

# **REPORT**

**on**

## **Australian Herpes-like Viral Outbreak**

**and**

## **Field Notes**

**Biosecurity** has two main goals: to protect the facility and the surrounding environment from the introduction of novel parasites and pathogens. Inherent in any biosecurity program is a record of the health history/status of all new animals entering a facility, control over movement of people and equipment, the ability to control water quality, a health monitoring program and standard operating procedures.

After visiting three abalone farms in Victoria, it is clear that varying levels of biosecurity have been achieved and are commented on below. Items considered important for a high level of biosecurity are outlined below. All items are considered important:

- **Knowledge of the health status of incoming abalone**
  - Health exam prior to collection of animals (within 30d of collection is best)
- **Quarantine of new brood animals**
  - Health sampling of all moribund or dead abalone
  - Health exam of all brood stock after use and prior to destruction
- **Staff training**
  - Quarantine procedures
  - SOPs
- **SOPs** for all aspects of animal handling (e.g. daily checks, mortality reporting and response, handling of samples for disease diagnosis, etc.)
  - Daily check sheets that must be signed by the individuals conducting the checks
- **Control measures:**
  - Foot baths (refreshed daily) and dedicated footwear of personnel
    - Especially for quarantine areas – brood stock and hatchery
  - Placement of screens on main drain to prevent escape of live abalone into drainage ponds and or natural habitat
  - Plumbing:
    - All tank drains should connect with the main floor drain (with an air gap) and not flood the floor and flow to the drain
    - Make sure an air gaps exists between tank drain and main floor drain to preclude an abalone from crawling from the drain into a tank
  - Dispose of all dead, moribund and any crawl-outs into an approved land fill (consider having dedicated freezer for weekly storage prior to disposal if this is possible under normal operating levels of mortalities).

- Remove all escaped abalone from drains (weekly to monthly) and discard into authorized land fill
- Survey near effluent pipe for escapees and visual health of nearby abalone populations – ie when pig pipes
- Monthly or quarterly rinse drains and effluent pipes with freshwater to reduce biota in drains
- **Routine health surveillance** for farm with a minimum of annual testing at the time of year most likely to pick up a disease or its agent
- **Ability to sanitize walkways** – Given the recent viral epidemic on farms and in some wild populations, it is recommended that the potential to keep walkways clean (devoid of tank sediment, dirt and feed) is important and may be best accomplished with concrete walkways or another material that is easily cleaned or onto which one can spray or spread a sanitizing solution that may only be freshwater or another solution deemed appropriate for the farm (human and abalone safe) and targeted pathogen(s).
- **Survey for people entering the facility and appropriate clothing to limit introduction of pathogens**
  - Use of dedicated footwear (such as boots that staff wear when on-farm) for staff and visitors in combination with footbaths to enter changing room and exiting this room to the farm (as well as during re-entry/exit)

Herpes viruses: ***Prior to the complete characterization of the virus impacting Australian abalone, it is important to consider this pathogen as a herpes-like virus.*** Although the virus in Australia appears to be highly virulent and pathogenic, the strategy of herpesviruses include the potential for latency as well as pre-clinical and patent infections. Thus it is imperative to collect samples even of apparently healthy groups should an outbreak recur, especially with respect to brood stock and their progeny (larvae and postlarvae or seed).

Brood stock and progeny sampling should be incorporated into protocols for animal movement and collection. Globally, there have been several instances of only examining one life stage that resulted in the failure to detect a pathogen in another life stage. This may have lead to the introduction of *Bonamia ostreae* via seed flat oysters from California imported into France.

The development of family lines creates an opportunity to collect important health information on each family produced, particularly given the potential to isolate each life stage in a separate room or building. Many herpes viruses have been demonstrated to have vertical and or pseudo-vertical transmission (from parent to progeny). Vertical transmission is suspected for the recently described oyster herpes virus 1 (See Renault and Novoa 2004). Whether transmission to progeny is via gametes or infective waters during fertilization is currently not understood. Therefore, systematic collection of samples for health monitoring needs to be collected and preserved for analyses. Samples from brood stock (and may include gametes prior to fertilization), larvae and seed need to

be collected for microscopic (e.g. histology) and molecular analyses, keeping in mind that only selected samples need to be examined initially for assessment of animal health. Archived samples are important to archive in the event of a disease or health problem. This is especially important when establishing a new facility or new family lines or stocks.

When an industry or facility plans to move animals among facilities or introduce animals from wild stocks, the potential for disease transmission is heightened. At this time a systematic health sampling is especially crucial. In the recent situation in which brood stock were being shared among farms, developing and implementing a health monitoring program would benefit all abalone industries.

