

Shellfish Production Aquaculture Technology

Global Perspective of Bivalve Hatchery Processes

A report for



by Ian Duthie

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Foreword

Shellfish (Bivalve) cultivation is a significant form of aquaculture around the world, with production at 13.9 million tonnes and continuing to grow at 5% per annum. (FAO – Facts). Bivalve cultivation is one of the earliest forms of aquaculture with records of oyster cultivation extending back to Roman times. Within Australia the commercial cultivation commenced with the Sydney Rock Oyster (*Saccostrea glomerata*), utilising wild ‘spat’ settlement, to support production. Pacific Oysters (*Crassostrea gigas*), Mussels (*Mytilus galloprovincialis*) and to a far lesser degree Flat Oysters (*Ostrea angasi*) also developed this way.

Oyster production extends from southern Queensland down the coast along New South Wales, across to Tasmania and South Australia. Mussel cultivation has a limited presence in NSW, and a well-developed industry in VIC, TAS and emerging in SA. Clam and scallop cultivation is not commercially relevant as yet although interest exists and with a commercial fishery and existing public demand for these shellfish.

In the late 1970s hatchery technology was adopted by the Tasmanian Pacific Oyster (*Crassostrea gigas*) industry to secure a reliable supply of ‘spat’ following inconsistent, short supply of ‘wild-catch’. This started the development of shellfish hatcheries within Australia. Further oyster hatchery development occurred, with oyster hatcheries now operating in NSW, TAS and SA. In 2006 a dedicated mussel hatchery was also commissioned to underpin ‘spat’ supply within Tasmania. The Victorian mussel industry followed this development with a government supported mussel hatchery also.

The development of industries farming these shellfish species, including the investment in the growout, processing, distribution and market development is all at risk, if a reliable consistent supply of ‘spat’ is not available to underpin the production processes. Therefore supporting hatchery development that is robust, and reliable in supplying sufficient quantity and quality of ‘spat’ is essential.

The Australian edible shellfish aquaculture industry is essentially made up of oysters producing 14,800 tonnes worth \$100 million, and mussels with 3,100 tonnes and a value of \$10 million. The direct employment generated by these industries is predominately in rural regional areas.

Australia has limited shellfish aquaculture development of its coastal area, particularly when compared with the intensity of development in countries such as China. The opportunity for Australia to extend its production of the existing species grown, and develop an industry with other indigenous species, particularly clams such as *Kateleyisia* sp. is immense (Internationally clams are worth as much as US\$300 million). *Kateleyisia* sp. currently sells for between \$18-22/kg at the Sydney Fish Market.

The continued growth and development of shellfish aquaculture in Australia is dependent upon its ability to meet ongoing challenges, and take advantage of the opportunities that present themselves. Underpinning this is a reliable, economical supply of 'spat'; it is recognised that shellfish hatcheries can meet this need. Identifying the technology, business structures, management and environment that allow hatcheries to operate as environmental and economically sustainable entities supporting the industries need for spat is critical. The technology utilised by the Australian shellfish hatcheries represents the best that is available around the world, with a mix of high intensity and low density culture practices for both larval and algae production. A focus on management that provides for reliability and efficiencies in production is still required. The 'devil is in the detail' and how these technologies are utilised. Australian oyster growers are paying as much as double for equivalent product i.e. five mm Pacific Oysters \$15.50AUD in America vs. \$26-32AUD in Australia.

The Australian oyster industry provides a good example of cooperation, with research into the development of Polyploidy, and Selective Breeding. This mirrors the best practice of industries around the world. This investigation however identifies that further cooperation will be necessary across areas such as disease management, water quality management, production difficulties and energy efficiencies.

The opportunity for the Australian shellfish hatcheries to diversify their production across a wider range of shellfish species was also very clear, with the majority of shellfish hatcheries visited supporting the production of a diversity of species, utilising essentially the same equipment and technology as exists in Australian hatcheries.

The diversity of species produced and vertical integration was also recognised as an opportunity for Australian shellfish hatcheries to mitigate risk and improve their profitability.

The ownership and management structure of international hatcheries was identified as influencing their efficiency and profitability. A number of facilities visited had 'management' buy-outs, or a variety of structures in place to retain skilled management, with 15-30 years continuous employment not being uncommon. This contrasts with the Australian experience

with more than 10 years continuous service being rare. Succession planning of key management is going to be a growing challenge around the globe, with the 'management' buy-outs referred to above going to become more difficult as the scale and value of the businesses continues to grow.

The scale of production by international hatcheries identified during this study dwarfs that of Australian hatcheries. This scale of production appears to be a major influencing factor in the efficiency of production, and the capacity of international facilities to offer considerably discounted spat prices compared to that on offer within Australia.

An economic study into the relative merit of species and product diversification should be undertaken, identifying the opportunity for Australian hatcheries to improve their international competitiveness, building efficiencies and improving profitability.

Investigations identifying and removing the impediments to develop a clam aquaculture industry within Australia are needed. Identifying further nursery and growout technology and investigating the economic, and social benefits of building this industry.

This Nuffield Scholarship Study Tour was supported by the Australian Fisheries, Research and Development Corporation (FRDC) with the intention of investigating what is the current 'state of play' of shellfish hatcheries and the industries they support, and what lessons are there for Australia.

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Abbreviations/Glossary

ABC Aquaculture Breeding Centre.

Algae: aquatic plants including Phytoplankton – microalgae.

Axenic indicates algae cultures that are free of any other ‘contaminating’ organisms.

Bivalve: mollusc having a shell of two valves that are joined by a hinge.

Byssus: thread-like filaments used by bivalves to attach themselves to a substrate.

Cilia: hair-like structures whose rhythmic beat induces a water current in bivalves.

Cultch: material used to collect bivalve spat.

Diatom: a single-celled alga of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains.

DNA (Deoxyribonucleic acid) is an [informational molecule](#) encoding the [genetic](#) instructions used in the development and functioning of all known living [organisms](#) and many [viruses](#).

Diploid: the normal number of chromosomes (2n) in cells.

Downwelling: in hatchery terminology, a growing system in which the flow of water enters at the top of a spat holding container (compare with upwelling).

D-larva: the early veliger larval stage of bivalves, also known as straight-hinge larva.

Embryo: an organism in early stages of development; in bivalves, prior to larval stage .

Exotic: introduced from foreign country or geographic area.

Eyespot: a simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light.

Eyed Larvae: the stage just before settlement and metamorphosis.

IFREMER: French Research Institute for Exploration of the Sea.

Metamorphosis: the stage at which the shellfish larvae transition from free swimming larvae to a settled spat.

