

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Report for Trip to USA

By: Tony Troup

### Introduction

Judd Evans and I attended the WERA 099 Broodstock Management, Genetics and Breeding Programs for Molluscan Shellfish meeting on Sunday the 6<sup>th</sup> of April and the National Shellfish Association Annual Conference, 6<sup>th</sup> to 10<sup>th</sup> of April, held in Providence, Rhode Island. We then traveled, via New York, to Newport, Oregon (the Hatfield marine Research Centre) and up to Shelton, Washington (Taylors' Shellfish) visiting an oyster farm and a hatchery along the way. We returned home on the 18<sup>th</sup> of April. The following is a report, in diary form, of this trip.

### Saturday March 29

I arrived in San Francisco in the morning and went to the Fisherman's Wharf. This was very touristy area that did not really have much in the way of seafood, except for the signature dish of clam chowder, which was sold everywhere. One restaurant was selling Pacific oysters for \$1.95 each.



*Pacific oysters for sale at Fishermans' Wharf, San Francisco, \$1.95 each*

I then visited the China Town area of San Francisco, where there was quite a bit of very interesting seafood for sale. One shop was selling a fairly large jar of "medium" sized Pacific oysters for \$3.10.

## Seafood CRC Report – Project 2008-707 WERA Bursary



### *Pacific oysters, China Town, San Francisco*

I had dinner at the Hong Kong Claypot Restaurant where large (>10cm long) Pacific oysters were on the menu for \$6.00 per half dozen.

I then spent the rest of this week with family.

### **Sunday April 6, WERA099 and NSA Conference Dinner**

After arriving in Providence the day before I met up with Judd at the WERA099 meeting. Each station/lab gave a report on what they were doing. The following came from my notes and may be adjusted when the minutes for the meeting are posted.

### **US East Coast**

#### **University of Maine (Paul Rawson)**

There had been a few commercial hatchery failures last year and the breeding program had sold some of their excess stock direct to growers to help the shortfall. They are dealing with three diseases (in *Crassostrea virginica*): MSX (*Haplosporidium nelsoni*), dermo (*Perkinsus marinus*) and ROD (*Roseovarius* oyster disease), which affect oysters grown from Maine south. They have two lines, one with MSX and dermo resistance (the NEH line) and the other ROD resistant (FNF) and are trying to breed an oyster with resistance to all three diseases. They crossed the lines and the resulting F1 was backcrossed with the original lines. They also introduced a somewhat naturally resistant wild line from the south coast. The best results to date for disease resistance and growth rates are the F1 backcrosses. They use mass selection as they don't have enough funds to run a single pair mating program.

# Seafood CRC Report – Project 2008-707 WERA Bursary

## University of Rhode Island (Gomez-Chiarri)

Rhode Island has lots of dermo and ROD and there are concerns about MSX. They are doing grow out trials comparing survival with line, age, environment and pathogen strain. They are using three wild strains of oysters as well as the resistant lines and crosses. They give seed to as many growers as possible to grow out the lines and also pay them a bit for their time. Also looking at matrix metalloproteinases which are zinc-dependent proteases that break down the extracellular matrix. MMPs have roles in tissue development, cell migration and innate immune responses. They have sequenced two MMP genes which produce antibodies against MMPs. MMPs are produced by oyster haemocytes. Using SNPs (single nucleotide polymorphisms, which can be used as genetic markers) they have found there is less variation in the MMP genes in the NEH line than in wild lines.

## Rutgers/Haskins Shellfish Lab (Ximing Guo)

The NEH line has been selected for 48 years and has strong MSX resistance and some dermo resistance. The FNF line has ROD resistance but little else. Hybrids do best growth wise. They are also trialing triploid lines, pure NEH and FNF triploids and hybrids, i.e. 4nNEH x 2nFNF. The 3n lines showed best growth and disease resistance, particularly the hybrid line, and have shown that triploidy is additive to growth rates achieved by selection and crosses (as has been shown with SRO). They are trying to develop marker assisted selection and have 239 microsatellite and 102 SNP markers to date. They are producing F2 and back cross families and collect samples before and after disease events and look for markers – clusters will indicate disease resistance. Will also try laboratory disease challenge, as this can give more specific results. Still a few years away from marker assisted selection.

## University of Delaware (Pat Gaffney)

Pat has developed a genetic map for the Pacific oyster, by fingerprinting BAC (bacterial artificial chromosome) clones, which involved tens of thousands of gels (see following poster). This is available on the internet. What is now needed is to put markers on the map, and this will need international involvement, as will using this to develop the Pacific oyster genome (see photo of poster). He has also been developing SNPs in *C. virginica* that seem to be line specific and scanning loci may be useful for other *Crassostrea* spp..

# Seafood CRC Report – Project 2008-707 WERA Bursary

## A BAC-based physical map of the Pacific oyster genome

Patrick M. Gaffney  
College of Marine and Earth Studies  
University of Delaware  
Lewes, DE  
pgaffney@udel.edu

Jacqueline E. Schein  
British Columbia Cancer Agency  
Genome Sciences Centre  
Vancouver, BC  
jschein@bcgsc.ca

**ABSTRACT**

As part of the Oyster Genome Consortium (OGC) effort to develop genomic resources for oysters, a BAC (Bacterial Artificial Chromosome) library was prepared using DNA from a single inbred Pacific oyster (*Crassostrea gigas*). A total of 64,403 clones were fingerprinted and assembled at the Genome Sciences Centre using the traditional high-resolution agarose fingerprint method. 61,825 of the BAC clones (96%) were assembled into 3,374 contigs, with an average of 18 clones per contig. The resulting map and BAC library will be available to the oyster research community, and will serve as a valuable asset for gene discovery, characterization of quantitative trait loci (QTL) and other genes of interest, and provision of genetic markers well spaced across the oyster genome. Future directions include the integration of physical, cytogenetic and genetic linkage maps for *C. gigas*, as well as comparative genomic studies.