Note on sample preservation for health exams - is best accomplished by storing parallel samples in 95% non-denatured ethanol and in formalin or, better **Carson's** (<48 hrs) and transferred into the appropriate buffer. If available, also store tissue/larvae at -80C (dry larvae), particularly if a pathogen is suspected. In reality, this is best accomplished by a dedicated person (not fertilizing gametes if collecting gametes for early specific testing of brood abalone should vertical transmission of the herpes-like virus occur) on farm or, better, from a local diagnostic facility. Ideally, brood animals and post-larvae would be collected, transported to a lab, and sampled by a health professional. If one is interested in assessing which parents (gametes) are infected and involved in transmission, gametes from individual parents may also be preserved for molecular and or electron microscopic analysis.

### **Wild Fishery**

The question was posed as to whether or not the newly observed herpes-like virus is likely to remain in wild stocks in the future. Although we cannot predict what will happen, it is highly likely that the virus will remain in local stocks. Over time, losses associated with viral infection are likely to dampen but given that there is little currently known about this virus, whether this will occur and the time frame over which the host-parasite relationship may equilibrate are unknown. Given current losses outside of SOM where losses of between 30-60% of the abalone have been suggested, combined with the common inter-annual variation in reproduction success, that losses of 50% of breeding stock from other species have resulted in reductions in abalone recruitment to that population, it will likely take many years before areas affected at this magnitude may recover. By recover, I refer to recovery of the population to pre-epidemic population levels. As mentioned above, the ability to visually observe affected abalone (ie epidemic) may wax and wane, but this is not considered recovery in the population sense of this report. Some may indicate that a population has recovered from active disease, referring to the end of an epidemic which differs from recovery of the population from a decline in density of individuals. Therefore, it is important to clarify the use of the term recovery in light of discussion of an active disease outbreak or epidemic and the population dynamics of various green and black lip abalone populations.

The response of WADA to monitor losses, attempt to reduce cross contamination of fishing sites, invitation of abalone and shellfish health experts, and open discussion with the aquaculture industry and managers is forward thinking and proactive; management of this disease will benefit from such actions and collaborations.

The potential involvement of commercial divers and universities may reduce costs associated with such surveys BUT must be conducted by all parties using standardized methods.

Discussion of farm locations in relation to the wild abalone resources should be discussed. Diseases in wild populations may affect farmed animals and disease outbreaks in farms may influence the health of adjacent wild abalones. The source of the abalone herpes-like virus is unclear but given the early observations of farm and wild abalone impacts in one location, a relationship is possible.

### **Processing plants**

Although the team did not visit any processing plants, processing was discussed by owners and participants. Potential areas of improvement were identified

- Disposal of shells and offal in authorized land fills is imperative
- Chlorination or ozonation of any effluent is needed
- Health history of all out of state and foreign frozen product for repacking is needed
  - Such movements may be disease vectors as illustrated by the introduction of white spot syndrome virus and Taura Syndrome Virus into a farm in Texas, USA from scavengers (eg birds) moving product from the plant onto the farm.
  - The examination of recently processed Chilean limpets is recommended.

### **Management**

The ability to compensate abalone farmers and commercial fishermen when stocks are destroyed or regions are closed due to disease is needed. A mechanism to put this in place should be a priority. This need is recognized by all sectors of the abalone industry and resource managers. The inability to compensate farms delayed stock destruction and relied on voluntary stock destruction and it was recognized that this lack impacted the ability to manage this viral epidemic.

It is also recommended that a set of reasonable and effective biosecurity measures are in place at farms prior to initiation. Given the recent outbreak of the abalone herpes-like virus, it should be recognized by both the aquaculture industry and management agencies that having biosecurity measures in place while designing a facility are typically less costly and easier to implement than trying the retrofit an existing facility. I understand that such measures are being developed and hope that the industry is involved in the development of such measures as they need to be willing and able to comply with these

measures. GSW has a well developed set of biosecurity measures as outlined above with a couple of added suggestions that may serve as a model from which to start these discussion.

Closure of the infected zone appears to be a prudent decision as opposed to applying heavy fishing pressure given that the relationship between size and infectivity and mortality is unknown. Whether or not apparently unaffected abalone are more resistant to the herpes-like virus is not known at this time (see research needs below).

As recognized globally, understanding stock health (abundance and disease presence/absence) is crucial to understand the epidemiology of a particular outbreak. The joint WADA-DPI conference in September shows that all interested parties are working towards a solution to this recent viral epidemic. The existing DPI stock surveys will be especially useful in assessing the impact of monitored stocks. It is recognized that such assessments are time consuming and expensive but given the current situation, stock assessments (stock abundance and demography) should be continued and expanded if possible. Increased surveys are currently planned (Dr. Harry Gorfine). Combining these surveys with health exams should be a priority as discussed at the WADA-DPI joint meetings. The addition of more expansive surveys (wider geographic range but, perhaps less frequently) is also needed and should be promoted Australia-wide as discussed at the joint WADA-DPI meetings.

The potential involvement of commercial divers and universities may reduce costs associated with such surveys but must be conducted by all parties using standardized methods.

