USA Trip Report on Recent Struggles Facing Shellfish Hatcheries and Industry on the Pacific North West of the USA, 2009

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Introduction

This study tour was undertaken to attend the WERA and 101st Shellfisheries Association Conferences held in Savannah, Georgia, USA, from 21st – 26th March 2009 and to visit shellfish hatcheries throughout Washington State and Oregon.

Unfortunately I was unable to attend the WERA conference because of flight delays however Peter Kube a quantitative geneticist from the CSIRO in Hobart attended and gave a presentation on the breeding programs conducted by Shellfish Culture Ltd. The NSA meeting was comprised of three and half days of industry and research presentations with a wide range of subject areas including shellfish, crustaceans, genetics, diseases, environmental impacts and restoration. The main purpose of my attendance and the visits to the NW hatcheries was to investigate the impact of *Vibrio* pathogens on hatchery production and recent advances in technology and management procedures to combat its affects.

The North West Coast of the US has a major shellfish industry worth $278 million in which 75% of the value is oysters and employees approximately 3000 people. The main suppliers of the oyster larvae and seed are Taylors Shellfish, Whisky Creek Hatchery, Coast Oysters and Lummi Hatchery. Whisky Creek supplies 75% of the Pacific oyster (*Crassostrea gigas*) eyed larvae to the independent oyster farmers throughout the region for 'remote setting' (figure 1). Both Taylors Shellfish and Whisky Creek hatcheries have experienced major problems in production of oyster larvae from 2005-2008 (figure 2 &3) resulting in undersupply to tideland farms from Southern California to Canada. This shortfall in seed supply will in turn impact on future mature oyster harvests. This report was compiled after several meetings with academic and commercial shellfish hatchery experts on the North West coast of the USA. The following documents the history of oyster larvae mortalities, the measures that have so far been put in place and what can be learned from an Australian perspective.

Figure 1. Tanks of oyster shells ready for eyed larvae input for 'remote setting'

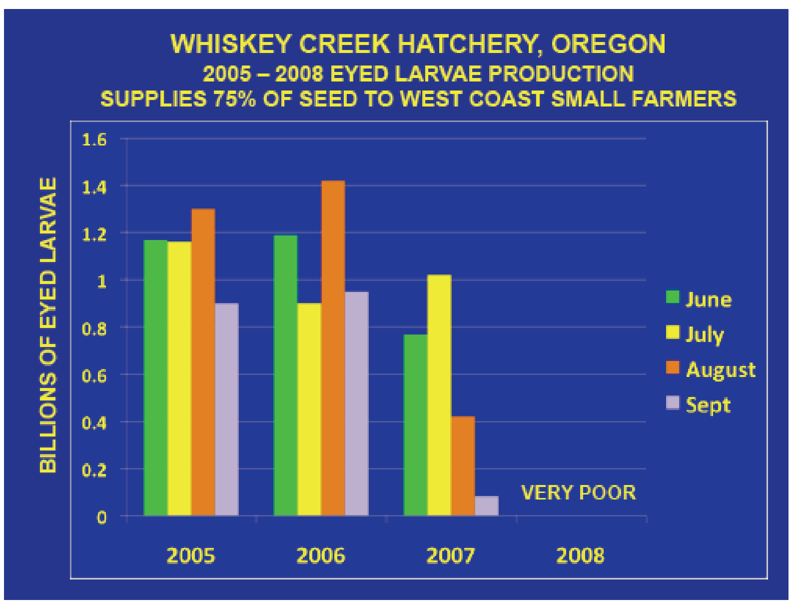

Figure 2. Shows decrease in oyster larvae produced at Whisky Creek Hatchery 2005-2008 (Langdon 2009)

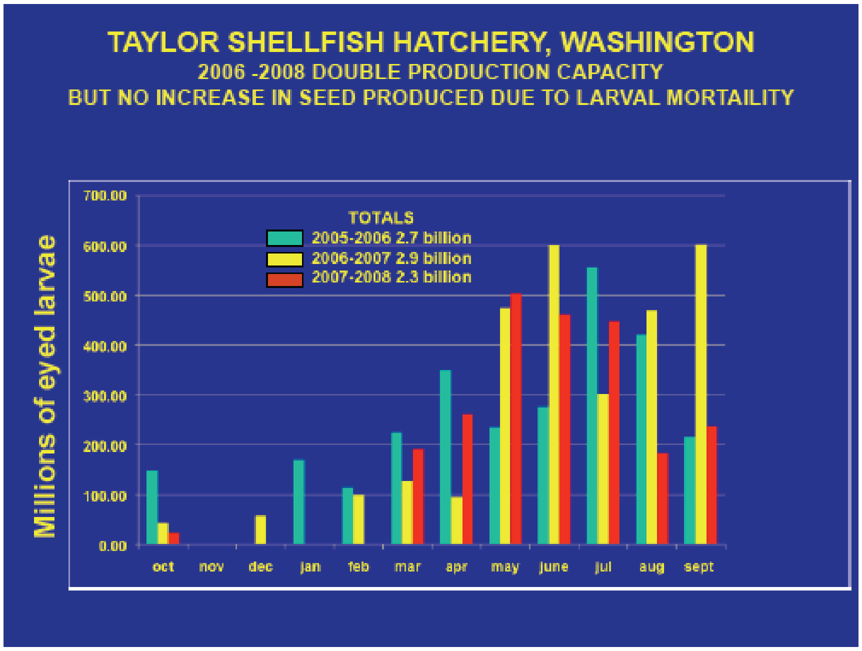

Figure 3. Shows reduction in oyster larvae produced by Taylors Shellfish Quilcine Hatchery from 2005-2008 (Langdon 2009). Note in 2006 Taylors doubled their capacity however they produced equal to or less larvae than in previous years.

- Where it all started.