OAT: Oyster Aquaculture Training

PCR: the polymerase chain reaction is a biochemical technology in [molecular biology](#) to [amplify](#) a single or a few copies of a piece of [DNA](#) across several orders of magnitude, generating thousands to millions of copies of a particular [DNA sequence](#).

Spat: the juvenile stage of a bivalve shellfish, following larval stage and settlement and metamorphosis.

Tetraploid: a polyploid animal with twice the normal complement of chromosomes (4n).

Triploid: a polyploid animal with an extra set of chromosomes (3n).

Upwelling: in hatchery terminology, a growing system in which a flow of water is induced through the base of a spat holding container (compare with downwelling).

VIMS: Virginia Institute of Marine Science.

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Executive Summary

Despite the Australian shellfish industry having developed hatchery technology for a variety of species, it has still been plagued by an unreliable and inconsistent supply, resulting in a shortage of supply of ‘spat’ to shellfish producers. This has stalled commercial development of both existing and ‘new’ alternative species to oysters and mussels. These ‘new’ species are closely related to those being commercially cultured around the world, with the production cycle starting within the hatchery, and strong existing domestic markets, such as with scallops and clams.

The cost of Oyster ‘spat’ to overseas growers is considerably cheaper than that offered by Australian hatcheries for equivalent ‘spat’. Investigations of the factors influencing this are important, as it may provide opportunity to improve the competitive productivity of the shellfish industry.

The need to study Shellfish Hatchery businesses from around the world, and identify both “World Best Practice” and the future trends in shellfish production is important for the future growth of the Australian Shellfish industry. Recognising the deficiencies of current technology and knowledge, that exist both in Australia and around the world is important. Looking for the business and industry structure that supports reliable, efficient and profitable supply of ‘spat’. Identifying current opportunities for technological transfer, and international collaboration and assess the structural and management systems employed by world leaders in shellfish hatcheries and integrated growers and processors.

Results

The commercial hatchery production of a diverse range of bivalve shellfish is being successfully undertaken around the world, predominately with oysters, clams, and to lesser degree mussels and scallops.

Hatchery production strategies are greatly influenced by the environmental, and social-economic environment; for example USA hatcheries utilise large scale tank production of larvae and algae, with low energy costs *compared to* UK hatcheries which utilise compact, high intensity systems for both larvae and algae, adopting energy efficient water heating and reuse, due to their relatively high energy costs. Species and product diversification was a common strategy for risk management, and optimisation of capital investment.

Collaborative efforts between, Industry, Universities, Government and Public associations have been essential in the continued development and success of shellfish businesses, with particular focus on initial species development, resource access, disease diagnosis and

management, education and training, technological development, and selective breeding and genetic (polyploidy) programs.

There is an evident shortage of supply of aquaculture-produced shellfish across all the countries visited, particularly oysters. The factors driving this include:

1. wild 'spat' catch difficulties,
2. hatchery production difficulty relating to environmental change and disease,
3. mortality and disease within the grow out of the shellfish.

There also exists across most species an almost insatiable demand for shellfish; some of this follows on from the 'heritage' of shellfish consumption as an artefact of wild fisheries. It was often commented that marketing and sales of shellfish is still really a function of 'taking of orders'.

Recommendations

Blanket recommendations relating to technological advancement for Australian shellfish hatcheries are not possible as there are too many individual considerations on existing business structures, and the various technology adopted by shellfish hatcheries overseas is dependent upon their existing cost structures and environmental location, and risk mitigation strategies, such as:

1. Species and product diversification should be adopted by shellfish businesses to mitigate risks and optimise human and capital resources. Vertical integration was also successfully demonstrated, as was synergistic 'joint-venture' relationships between shellfish hatcheries/nurseries and grow-out producers sharing similar values and attitudes.
2. Australian Industry and Government should support coordination in the areas of disease diagnosis and management, education and training, technological development, and selective breeding and genetic (polyploidy) programs.
3. New species and product development also represent important opportunities, as does diversification into clams as a strategy to insure the industry against the threat of demise of a single species or product through disease or other misfortune.
4. Building strong on-going relationships with key staff, as they are the fundamental to business success.
5. Developing a network of international shellfish hatchery producers, providing for opportunities to interact, not just at the manager level, but also between technicians, encouraging discussion of issues influencing "success".

6. Recognising the advantages of using ‘Collective Intelligence’ between hatcheries where they collaborate to find solutions to common problems. Well executed this could improve the speed at which problems are overcome, and improve the use of available resources. The difficulty of adopting a culture of information sharing and collaboration between companies in resolving common problems can be countered by investigating the real competitive advantages of different hatcheries. These typically relate to the “operational” processes that are performed by staff, and relates to technical knowledge and skill of individual staff. Competitiveness results from the overall cost structures, attention to quality and species/product produced. This allows then for the collaboration on ‘bigger’ issues that influence all producers such as: disease, water quality and treatment, selective breeding, education and training, technological development i.e. water quality treatment and management, energy efficiencies in water heating/cooling, or LED technology for algae production.
7. Encouraging staff exchanges, secondments between national and international colleagues within the shellfish industry, where issues of direct competitive pressures are not significant.

Introduction

I openly confess that I have a shellfish aquaculture addiction, and hold a passion for all aspects of the industry. I enjoy being involved with the shellfish seed from its creation within the hatchery, to the day a crop is harvested, having nurtured and cared for them throughout their time on the farm.

I have immersed myself into the Australian shellfish industry since I started studying Aquaculture at the University of Tasmania. I've worked extensively within the industry with a variety of businesses and roles from a general farmhand and research technician through to manager/owner. I also have held roles in the broader industry such as Chairman of the Tasmanian Oyster Research Council, Oyster Tasmania – steering committee, representative to the Oyster Consortium – Seafood CRC, an Honorary Associate of the School of Aquaculture, University of Tasmania, and advocacy roles as Chairman of Circular Head Shellfish Growers, and Tasmanian Shellfish Executive Council.

I have a specialised interest/experience within the hatchery production of shellfish.

My work experience has allowed me to have a significant role in bringing efficiencies and trouble shooting problems for all the Tasmanian hatcheries, and establish good working relations with South Australian, Victorian, New South Wales, Queensland and New Zealand hatchery producers.

I have a full understanding of the complexities of establishing a new operation and some of the difficulties in reforming and adjusting existing businesses to existing and future challenges and opportunities.

I was working with the University of Tasmania on Australia's initial investigations into developing technologies such as triploidy and selective breeding programs, investigations of previously uncultured clam species, developing hatchery protocols (Spawning, settlement, and growth of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849) in culture).

My most recent challenge was developing the cultivation of mussels through the hatchery and nursery in commercial quantities, developing processes and technology that provides for reliable and efficient production outcomes. Spring Bay Seafoods Pty. Ltd. provided me with the opportunity as hatchery manager to do this by letting me design, build and then operate a new hatchery specifically for this task.