### **Research Needs:**

The need for specific research was discussed within the team (WADA and invited guests) and with local researchers and managers. Below is a list of needs that generally reflects consensus among the groups.

- Isolation of virus for genome sequencing
- Development of molecular tools
  - Polymerase chain reaction (PCR) test to identify viral DNA in tissues or environmental samples (less expensive and faster test than histology but not confirmative and qualitative data, present or absent)
  - *In situ* hybridization test to visualize virus in tissue sections or on filters
  - Quantitative real-time PCR (Q-PCR) test to enable simultaneous identification and quantification of viral load in tissues or environmental samples
- Coincident with molecular tool development
  - Survey methods
  - Surveillance of disease presence or absence in populations
  - Sample collection and archiving for histology and molecular tests

- Tissues
  - Water samples
  - Alternate hosts
- Laboratory studies
  - Transmission trials using 0.22  $\mu\text{m}$ -filtered infective waters or tissue homogenate to preclude any potential bacterial contamination (bacteria can pass through 0.45  $\mu\text{m}$  filters)
  - Differential susceptibility of different life stages
    - Larvae, post-larvae, juveniles and adults
  - Further pathogenesis studies to better understand the disease course, entry portal, tissues infected, etc – TEM, histology, molecular tools
  - Determination of LD<sub>50</sub> and minimum infectious dose
  - Thermal tolerance of virus
  - Disinfection of virus
- Field-lab studies
  - Quantify viral load during an epidemic (temporal and geographic range)
    - Seawater and mucus/degrading tissue (if these can be separated via size selection filtration – capture mucus and degrading tissue (for analysis) and let sea water pass through for subsequent collection and analysis by Q-PCR)

## NOTES SENT TO SPECIFIC FARMS

**Day 1: September 16, 2006**

### **Great Southern Waters (GSW)**

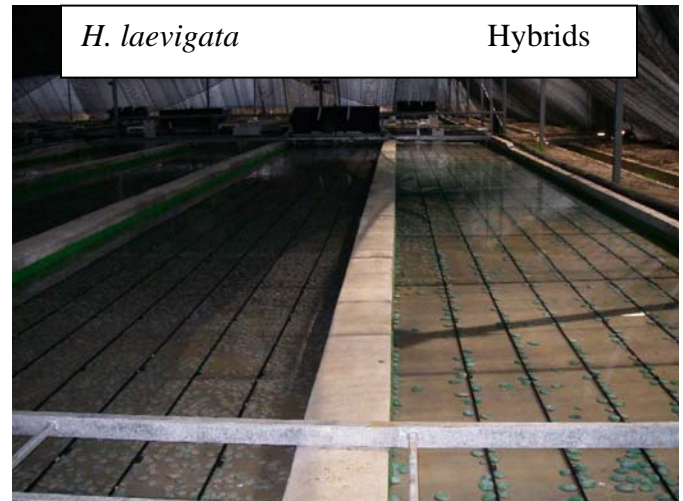
The team visited GSW to discuss the mortality events at that facility. The team was welcomed by Managing Director, Steve Rodis; Operations Manager, Anton Krsinich, and Development Manager, Rodney Roberts. All visitors must complete a survey form indicating recent movement of individuals regarding potential exposure to abalone pathogens prior to entry into the farm as part of their biosecurity procedures.

Rodney Roberts then presented a summary of previous and current biosecurity measures at this facility. Measures discussed included:

- Presence or absence of health status of incoming abalone
- Quarantine of brood animals
- Staff training
- SOPs for all aspects of animal handling (e.g. daily checks, mortality reporting and response, handling of samples for disease diagnosis, etc.)
- Control measures: foot baths and footwear of personnel
- Handling of animals used in the development of their family lines
- Survey for people entering the facility

We discussed the above biosecurity measures and also highlighted the need for routine health sampling to complement their current protocols to examine animals only in response to problems. GSW has implemented routine disease surveillance, with moribunds being accumulated and analyzed quarterly. The ability to include microbiological sampling on site and in conjunction with local labs was also discussed. The utility of collecting samples for multiple analyses (such as histology and molecular analyses) was also relayed as an important component of health monitoring for the facility particularly in light of the herpes virus epidemic and potential future outbreaks, of this or an alternate pathogen, should they occur.

After a discussion of GSW's biosecurity measures and future plans, we toured the facility which contained a high number of grow out abalone – *Haliotis laevis* and hybrids (*H. laevis* x *H. rubra*; see images below).



