whiskey Creek, using a large pool of crosses derived from MBP broodstock. This pool of MBP crosses is currently in production at Whiskey Creek in large 6000 gallon, and is being treated as a normal group of larvae in our production cycle. In this way, they hope to expose the larvae to the stresses typically encountered at the hatchery during periods of poor water quality. At harvest, Mark Camara will use genetic techniques to identify specific crosses that harvest quickly and show high survival. This technique will allow for the rapid detection of resistant crosses, and within 2-3 months could identify a subset of 'super-crosses' which show elevated performance in the larval stage.

Strategy for use of the seawater treatment system in 2009

Limited use at the beginning of the season- In 2008, it was very difficult to make progress toward improving the seawater treatment system because it was impractical to continuously run side by side comparisons of treated and untreated seawater. Because of the extreme variability observed in our incoming seawater, we often missed the start of poor water quality events in commercial scale bioassays. Therefore, we decided to set up a redundant set of plumbing in part of the hatchery (where small larvae are typically grown), so that side by side experiments could be conducted with small larvae in more controlled conditions. These experiments (treatment vs. control) will be run continuously throughout the 2009 season. Over time, individual variations in water masses will average out, and provide a clear picture of the benefits conferred by seawater treatment.

Individual tests of additional treatments- A number of potential additions to the treatment process were discussed over the winter, some of which had shown positive results in late 2008. However, before adding them wholesale to the treatment system, each component was tested individually to understand its potential impacts on seawater quality, and the results are discussed in the following section. Essentially, our goal in these experiments was to ensure that each additional treatment was not *harmful* to larvae. As stated before, water quality in Netarts Bay is extremely variable, and discarding a treatment because it didn't help *this week* could eliminate treatments that could potentially be very important later in the season. This 'kitchen sink' approach to seawater treatment should provide our best defense against a variety of stressors, and the individual trials of each component will insure that these additions do not negatively impact the overall performance of the system.

Improvements to the treatment system

Installation of a pH control system- Low pH of seawater remains a relevant concern for the hatchery, particularly during extreme upwelling events, and buffering experiments in 2008 met with some limited success. Therefore, we took steps this spring to automate the process of buffering incoming seawater. Bill Robertson designed an efficient and effective system for the hatchery, which is currently in use at the front end of our seawater treatment system. Thus far, the system has functioned flawlessly, and insures that treated seawater never drops below a pH of 7.9.

Pre-heating seawater prior to treatment- In 2008, cold seawater was passed through the treatment system, then heated afterward. However, because of concerns about gas saturation, the process was reversed over the winter, and seawater is now heated prior to entering the treatment system. This change should help in our efforts to drive off gasses, since gasses are much less soluble at high temperature. As the data presented earlier shows, the treatment system now seems to function as an effective degasser (at least for volatile gases like oxygen, nitrogen, and methane), and reduces O_2 saturation to equilibrium concentrations.

CO_2 scrubbers- As was mentioned earlier, offgassing of CO_2 can take much longer than many other gases. In further efforts to reduce the pCO_2 of treated seawater, all air entering the treatment system through the protein skimmers and the bioreactors is passed through air scrubbers. These scrubbers reduce the concentration of CO_2 in air to near zero. This increases the concentration gradient between air and water, and should speed up the removal CO_2 from the incoming seawater. CO_2 scrubbers have also been tested on the air supply to individual tanks in the hatchery, and the preliminary results have been quite promising. The following data shows the size of larvae in 6000 gallon tanks after eight days, and shows a marked improvement in the size of larvae when continuously aerated with CO_2-free air.

Treatment	Size range	% on 100um screen
Untreated Seawater	110-130 microns	trace
Untreated Seawater, w/ CO_2 air scrubber	120-150 microns	~30%

In a subsequent bioassay, however, no improvement was observed with use of the CO_2 scrubber, and both groups of larvae were identical in size after eight days. Potentially, the water masses were quite different in the second experiment, and rendered the treatment unnecessary. For our purposes, that's okay. The treatment shows promising results in some conditions, and does not produce negative effects in others, so we will continue to proceed cautiously with the use of CO_2 scrubbers in seawater treatment.