Problems with larval mortality were first noticed in 2005 at the Oregon State University (OSU) which caused the shutdown of the Molluscan Broodstock Program. This program run by Professor Chris Langdon plays an integral role in the industry by providing superior performing broodstock and product through genetic selection. For the first time since the program was established, mass mortalities occurred through the larval stages. Not only progenies of selected crosses were lost but the loss of parents that had to be sacrificed for stripped spawning was a further setback for the breeding program.

Through an investigation in collaboration with Dr Ralph Elston from Aquatechnics it was found there were unusually high numbers of the bacteria *Vibrio tubiashii* in the seawater. *V. tubiashii* can be highly virulent and can cause serious disease in oyster larvae. Not only were the numbers extremely high (in some tests up to 1 million/ml), *V. tubiashii* was also extremely dominant over other bacteria species, at times making up the entire bacterial population (Table 1).

	Vibrio tubiashi as % of total 24 hour MA counts	
Date Sample Taken	Incoming Tide	Outgoing Tide
6/27/2006	**100%**	**0%**
7/31/2006	**50%**	**38%**
8/2/2006		33%
8/5/2006	**58%**	**13%**
8/9/2006	**52%**	**75%**
8/14/2006	**48%**	**19%**
8/17/2006	**10%**	**0%**
8/21/2006	0%	0%
8/28/2006	**67%**	**54%**
9/1/2006	**64%**	**0%**
9/8/2006	0%	0%
9/13/2006	0%	0%
Average:	**56%**	**25%**

Table 1. Shows total amount of *Vibrio tubiashii* on marine agar compared with other marine bacteria (Langdon 2009)

Vibrio tubiashii has been associated with larval disease since it's identification by Tubiash et al in 1965. There are two known pathologies for *V. tubiashii*. Firstly, *V. tubiashii* can produce extracellular toxins such as haemolysons, metalloproteases and cilaristatins, and secondly it can also be invasive. Both pathologies result in rapid reduction in larval motility and necrosis of soft tissue leading to death (see Figure 4)(Hasegawa et al 2008). Unfortunately for hatcheries in Australia it has been identified in its ocean waters, however more investigation is needed to establish its virulence.

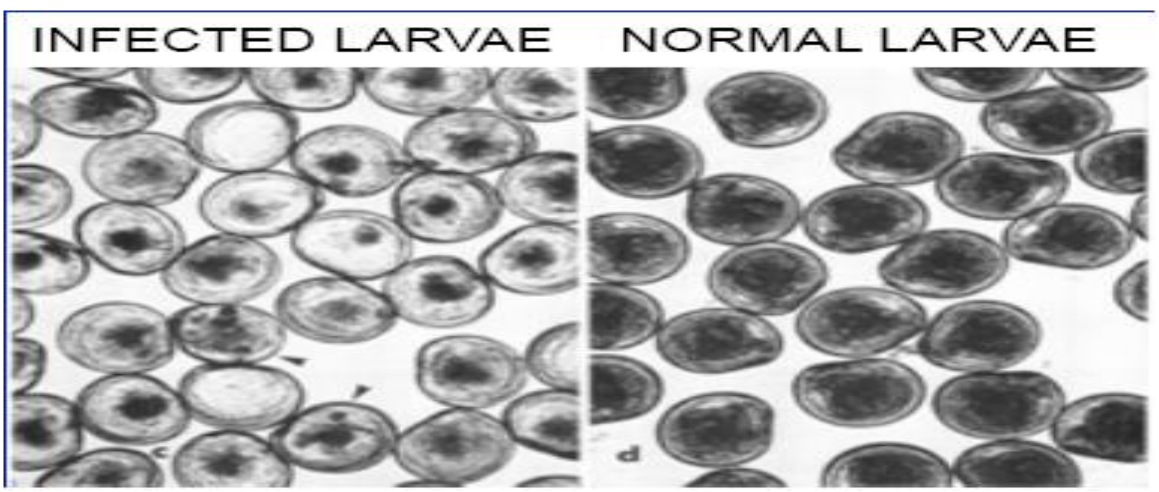

Figure 4. *C. gigas* larvae that are infected with *V. tubiashii* (left) and healthy larvae on right (Langdon 2009)

The high numbers of *V. tubiashii* were found to be associated with dramatic changes in water chemistry off the Washington and Oregon coasts. These changes coincided with unusual upwelling events which brought to the surface cold water that was found to be acidic, nutrient rich and hypoxic (< 1.4ml of O_2 per ml of seawater). These 'dead zones' have been steadily increasing in distribution since their discovery in 2002 (Figure 5)(Langdon 2009). These hypoxic zones are very unusual in shallow water close to the coast line. However "dead zones" are common on the sea floor on the middle to outer regions of the continental shelf, and it's the upwelling in summer time that brings it to the surface. The reason for the dominance of *V. tubiashii* over other bacteria in these dead zones is not fully understood. One hypothesis is that *V. tubiashii* being a facultative anaerobe (ie. prefers aerobic respiration to grow but can switch to anaerobic respiration when oxygen levels drop) it can out compete other bacteria that are not able to utilize the nutrient rich water to proliferate under hypoxic conditions (Chris Langdon, personal communication).