A number of questions were posed regarding GSW's avoidance of farm-wide infection:

- Luck of not getting infected brooders
- Current biosecurity and hygiene measures
- Or combination of above

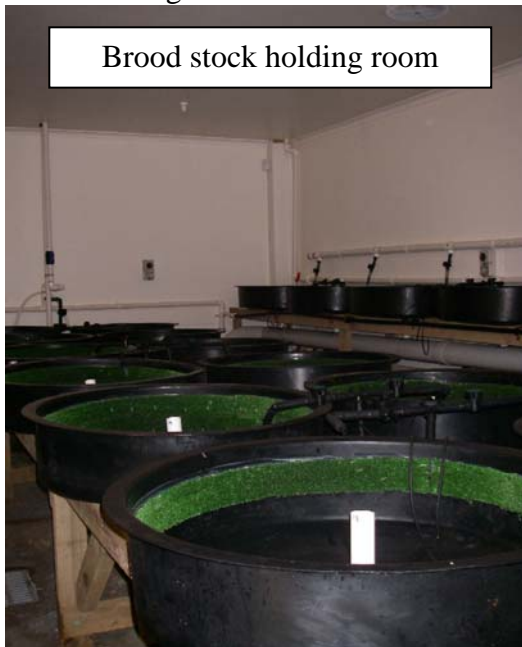
The chronology of events on farm relayed by Anton and colleagues mirrored that reported by Dr. Paul Hardy-Smith. Unlike the two farms affected by the virus, GSW did not take wild broodstock from South Australian waters. This could be why this herpes-like virus was not observed at GSW. Although some brood stock from the selection program died, such losses typically occur during the handling and spawning process annually. If some brood stock were in fact affected (the health status of brood abalone is not known), the reason the disease did not spread throughout the farm due is likely due to the already high level of biosecurity (which has recently been further improved) at this facility.

In place methods that were helpful at GSW included:

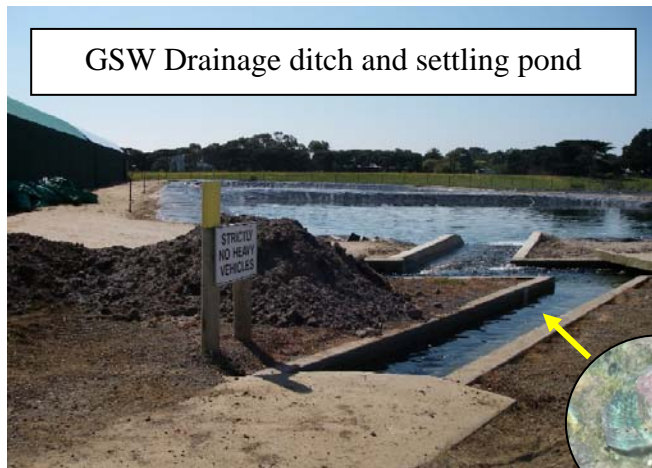
- Quarantine of wild brood stock while on farm
- Immediate destruction of animals that died
- Separation of each level of operation on site
- Highly trained personnel

Suggestions to improve biosecurity:

- Implementation of a health management program
- Boot area in office building: the use of specific foot ware while on-site is an excellent idea. However, for better efficacy of this control measure, the separation of street shoes and farm boots is recommended. This may be accomplished by the use of separate rooms for these foot ware in conjunction with foot baths. Should this be difficult to construct, the use of foot baths for all foot ware prior to entry and upon exit of this room is an alternative control measure. The former suggestion is considered the highest level of biosecurity.
- Avoid having all brood stock and larval tanks drain onto the ground prior to entering the central drain – see images below (arrow highlights tank drain)



- Consider having individual tank hoses to connect each tank to the drain via a PVC pipe drain beneath each row of tanks (Note: given the current drain configuration it is surprising that the viral infection, if it was really present in the some of the brood stock did not spread within the quarantine room)
- Collection of samples from all brood stock and family lines (larvae and seed) for health assessment.
- Removal of escaped animals in drains (see images below)



GSW Drainage ditch and settling pond



GSW off shore area

The need to be able to collect health samples from brood stock after use and prior to destruction was found imperative!

This health information is crucial for effective biosecurity and is an established component of ICES recommendations including.

- There was mention that GSW saved small tissue samples for genetic assessment (pedigree); these samples might be useful for PCR testing for the putative herpes virus once this test is developed.

Future plans: In order to reduce the need to routinely collect wild brood stock this facility (and the Victorian abalone aquaculture industry – GSW, SOM and CS at least) plan to create a suite of family lines produced via multiple half-sib crosses for use as future brood stock. To accomplish their goals GSW plans to collect wild abs for one to two additional years. GSW plans to remove all escapees from drains prior to intake of new brood stock.