**INTRODUCTION**

The Pacific oyster *Crassostrea gigas* is cultured worldwide, with the highest annual production of any freshwater or marine organism (4.2 million metric tons, worth \$3.5 billion). In addition to the obvious economic importance of oysters, there are several other reasons for increased interest in understanding their genomic architecture: 1) they are members of the Lophotrochozoa, an eukaryotic supergroup of the eukaryotes; 2) they play a sentinel role in estuarine and coastal marine habitats, where the majority of humans live, environmental degradation is substantial, and oysters may be associated with risk to human health from infectious diseases; 3) the genome of the Pacific oyster, at 1C = 0.89 pg or ~824 Mb, ranks in the bottom 12% of genome sizes for the phylum Mollusca. A strong case for sequencing the oyster genome has been made (Hedgecock et al. 2005), and it is possible that whole-genome sequencing will be undertaken in the next few years. In the interim, a number of important steps have been made towards developing the infrastructure needed for genetic improvement of this major aquaculture species.

An international community of scientists, self-organized as the Oyster Genome Consortium (OGC), has made substantial progress towards the development of genomic resources that will serve a variety of ends, ranging from fundamental biology to commercial aquaculture. These resources include extensive transcript sequencing (expressed sequence tags, ESTs), genetic maps using various markers including microsatellites, amplified fragment length polymorphisms (AFLPs) and single-nucleotide polymorphisms (SNPs), construction of large-insert bacterial artificial chromosome (BAC) libraries, and full sequencing of >50 BAC clones. We describe here the next step in development of oyster genomic resources, the construction of a physical map of the oyster genome based on the assembly of individually fingerprinted BAC clones into overlapping sets (contigs).

**OBJECTIVES**

- Construct a BAC library from a single inbred Pacific oyster (*Crassostrea gigas*).
- Use agarose gel-based restriction enzyme fingerprinting to construct sets of overlapping BAC clones (contigs).
- Make the resulting physical map and arrayed BAC clones accessible to the scientific community.
- Integrate the physical, genetic and cytogenetic maps to provide a resource for gene discovery, marker development and comparative genomics.

**METHODS**

**BAC Library Construction.** Sperm was stripped from a single inbred Pacific oyster (05x7-T-G4-CL051, inbreeding coefficient 0.594) at Taylor Shellfish, frozen in liquid nitrogen and shipped on dry ice to Amplicon Express (Pulman, WA), where high molecular weight DNA was extracted and partially digested with *Hind*III. Digestion products were ligated into the *Bam*HI site on pcc1BAC vector and transformed into DH10B *E. coli* cells. Transformants were robotically picked and arrayed onto 384 well plates, which were assigned a bar code and recorded in an Excel spreadsheet.

**BAC fingerprinting and contig assembly.** Arrayed transformants were provided to the Genome Sciences Centre (Vancouver, BC), where they were cultured as described by Mathewson et al. (2007). DNA was extracted, purified, and double-digested with *Eco*RI/*Eco*RV. Fragments were separated on high-resolution agarose, stained and scanned. For automated identification of the restriction fragments, fingerprint data generation and automated contig assembly were facilitated by FPC (FingerPrinted Contigs) software (Soderlund et al. 2000), with further contig map processing using automated tools available developed at GSC.

**Fingerprinting Process**

**Map construction**

- compare BAC fingerprint patterns of each clone
- identify clones containing highly related DNA and reconstruct contig regions of the genome (FPC)

High resolution agarose electrophoresis

Scanned restriction fingerprint is subjected to automated band calling

ICE screen shot showing oyster contig 1602, containing 100 clones

**RESULTS**

The BAC library contained 73,728 clones (estimated =10X coverage), with an average insert size of 134.5 kb. Two copies are currently in existence (one at GSC, the other at Clemson University Genomics Institute). The clones have also been arrayed on a nylon filter set.

Fingerprinting yielded an average of 61 fragments per clone. 65,146 (88%) of the clones passed quality filters, and 64,403 were assembled to yield 3,374 contigs containing 61,825 clones (96%) and 2,578 singletons (4%). The average contig contains 18 clones.

Difficulties in assembly resulting from haplotype-specific clones and/or duplications appears to have been minimized by the use of DNA from a single highly-inbred individual.

The BAC map is presently hosted on the GSC server and accessible (password protected) using ICE (Internet Contig Explorer).

Placing currently available genetic markers and sequenced BAC clones onto this BAC map will be an essential next step for the oyster genomics community.

Poster of BAC research (from NSA Conference)

4 | Page

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Virginia Institute of Marine Science (Kim Reece)

Oyster restoration project on Great Wicomico River. Placed 800k and 400k of *C. virginica* seed, in 2003 and 2004 respectively, and planned to put out 15 million in 2005, but have seeded oyster reefs with less – 12 million to date. They used a strain of oysters originating from Delaware Bay (DEBY) that has MSX and dermo resistance. This strain has high frequency of B allele (around 35%) compared to wild oysters of less than 2%. The measure of success of the project was to be the increase of the DEBY signature in wild population. To date there has been no observed change. Why? 1. Possibly high predation on seed when released – this certainly was a problem in the early days but thinks they fixed this problem. 2. The genetic signal may take time to appear in the wild populations - unfortunately if this is the case they probably won't find this as money for the project has run out. 3. The release site may not have been appropriate – it was chosen as historically it was thought to be a major source of recruitment for the area, but perhaps recruitment came from further upstream and this was just an accumulation site so spawn may have been taken elsewhere. They have also been testing microsatellites to discriminate between hatchery lines. This work showed that commercial hatcheries had not done crosses of lines, to give multi immunity and best growth, as they were asked, but had produced oysters from the individual lines and then mixed the results.

Also have a project underway to introduce the Suminoe oyster, *C. ariakensis*, into Virginia for oyster growers as MSX and dermo still restrict the viability of commercial farming of *C. virginica*. They originally looked at Pacific oysters as well but found *C. ariakensis* to be better suited to the area. When they collected what was supposed to be *C. ariakensis* (in 1999) from Asia they turned out to be eight separate species. There are already stocks of *C. ariakensis* in Oregon, on the west coast, (introduced in the 1970's), but these show less variation than those sourced from China. Currently none have been released commercially due to concerns about an introducing an exotic species (even though they will only release triploids) and disease transmission.