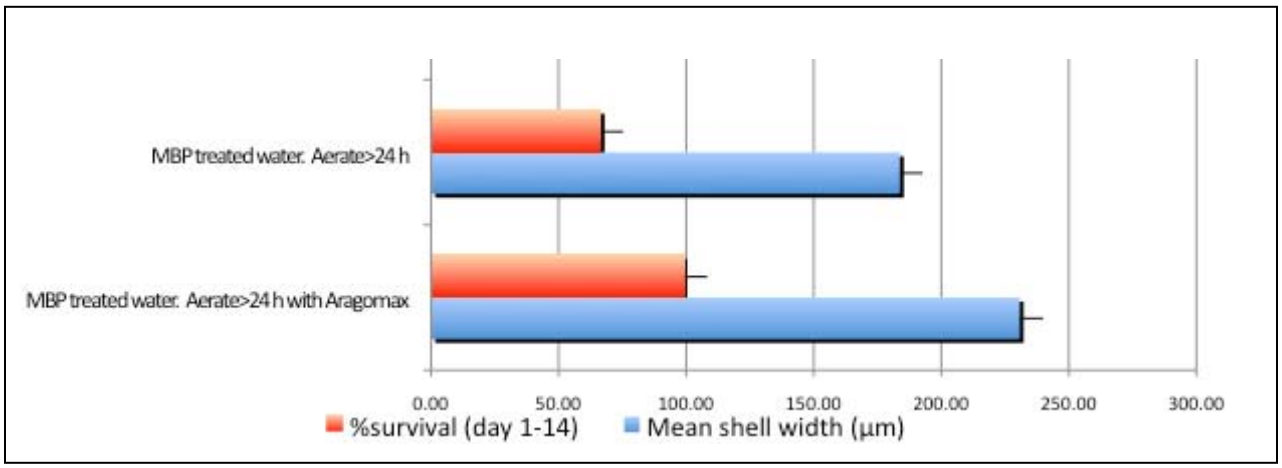
Venturis- In many commercial locations (fish farms, dams, etc), degassing of water is an important process, and a number of simple technologies exist to remove gasses from seawater. We have adapted one such technology for filling individual tanks in the hatchery, and measurements of $\%O_2$ saturation show an improvement over tanks filled without the venturis.

Source	% O_2 (3/29)
Heated Seawater (25 deg C)	113%
Heated Seawater in tank immediately after filling	94%
Heated Seawater in tank immediately after filling w/ Venturi	89%
Heated Seawater in tank filled overnight	84%

These venturis are currently being used to fill most tanks in the hatchery, and certainly show no adverse effects on larvae. Although treated seawater $\%O_2$ saturations are already reduced to equilibrium levels, venturis are also being used when tanks are filled with treated seawater. The technology is simple and

cheap, and has been shown not to harm larvae, so we are including it in our comprehensive set of treatment options.

Coral sand- Another treatment that has shown great promise in both small scale and large scale bioassays is passing seawater through coral sand as it enters the tank. Large scale experiments in fall of 2008 showed a marked improvement in larval growth, and follow up experiments at MBP supported this assertion in replicated bucket trials, the results of which are shown below.



Large scale experiments in 2009 also show the positive effects of coral- the table below shows size measurements of larvae after ten days, and shows a big improvement in the growth of larvae when seawater is passed through coral (and a venturi).

Treatment	Size range	% on 100um screen
Untreated Seawater	100-130 microns	trace
Untreated Seawater, w/ venturi and coral	110-150 microns	~40%

A second bioassay in 2009 showed no improvement with the addition of a venturi and coral to untreated seawater, and larvae in both the treatment and control were identical in size after 10 days. However, no negative effect was observed, and repeated experiments in the past year (five trials with positive results in 2008 and 2009) give us confidence that the addition of coral to the treatment system can significantly improve larval growth and survival.

'Why' adding coral helps is unclear, but there are at least two plausible explanations. First, the coral may simply be helping in the degassing process, by further agitating water as it falls in the tank. Second, some evidence suggests that larvae need small crystals of aragonite to be present in seawater, to aid in shell formation. During acidified conditions, these normally-occuring particles may have dissolved, and need to be replaced in our larval tanks. These particles could either be used directly in shell formation, or serve as seed crystals for non-biogenic precipitation of calcium carbonate, and make it much easier for larvae to build their shells. Regardless of the reasons, repeated bioassays show that coral helps, and it has therefore been included as a final step in our seawater treatment process.

Moving forward with the modified treatment system

The set of changes detailed above have resulted in a much more comprehensive approach to seawater treatment. The system, as best we are able, addresses the four major stressors we feel are affecting shellfish larvae: *vibrio tubiashii*, direct effects of low pH, indirect effects of elevated pCO_2 on shell formation, and degassing of gases such as carbon dioxide, methane, and nitrogen. To be sure, some additional tweaking of the system will follow in the coming months, but we are now essentially at the end of the list of available treatment options. We will continue limited use of the treatment system in side by side comparisons with untreated seawater throughout the growing season. If seawater conditions deteriorate in the coming months, we will switch over full production to treated seawater whenever it becomes necessary. Certainly, we do not have complete confidence that the new treatment system will solve all of our water quality problems, but by address a number of potential stress factors we may provide enough advantage to significantly improve production in the hatchery.