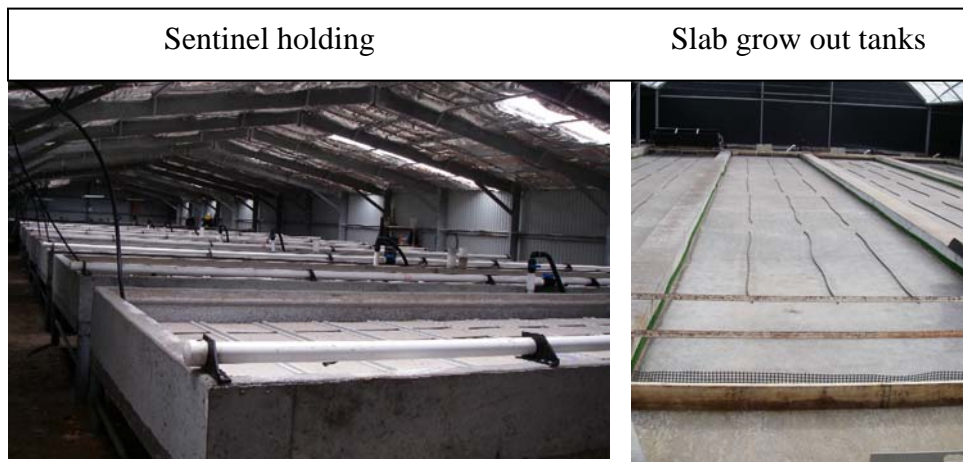
Personal notes: Drs. Friedman and Renault have visited many abalone culture facilities in the United States, Canada, Chile, France, Japan, China and Australia. GSW is a model aquafarm regarding facilities, training, biosecurity plans, and open attitude.

**Day 2: September 17, 2006**

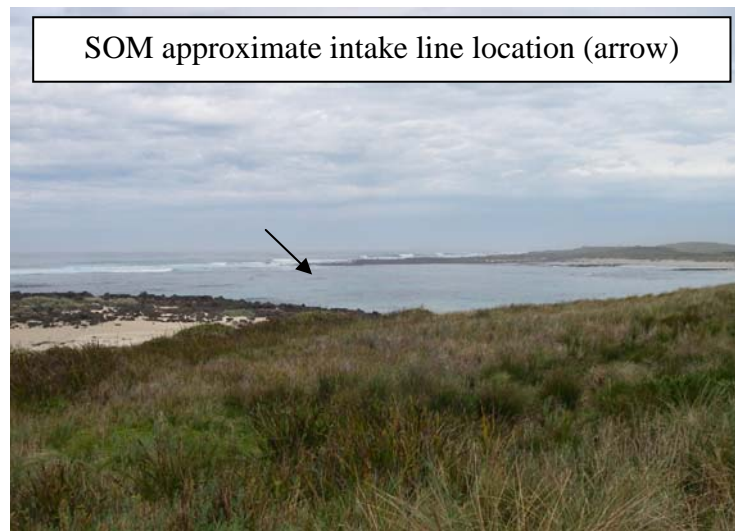
**Sunday Southern Ocean Marine (SOM)**

The team was met by Hamish Ebery who relayed the disease and loss pattern at SOM and gave us a tour of the facility, the oldest farm in Victoria. The chronology of events on farm relayed by Hamish and mirrored that reported by Dr. Paul Hardy-Smith.

No abalone were present on site (See images below) other than sentinels in one building. We did not enter the building but looked in the door. A chlorine foot bath was placed at the entrance to the building holding sentinel abalone. Sentinel abalone are being used to test for persistence of the herpes-like virus in the facility or affluent waters.