It was during this process that I recognised that there is still much to learn, not just with the technology, but also with the overall business philosophy. Factors not unique to a shellfish business, and common amongst other primary producers factors such as; vertical integration, risk mitigation, scale of operation, diversification, product development, research and training, future growth and cost of operations and profitability. All this sits alongside the governmental and social environment, further increasing the complexity. A global perspective, as offered through undertaking a Nuffield Scholarship, was what was needed.

Having worked with and visited all the major shellfish hatchery producers within Australia, I recognised that there is a gap in the knowledge and processes in bringing the shellfish from the hatchery through to the nursery, delivering them to the grow-out farmers. Efficiency gains in this area, I believe, will strengthen the overall industry by providing for enhanced production at a lower cost, and improve continuity of supply. This then allows hatcheries to further investigate and support the cultivation of new species, allowing for species/product diversity for existing and new producers into the future.

Traditional means of investigation, such as research papers, trade journals, and more recent methods such as use of the Internet and 'YouTube' were all investigated, but the best way to really see what is happening is to get out there and visit people, building the relationships and opening discussions into factors influencing successful shellfish production.

I travelled extensively during this Nuffield scholarship, visiting eleven countries specifically to investigate shellfish aquaculture. These included; United States of America, Norway, Sweden, Portugal, Spain, New Zealand, Canada, France, Guernsey, England and Scotland.

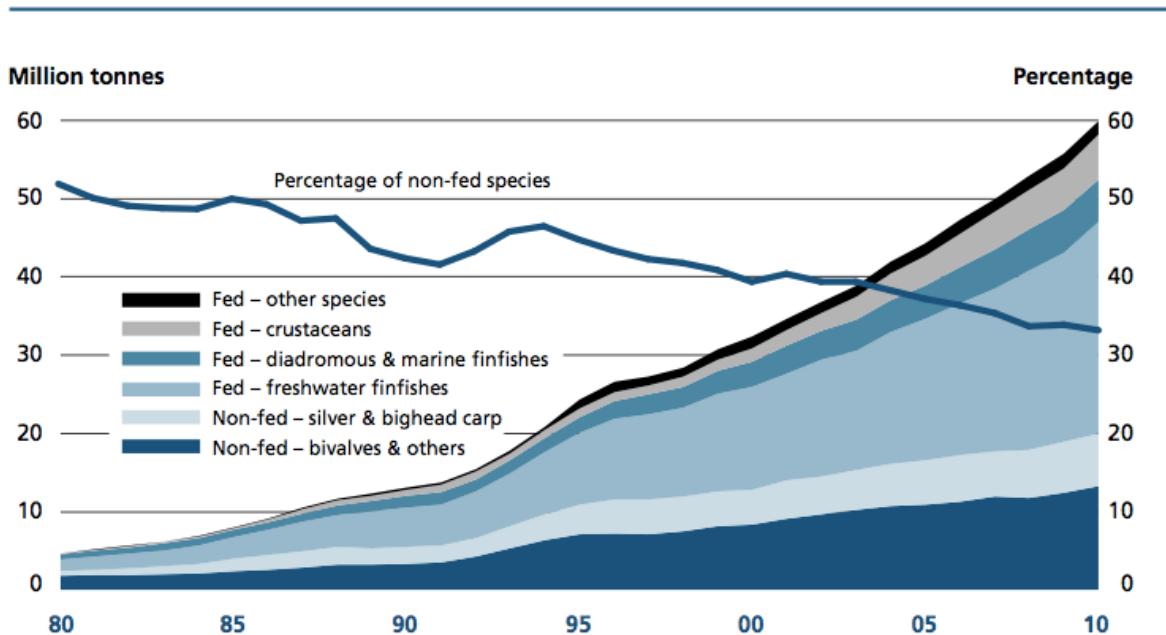
How to report or organize what was observed during this tour is a difficult challenge, there was so much across such a broad sweep of the industry. People were generally very open and sharing at the personal level, but held some concerns as to how widely distributed the information and discussions we held would be. This leaves me with a responsibility to 'generalize' some of the findings of this tour.

Chapter One

Global Aquaculture –Shellfish (bivalve) Production.

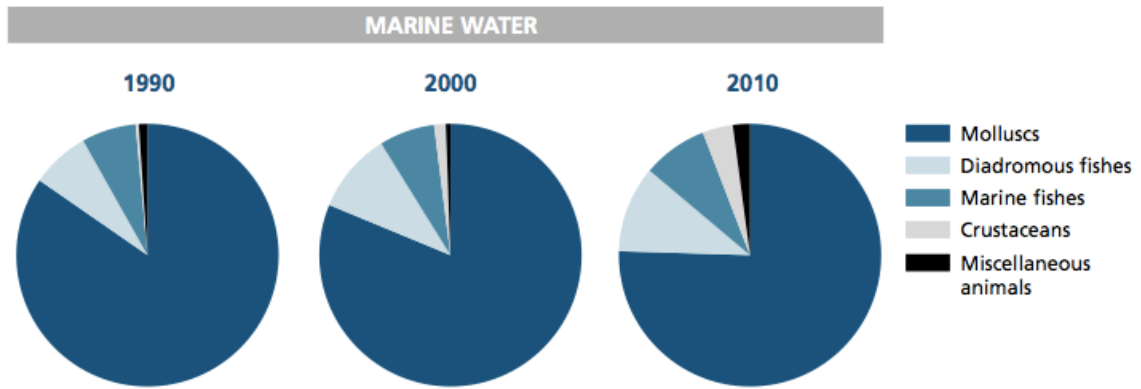
The Food and Agriculture Organisation of the United Nations (FAO) reports that global aquaculture production has continued to grow in the new millennium. Aquaculture has developed rapidly over the last half century or so, now reaching production comparable with capture production in terms of feeding the worlds population. Aquaculture has continued to evolve in terms of technological innovation and adoption to meet changing requirements. World aquaculture reached an all time high in 2010, with 60 million tonnes of edible production, valued at US\$119 Billion. One-third of this was achieved without the use of supplementary feeding, through the production of bivalves and filter feeding carps.