In the meantime, we will continue to improve our understanding of seawater chemistry, and build on collaborations recently established with researchers around the country.

Seawater Monitoring Program

By the end of 2008, we came to understand that our knowledge of our coastal bays and estuaries is at best imperfect. It also became clear that the difficulties we are facing are part of a big, global problem, from conversations with hatchery managers as far away as Australia, England, and Chile, and from current research showing the decline in growth of corals and shellfish around the world. Most of all, it became clear that we need to build collaborations with a diverse set of researchers if we hope to fully understand and correct these water quality problems.

To that end, the PCSGA seed supply committee developed and secured funding for a comprehensive monitoring program in both Netarts Bay and Willapa Bay, which will be managed by Dan Cheney and researchers at the Pacific Shellfish Institute (PSI). Data loggers deployed in each location will record hourly measurements of pH, temperature, salinity, dissolved oxygen, carbon dioxide, and oxidation/reduction potential. Each week, water samples will also be sent to Burke Hales, a chemical oceanographer at OSU who specializes in ocean acidification research. Burke will provide redundancy to check the accuracy of our data loggers, and will also obtain accurate measurements of pCO_2, dissolved inorganic carbon (DIC), and total alkalinity, as well as total nutrient concentrations. These samples will take the guesswork out of our efforts to define water quality problems, and Burke's lab will provide the expertise required to pinpoint exactly what is wrong with seawater in our coastal bays.

In addition, weekly samples of treated seawater from the hatchery will be sent to Burke's lab, and will allow us to understand whether our treatments are sufficient to return water quality to 'normal'. By correlating Burke's measurements with the performance of larvae in both treated and untreated seawater, we should gain valuable insight into which parameters are of most concern to shellfish larvae.

This monitoring program will also include sending weekly water samples to Ralph Elston, in a continued effort to monitor outbreaks of *vibrio tubiashii*, and to monitor total bacteria counts in the bay. Hopefully, comparing these results to detailed measurements of seawater chemistry can determine the environmental factors that trigger *vibrio* blooms and the production of toxins that cause mortality in shellfish larvae.

In addition, this program will include the work already being conducted by Alan Trimble, which includes routine plankton samples in Willapa Bay. It is hoped that comparison of this data with Burke and Ralph's measurements will help identify which environmental factors are of greatest concern for shellfish larvae in the natural environment.

We now have a lot of heads together on this issue, and the collaborations fostered by the seed supply committee have brought together researchers with a wide range in areas of expertise. By combining their talents and overlaying oceanographic measurements, bacteria monitoring, plankton sampling, and larval performance data from the hatchery, we hope to obtain a clear picture of the changes affecting our coastal bays and estuaries, and take great strides to mitigate their effects on the Pacific Northwest shellfish industry.

References

Catlip, K. Rising Acidity of Estuary Waters May Spell Trouble for Oysters and Other Shellfish. *Inside Smithsonian Research*, spring (2008).

Feely, R.A., Sabine, C.L., Hernandez,-Ayon, J.M., Iansun, P., and Hales, B. Evidence for Upwelling of Corrosive "Acidified" Water onto the Continental Shelf. *Science*, 320, 1490 (2008).

Gazeau, F.., Quiblier, C., Jansen, J., Gattuso, J.P., Middleburg, J.J., and Heip, C.H.R. Impact of Elevated CO_2 on Shellfish Calcification. *Geophysical Research Letters*, 34: L07603 (2007).

Kurihara, H., Kato, S., Ishimatsu, A. Effects of Increased pCO_2 on Early Development of the Oyster *Crassostrea gigas. Aquatic Biology*, 1: 91-98 (2008).

Langdon, C., Broecker, W.S., Hammond, D.E., Glenn, E., Fitzsimmons, K., Nelson, S.G., Peng, T.H., Hajdas, I., and Bonani, G. Effects of Elevated CO_2 on the Community Metabolism of an Experimental Coral Reef. *Global Biogeochemical Cycles*, 17, no. 1011 (2003).

Lee, S.W., Hong, S.M., and Choi, C.S. Characteristics of Calcification Processes in Embryos and Larvae of the Pacific Oyster *Crassostrea gigas*. *Bulletin of Marine Science,* 78(2): 309-317 (2006).

Weiss, I.M., Tucoss, N. Addadi, L., and Weiner, S. Mollusc Larval Shell Formation Amorphous Calcium Carbonate is a Precursor Phase for Aragonite. *Journal of Experimental Zoology*, 293: 478-491 (2002).