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Shellfish industry and research tour in Australia

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Report to the Australian Seafood CRC

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Pierre Boudry and Helen McCombie visit Australia, August 2008

Pierre Boudry, a research scientist at the French Marine Institute Ifremer and Helen McCombie, an independent European researcher, came to Australia in August 2008 to meet with shellfish research community and industry. We visited hatchery, oyster culture and research facilities while travelling though Queensland, Tasmania and New South Wales. Throughout our trip of nearly one month, we were delighted with the reception and hospitality we met. Discussions and idea sharing allowed us to look at French and European research in the context of another country, and notably to see how the shellfish industry interacts with research providers in Australia. We also hope that in return we made a positive contribution with our advice and exchanges with research and industry in Australia and that this may lead to continued productive relations between institutions in our countries.

Australasian Aquaculture Brisbane 2008

We were invited to participate in the AA08 conference that gave us a fine example of the integration of shellfish industry and research accomplished in Australia both in the outlook of many of the presentations which we saw and by the combination of international conference and trade fair, with elements of interest for everyone from producers to scientists. Pierre gave two presentations, one on oyster selective breeding and the role of genomics, and the other on the influence of genetics on summer mortality. Helen also presented two talks, one about the Blue Seed mussel hatchery technologies project, and the other an overview of the Moresst oyster summer mortality project with updated reflections on the 2008 crisis. This meeting gave us the opportunity to see presentations of Australasian research and industry, concerning both species known to us, like *Mytilus galloprovincialis* and *Crassostrea gigas* and those more exotic like Abalone, *Saccostrea* and *Perna* species. It also allowed us to meet researchers, producers and industry working on subjects similar to our own and examining similar questions.

Subjects that particularly interested us were the selective breeding programs described on oysters and green mussels, economically-based selection criteria, successful structures for mussel settlement, the welfare management aspects, and investigations into the *Martelia* problem in the Sydney rock oyster and viral threats in abalone. There are often parallels with situations in European waters. Questions that bring the two continents into direct contact are those of trade and shellfish safety on which we learnt some interesting new perspectives.

We strongly encourage the participation of European research and Industry representatives in future Australasian Aquaculture meetings.

Visits in New South Wales

In NSW we had Dr. **Wayne O'Conner** as our guide. He organised visits to the Sydney rock oyster (SRO) industry and sites and businesses rearing *C. gigas*, *Ostrea angasi* and Akoyas.

Notable differences with Europe that struck us included the systematic presence of depuration facilities on the oyster farms (even if not in use), complete records on oyster stock movement and active industry participation in disease detection in the Sydney rock oyster.

C. gigas as a non-native invasive species in NSW and we saw how triploids are used principally as a precaution against spread. We learned and how the notion the 'native' and the 'alien' species is strong among producers.

We drove from Brisbane down the coast to **Port Macquarie** where we paid a visit to Bale Brothers oysters on the Hastings River. Stewart Bale showed us an example of specialisation in Sydney rock spat rearing to supply farms in other areas of the state. On this and other farm visits we saw the demonstration of a number of techniques and original solutions to familiar problems, including capture structures with thin layers of cement to aid spat removal.



One capacity of the Sydney rock oyster that surprised us was its ability to withstand weeks of out of water when crates or sticks are lifted onto rafts to stimulate growth and kill off overcatch of younger and thus more sensitive cohorts settled on the shells.

We are used to an almost universal use of net oyster bags in France. In Australia we saw a variety of systems: oysters grown in stacked crates hung underwater from lines, on sticks, in baskets or in clipped-on closed rolling cage systems to encourage an even shell shape.



At **Camden Haven** Tony and Jonine Troup showed us their enterprise where they grow-on SRO from 1mm hatchery spat to market size. We also saw one of the rare examples of *Ostrea angasi* culture.



On the **Wallace Lake** near Forster, Anthony Sciacca took us out on the river on his boat, Upriver to a catchment site where sticks and drainpipes are used as collectors. There appears to be a relationship between the lower salinity and settlement/seed development success. Growing-on is done downriver where we saw the oldest oysters growing on leases separated by stakes. An important management choice was whether to leave oysters on the sticks for growing on or not as this leads to more irregular shapes than growth as single seed.

In this area alien *Crassostrea gigas* is considered a pest and great efforts are made to separate it from the Sydney rock oyster spat of approximately the same size and shape.