World aquaculture production of non-fed and fed species

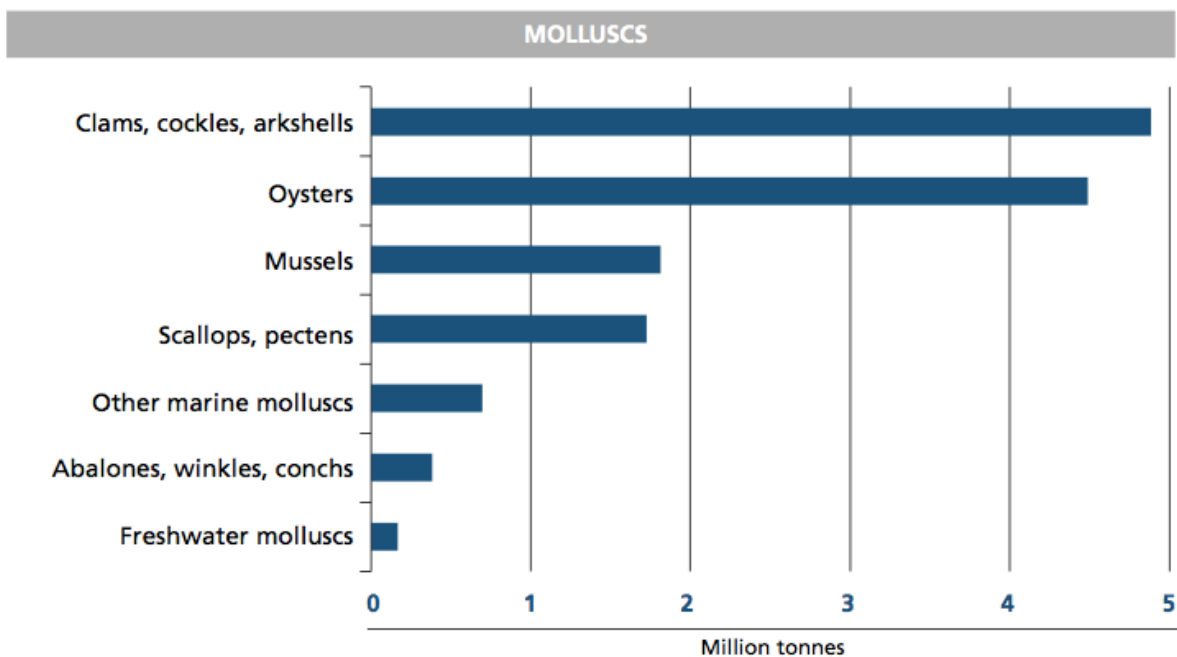


Source: FAO (2012)

Marine molluscs account for 13.9 million tonnes, or 75.5% of marine aquaculture production, although this has declined from 84.5% in 1990, and this is a reflection of the rapid growth in finfish culture in marine waters, which is growing at 9.3% per annum. Bivalve production is still growing at respectable rate of 5% per annum. Production of clams has increased much faster than that of other species groups. In 1990, clam production was half that of oysters, however by 2008 it had exceeded oysters and now represents the most produced species group of molluscs.



Source: FAO (2012)



Source: FAO (2012)

A significant part of the global production of marine molluscs, particularly in Europe and America, relies on the widely introduced Manila clam (*Ruditapes philippinarum*) and Pacific oyster (*Crassostrea gigas*). China also produces large quantities of the Pacific oyster and the Atlantic bay scallop (*Argopecten irradians*) and Yesso scallop (*Patinopecten yessoensis*).

The FAO also reports that over the last five decades, world food fish supply has outpaced global population growth, and today fish constitutes an important source of nutritious food and animal protein for much of the world's population. In addition, the sector provides livelihoods and income, both directly and indirectly, for a significant share of the world's population. In fact, in the last five years for which data are available, the number of people engaged in fish farming has increased at 5.5 percent per year. This growth is faster than the

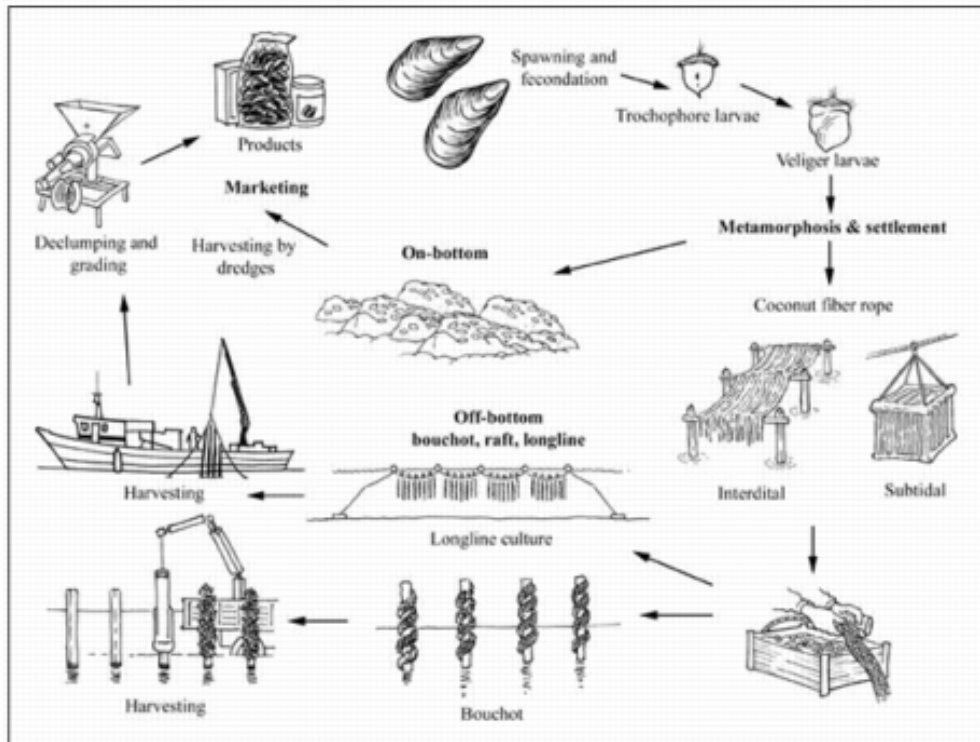
0.5% growth in employment that is seen in traditional agriculture.

Aquaculture production keeps on expanding, with the FAO predicting it to remain one of the fastest-growing animal food-producing sectors into the next decade. A growing global population with finite natural resources, facing the continuing economic and environmental challenges will be well served by the aquaculture production of bivalve shellfish. Bivalves do not require feeding, as they filter algae and other material from the natural environment, providing the opportunity for an on-going sustainable way of providing sustenance into the future. Hatchery production of the 'spat' will continue to have an increasing importance to securing this opportunity to feed the world.