## Virginia Institute of Marine Science (Stan Allen)

Their program to develop aquaculture in Chesapeake Bay began in 1998 as farming or harvest of wild stocks of was not economical due to impacts of disease and overfishing (the work with *C. ariakensis* is also part of this program). They use disease resistant lines: MSX resistance relatively easy to develop, three generations only required, Dermo more difficult have found that 'outgrowing' the disease may be possible. 60 -70% of oysters produced are also triploid. They are using the DEBY strain and introducing wild strains of oysters into the lines, including some that show natural resistance to Dermo from Louisiana. They have found environment has a big effect on the success of different lines so have their own 'farms' at four locations with different environments with respect to salinity, temperature etc. They will have eight lines, four of which are site specific, at each site this year. They use mass selection and have a system of line rotation to reduce inbreeding. Demand is increasing rapidly – in 2004 they needed 500 animals for the commercial hatcheries for broodstock, but by 2007 they needed more than 7000. Stan predicts the industry will need a billion eyed larvae per annum in the near future which will require tens of thousands of broodstock. (Note: most, if not all, hatcheries in the US use strip spawning and much of hatchery production goes to oyster farmers as eyed larvae (ready-to-set) which they set themselves on oyster shell cultch.)

# Seafood CRC Report – Project 2008-707 WERA Bursary

## US West Coast (Kristina Straus)

Doing work with abalone, wild populations have been in decline for quite a while and here has been no fishery for the last 15 years. They have started restocking with resistant (nearly 100% to withering disease) animals, 400 to date.

They have been having problems with oyster herpes virus in oyster spat and are using Quantitative PCR to identify the disease to better understand its effects.

## Molluscan Broodstock Program (Chris Langdon)

Chris first talked about the Kumamoto (*C. sikamea*). This is an important species for the west coast and there have been concerns about inbreeding in the local populations so two years ago he and Joth Taylor went to Japan to get some more broodstock. They brought back what appeared to be Kumamoto oysters but were in fact something else (possibly *C. angulata*). The original oysters looked like Kumamotos but the F<sub>1</sub>s didn't and don't look like their Pacifics either. This year, Mark Camara collected oysters from Japan but he used genetic markers he had developed to identify Kumamotos to avoid a repeat of the previous trip. Chris has the F<sub>1</sub>s set on culch but they are still small. All oysters that are brought in from Japan, and their F<sub>1</sub>s, have to be isolated in a quarantine facility and destroyed once they are no longer needed. F<sub>2</sub>s also have to be isolated until they are mature and have shown no sign of disease.

The Molluscan Breeding Program began in 1996 with 6 founder cohorts, of 100 Pacific oysters each, collected from different locations on the west coast. It now has two cohorts of fifty families each kept in four industry sites (California, Oregon, Washington and Alaska). In spite of the large geographical distribution there is no environmental selection, as used in Virginia, and parents are single pair mated. They have a repository of cryopreserved gametes. Selection is focused on yield, which is the sum of survival and growth. They have managed to improve yield by an average of 20% per generation, but this result comes from increased survival (due to summer mortality resistance) not growth improvements. They have not been able to increase growth rates and think this may be due to 'corner effects', ie the biggest oysters selected are not big because of their genetic make-up but because they had the best position in the growing receptacle.

They have also been doing work with shell colour, which they have found to be highly heritable.

They have done a customer survey and found that people prefer multicoloured oysters to either dark or white only oysters.

## USDA, Ag Research Service (Mark Camara)

Mark has been working on differentiation of oyster species and races. He has sourced Pacific oysters from New Zealand to see what race they have there and compare to those on the US west coast. He asked what race/s we had in Australia but unfortunately neither Judd nor I knew. He would be interested in obtaining some Australian oysters.

He is also developing software assist in developing family breeding programs, mapping quantitative trait loci for commercially important traits (see 'Mixed family breeding' in NSA report, pg 9), using microarrays to identify proteins involved in summer mortality and using microsatellites to identify sub-species of the native Olympia oyster (*Ostrea lurida*).

# Seafood CRC Report – Project 2008-707 WERA Bursary

## **University of Southern California (Dennis Hedgecock)**

Dennis is the US rep for the Pacific oyster genome consortium which involved 70 scientists world-wide but unfortunately didn't start. The BAC project started by Pat Gaffney and being finished by the French should result in the project being much easier and cheaper. The Pacific oyster is the most cultivated aquaculture species, even bigger than Atlantic salmon, so should be done and benefits would be large.

He has been doing EST (Expressed Sequence Tags) sequencing but is worried that polymorphism may be a problem.

## **International Participation**

Unfortunately ours was the only international participation at WERA099 this year. I delivered a PowerPoint presentation that covered the topics suggested by Graham, fortunately I had 15 minutes. I started with the first few slides of Graham's 'Breeding for Profit' theme business plan to introduce the CRC and then used the Australian Oyster Breeding Program presentation supplied by Wayne O'Connor. It seemed to be well received and prompted and some questions.

## **USDA and Northeast Coast Aquaculture Centre (Gary Jensen and Fred Wheaton)**

This was a combined presentation by people that are responsible for much of the grant money that keeps many of the breeding/research programs going. The gist of the presentation was that the funding bodies were going to look for more collaboration amongst the research bodies and would no longer fund work that appeared to be a duplication of something that was being done or was similar to what was being done elsewhere. This did not seem to be very popular with the researchers in the room.

## **Workshops and Discussions**

There was little time left for much discussion and much of what was talked about was covered in reports earlier in the day.

## **President's Reception/Conference Dinner**

The dinner was well presented with freshly shucked local oysters from a few different growers, as well as plenty of other seafood. I had the chance to talk to Ximing Guo, Stan Allen and Chris Langdon, all of whom invited us to see them.

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Monday, April 7 – Thursday 10<sup>th</sup>, NSA Conference

There was much to see at the conference and Judd and I often went our own way. I will give a brief report on topics I found interesting. I have named only the initial surname on the in the presentation, two or more names indicates more than one presentation.

### ***Bonamia* sp. in *C. virginica* - Wilbur, Audemarde**

*Bonamia* was found *C. virginica* and was found to be responsible for mortalities in *C. ariakensis*, predominately in oysters of less than 15mm. They showed that transmission occurred from oyster to oysters in the lab and disease increased rapidly in infected oysters in the field regardless of disease load in the environment. The *Bonamia* sp infecting the east coast oysters seemed to be closely related to *B. austral*.

### **Shell Formation – Johnstone, Saiger**

Shell formation the same process as wound healing in higher animals which involves folian proteins. There is a connection between shell formation, cell repair and disease response. Matrix metalloproteinase (2 genes in oysters, 26 in mammals), MMP, may activate cells that carry folian proteins.