At **Port Stephens** we visited the **NSW DPI** hatchery where Sydney rock lines are bred. A new CRC project involves pair mating and gamete cryopreservation, and is now integrating triploids into the genetic improvement program, closely linked to SoCo and the industry. We also learned about the CRC hatchery network.

Larval rearing facilities at the NSW DPI consist of large volume paired batch tanks. Other species reared in these facilities include flat oysters, scallops, Pacific oysters and pearl oysters. Other work of the centre involves probes for PSP toxins, study on QX in the natural environment, effects of oestrogens and nanophenols, algal biofuels and *Donax* population enhancement. Work in other parts of the centre involves aquacultured fish species, like kingfish and red snapper in recirculating systems.



Oyster condition (i.e. flesh content) is the subject of new research in both Sydney rock and Pacific oyster using proteomics to trace changes in reproduction and condition.

Wayne told us about the management of his breeding program management and the new focus of work with the Sydney rock and the procedure of giving families to farmers to test and choose their favourites - which would then be used to breed subsequent generations. This program is one way in which this government department is close to the industry and its needs. Pierre and Wayne discussed about maintaining and monitoring genetic diversity in breeding programs and means of marking individuals. Microsatellite markers developed in the European flat oyster (*O. edulis*) could make suitable tools for *O. angasi*.

We also discussed what form a potential French-Australian cooperation could take, and concluded that reciprocal visits of students or staff would be the most practical.



At **Tomaree point** we visited Southern Cross Shellfish where Greg Kent showed his outdoor hatchery in which he raises SRO families and Pacific oyster, we also talked about gynogenesis on which Greg and Helen had previously done similar experiments.

At **Cromarty Bay**, Geoff Diemar farms both SRO and Pacific oysters (diploids and triploids) and told us about their complementarities. We also saw pearl harvesting taking place from oysters farmed in nearby Salamander bay.



Overall we were very impressed by the close communication between research and shellfish industry in NSW and the resulting efficacy of the support offered by research to the local industry. SRO is a traditional production species facing severe problems related to diseases and, more recently, competition from the non-native Pacific oyster. This situation is in some ways similar to that of the native flat oyster in France, although production of SRO and extent of natural populations is still greater. One can hope that the combination of selective breeding and disease monitoring will successfully support sustainable production of SRO. Monitoring of genetic diversity and its evolution in selected lines and wild populations of SRO and *O. angasi* with molecular markers is to be encouraged. Further characterisation of selected lines would provide valuable information regarding host-pathogen interactions and the molecular basis of disease resistance.

Visits in Tasmania

Oyster Mortality Workshop

Our visit to Tasmania started with a workshop on oyster summer mortality, hosted by the Peter Kube and Nick Elliot of **CSIRO**, in **Hobart**, and involving research and oyster industry representatives. Mortality crisis events have happened in areas of Australia for reasons linked to heat stress, freshening, acid sulphate and disease. The French 'Morest' project, presented by Helen, with its integrated approach was therefore an interesting topic of discussion. The Australian perspective covers not only *C. gigas* but Sydney rock oyster and *O. angasi*. Though clearly the geographic distances and environmental differences between sites differed vastly between France and Australia, this meeting brought together many common ideas.

For NSW Wayne O'Connor talked about QX (*Martelia sydneyi*), winter mortality (*Bonamia roughleyi*), and limeburners syndrome. Farmers' participation is encouraged by issuing report forms on the internet and descriptive picture pamphlets to farms, extension education work is done and fixing solutions given to some farmers to preserve specimens. Local government even paid for a microscope for one farmer. We were very impressed by these reports given by farmers even though not mandatory. Industry involvement is fostered by a research-based response to the farmers *e.g.* farmers wanted selection against winter mortality and the DPI responded with a selection program, but needed the farmer's reports to justify funding. The SRO selection program addresses both QX and winter mortality problems. Discussions were made on the suitability of Phenoloxydase as an efficient marker for selection against disease resistance, the relationship with QX is not direct or fully understood and SOD and carvortin may also be relevant.

Mass mortalities of *C. gigas* like those seen in France are unknown in NSW, nor has *Bonamia* been shown to kill *O. angasi*, though this is not a major commercial species and natural populations have clearly been reduced by the parasite.