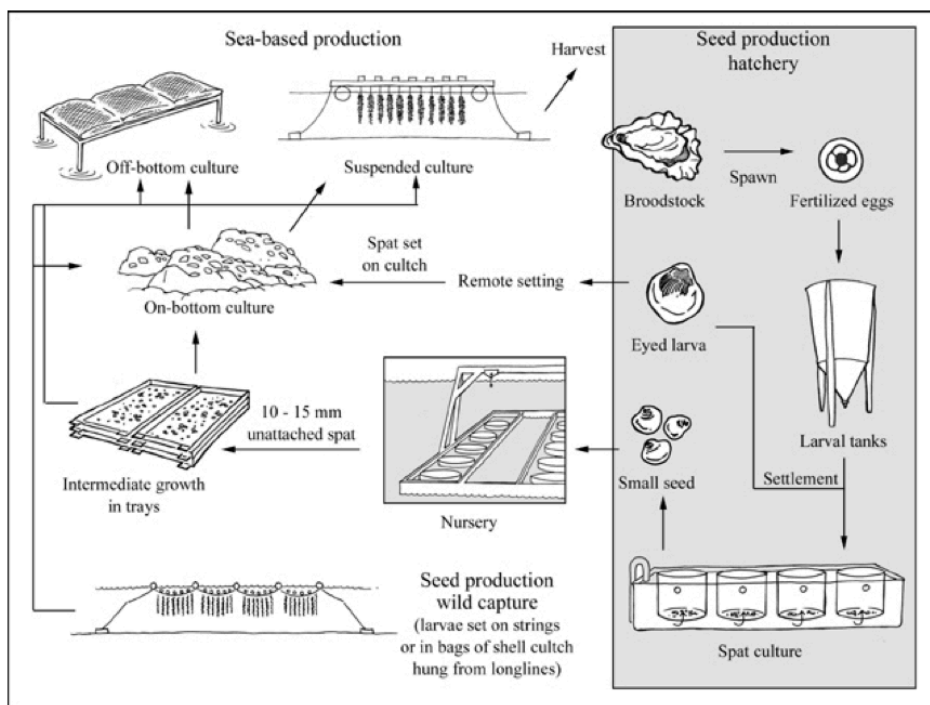
Chapter 2

Life Cycle of Bivalve Shellfish.

The life cycles of bivalve shellfish are remarkably similar. Generally the sexes are separate and sexual maturity is attained at a range of sizes from 20mm for clams through to 40-50mm for oysters and mussels. In the spring, as the water temperatures begin to increase, the gonads of the male and females begin to ripen. Spring typically brings increased phytoplankton levels which helps with the production of gametes. The temperature increase also stimulates the conversion of 'fat' or body reserves into gamete production. Once the individual shellfish are ripe, some stimulus, often a rapid change in temperature, but also increased quantities of phytoplankton, or current flow, will trigger a spawning. Eggs and sperm are released into the water where fertilisation occurs. Other individuals are often triggered to also spawn through the presence of gametes that have been released. The fertilised eggs develop into straight-hinge, free swimming larvae within 24 hours, which have two half shells. The typical size is between 70-90 micron shell length. These larvae are sometimes referred to as 'veligers' because they have an organ called a velum, and this lets them swim, feed and 'breathe'. The larval stage typically last between 2-3 weeks, and the larvae grow to a shell length between 200-350 micron. At this point they are referred to as 'pedi-veligers' or 'eyed' larvae, because they have developed an 'eye-spot', and a 'foot' in addition to the velum. This allows them to both swim and crawl, with their foot looking for a suitable location to 'settle', or attach. At this point the strategies between oysters and clams/mussels/scallops differ. Oysters will 'cement' or adhere themselves permanently to a single place, undergoing metamorphosis, and developing gills and losing the function of both the velum and the foot, thus starting a sedentary life. Clams, mussels and scallops typically do not 'cement' themselves, but attach to a suitable substrate using 'byssal threads'. They then undergo metamorphosis, also developing gills for the function of feeding and respiration, losing the velum. However, they keep the foot, and often retain the capacity to break the byssal attachment, crawl or 'pedal-drift' and reattach themselves to a different position. Mussels though keep the byssal threads to adulthood, but often clams and scallops lose this function as they grow into juveniles.

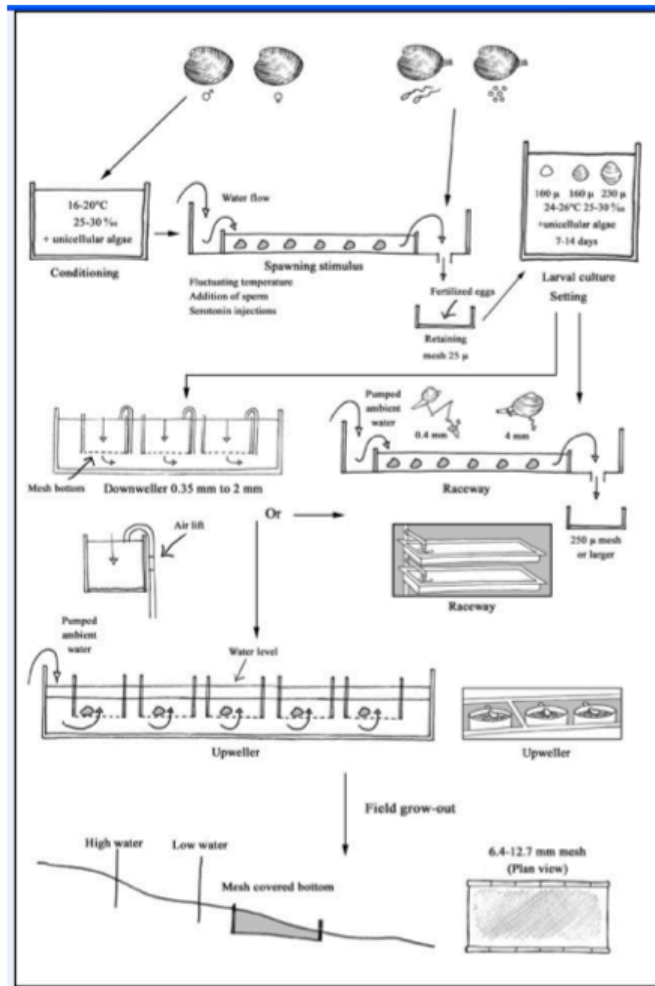


Generalisation of the Production Cycle of Mussels (FAO-website)



Production cycle of Crassostrea gigas

Generalisation of the Production Cycle of Oysters (FAO-website)



Production cycle of *Mercenaria mercenaria*

Generalisation of the Production Cycle of Clams (FAO-website)

Chapter 3

The Advent of Hatchery Propagation of Shellfish.

Aquaculture of bivalve shellfish developed through the manipulation of 'wild' caught spat. Moving shellfish from areas of high recruitment, and thinning out to improve growth and returns. This progressed to enhancing stock numbers by catching spat with additional substrate, initially being rocks, branches and bamboo following the natural larvae life cycle within the natural environment. This evolved into a variety of different substrate types, both natural and artificial, e.g. 'shucked' oyster shells for oysters & polypropylene ropes for mussels.