### **Roseovarius Oyster Disease in Rhode Island in 2007 - Markey**

Roseovarius occurred in juvenile oysters, 4-20mm in height, and mostly in upwellers. Three sites were surveyed and one had 100% mortality in oysters <10mm supplied from 'hatchery A', site 2 had similar mortality from 'hatchery A' but less from 'hatchery B' while site 3 had little mortality from either hatchery. Low mortality site temperature stayed below 25°C and had less temperature fluctuations.

### **Haemocyte response to toxic or harmful algae – Wikfors, Haberkorn**

There are two types of haemocytes; transport and defense. Defence haemocytes surround *Prorocentrum* sp in the gut and the pass through the epithelial wall. Mussel haemocytes respond to a toxin that *Prorocentrum* releases and act more quickly than oysters. *Alexandrium* sp. rapidly form cysts when ingested and are passed as viable spores. Transport haemocytes accumulate the toxins from *Alexandrium* and are passed through the epithelial wall. Triploid Pacific oysters accumulate around twice as much *Alexandrium minutum* toxin as did diploids when exposed to the same bloom. Oysters exposed to *A. minutum* have less free fatty acids and less digestive activity than unexposed oysters. There is also an increase in phenoloxidase activity and number of haemocytes in *A. minutum* exposed oysters.

### **Effects of 'Brown Tide' on clams – Bricelj**

Brown tide, *Aureococcus anophagefferens*, causes paralysis of cilia and the cessation of feeding in clams. One strain was very toxic but over the last few years it has become less so. Clam larvae fed the toxic strain of the algae showed 27% less growth than those fed the non toxic strain, but both had low triglycerides and which reduced the set in both groups. This has implications for clam recruitment.

### **Death by dissolution – Green**

Generally ocean water is supersaturated in CaCO<sub>3</sub> down to 1000 meters, but often the top 1-2 cm will dissolve CaCO<sub>3</sub>. A pH of 7.34 is ok for clams but the shell of juvenile clams begins to dissolve at a pH of 7.2.



# Seafood CRC Report – Project 2008-707 WERA Bursary

## **Heat shocked oysters show increased resistance to Dermo – Kang, Lund**

Oysters, *C. virginica*, showed an increased resistance to dermo when heat shocked, 40°C for one hour, or were air exposed every 6 hours to imitate low tide when compared to oysters that were not exposed.

## **Retention of HEV by oysters – Provost**

Human Enteric Viruses (HEV) are acid stable and are retained in haemocytes and are found for up to 20 days using PCR. This may be due to the viruses leaching from other tissues to haemocytes. Silica can reduce level of HEV in chickens this may also be effective in oysters. There is evidence that there was a reduced uptake of HEV by oysters injected with silica.

## **Solar powered FLUPSY- Leavitt**

This was a presentation by a group of engineering students who won a prize for redesigning a Floating UPweller SYstem (flupsy). They improved the efficiency of the pumping system and added solar panels but at \$9500 just for the power system it did not seem to be something the growers would take up.

## **Temporal expression of genetic load – Plough**

They bred pairs of inbred lines of Pacific oysters and then crossed F<sub>1</sub> siblings to produce two F<sub>2</sub> families which were analysed for microsatellite marker distortion, caused by lethal gene expression, from 4 hours post fertilization to metamorphosis. In one family 28 of 48 markers was significantly distorted, with 12 expressed during or immediately after metamorphosis. Quantitative Trait Locus (QTL) mapping can show where, how many and the effect of potentially harmful mutations.

## **Mapping QTL controlling growth and body size in Pacific oysters – Perry**

Inbred crosses perform well and all line crosses perform better than their parents, i.e. classic heterosis. Females are overall heavier than males. They detected mostly non overlapping sets of QTL for exponential and asymptotic growth phases. Three negative dominant QTL were detected for the exponential phase and a total of five QTL, three negative dominant, on the asymptotic phase. This doesn't explain why heterosis occurs, it must be gene interaction that results in better growth.

## **Mixed family breeding - Matson**

Breeding programs generally have many families and breed from the best performing families. Variance in reproductive success and high genetic load can result in loss of variation and result in inbreeding. Family effects can be affected by microenvironment, this can be rectified by replication, but this is expensive. Genotyping allows mixing of families, or mixed family selection (MFS), reducing handling costs and environmental effects. They have DNA marker pedigree reconstruction for their MFS model of the PO breeding program and are testing this alongside separate-family selection method. They have developed a software, P-LOCI, that identify the best and minimum number of markers to use by taking the parental genotype, simulating the offspring genotype, assessing the offspring, ranking the markers and then assigning the best set of markers to use. Also, experiments have shown the best time to mix is after set, when oysters are about 1mm.

## **Retail market study for pathogens – DePaolo**

This study involved nine states sending two samples twice a month from retailers and wholesalers to 4 different testing labs (which were assigned samples from all states to avoid lab influence). They were looking for seasonal trends, correlation between indicator and pathogens, gaps in *Vibrio parahaemolyticus* (Vp) assessment and to assess the NSSP effectiveness.

They tested for Vp with MPN (Most probable number), one DNA probe and qPCR, *V. cholera* and *Salmonella*, if PCR positive then to a culture confirmation to show if active pathogen present, HEV,

## Seafood CRC Report – Project 2008-707 WERA Bursary

*Norovirus* and Hepatitis A, qPCR if possible or PCR and indicators, faecal coliforms, *E. coli* using MPN and male-specific bacteriophage (MSB). 60% of samples were from retail, 2% wholesale and 48% restaurant. They found most storage and handling was done at <50°F so not an issue. They found the Gulf of Mexico states had low Vp in winter, <10/g, but higher, 1000/g in summer. Mid Atlantic <1000 in summer but had more pathogenic strains and appeared to be over model with Vv so they may see some cases turning up. Vc, 1 positive in Washington state and 3 in Gulf region, which is not unusual for there. *Salmonella*, 1 positive from Canada (accidentally included in survey) and the rest from Florida – implies that some oysters are being harvested from non classified areas. 1 positive for Noro in Rhode Island and no positives for Hep A.

Correlation with indicators was not perfect, especially faecal coliforms as they multiplied after harvest, but still no ideal replacement.

Imported Korean oysters had recently caused a Noro event and there were 30 cases to date.

They have developed a model based on results in which you enter water and air temperature at harvest, time to cooling and time to consumption which forecasts pathogen load in oysters.

Next: Need to relate exposure (from model) to epidemiology, validate risk assessment, develop control plans for Vv and Vp, adopt qPCR techniques but these will need verification and compare results to other countries.