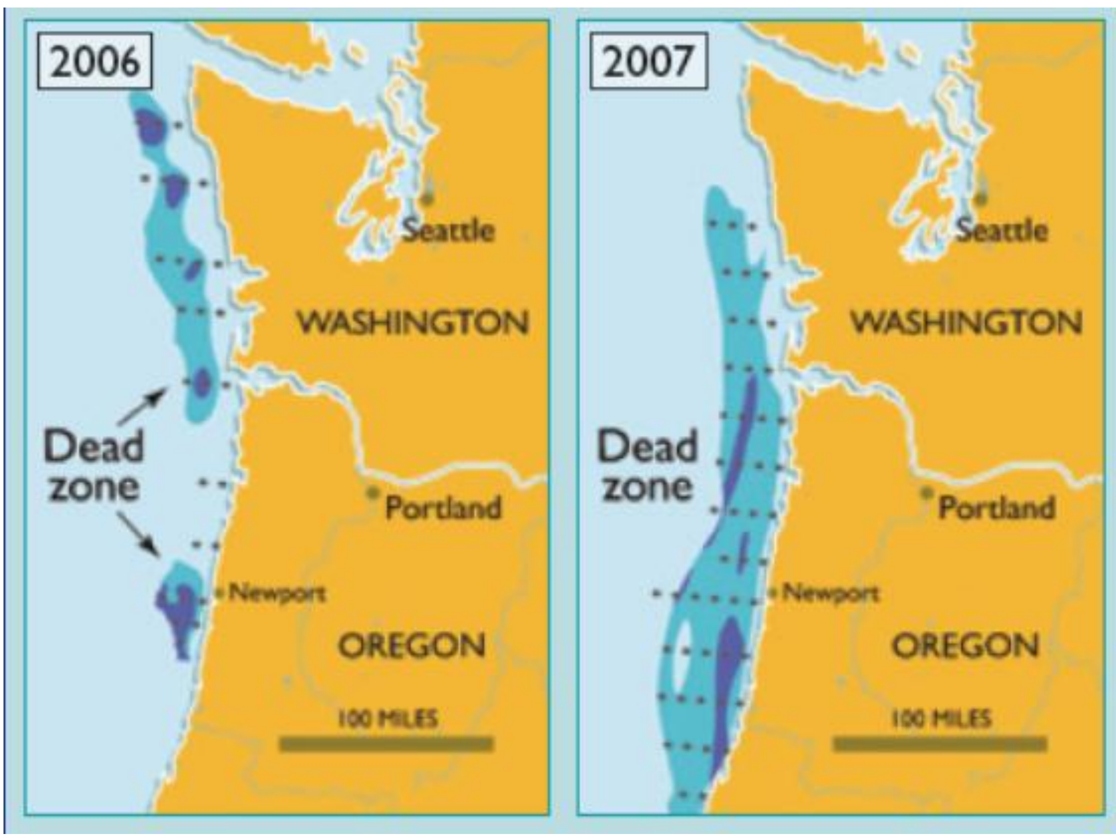

Figure 5. Recent expansion of the hypoxic 'dead zones' caused by upwelling during summer off the coasts of Washington and Oregon (Langdon 2009).

- Solving the vibriosis problem

To combat the effects of *Vibrio tubiashii* Chris Langdon and Alan Barton from OSU developed a filtration system in 2006 which would remove all incoming bacteria. They used a combination of mechanical filtration (sand and cartridge filters) as well as foam fractionation and ultraviolet radiation. The sand and cartridge filters were important in taking out the major proportion of detritus material from the water, also the foam fractionation was very important in removing the colloidal material that bacteria are attached to (Figure 6). However, Alan stressed that in seawater sterilized using UV, the larvae could not grow and died within several days from hatching. The same observations were made in Australia on *Crassostrea gigas* larvae (Garland et al. (1986). To overcome this problem Langdon and his colleagues decided to develop a biological filter to re-colonize the water with bacteria that would not cause disease in oyster larvae. Further collaboration with Ralph Elston led to the isolation of a bacterial species that was not only non pathogenic to shellfish larvae but also had a probiotic affect against *Vibrio tubiashii*.