Shellfish hatchery methods have evolved significantly from the first report of successful artificial oyster spawning occurring in 1879 when Brook produced "free-swimming" oyster larvae by stripping eggs and sperm from ripe adult oysters. Attempts to grow other shellfish larvae continued, however W.F. Wells reported having successfully reared and set oysters in 1920.

The 1960s saw the development of shellfish hatcheries across Europe and the United States, pioneering the technology necessary to grow a variety of species. Knowledge of the biological requirements and technological capacity has continued to develop, with intensive production strategies being developed within purpose built hatchery and nursery facilities to support the sea-based growout.

The typical shellfish hatchery has five main components.

1. Broodstock are essential as this is the basis for all production, and are typically selected to exhibit traits that producers want to see in cultivated stock, i.e. growth, disease resistance, quality –shape, colour.
2. Larvae form the production centre of the hatchery where the shellfish are allowed to develop through their 'free-swimming' stage.
3. Settlement is a critical stage where the shellfish undergo metamorphosis and become spat, no longer free swimming.
4. Nursery is the stage where the spat are on-grown in preparation for transfer to the growout production stages.

5. Algae production underpins the entire production process delivering ‘food’ for all of the above stages.

The United States and Canada have adopted an alternative production approach to the fully integrated five stages of the hatchery process, with their oyster production. Given the scale of their production and a transition from a ‘wild’ catch farming model, they developed a process called ‘Remote Setting’ to replace the unreliable and costly collection of natural spat. This effectively married the advance hatchery production process to the existing substrate based farming systems. Jones & Jones (1988) report that Bill Budge of Pacific Mariculture did the first commercial transfer of eyed oyster larvae in the early 1970s. They were producing their larvae at Pigeon Point, California and moving them in buckets of seawater to their setting facility at Moss Landing, California. The method didn't come into widespread practice until 1978 when Lee Hanson built the Whiskey Creek Oyster Hatchery near Tillamook, Oregon, exclusively for the production of larvae for remote setting operations.

The remote setting process, as the name suggests, has the settlement occur away from the hatchery. The eyed larvae are shipped out of water in chilled moist packaging, and then added to cultch at a site remote from the hatchery, where they settle. This has allowed the centralisation of hatcheries, shipping billions of larvae around the country without the expense and risk of moving vast quantities of cultch also. Gordon and Bruce Jones (1988) provide a thorough account of the background and process in their publication, “*Remote Setting of Oyster Larvae*”.

The alternative strategy to remote setting is setting onto cultch at the hatchery, or production of ‘single-seed’ which involves settlement onto ‘micro-cultch’, very finely ground shell, of the same approximate size as the settling larvae, or using induction techniques that enable metamorphosis without the oysters attaching to a substrate. Single seed can also be produced using a substrate and then once spat being detached from the cultch, i.e. plastic slats, French tubes.

Shellfish hatcheries are effectively trying to manage biological processes, such as the husbandry of the selected species through development from egg to spat, and also the culturing of the microalgae to provide sustenance through this process. Finding the balance that meets these biological considerations while still meeting economic business requirements can be challenging.

Multiple variables need to be managed with many people referring to operating a successful hatchery as being as much about the *art* of running a hatchery as the *science*. The facilities visited demonstrated this with some being run as much by '*feel*' as by *science*, I am of the opinion that the more we discover about the factors influencing success within the production of shellfish spat, the more we can take a '*recipe book*' or scientific approach. Relying less on the '*art*' of producing shellfish spat. However, the issue still remains that, like traditional farming, it will always require a certain tenacity and resilience to really excel.

Chapter 4

Broodstock.

Broodstock are the animals from which gametes are produced, and are the start of the shellfish hatchery process. The facilities visited had a variety of strategies to ensure sufficient good quality broodstock were available across the range of species being grown.

Typically broodstock conditioning is required to extend the availability and ensure the best quality gametes are available for the hatchery production process. This often involves the temperature manipulation of the seawater to either bring on early maturation, or hold condition or development, so it can be delayed, and managed to match production scheduling. In addition to temperature control, supplemental feeding utilising a mixed microalgae diet is also undertaken.

The strategies adopted by facilities visited weren't significantly different to Australian practice. There are a number of factors that influence these strategies that may have an influence on the future production of shellfish at Australian hatcheries. These include disease screening, and establishment of quarantine facilities, allowing broodstock to be brought onto site from field sites, or farms where a variety of diseases maybe present or suspected of being present. These quarantine facilities allow stock to have initial conditioning undertaken, while they are screened or examined for a variety of applicable diseases. Horn Point Hatchery, IFREMER, ABC @ VIMS, and Hatfield Marine Science Centre are all good examples of 'public' agencies that had these facilities.

The quarantine broodstock facilities typically had a level of physical separation between themselves and production areas. This was reinforced with appropriate physical and biological security measures that extended from restricted access and signage through to disposable overalls, boot/shoe covers, footbaths, cleaning/disinfection stations. The water and material exiting the facilities underwent a variety of treatments dependant upon the assessed risk, and included water treatment with chlorination/de-chlorination, UV, ozone and in some circumstances this water and material was then collected and removed to secured landfill sites, i.e. pumped out much the same as a septic pit.

Beyond quarantine, careful consideration was also necessary with regards to the location that broodstock were collected from, particularly where the shellfish were indigenous species, necessitating an awareness of the risks of influencing the genetic 'pool' by introducing

genetics from animals from a different geographical region. The American Oyster (*Crassostrea virginica*) grown in the Chesapeake area also exhibits a geographical variation of stock to adapt to low salinity or disease. This species is also the target of natural rehabilitation for environmental and conservation purposes, so management of genetic diversity is important and catering for this within the broodstock systems is critical.

The fecundity of the cultured shellfish species also influences the infrastructure and management of the broodstock systems necessary to meet production targets. The American Oyster along with the issues discussed above has a relatively low level of fecundity, particularly when compared with the Pacific Oyster. This impacts the resources, both physical and in terms of general husbandry and food production. Mussels have a similar issue with low fecundity, and a common strategy is to adapt the scale of hatchery production to optimise the natural availability of 'farm' conditioned broodstock. The intensive production of microalgae and infrastructure requirements necessary for managing temperature required to condition the Blue Mussel (*Mytilus edulis*) was found by the European project Blue Seed to make broodstock conditioning un-economic, and threatened the economic viability of hatchery production of this species. The New Zealand mussel industry growing the Greenshell (*Perna canaliculus*) is still predominately wild caught spat. However, investigations into the hatchery production of this species have identified the same difficulties relating to low fecundity, and the impact this has on the economic production of Greenshell within the hatchery and the subsequent ability to adopt selective breeding and triploid production. The approach they have taken is to use seawater ponds to culture a 'natural' mix of microalgae and use this to feed their mussel broodstock.