*Based on results and using model, not epidemiology, the expectation is for time to cooling to be reduced from 10 hours to five due to Vp levels, but in the Gulf states it could be reduced to <1 hour due to Vv levels.*

### **Role of sponges in regulating algal blooms -Wall**

Some areas of the Florida Keys have had their sponge populations destroyed by hurricanes.

Cyanobacteria blooms in these areas are causing the seagrass beds in the area to die. Areas with normal populations of sponges take three days to filter water column, but it takes 12 days in sponge depleted areas. The areas with few sponges regularly have blooms of >100,000 cells/ml, and at these concentrations sponge survival is poor, so natural recovery from damage has been slow or even non-existent.

### **Carrying Capacity and flow rates – Newell**

There appears to be a maximum biomass/m<sup>2</sup> for given site, so it could be fewer large animals or smaller animals. Determining this can have a big influence on productivity and returns. They have developed a model to help determine this using flow rates and patterns, which determines best species for a given site and best configuration for structures on a site. Based in US but doing presently work Ireland.

### **Impact of intertidal oyster farm on macrobenthos – Lu**

Surveyed macrobenthic infauna in an intertidal bottom bag culture oyster farm and on adjacent tidal flat. Found greater overall diversity outside the farm with different community structure between the two groups. In farm had more polychaetes and outside had more bivalves.

### **Benthic communities associated with raft culture – Barnes**

Two sites were studied, one well ventilated and her other not so well. They expected the organic input of pseudofaeces of the oyster farm to cause eutrophication of the benthos but although the poorly ventilated site had higher sulphides than surrounding areas this didn't decrease biodiversity of the site. Biodiversity of the raft sites was similar to surrounding areas but community structure different, mostly due to harder substrate from shell dropping from raft. So eutrophication not an issue, shell drop changing substrate is, but is impact negative.

## Seafood CRC Report – Project 2008-707 WERA Bursary

**Wednesday, 9<sup>th</sup> of April**

On Wednesday morning Judd and I were invited, with Rowan Jacobsen – author of *A Geography of Oysters*, to see Bill Silkes farm and processing operation (American Mussel Harvesters, Inc.), situated in Providence. Bill's processing operation involves processing mollusks that he buys live from all over the US, as well as his own oysters and mussels. He bought some live NZ Pacifics but did this was not successful as he experienced large losses. He was very interested in trying Sydney rocks, particularly when he learnt of their shelf life. Bill usually keeps what he grows and purchases in wet storage, which used flow through UV treated water, prior to processing to keep the product fresh.



*Bill Silkes' processing plant*

## Seafood CRC Report – Project 2008-707 WERA Bursary



*Bills wet storage facility and oysters coming out of wet storage*



*Today's delivery from Virginia, Washington and Alaska*

Bill has a mussel harvesting boats in the north as well as an oyster farm in Rhode Island. The RI farm started as a mussel farm but even though there is a large natural catch of mussels which grew well

## Seafood CRC Report – Project 2008-707 WERA Bursary

on his farm he found that the mussels became infested with commensal pea crabs in their second year which rendered the mussels unsalable. He switched the farm to oysters, *C. virginica*, which grows subtidally on longlines. He uses a stackable tray that has recessed corners for taking the suspension line. This makes assembling and pulling apart the stacks much easier than using internal lines.



*Bill's oyster farm*

*Handling tray stacks ashore*



*A stack of trays showing external suspension lines*

Bill doesn't handle the oysters over winter and the trays become heavily encrusted with tunicates during this period, but this does not seem to affect the oysters. At about this time of year he starts to bring in the oysters to be cleaned up and grade in preparation for harvest.

## Seafood CRC Report – Project 2008-707 WERA Bursary



*Cleaning a tunicate infested 'module'*

Bill sources his oysters from two hatcheries which use broodstock he supplies. The original broodstock come from Stan Allen's breeding program but Bill prefers to use his own now as he believes as he is selecting the best oysters from his farm he will get oysters best adapted to his conditions. He also uses triploids which are crosses from his 2n oysters and Stan's 4n. The hatcheries will do runs as small as 1 million.



*Bill's two year old broodstock*

Bill generally buys spat at a relatively large size from the hatcheries, >15mm, but last year one of the hatcheries had some problems (caused by ROD) and the spat were smaller than usual on delivery which resulted in heavy losses and a smaller oysters for crop for the coming year. Bill usually sells his

## Seafood CRC Report – Project 2008-707 WERA Bursary

oysters after he has had them for 12-18 months. This year he is installing a flupsy and will take delivery of his oysters at a smaller size to avoid the problems he had last year.

### Thursday 10<sup>th</sup> of April, Perry Raso's oyster farm

Perry's farm is a seven acre 'rack and bag' subtidal oyster and bottom clam farm in a 'salt pond'. Salt ponds are quite common on the northeast coast and were formed after glacial retreat from the last ice age and are similar, but smaller, to the barrier lakes of the NSW coast. Perry uses a home-made plastic mesh bag which is held onto his 1" pvc pipe rack with elastic shock cord. He buys his stock from a hatchery and it generally takes two or three years to get the oysters to market size. During summer the bags are placed on top of the racks but in winter they are suspended under the rack to avoid damage and losses from winter ice. Perry was also affected by the local hatchery problems and his 2007 crop is behind where it should be for the time of year. Perry sells most of his oysters to a retailer in Boston, where he gets \$0.40 to \$0.50 'a piece'. A license, which costs around \$30,000 pa, is required to sell shellfish to the public, so most growers have to sell their oysters to a wholesaler. Perry also grows hard shell clams, *Mercenaria mercenaria*, which are set in rows on the bottom and covered in mesh for the first twelve months. They take 4-5 years to reach maturity/sale size.



*The salt pond*

*Houses surrounding the pond*

## Seafood CRC Report – Project 2008-707 WERA Bursary



*Perry with bag of oysters*



*Pipe rack with hold down cord*



*2006 crop, for sale this year*



*2006 crop*

### **Rhode Island shellfish harvesters' meeting**

Judd and I were invited, via Marta Gomez-Chiari, to a meeting of shellfish harvesters. The meeting was called as the wild harvesters had concerns over the number and size of leases being granted for oyster and clam growing, particularly in the salt ponds. The meeting was interrupted for us to answer any questions, the growers and fisherman were a little hesitant at first but once they started asking us about oyster growing in Australia they become very interested.