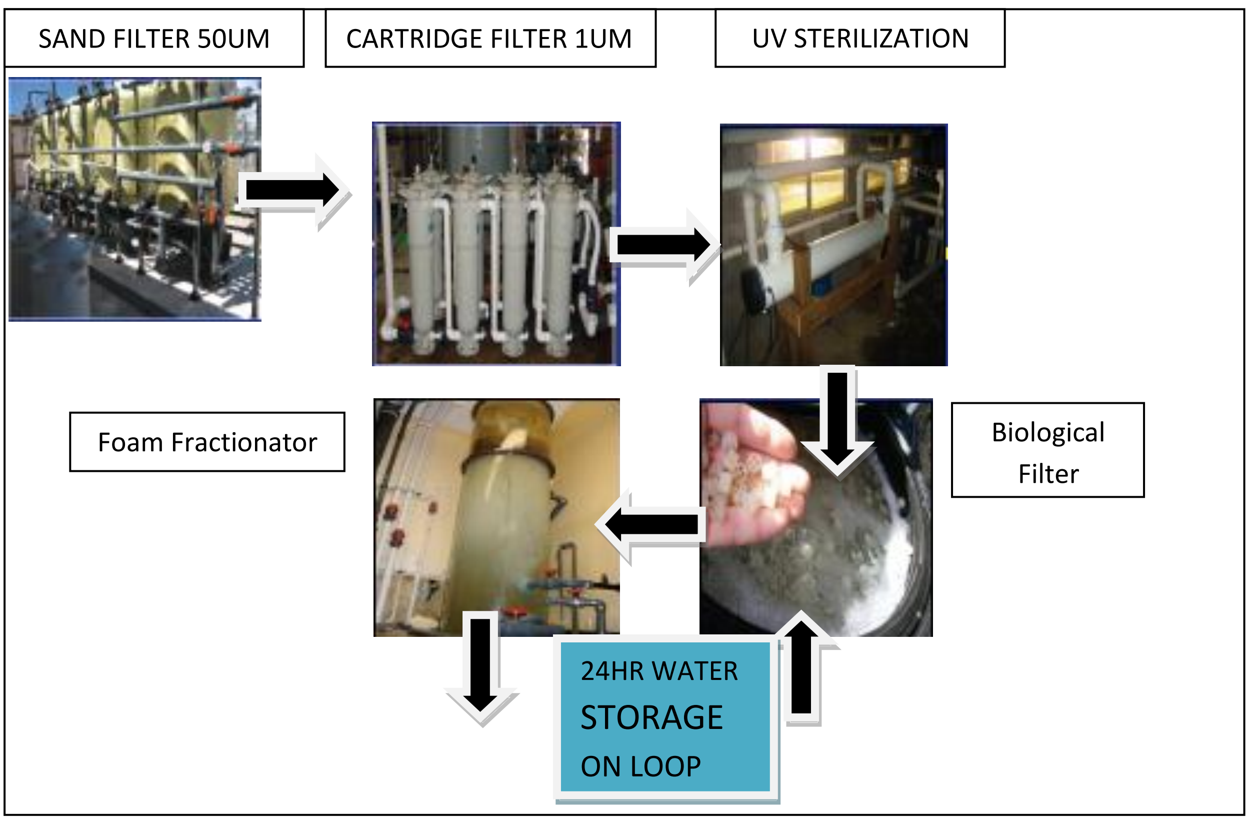

Fig. 7. Molluscan Broodstock Program filtration system developed by Chris Langdon and Alan Barton

The new advances produced positive results and larvae could be produced again at experimental levels. Following on from this R&D success, the filtration technology was transferred to the Whisky Creek hatchery in 2008 at a cost of $180,000 USD, which was only sufficient to acquire a filtration system for half of the production capacity of the hatchery. Researcher Alan Barton saw this as a unique opportunity touse the site as a proof of concept and with the support of hatchery owner Sue Cudd, Alan was able to compare production success of commercial larval batches raised with filtered water against unfiltered water control. From February till June 2088 the filtered water showed it produced consistently better larval growth and survival compared to untreated water (Figure 7).

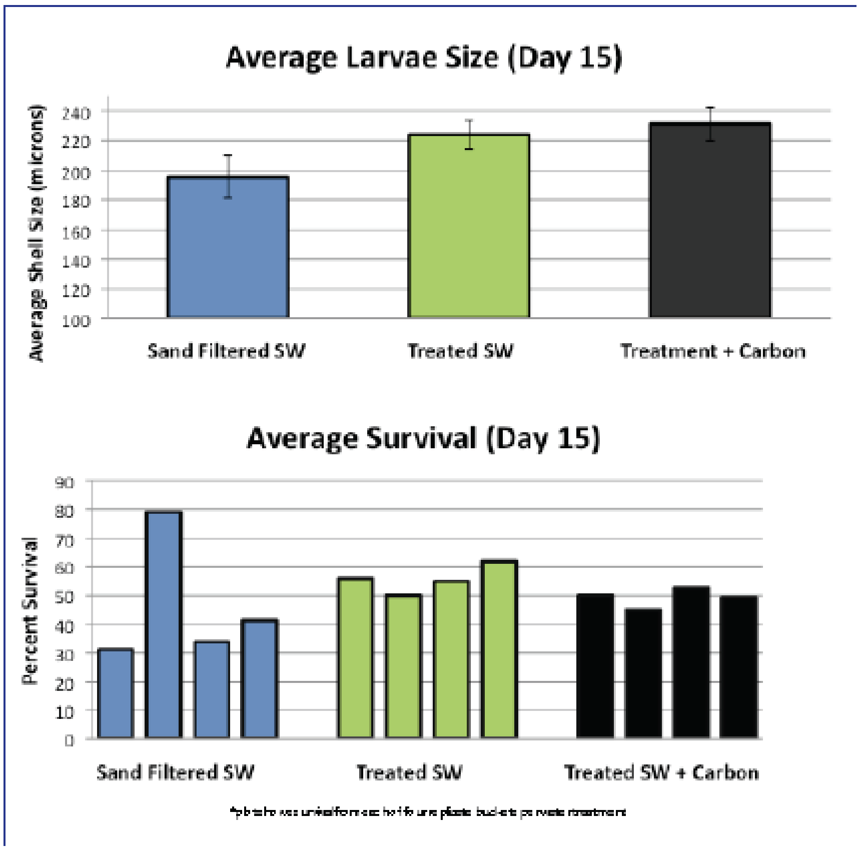

Figure 7. Average growth and survival of oyster larvae between treated and untreated seawater at Whisky Creek Hatchery (Langdon 2009).

Unfortunately reality struck in July of that same year when Sue Cudd and Alan Barton started to notice problems again not just in the untreated water but in the treated water as well. High mortality and slow growth in the early stages of development was seen across the board and the problem worsened until mid September when commercial production was stopped to save money.

The difficulties experienced at the Whisky Creek Hatchery were also echoed by Taylor Shellfish, another large oyster seed producer. So, by the end of 2008, after having solved the *V. tubiashii* problem, commercial shellfish hatcheries on the north west coast of the USA were back to square one, struggling to supply the growers.

- What now?

Chris Langdon and his team at OSU went back to work and what they found was a dramatic shift in the chemistry of the seawater used in the hatcheries. The pH had dropped from 8.2 -7.4 for Taylors Shellfish and 8.2- 7.6 for Whisky Creek and overall - C below average. The shift was sudden and corresponded with the drop in larval growth and survival observed during commercial production

The drops in pH and temperature pose two separate and significant problems for shellfish hatcheries. Firstly, the upwelling is bringing to the surface acidic water is corrosive for calcium carbonate ($Ca\ CO_3$). Shellfish incorporate $Ca\ CO_3$ from dissolved calcium (Ca^{2+}) and carbonate ions (CO_3^{2-}) through the reaction $Ca^{2+} + CO_3^{2-} = Ca\ CO_3$ to form their shells. More specifically Pacific Oyster larvae along with other commercially important shellfish use a crystalline form of $Ca\ CO_3$ called aragonite to form their shells (Salisbury et al 2008). When the pH of seawater decreases the solubility of aragonite increases (figure 8), which according to Chris Langdon and Alan Barton could dissolve larval shell or inhibit shell growth making them more susceptible to disease and infection.