Chapter 5

Larvae Production – Technology.

Larvae production and the technologies surrounding it focus on providing for the requirements of the free-swimming life stage of shellfish. Maintaining water quality that matches the requirement of the species being grown is essential, and this is usually addressed when first selecting a suitable site for the hatchery, with water available that has the appropriate salinity, and is free of ‘toxic’ compounds, such as heavy metals. The temperature, dissolved oxygen, clarity, microbiological and general physical parameters can all be manipulated within a hatchery. The level of ‘control’ or manipulation depends upon the site selected, the species being cultured and the resultant economics.

Water Filtration:

Shellfish hatcheries typically undertake physical filtration of the water being used for larvae culture to remove sediments, algae, and other invertebrates. The hatcheries visited used a variety of physical filtration systems, from sand wells, sand and multi-media filters, disc filters, filter screens, filter bags and cartridges. One hatchery even used shellfish themselves in combination with a ‘biological’ substrate filter. They first collected water that had passed through juvenile shellfish, effectively ‘cleaning’ the water of almost all sediment and algae etc. before running it through the biological filter, then stored this water and used it for larvae culture.

Sand filters were commonly used but there was a growing awareness of the issues of using this style filter, with great care required to maintain their effective operation and avoid build up of detritus, with both bacterial and biological consequences, such as dissolved oxygen drops, fluctuations in pH and microbiological population growth and species alteration. Cartridge filters were also used extensively by some of the public facilities, although the on-going cost of replacement and labour for management of these was recognised as being considerable.

Temperature Control:

Typically larvae water is heated to the ambient temperature. The hatcheries in the USA had been operating in an environment of low energy costs, with water heating for larvae culture consisting of diesel and propane boilers, and the occasional electric heater. Following escalating fuel costs, (still low compared to other nations, i.e. less than \$1/L diesel vs. \$2/L for diesel in the UK) the trend is to adopt heat recovery technology, and investigate the use of

electric heat/chill style systems. This need to heat the water and the implications of the cost of power is driving the adoption of heat recovery technology that has been long practiced in the UK, and Australia. The water that has been heated and then used for larval production is captured and directed through titanium heat exchangers, with new 'incoming' water being pre-heated as it runs counter-current to the out-going water. The adoption of this heat recovery has also led to a change in the management of larvae rearing tanks (LRTs).

Typically LRTs were static batch-culture tanks, with seawater and larvae contained. The volumes in the USA started around 10,000L and went up to 50,000L. The larvae were grown at relatively low densities, with between 0.5-1 larvae/mL at settlement. The water would usually be changed every second or third day, and the larvae captured on an appropriately sized mesh screen. This compared with European practice, where a similar static batch approach was taken, but with much smaller tanks of 100-500L, usually kept within a temperature-controlled room. This static, batch-culture method of growing larvae does not optimise the opportunity to capture the heating already put into the larvae, with tanks needing to be drained at the same time as new tanks are filled, just adding to the complexity of the process, juggling tanks.

The solution to this was to adopt a continuous flow larvae production system, and this followed the development of 'High Density' systems by John Bayes of Seasalter Shellfish, with densities of greater than 130 larvae/ml at settlement possible, in LRTs of 200L. Hybrid systems have now been developed ranging in volume from 200-50,000L, with the bigger volume LRTs still keeping a relatively low density, around five larvae/ml at settlement. The advantage with regards to heat recovery has been achieved, in addition the duration between draining tanks has also been extended. This development significantly affects the two greatest costs associated with a hatchery: labour and power, making savings on both of these.

Feeding strategies across North America and Europe were consistent with the concept of 'feeding the water', that is maintaining an algae cell density within the culture water. This was typically around 20-80 cells/ μ l. The maintenance of this feed level was adjusted by some facilities dependent upon the age and stage of development, with the 'green-thumb' approach being just looking into the tank. Other places used more sophisticated electronic equipment from a 'fluro-meter' through to coulter counters and similar particle size analysis equipment. Manual inspection and adjustment was common, although there is ongoing development of automated systems, working on a feedback from the sensor and using computer programming to then determine and deliver the required amount of algae culture to the larvae.

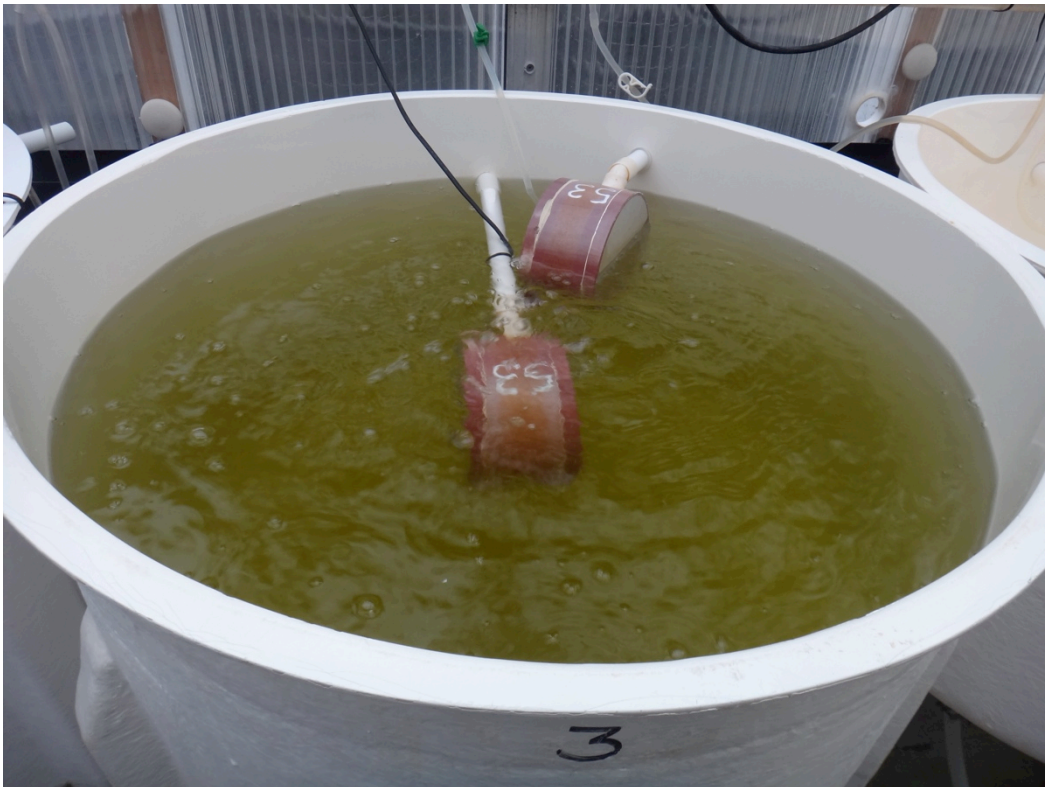
This strategy of ‘feeding the water’ is very different to the commonly adopted ‘feed charts’ that Australian hatcheries use. These were developed following numerous replicated larvae cultures, with the number of algae cells being delivered to LRTs being carefully measured. This along with the size and number of larvae being cultured allowed the larvae consumption of algae cells to be determined following assessment of remaining ‘un-eaten’ algae. This is typically used by Australian hatchery producers to predict the consumption and required feeding of algae to larvae, and is used in conjunction with microscopic inspection of the ‘residual’ algae density, and condition of the larvae. This understanding of consumption when used properly provides the Australian hatcheries with a greater control of the larvae conditions, optimising growth and survival.