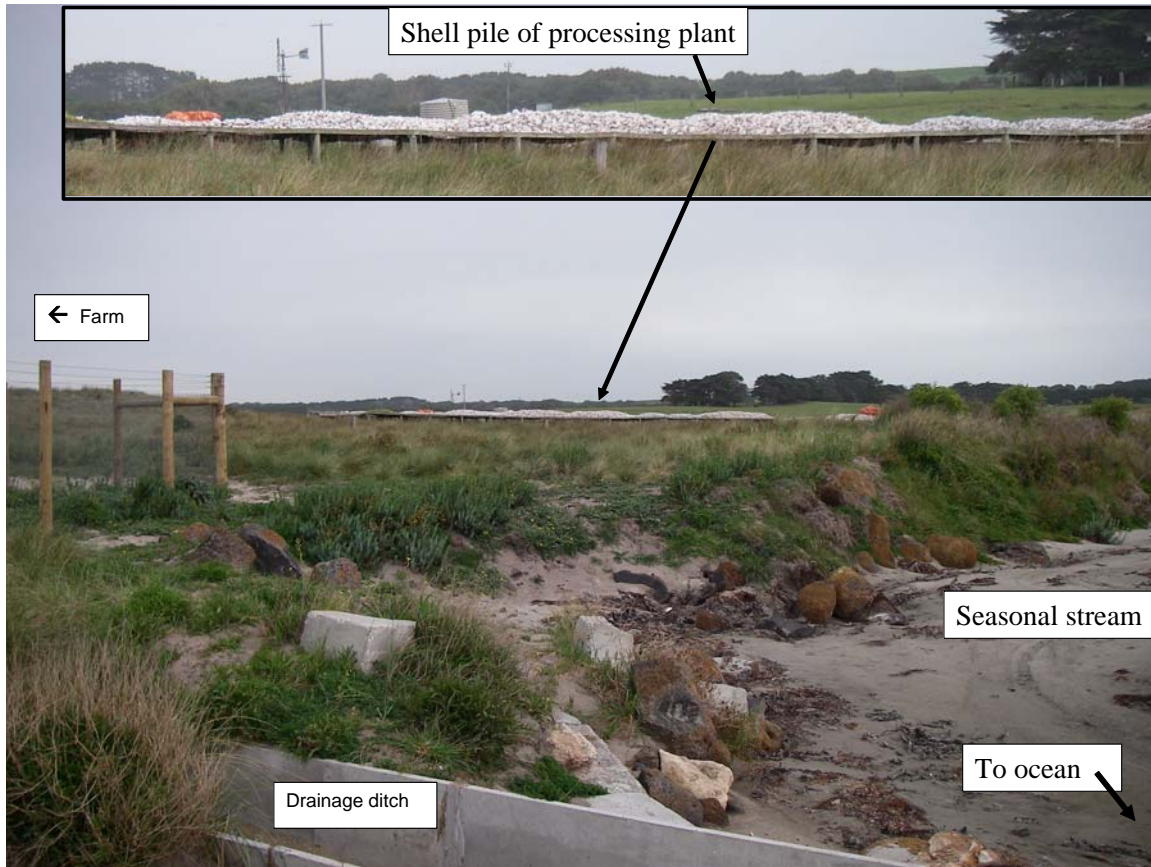


The bay surrounding SOM, where the intake line is located (Taylor's Bay; see image to right) was previously inhabited by high numbers of abalone. The effluent pipe is located around a point from the intake in a nearby embayment (Drain Bay). Mortality was observed in wild abalone around the intake line before those near the outlet area. Hamish indicated that the bay was not completely surveyed and there was little ability to monitor wild animals, thus the timing and origin of losses in wild animals is not able to be confirmed.



Suggestions to improve biosecurity:

- Shell pile from processing plant adjacent to farm and to wild abalone resource – potential source of disease agent (See images below)



- General practices
  - Replanting abalone removed from drains and settling pond
  - Introduction of non-certified brood stock into farm
  - Mingling of new brood stock with grow-out animals
  - Locating harvest bins near grow out animals
  - Burial of dead abalone on site (10 ft deep, limed and covered with 5 ft of dirt/sand – although liming is a good precaution, there is still the potential for leakage of infectious material and removal of shells by scavengers)
    - It was suggested that burial methods evolved and that scavenging of mortalities was noted.
  - Apparent absence of SOPs regarding responses to an abnormal mortality event
- Lack of physical barrier (air gap) between main drain and tanks – thereby allowing an abalone to move between tanks (See image below – circle highlights that tank drain is submerged in main floor drain water)



- Need to develop standard operating procedures (SOPs) for all aspects of farm activities as well as for dealing with problems such as elevated mortality and disease
- Need to establish a health monitoring program with a particular diagnostic laboratory
- **Ability to sanitize walkways** – Given the recent viral epidemic on farms and in some wild populations, it is recommended that the potential to keep walkways clean (devoid of tank sediment, dirt and feed) is important and may be best accomplished with concrete walkways or another material that is easily cleaned or sanitized.