In Tasmania, the main cultivated oyster is *C. gigas*. Hayden Dyke told us about a mortality event that affected the entirety of his stock at the peak of their reproductive maturity on Boxing Day 2005 when an extreme freshening after heavy rainfall in summer. This appeared at first to have very similar characteristics to mortality observed in 2-year-old oysters in the Morest project in France: high trophic level leading to heavy reproductive development but

not yet spawning, high temperatures and freshening. However the salinity shocks involved were extreme, notably when compared with the French experience where the relationship (though not necessary a direct cause) has been established between cumulative freshening in winter and subsequent summer mortality. In this particular event in Little Swanport, Tasmania not only oysters were killed but also mussels and barnacles, pointing to the effect of extreme salinity shock or even pollution. One farmer in the area released his oyster baskets and let them sink to the bottom, where salinity remained higher: these oysters survived. Freshening events were also associated with mortalities in 2002. Floods may be predicted as there is a delay between rain and elevated discharge and stocks can be saved in time.

Mechanical handling stress is also an issue for mortalities, with the increased mechanisation of oyster farming that may disfavour larger oysters in particular.

Discussion then centred on mortality events when there *were* surviving oysters and what distinguished these individuals. Pierre talked about the diploid *versus* triploid difference observed in Morest and Bob Cox about differences in reproductive development.

An interesting consumer difference between Australia and France is that oysters with a fully developed gonad are considered highly palatable in Australia but not so in France.

As with the mortality episodes in France samples are collected and analysed afterwards. The aim is diagnosis of known pathogens. The Tasmanian DPIW and Graham Knowles, who survey 40 farms across the island, find mortality often associated with freshwater floods and very few pathogens. Herpesvirus (OsHV-1), which was of interest to us because of its implication in present summer mortality in France, can be detected by histopathology followed up by electron microscopy if necessary. PCR tests are not fully standardised but PCR testing will soon start for abalone as we heard at AA08 in Brisbane.

The ability of oysters to defend themselves in such circumstances by remaining closed is also a matter of interest. It is hoped that this is one parameter on which the genetic lines can be tested. During the Morest program some co-workers developed an index of muscular strength which could be relevant.

Pierre made the comment that salinity is an easily controllable factor for imposing selective pressure, assuming heritable variation exists for survival to low salinity stress (which remains to be demonstrated).

Pollutants are also an issue in Tasmania, mainly due to Forestry (Triazines, Pyrethrin and Organo-chlorines) but also from TBT.

For South Australia Barry Ryan told us how *C. gigas* mortality was also associated with the 'conditioning' (pre-sale fattening) period when growth rates were high. Losses were sudden and had been stronger in 2007-2008 than other years. Though studies have examined heat stress during low tide, which may reach 40° C and cause high stress to oysters during high tidal coefficients, there has not been a large scale evaluation of the mortalities.

We discussed if the could be associated with an outside agent or a predisposed sensitivity and Ian Duffie suggested sampling before mortality events to try to understand what subsequently went wrong if such events occurred. Five individuals are indeed already collected and stocked per site.

Helen had already talked with Gary Zippel at AA08 about the characteristics of his mortality episodes. Acid sulphate could be a problem but is also a question of dilution and an interaction with hot weather and rainfall.

Pierre presented how genetics and experimental selective breeding was used in the Morest program to look at how resistance could be selected for and the correlated characteristics of the selected line, which subsequently tell us things about the patterns of mortality such as reproductive effort. One of the genetic results of this research have been QTLs which were *a posteriori* found to be related to herpes virus presence and resistance. Pierre discussed with Peter Kube and Nick Elliot whether highly correlated phenotypic traits in segregating families can lead into the identification of different QTLs.

Selection criteria discussed included: PK the usefulness of an artificial stress system (is opposed to pathogen challenge) Pierre said the French situation was not yet at this stage but emphasised that for summer mortality, selection on disease resistance alone was unlikely to be appropriate as it was generally known that disease was not the only factor in summer mortality as effects were not direct (this is emphasised by the lack of correspondence between vibrio challenge results on the resistance and susceptible line in Morest). However, the role of the herpes-like virus in summer mortality in France, while strongly suspected, remains to be further documented.

Would selection for an earlier or later onset of reproduction help? Or rate of gametogenesis? Pierre talked about the gene expression work done in his present lab on the genes expressed and therefore indicative of different stages by non destructive RNA sampling.

General Discussion There had already been a workshop in Australia in 2006. Research work is regionalised with for example the Tasmanian oyster research council funded by a levy and linked to the DPI. Scott Parkinson mentioned that this means that data are not united and compared nationally. There are not sufficient funds for a field officer so discussion turned around the idea of doing such work in the industry itself. There are however tensions between the hatchery and industry on the quality of the seed in cases of mortality.