### **Friday 11<sup>th</sup> to Sunday 13<sup>th</sup> April**

We travelled to the west coast via New York where we had hope to visit the some seafood wholesalers, such as the Fulton Fish Markets which have just been rebuilt for a cost of \$83 million, but unfortunately these markets were closed for the weekend. We arrived in Newport Oregon on Sunday evening after flying from New York to Portland and then driving the 3 hours to Newport.



# Seafood CRC Report – Project 2008-707 WERA Bursary

Monday 14<sup>th</sup> April

## Hatfield Marine Research Centre.

Chris Langdon showed us around the centre which houses the hatchery for the Molluscan Broodstock Program. A few years ago the hatchery started suffering mortalities which they found was due to *Vibrio tubiashii* which was coming from the ocean in large numbers. Chris has been working on systems to overcome this problem. The hatchery pumps water from the Yaquina River into storage tanks at high tide only as this is when the salinity is highest and the water quality is likely to be best. Salinity rarely drops below 20‰ has not been a problem in the past. The water is then passed through 3 stage sand filters and then membrane filters down to 1µm and then UV light treated. Once this is done the water is put into a loop with a biofilter and a fractionator for at least 24 hours before being used in the hatchery.



*Sand and membrane filters*



*Biofilter*



*Bioballs in the biofilter*



*Fractionator*

## Seafood CRC Report – Project 2008-707 WERA Bursary



*'Clean' water storage tank*



*Upweller with 'ice cream bucket' screen*

The centre only used intensive larval rearing tanks and only batch algal systems. They use 'ice cream buckets' for screens in their upwellers which would be a lot cheaper than the PVC screens used here. They use electric field heaters for heating water which do not affect water quality. They have been experimenting with their algae and have found that using *Tetraselmis* with a bit of added probiotic helps keep vibrio numbers down - the *Tetraselmis* is 'sticky' and adheres to pipework etc. They have also been lowering the thiosulphate concentration, from 80ppt to 75ppt, post chlorination. The centre also has a quarantine section where they are holding the stock brought in from Japan.



*Electric field water heater*



*Chlorination channel in quarantine area*

After the tour Chris introduced us to Ford Evans and we had a discussion about our different breeding programs and they were impressed by the gains we had made, particularly with growth. They have been increasing yield, as stated earlier in the report, but think their gains have relatively slow as they haven't been able to apply enough selection pressure due their growing methods (i.e. bag on bottom).

Chris made contact with Xin Liu, Oregon Oyster Farms, and Sue Cudd at the Whiskey Creek Shellfish hatchery and organised for us to see them on our way north.

### **Oregon Oyster Farms Inc**

This farm, co-owned and managed by Xin Liu and sited about 4.5 km up the Yaquina River, is the largest in the area but relatively small for the northwest. It employs 20 people and processes and retails part of its crop locally as well as wholesaling oysters in New York, Taiwan and China. All, but a

## Seafood CRC Report – Project 2008-707 WERA Bursary

very few native Olympia oysters, *O. lurida*, are purchased from one of two hatcheries that are available. The oysters are purchased, in lots of 5 million, as eyed larvae. They are placed in settlement tanks with 100 bags of cultch, each with 300 oyster shells. The tanks have air pumped through them at a rate that is enough to circulate the water, no feeding or flow through is required. The spat will settle over four days and then the cultch will be assessed for the number of settled oysters. If around 10 spat have settled the cultch is used for seeding shell beds, while a settlement of around 20 or more will see the cultch being strung onto a three foot length of rope and suspended under a raft, each raft has around 30,000 oysters. If the average settlement is 15/cultch this means there will be a total settlement of 450,000 from the 5 million eyed larvae.



*Spat settlement tank*



*Rafts at processing shed lease*

The shell bed oysters are left for three to four years while the raft culture spends one summer in a lease lower in the estuary where salinity and flow rates are higher resulting in fast growth but much overcatch barnacles before being towed to the upper estuary site (pictured) where any overcatch is killed by the low salinity associated with winter rainfall events. The rafted Pacific oysters are harvested after 12 to 18 months of age. The farm produces both Pacific and Kumamoto oysters, with a large portion of the Pacifics being triploid. The harvested oysters are placed in bins which are tipped into a washing machine which then feeds the oysters by conveyor to oyster cullers. The Pacific oysters with good shape are kept for the half shell market and the rest sold as bulk meat. The large Pacifics are exported to Taiwan and China. All the Kumamotos are sold in the half-shell; most go to New York where they command a premium price.



*Oysters being loaded in to washer*



*Culling 'Kumos' off their rope*

## Seafood CRC Report – Project 2008-707 WERA Bursary

The farm only opens bulk meat oysters, all half shell oysters are sold live. The farm has been involved with a few *V. parahaemolyticus* outbreaks which were caused by restaurants not handling live oysters correctly. As a result of this oysters are not harvested if the water temperature rises above 64°F (18°C), which happens very rarely, and half shell oysters are only sold to trusted suppliers. Their oysters do not spawn, due to the low summer water temperatures, and as a result have good condition year round.



*Extra large POs destined for Taiwan    The three grades of Kumos*



*Opening bulk meat oysters*

*Kumo and 'local' half shell PO*



*Large PO condition*

*How the large POs are generally served*

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Whiskey Creek Hatchery – Sue Cudd

The hatchery, which was the first to be established in the US, is sited in Oregon about 150km north of Newport on Netarts Bay. This is a high salinity site with almost no fresh water inputs. When in full swing the hatchery produces around 80 million clam, mussel or oyster larvae per week and employs four people. The larvae are sold 'dry', i.e. wrapped in a wet cloth and placed in a foam box and posted to the grower. Sue says three days in these conditions is no problem for the larvae and once had to have shipment returned that was five days out of the water and they were fine. They are also producing geoducks, *Panopea abrupta*, which are the only animal they set. They heard of Chris Langdon's vibrio problems but had hoped they were isolated to his area but last year they began to have failures and initially tried to increase production to overcome the shortfalls they were experiencing. Sue says that this, in hindsight, was a mistake and following Chris' advice has shut down to install and trial the new water treatment system. Prior to the new system they used only basic swimming pool sand filters. The vibrio counts are often in the millions per millilitre and if the water is only UV sterilised it rapidly becomes cloudy with the bacterial bloom that takes place after treatment. Only oysters and geoducks are affected by the vibrios.