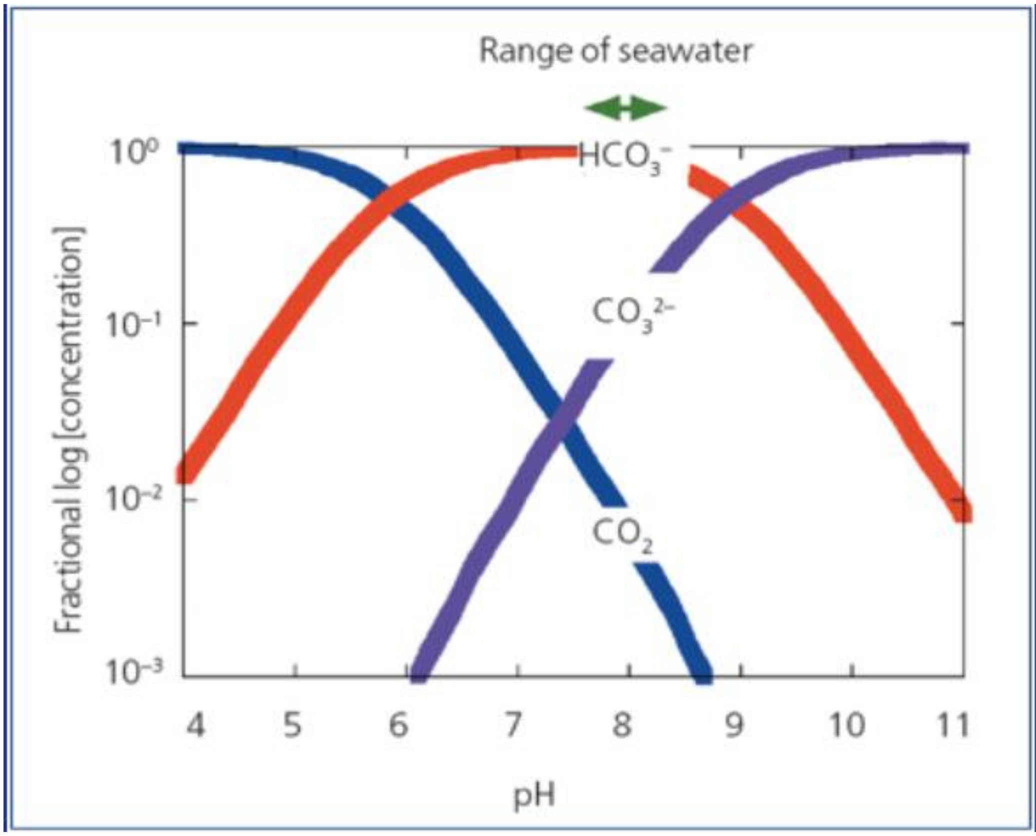

Figure 8. Shows solubility of Ca CO_3 with varying pH in seawater.

The profile of pH and aragonite solubility at varying depths can be seen in Figure 9 and illustrates the impact that the upwelling has in bringing this cold acidic water to the surface. It also brings to light a bigger and sometimes contentious issue of increasing atmospheric CO_2 resulting in acidification of our oceans. With increasing diffusion of higher amounts of atmospheric CO_2 into our seawater The Royal Society (2005) suggests that the average pH will decrease by 0.2-0.5 units by the end of the 21st century. The impact of this on all marine life and coastal zones remains to be seen and is a larger issue outside the scope of this report but is worth noting.