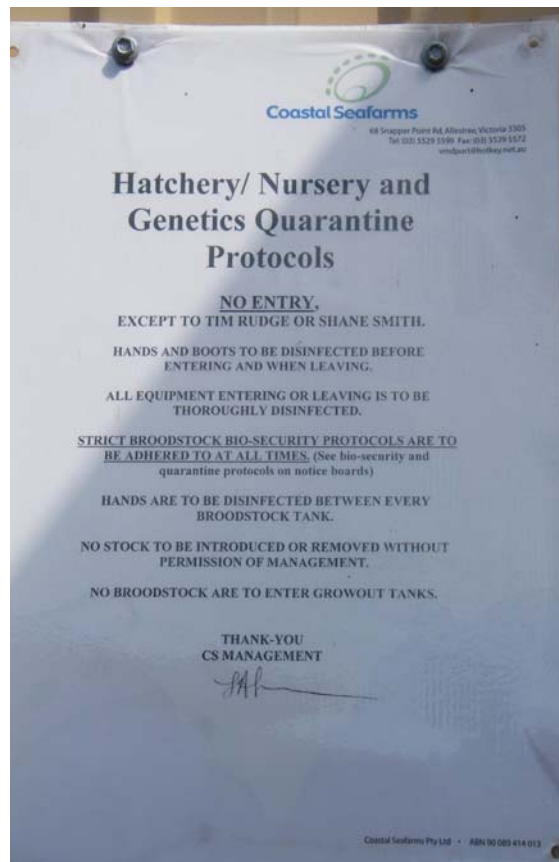
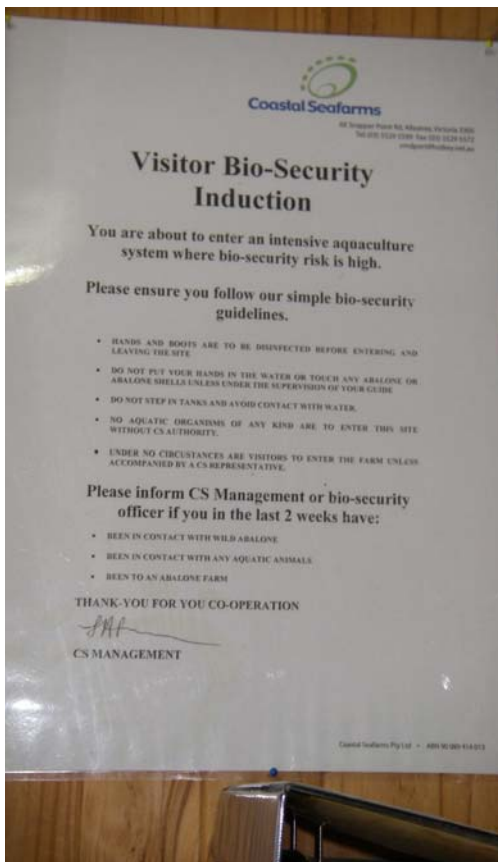
Hamish generously provided us with several files regarding tank losses, their mortality prevention issue (January 1, 2006), their protocol for identification of affected abalone and sample collection (See attached protocols). Strict adherence to SOPs and further development of these documents with Dr. Paul Hardy-Smith and DPI is recommended, especially given the recent observation of ganglioneuritis in local waters off the farm.

## Coastal Seafarms (CS)

The team was met by Tim Rudge and Shane Smith who relayed the sequence of mortality events at CS and gave us a tour of the facility. No abalone were present as this facility and plans to use sentinel abalone (as at SOM) to assess presence of virus are being developed (see image).



Biosecurity: CS has several SOPs posted regarding biosecurity measures (see images below).



This facility, like GSW, has good potential to have a high level of biosecurity as they have separate brood stock (A), spawning (B) and larval rearing rooms (C; see below).



Although, the ability to separate grow out animals is more limited, there were specific tanks for family line development allowing more control (separation among families and between the family lines and general grow out animals; see above D).

Suggestions to improve biosecurity:

- Create a separation between tanks (which allows for aerosol cross contamination) and allowing effluent tank water to flow along the floor to a central drain may facilitate transmission of an infectious agent.
  - The addition of lids, barriers or more space between tanks may reduce aerosol contamination.
- The addition tubing to allow effluent water to flow directly from the tank drain to the main floor drain will reduce splashing of infectious water between tanks and to other parts of the facility.
- Add an air gap between grow out tanks and main drain

Mortalities were handled as at SOM – buried on site (no mention of lime used here). At all farms it is critical to have a proper disposal plan for daily losses and major disease event. Ideally abalone will be disposed in a land fill.

Like at SOM, Tim and Shane generously shared documents with us regarding loss data. CS has a temperature probe near their intake line. It is recommended that this is retrieved

and examined in relation to mortality trends. This may be particularly important as Tim indicated that losses appeared to dampen when temperatures exceeded 20 or 21C. The temperature above which losses were reduced needs to be confirmed. Anna Mouton noted that at higher temperatures management changes to reduce losses expected from vibriosis such as increased cleaning, culling etc.

Note for all farms: the open discussion among farms and managers and health specialists was and is very beneficial to understand the disease, types of protocols needed, and proper responses for daily management and specific disease avoidance, management and response. This open dialog should be encouraged.

### **Day 3: AAHL Meeting with Drs. Serge Corbeil and Ken McColl**

Serge presented the activities that occur at AAHL, followed by sharing of transmission electron microscopy (TEM) images. The TEM images were consistent with herpesvirus morphology including nucleocapsids and capsids in the nucleus and fully formed, enveloped virions in the cytoplasm of infected nerve cells.

Ken presented images from histological sections from their transmission trials and included temporal samples after exposure (1, 2, 3 and 4 days after bath exposure). Tissues examined in this study were restricted to the head and foot containing main ganglia. Two days after exposure visible signs of disease were observed (weakness and inability to attach to the substrate). Neural lesions were observed two days after exposure and included peri-neural edema, hemocyte infiltration, morphological changes in the grey matter (increased cellularity and possible apoptosis), some changes in the white matter (hemocyte infiltration). Peripheral nerves were often swollen with an increase in cellularity as well possibly due to hemocyte infiltration or glial cell proliferation, etc. A diffuse increase in cellularity was also observed in connective tissues around the nerves. By day four after infection, almost total destruction of white matter and infiltration/necrosis of grey matter was observed.