*New sand filters*



*Large fractionators*

The hatchery uses both 'old' and 'new' technologies for algae and larvae production, but Sue says if they were setting up now she wouldn't use the older methods and is considering converting all production over to continuous flow algae and high density larval production. The hatchery has an extensive heat exchange system that uses waste water to preheat incoming water. The hatchery replaces all pipe-work every year as part of its normal maintenance practice.

## Seafood CRC Report – Project 2008-707 WERA Bursary



*Batch algae tanks*



*Continuous flow algae bags*



*Low density larvae tanks*



*High density larvae tank*

**15-16<sup>th</sup> of April**

### **Taylor's Shellfish Farms**

The visit to Taylor's was a non conference activity Judd and I had organised prior to leaving Australia. I contacted Paul Taylor via Bruce Zippel and he immediately invited Judd and me for a two day tour of their operation at Shelton, Washington. This was about a three hour drive north of Whiskey Creek Hatchery, on the way we passed a number of large oyster farms and stopped at one stage to take a photo of some cultch. Taylor's is the oldest and largest shellfish farm in the US employing 400 people. They now have a number of operations around the world but the main operation is in Shelton. Both Bill and Paul Taylor met us, showed us to their flupsy farm, took us to lunch and organised a two day tour of their operation in the surrounding area. They grow oysters (Pacific, Kumamoto, Virginica and Olympia), manilla clams, mussels and geoducks locally. Unfortunately the tides were high and we were not able to see any of the intertidal sites. All the sites are located in Puget Sound.



*Oyster shell cultch*

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Processing Plant

Shelton is mostly a processing plant and the operations head offices. They have a large wet storage facility that uses UV sterilisation but as the plant is well removed from any water source the water is re-used and only topped up as necessary. The water is recirculated through a fractionator and biofilter (which uses oyster shells instead of bioballs) as well as the UV steriliser.

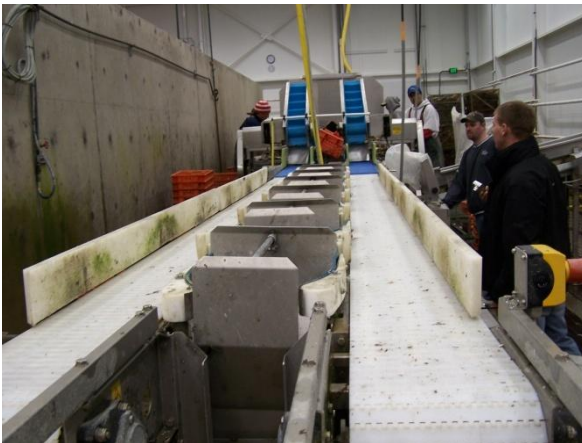


*One of Taylors' processing sheds*



*Wet storage oyster shell biofilter*

The processing plant is highly mechanised with, amongst other things, grading machines for oysters, mussels and clams, mussel debearding, vac-packing and cooking machine, oyster meat washing machine, nitrogen tunnel freezer and large freezer room. The oyster grader weighs each oyster 12 times and can be programmed to vary grades for different harvest areas, species etc. Oysters are still shucked by hand and about 25% of the crop is still sold as bulk meat although the market is changing rapidly to half shell.



*Oyster(weight) grading machine*



*Oyster shuckers*

# Seafood CRC Report – Project 2008-707 WERA Bursary



*Oyster meat washing machine*



*Washed oyster meat*



*Kumos, (\$8.00 per dozen)*



*Pacific oysters*



*Locally grown virginicas*



*One side of the freezer room*

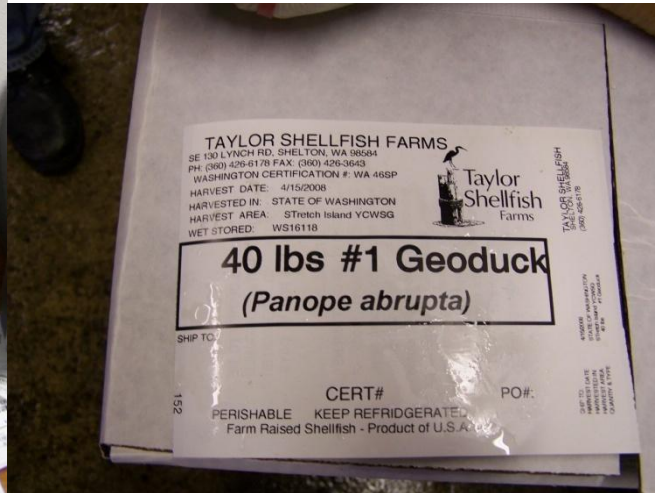


## Seafood CRC Report – Project 2008-707 WERA Bursary

The harvest/processing trail is computerised with every bag, box or jar having a unique label with all harvest and processing details printed on it.



*Label printer*



*Example of a label*

### **Flupsy Farm (Floating Upweller system)**

After the tour of the processing shed Bill and Paul took us to their 'flupsy farm'. Each flupsy is about 18m long by 6m wide and has twenty 0.9m square screens. The screens are stocked with from 750,000 4mm oysters to 100,000 25mm oysters. The oysters are sent from the oyster set hatchery in Hawaii at 4mm and are generally 'planted' out on the farms at 25mm in size. There are ten screens on each side of a middle channel that has a paddle wheel on one end that forces water out of the channel. Water can only enter the channel by coming up through the screens.



*Flupsys*



*Flupsy 'paddle wheel'*

## Seafood CRC Report – Project 2008-707 WERA Bursary

The screens are lifted with an overhead crane and washed daily using a high pressure cleaner. The oysters are graded using a shaker grader that has a belt fed pre-grading washer. Their hatchery has been suffering some mortalities, due to *Vibrio tubiashii* that decimated the hatcheries further south, which has meant that they have had to carry smaller oysters than normal through winter in the flupsys, which has also resulted in some losses.



*Washing a screen*



*Shaker grader*



*Ungraded oysters with dead shell*



*Graded oysters*

### **Mussel Farm**

In the afternoon we visited a mussel growing site. Taylors' grow the Mediterranean mussel, *Mytilus galloprovincialis*, which has been growing in Puget Sound for some time. The farm uses only hatchery reared stock that is grown on ropes suspended under rafts. The seed is mostly set on screens and then placed into 'socks' with the oyster rope but they are trialling setting mussel spat directly onto socks. They are also trialling a sock with a cotton seam that rots away after a while allowing the sock to expand with the growing mussels/oysters. A problem with the Mediterranean mussel is that it prefers to attach to other mussels more that substrate which can lead to large clumps forming which then dropping off the ropes.