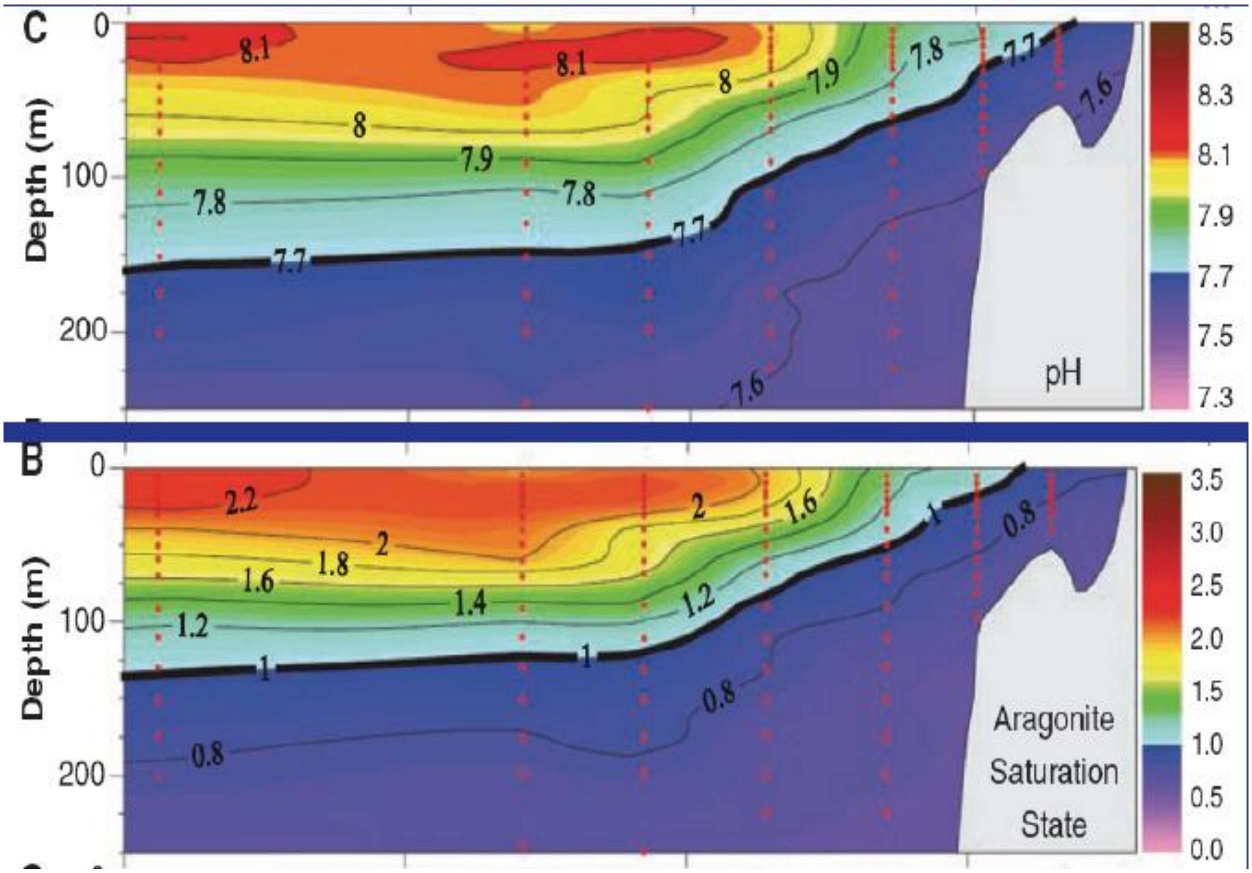

Figure 9. Profile of pH and aragonite saturation changes with depth of ocean water (Feely et al 2008)

Chris Langdon also highlighted that the impact of the upwelling is not only affecting the hatcheries but also on recruitment of shellfish in the wild. As opposed to the Australian oyster industry, which is supplied entirely with hatchery seed, the US industry relies on both wild recruitment and hatchery produced seed for their ongrowing. Wild spat is collected on oyster reefs constructed with oyster shells that provide the free swimming larvae with a setting substrate. Following set the shells are removed and distributed across their tidal leases. However in the past 3 years there has been no recruitment. Chris Langdon added that the natural fouling of barnacles on oyster reefs, intake pipes and boats have been non-existent in this period, which is a concern for the eco-system as a whole.

Lower pH in the ocean is not the only factor influencing low recruitment, cold water temperatures could be a factor as well. Pacific Oysters spawn once a year in estuaries during the summer time which coincides with the upwelling events seen over the past few years. Oysters convert their winter reserve to gonad during spring when the water temperature and food availability increases. The warmer summer water temperatures (- C) will induce final gonad maturation and trigger spawning events. Over the past few years particularly in 2008 summer water temperatures have been - C below average, due to upwelling events. These cool temperatures have delayed gonad maturation and have failed to trigger spawning in the wild, thus no recruitment has occurred in 2008 (Figure 10). Naturally this has put more pressure on the hatcheries especially Whisky Creek to produce the larvae that the farmers could not obtain through wild spat fall.

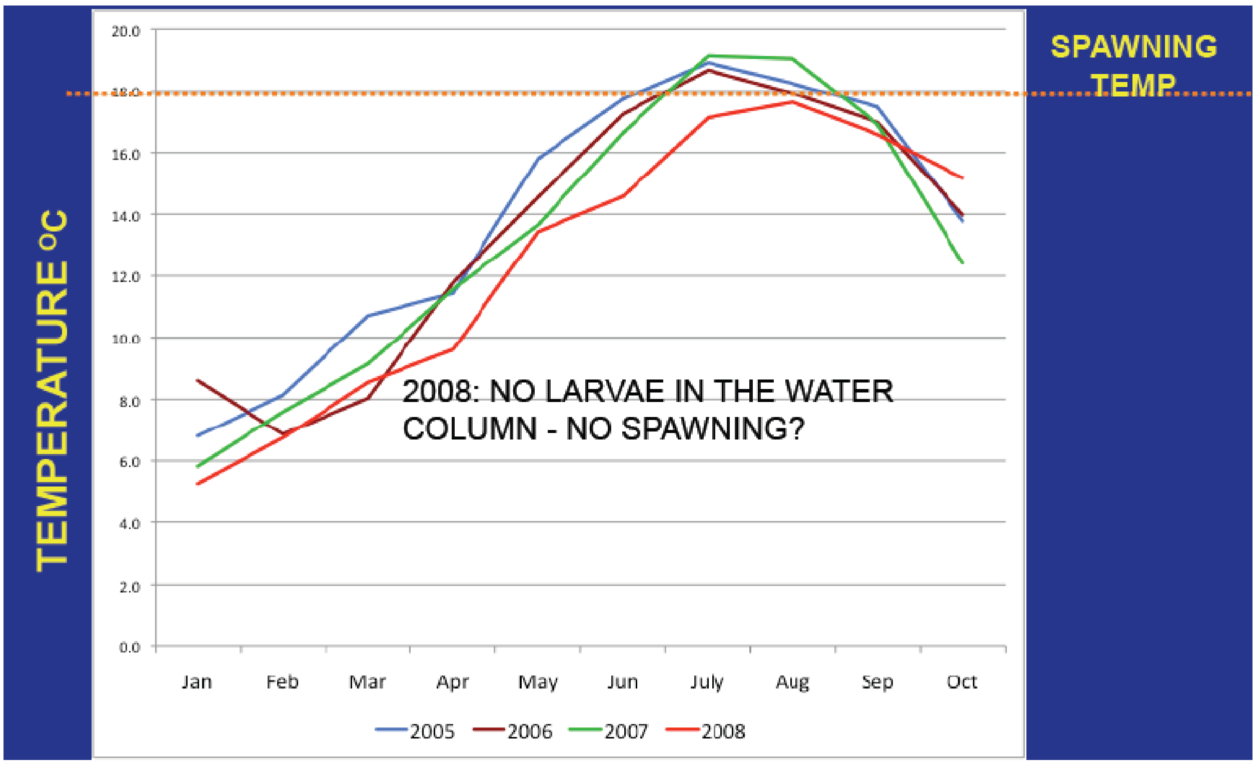

Figure. 10. Average monthly seawater temperature in Willapa Bay on Pacific North West of the US (Langdon 2009).