Other questions also raised in discussion included:

- Could stress indicators evaluate the quality of sorting machines?
- Can data be translocated out of the individual states and applied elsewhere?
- Could phenoloxydase, studied in SRO, be a useful marker in *C. gigas* ?
- Environmental characteristics of importance include duration of exposure to air, use of NIR technology.
- Selective breeding

On this final point, selection for survival of lines bred in Tasmania could be done where they are grown in SA. However, the survivors from the field cannot be used as parents in subsequent generations as for sanitary reasons they cannot be transported back to Tasmania and hatcheries in SA are not yet involved in the program.

Pierre commented that the first work of a program on mortality is which characters to study and thus a search for indicators. Sources of mortality risk factors differ with the important

regional geographic differences across Australia. Areas involve are larges as are the distances between them.

It was discussed whether the environmental optimum for selected oysters be narrower? The present plan of ASI is to breed good all round oyster lines suitable for many sites.

Much of what we heard in this workshop again showed the direct relation that exists between research and industry on the state scale and the cooperation which appears to be actively sought on both sides.

Continuing our visit to **Tasmania**, we spent a day at **Shellfish Culture**, Clifton Beach, where we visited the hatchery where ASI lines, triploids and tetraploid broodstock are reared before nursing in local waters. We saw parental ripening and the intensive micronursery that allows in large numbers or spat to be produced in a small space under careful monitoring. Shellfish Culture sometimes works with the University of Tasmania to whom they send samples for bacterial analyses.



We had a meeting about broodstock genetic diversity management, with particular reference to polyploids with Scott Parkinson and Michel Bermudes and Richard Pugh from Shellfish Culture and Peter Kube and Nick Elliot from CSIRO. Pierre indicated that parentage analyses using microsatellite markers are currently used in France in triploids to study the genetic basis of reproductive effort in so called “sterile” Pacific oysters.



At **Spring Bay Seafoods** we saw modern mussel hatchery facilities, and heard about production in this very complete business which takes *Mytilus galloprovincialis* from gametes right to supermarket-ready packs of washed mussels.

The equipment in the hatchery realised many of the ideas we had independently had in Europe during Blue Seed, but we did not yet test on such a large scale. It was also clear that the motivations behind the research were similar: unreliability of natural seed supply, search for a ripening conditions perfect

settlement substrate, metamorphosis fragility and survival. Field growing is done deeper than in Europe as the method was originally conceived for scallops. Spring Bay Seafoods was also a business with relationships with the university: they had project students to examine a number of questions on conditioning and settlement. Technologies had also been specifically developed around packaging.

We then went with as group from research and industry to visit Hayden Dyke's farm **Oyster Bay Oysters** at Little Swanport. We enjoyed a Gourmet seafood Lunch prepared by Jane Dyke.

Here Hayden's father Colin told us about the foundation of the farm on the principles of trophic resource management for oyster density and immersion. Water quality management is also closely surveyed at the level of the estuary and river where there are sensors for salinity and temperature. Freshening can therefore be predicted and



stocks moved if necessary, this is not just a question of potential low salinity shocks for the oysters but also sanitary questions and the need to depurate. The farm also has a close relationship both with environmental organisations and state regulators emphasising a openness of information, notably about water purity. On the farm we saw the ASI lines that leave an impression not only for their taste but the visual attractiveness of their deep dark shells and thickness and whiteness of their nacre.



Concluding remarks

There are many similarities between French and Australian oyster industry and research. In both countries, native species suffer severely from parasites, and research has led to the production of resistant lines by selective breeding. For *O. edulis* (in France) and *O. angasi* (in Australia) the relatively small size of the industry has clearly been a limiting factor. For SRO, significant progress has been achieved and transfer to industry is effective. The improvement of selective breeding, from mass selection to marker assisted family-based breeding should contribute to improved production in this species. Pacific oyster has also benefited from genetic improvement in Australia, following development of cytogenetics (triploids) and selective breeding. Until now, mortality in this species appears to be mainly related to fluctuating environmental factors such abiotic environmental factors, pollutants or salinity. As emerging pathogens (vibrios, herpes-like virus) can lead to severe mortality in this species in other parts of the world, special attention should be given to the ability to face such potential new problems.

Overall, we were most impressed by the constructive and effective dialogue that exists between research and the shellfish industry in Australia.

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