Lesions observed were limited to nervous tissue during the trials. It would be interesting to further examine the slides to assess if lesions may be observed in non-neural tissues (such as gills and oral epithelia) as well as probing these tissues with an *in situ* hybridization assay when available.

The group discussed the difference between necrosis and apoptosis (or programmed cell death) as apparently apoptotic cells were observed within lesions. Apoptosis is an important host cell defense mechanism against viral infection. In addition, some viruses including herpes viruses are able to modulate apoptosis to impact the host immune response and to replicate in target cells. AAHL does not currently plan to examine the presence or absence of apoptosis in affected abalone and an examination of apoptosis in conjunction with challenge trials may be insightful. Such a study could be conducted by a graduate student who could work with preserved material generated at AAHL.

## APPENDICES

### 1) SOM MORTALITY PREVENTION ISSUE 11-1-06

#### CLEANING PROTOCOL:

- Minimum sweeping. Brooms are a major contamination source, therefore if you can get away with not sweeping a tank then that is fine. Also, if a tipper can be left on a tank for longer to get away with not sweeping then that is fine also. Most of the A side of 2-year olds, (except for ungraded tanks), are fine not to be swept.
- If a tank has to be swept, use a broom from a chlorine bath, rinse in the outlet of the tank to be swept, and sweep. Rinse the broom in the outlet again before placing back in a chlorine bath, and take a new broom. In practice this will mean cleaning will take longer, so think practically and be aware of what we are trying to prevent here; chlorine bath the brooms as much as possible.

#### MORTALITY PROTOCOL:

- You will need a water-proof bucket, (no leaks), gloves, and a spray bottle filled with a chlorine mixture. **(Use 20ml per Litre => 10ml per spray bottle).**
- Pick up and count morts from the outlet side of the tank using gloves.
- Rinse gunk off the gloves in the outlet.
- Spray gloves with chlorine mixture.
- Repeat process for the next tank.

#### GENERAL PROTOCOL:

- Use foot baths and try to rinse boots before dipping.
- Use broom baths as much as possible.
- Remember we are trying to stop the spread, firstly between sheds, and secondly within sheds.
- It is also handy to remember that it hits the 1-year olds hardest, then the 2-year olds, then the 3's, so take particular care with those relative tanks. It is easier to chlorine bath the brooms after sweeping each tank of the 1-year olds, so please do so; there are only 7 tanks in A&B, and 1 in 1-45.
- Don't cross tanks by stepping through the water, you will have to use the walk-ways and walk around.
- Only use walk-ways to feed tanks; you shouldn't have to step in them.
- Be aware of drips from brooms and splashing; minimize.
- Take care and a little more time with checks. If a slab tank is hit then it would be good to know about it immediately so that it can be quarantined.

### 2) SOM Notes on the identification and collection of diseased abalone specimens.

The herpes like virus recently found in abalone causes "neurogangliitis". This basically means that the virus replicates and therefore impairs the function of the nervous system of the abalone. The "brain" of the abalone is located in the head region near the mouth and the nerves run back along the foot muscle towards the tail and outwards to the sides

of the foot. The effect of the virus results in the abalone losing control of its foot muscle and causes both curling of the edges of the foot and protusion of the mouth as shown in the attached photos.

### **Signs to look for.**

For the diver the signs to look for of a recent infection are:-

New abalone shell on the seabed.

Moribund abalone, meat still intact but with the abalone making no attempt to turn over.

On passing your hand over abalone you would normally expect them to clamp firmly onto the rock, with infected abalone you can go back over these animals and pull them off the rocks relatively easily.

Easy chipping of abalone off the rocks.

Infected stock is likely to be found in patches.

Infected stock may only be 5% of the population.

### **Identification of the disease.**

For the diver and the deckhand to identify the stock as being infected firstly examine the response of the abalone, in infected stock very little extension of the foot is seen.

The edges of the foot tend to curl inwards (this seems to get less as the abalone get larger.

The most obvious clinical sign is the protusion of the mouth.

### **Preservation of specimens.**

Preservation of all diseased abalone for whatever disease can be done in the same manner. A 10 % formalin solution, available from chemists and agricultural stores (1 part formaldehyde to 10 parts water) is made up (following all the relevant safety instructions) and kept on the boat in a 1 or 2 litre plastic or glass fully sealable container.

The suspect abalone is preferably shucked from the shell with the gut and gonad left attached to the foot and put into the solution. If collecting a few specimens the edges of the foot (keep the mouth and head region) can be cut away and discarded, with the rest put into the solution. It can also help to make a single cut through the gut and gonad with a sharp knife as this allows the formalin to be absorbed through these organs.

This same method of fixing can be used for storing any other abalone which look diseased for example those with pustules, lumps, nodules etc.

The local DPI office will collect samples and forward them on for examination.