Researchers have also found that lower temperatures are also influencing larval survival at Whisky Creek hatchery because of gas super-saturation. Gas supersaturation can occur through a variety of ways. One of the common causes of gas supersaturation is heating cold water quickly to greater than six degrees above ambient water temperature. When water is heated the gases decrease in solubility forming gas bubbles. Sue Cudd C for optimal larval growth. However as the incoming water is C gas supersaturation occurs. Alan Barton did some trials on larval growth and survival and found that as oxygen saturation increased survival and growth decreased (Figures 11 and 12).

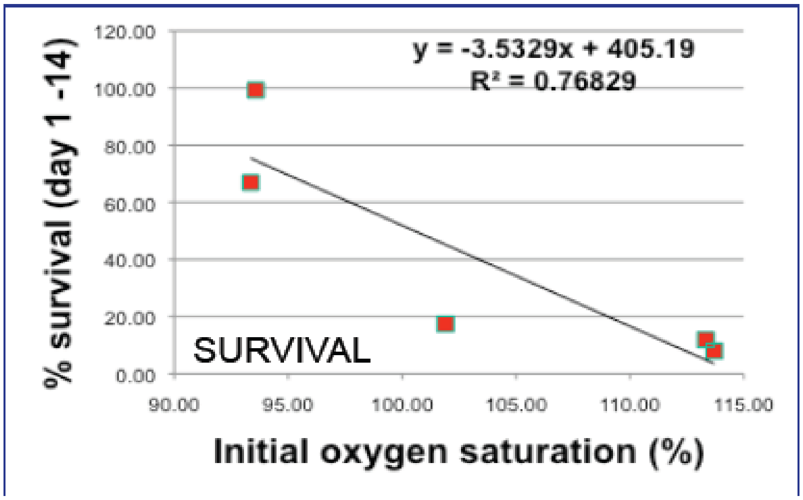

Fig. 11. Percentage survival of C. gigas larvae versus oxygen saturation at Whisky Creek hatchery (Langdon 2009)

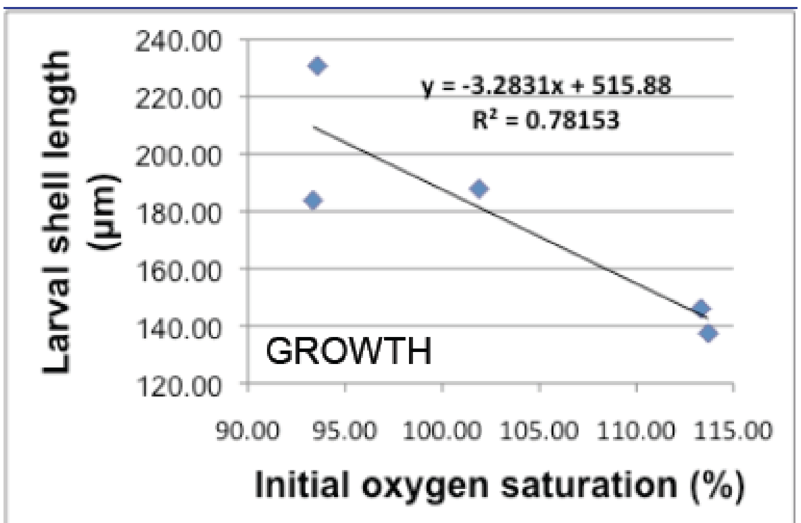

Fig. 12. Growth of C. gigas larvae versus oxygen saturation at Whisky Creek hatchery (Langdon 2009).

Conclusion – Lessons for Australian hatcheries

Changes in oceanic water chemistry that have taken place off the Pacific north west of the US have severely impacted on the production of shellfish both wild and in hatcheries. Researchers, government and industry leaders have been working closely together to investigate and solve the issues preventing shellfish larval growth and survival. It has been found that the source of poor production is not just related to *Vibrio tubiashii* but on a wide range of water quality changes, which are not yet fully understood. If these changes were to occur in Australia it could potentially collapse the oyster seed supply chain, which relies solely on hatchery production. This in turn would put the entire Australian shellfish industry at risk being pearl oyster growers in Western Australia, Pacific oyster growers in Tasmania, NSW and South Australia and Sydney Rock oyster growers in NSW and Queensland. For this reason hatcheries in Australia need to take a proactive approach in developing reliable seawater monitoring and treatment processes to insulate themselves from these changes if they are to have reliable larval production in the future.

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Paul and Bill Taylor – Taylor Shellfish

Dr Benoit Eudeline, Dr Joth Davis and Ed Jones – Taylors Shellfish Hatchery

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Abstracts from presentations conducted at the 101st National Shellfisheries Conference relevant to the scope of this report

SEARCHING FOR SOLUTIONS: IMPEDIMENTS TO SHELLFISH HATCHERY PRODUCTION IN THE PACIFIC NORTHWEST, USA. Kevin Amos and **Dan Cheney.** National Oceanic & Atmospheric Administration (NOAA), 1315 E-West Hwy., SSMC #3-13th Fl., Rm. 13113, Code:F Silver Spring, MD, 20910, USA; Pacific Shellfish Institute, 120 State Ave. NE #142, Olympia, WA, 98501, USA

Bivalve molluscan hatcheries and wild spat sets have been severely impacted the past few years in the Pacific Northwest. Significant mortalities in shellfish hatcheries are due to bacterial infections of Vibrio tubiashii, ocean acidification, and a combination of unknown environmental factors. This situation has reached emergency proportions as shellfish hatchery operators consider the very real possibility of going out of business. Consequently, growers who depend on hatcheries and wild set seed will be unable to meet buyers demands in the coming years. In response to this emergency, the NOAA Aquaculture Program, in co-operation with the Pacific Coast Shellfish Growers Association and the Pacific Shellfish Institute has brought together growers and researchers to develop a strategic plan to investigate these problems. Information will be presented on the status of the plan development and implementation.