# Seafood CRC Report – Project 2008-707 WERA Bursary



*Mussel rafts*



*Mussel set screens*



*Recently 'socked' mussels*



*More advanced stock*



*Ready for harvest*



*Sock with cotton seam*

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Taylor's Hatchery

The hatchery produces oysters, mussels, clams and geoducks for in house use only, using a mixture of techniques similar to the Whiskey Creek hatchery. The hatchery mostly produces oysters and clams only to the larval stage, which are then set at their Hawaiian site or, in case of oysters, set on cultch. The mussels are set on screens or socks and the geoducks are grown in raceways until large enough to be 'planted' on the growing site. The hatchery has been affected by *Vibrio tubiashi*, but not to the extent that the areas further south have. They have installed sand large three stage sand filters but have not as yet installed UV or biofilters except for the geoducks which seem to be particularly sensitive. A diesel powered boiler produces steam for water heating and they have installed a computer controlled heat exchange system.



*Natural light algal tanks*



*Natural light algal bags frames*



*Three meter deep algae tanks*



*Continuous flow bags*

# Seafood CRC Report – Project 2008-707 WERA Bursary



*The geoduck hatchery*



*Miniaturised 'Langdon' system*



*Large larvae tanks (12)*



*High density larvae tanks*



*Heat exchanger*



*Algae flow regulator*

## Seafood CRC Report – Project 2008-707 WERA Bursary

The hatchery uses recycling upweller/downwellers for raising set seed that use and airlift system for moving water. They have a research facility and have their own family line breeding program that they were setting using epinephrine when we were there. All broodstock are strip spawned to ensure the timing of line production is the same.



*Larvae, 30million produced per week*



*Recirculating upweller*



*Family lines being set*



*Geoduck broodstock*

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Summary

### Itinerary

Judd and I had retained fairly flexible itinerary when we left Australia to allow adjustment of our schedule depending on who we met at the conferences and this worked well, though a little stressful organising flights at times. We did have our tour of Taylors' Shellfish Farm organised, and this was fortunate as no one from Taylors was at the either conference this year. We did have Marta Gomez-Chiari and Bill Watson organised to help us with our time on the east coast, which was a great help. I was surprised at how long flying in the US took compared to here, which restricted us a little – it would have been good to visit Stan Allen's but by the time we had flown to Philadelphia on the Friday available, there would have been little time to see much.

### US Shellfish Farming

On the whole much of the farming practices used in the US are fairly low tech. This is not to say they are not efficient, in the same way stick growing methods in NSW are still a very cost effective way of growing oysters in the right situation. Much of the oyster growing can be done on the bottom with little or no infrastructure and this reduces the costs of growing oysters dramatically. In some areas this method is becoming difficult with either: increased prevalence of disease, which appears to be exacerbated by on-bottom growing; and changes in the bottom, such as, on the west coast, increased burrowing ghost shrimp numbers which soften bottom sediments and cause the oyster to sink and suffocate. Also the growing demand for half shell oysters may see an increase in the use of more infrastructure. Most US farms appear to grow more than one species of shellfish, which may be something that more Australian growers should consider.

The industry is growing quite strongly and there are issues over expanding the shellfish growing areas. The meeting we attended in Providence attested to the conflict between farmers and fishers and Taylors are battling to retain a geoduck growing area that was given a five year experimentation lease which has not been renewed. All problems that we have had at some time in Australia. The food safety aspect of growing shellfish is highly vibrio focussed, unlike Australia to date. I don't know if this can be fully attributed to their growing techniques or not. It surprised me to learn that even Alaska has been having problems with *V. parahaemolyticus*. The results of our vibrio work will make an interesting comparison.

### Breeding Programs and Genetics

Our oyster breeding programs here compared very well to those in the US. They have been selectively breeding for a long time now and have some long established lines, especially with *C. virginica*, but they don't seem to have made as much progress our programs. This may be a reflection, as Chris Langdon thinks, that their growing methods haven't put as much selection pressure on the selected oysters ours do here. They are rapidly moving towards marker assisted selection so this may change.

They have been doing lot of work with genetic markers not only for their shellfish but also for the shellfish diseases. To date I don't think the benefits of this have been seen on farm, but are not far away. Pat Gaffney is looking for international participation in putting markers on his BAC Map and I think this is something that the consortium should follow up on. I had a chance to speak to Dennis Hedgecock about the Oyster Genome Project and he said it was difficult as no one was taking the lead, but that the cost of the project should have come down, to as little as 10% of the original cost, by using the BAC map. I told him that although I could not speak for the consortium that we would like to be included in the discussions and may be able to contribute.

# Seafood CRC Report – Project 2008-707 WERA Bursary

## Marketing

I was surprised how little even relatively large oysters cost, particularly Pacifics on the West coast. I was also surprised at how many, admittedly oyster informed, people knew about Sydney rocks. They even rated a short section in Rowan Jacobsens's book (The Connoisseur's Guide to Oyster Eating in North America, *Bloomsbury*, 2007) in which he calls them the "Yukon Gold of shellfish" (we met Rowan, and bought his book, at Bill Silke's). This being the case, I believe that the export of SROs is more likely to be viable than POs even given that the quality of the Australian POs appears to be higher than those in the US, especially in the US winter, when the US oysters are at their poorest.

## Aims of the Report

On applying for this bursary the successful participants were asked to prepare a report with the following aims:

- a. *Issues that need to be considered by any bursary member prior to, during and after WERA/Conference to maximise the benefits of participation;*

I think it would be necessary for one of the next participants to be directly involved in one of the breeding programs. Our relatively unplanned itinerary worked well but could have become complicated had we missed out on flights etc. Given the short amount of time that most can afford it may be better to have more of the itinerary planned, but still leave some 'free' time.

- b. *Their own learning from the experience; and*

I have learnt much from this trip. I may not be able to apply much to my own farming practices in the short term but it has widened my understanding of the international oyster industry and how our industry here compares.

- c. *Relevant information for the Seafood CRC and its members.*

I hope some of the information included in this report will be of interest to some members of the CRC.