PHAGE AS A POSSIBLE INTERVENTION FOR *VIBRIO TUBIASHII* IN SHELLFISH HATCHERIES. Gary P. Richards. USDA, Agricultural Research Service, James W. W. Baker Center, Dover, De, 19901, USA.

Vibrio tubiashii is a bacterial pathogen that is highly lethal to larval and juvenile shellfish. It has dramatically reduced the production of bivalve seed stock for commercial shellfish growers on the US West Coast. A potential remediation plan was developed to isolate and characterise a phage against *V. tubiashii*. Pages are bacterial viruses that are being increasingly used in food processing to rid products of bacterial contamination. For instance, the addition of *Listeria* phages to meat products has been accepted by the US FDA as an intervention against *Listeria* contamination to reduce human illness. There are two general types of phages: lytic and lysogenic. Lytic phages offer some promise as a remediation for *V. tubiashii* in shellfish hatcheries. Although phages against some *Vibrio* species have been isolated, there are no known phages against *V. tubiashii.* This presentation recaps our plan and progress over the past year to develop specific assays for *V. tubiashii* phages in seawater, to pick and confirm presumptive virus plaques, to introduce phages in hatchery settings, and monitor *Vibrio* levels using our novel (COPP) assay for total vibrios. The viability of the West Coast shellfish industry may well depend on success in controlling this important pathogen.

ASSESSING SELECTED PACIFIC OYSTER STOCKS FOR DISEASE RESISTANCE IN TOMALES BAY, CALIFORNIA. Colleen A. Burge, Carolyn S. Friedman , Paul G. Olin , Terry Sawyer , John Finger, and Drew Alden. University of Washington, Box 355020, Seattle, WA, 98195, USA; University of California Davis, One Shields Avenue, Davis, CA, 95616, USA, Hog Island Oyster Company, 20215 Highway 1, Marshall, CA, 94940, USA; Tomales Bay Oyster Company, PO Box296, Point Reyes Station, CA, 94956, USA.

Summer seed mortalities (SSM) of Pacific oysters, *Crassostrea gigas,* have occurred in Tomales bay, California for 13 of the past 15 years and an oyster herpesvirus (OsHV) has been identified and associated with SSM in Tomales Bay as early as 1995 (earliest test date). OsHV has been associated with mortalities in larvae and/or seed oysters in New Zealand, France and Spain and ahs been detected in oysters from multiple Asian countries. Differential survival has been reported among stocks reared in Tomales Bay, indicating some stocks may have natural resistance to OsHV. Oysters selected for OsHV resistance may provide oyster growers with stocks with increased survival. Potentially OsHV resistant Pacific oyster families were produced in April and July of 2008 at the Bodega Marine Laboratory from survivors of SSM in Tomales Bay. Three families of oysters produced from the April 2008 spawn were planted at two sites in Tomales Bay and monitored from June-September 2008 for mortality, disease (OsHV and other diseases), and growth compared to a control stock. Growth and mortality of the April and July 2008 (planted Fall of 2008) spawns will be monitored through Summer of 2009 to judge the utility of these oysters as farm product.

UPDATE ON RE-EMERGENCE OFVIBRIOSIS IN SHELLFISH HATCHERIES AND NURSERIES. Ralph A. Elston, Karen Humphrey, and Ildiko Polyak. Aquatechnics, 455 West Bell Street, Sequim, WA, 98382, USA.

During 2007, we documented the re-emergence of a severe episode of vibriosis caused by *Vibrio tubiashii* in shellfish hatcheries. During continuing studies in 2008, we have identified chronic and sporadic outbreaks at the N.E.L.H.A. site in Hawaii, the west coast of N. America and elsewhere. The west coast episode in 2007 appeared to be driven by unusually warm sea surface temperatures (SST), in conjunction with intermittent upwelling. Shifts in key oceanic and estuarine water chemistry parameters that have negatively affected natural and farmed early life stage shellfish seem to have also occurred, but these are incompletely known and need further elucidation. Environmental conditions may favour outbreaks of vibriosis, but the disease has caused documented losses since at least the 1970s.

In summer 2008, along the west coast of N. America, SSTs were in a more or less typical range until mid-summer, followed by a warming event, that was associated with another lesser vibriosis event. There is a need to standardize and upgrade sanitation procedures for hatchery and nursery production facilities. In addition, water quality standards for the raring of healthy bivalve larvae and juveniles are needed, along with improved prevention methods for vibriosis and better application of known management controls.

RECENT STRUGGLES OF WEST COAST COMMERCIAL HATCHERIES TO PRODUCE PACIFIC OYSTER LARVAE. Benoit Eudeline , Alan Barton , and **Chris Langdon.** Taylor Shellfish Inc, 701 Broad Spit Road, Quilcene, WA, 98376, USA; Whisky Creek Shellfish Hatchery, 2975 Netarts Bay Road, Tillamook, OR, 97141, USA; Oregon State University, 203 Southeast Marine Science Drive Newport, OR, 97365, USA.

Much of the West Coast shellfish industry depends on hatchery production of larvae; however, environmental conditions in the ocean off the Oregon and Washington coasts and adjacent Hood Canal have recently severely impacted production of oyster larvae. In the last few years, increased areas and intensities of deep acidic hypoxic water have been reported in those regions, contributing to the formation of 'dead zones.' Coastal upwelling can bring this deep hypoxic water to the surface waters of coastal bays and into hatcheries. These changes in seawater properties influence complex chemical interactions, many of which are not fully understood. However, recent research has identified at least three potential stressors adversely affecting oyster larvae: 1) low p

C of upwelled cold water may exacerbate problems with gas super-saturation that may adversely affect oyster larval growth; and 3) increased prevalence of the pathogenic bacterium *Vibrio tubiashii* in hatcheries has been positively linked to massive larval mortality events. Hatcheries have responded by undertaking research to better understand these stressors and implementing seawater treatment and management protocols to reduce their effects.