A common opinion offered was that mussels consume more algae than oysters. Having undertaken the studies necessary to prepare these feeding guides, it is apparent that oysters actually consume 33% more algae than mussels at settlement, and that the assumption that mussels eat more is based around them being grown at a greater density within the larvae tanks, which results in more mussels/LRT. Having a greater understanding of the specific larvae requirements is an advantage: determining the feeding strategies and requirements of species grown overseas requires interpretation the data available, such as number of larvae, and amount of algae feed and its density.

The grading and handling of larvae followed a similar process to that employed in Australia. However, handling at the larger hatcheries puts a greater focus on the efficiency of handling larvae, using volumes and weights of larvae rather than enumeration through counts. The handling of large numbers of larvae and managing numerous big LRTs has led to the use of the refrigerator as a means to store larvae. This ability to refrigerate larvae for extended periods is the basis on which the eyed larvae oyster market is based. Mussels and clams can also be handled in a similar way.



Sue Cudd (owner) inspecting her large static larvae tanks at the Whiskey Creek Shellfish Hatchery in Oregon, USA.



Small Larval rearing tanks – continuous flow.

Water Quality:

Water quality for larval rearing normally means having the right salinity, clear seawater low in bacterial concentration with few or no *Vibrio* sp. (this genus contains a number of pathogenic species). This is not the whole story anymore. The carbon chemistry, and pH of the water has been identified by a number of shellfish hatcheries on the west coast of the USA to be critical to successful larvae production. Hatcheries that had 30 years of continuous reliable production, with some seasonal variability, with consistency of process and management derived through continuity of the same staff, are now finding that production is severely impacted by these changes in water chemistry. Production was reduced to 25% of normal levels, and given they supplied 75% of independent growers, this was significant to the whole industry. Other hatcheries, experiencing this same problem, responded by doubling larval capacity allowing them to *almost* maintain regular production. ‘Wild’ settlement of oysters in Willipa Bay, a major source of cultch oysters has also been significantly affected. This change in carbon concentration and resultant lower pH has dramatic consequences for ‘hatching’ and early larvae, with stunted growth occurring and an inability to complete the free-swimming larval stage. This alteration in carbon chemistry and ‘acidification’ of the seawater occurs in conjunction with ‘hypoxia’, which is brought about by low dissolved oxygen concentration, and high organic loads of a process known as “upwelling.” The fate of shellfish larvae and those trying to grow them is grim, when these conditions prevail.

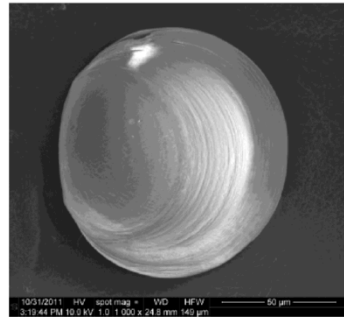
While investigating these problems a variety of other water treatment processes were investigated to improve water quality for larvae, including foam fractionation, carbon filtration, and biological filters. The management of this problem has required real-time monitoring of the parameters that influence larval production success; these extend beyond just the water quality, to the weather patterns and wind conditions that influence the upwelling events. Treatment of water by ‘buffering’ the seawater and allowing the pH to increase has been successful to some degree, but to get full availability of the appropriate pCO₂ and available calcium carbonate can take a number of days post-treatment.

The experience of the West Coast USA shellfish hatcheries demonstrates that securing ‘good’ water quality has become more difficult, and that continued effort will be necessary to understand the growing complexity of the environment in which the hatcheries operate. Some parameters are much easier to understand and respond to, with one facility going as far as to manipulate the salinity of the water, as their production had been impacted by extended

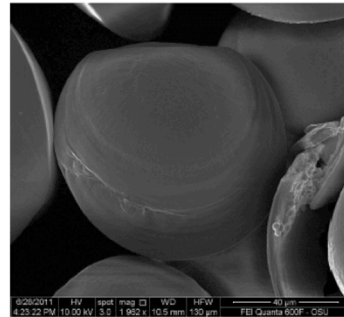
flooding, and low salinity. (Appendix 2: Ocean Acidification and Ocean Upwelling, provides further information.)

Larval oysters are impacted by ocean acidification

Larval bivalves like these 9-day old Pacific Oysters from Whiskey Creek Shellfish Hatchery in Netarts Oregon are negatively impacted by corrosive seawater caused by ocean acidification. High levels of CO₂ in water are correlated with developmental abnormalities, reduced fertilization success, slowed growth, and the precipitation of weaker thinner shells. Scientists at Oregon State University are trying to understand the mechanisms behind these deleterious effects.



Normal larval shell



Abnormal larval shell

Photographs taken at Oregon State University by E.L. Brunner
PI: G.G. Waldbusser – NSF-Climate Research Initiative/Ocean Acidification #1041267



Larval Oysters are impacted by Ocean Acidification – Alan Barton, Whiskey Creek Shellfish

Settlement:

The settlement technology identified during this study tour can be separated into two general categories.

The first category represents an extension of ‘natural’ settlement, where the shellfish are allowed to attach to a substrate, such as cultch in the case of oysters, or rope/mesh/natural fibres for mussels.

The second methodology aims to provide for ‘single-seed’, and typically takes the form of using a *downweller* with shellfish retained upon a screen, and seawater directed down through the shellfish and mesh. This may also have ‘micro-cultch’ in the case of oysters to encourage a single-seed. This process was also being used for clams and scallops.

The recovery percentage of eyed larvae to ‘spat’ varied from about 12.5% through to 80% dependent upon the species and the technology being employed.

The use of *epinephrine* as a settlement inducer for single-seed oysters wasn't widespread, which was surprising, given the efficiency gains this provides. The facilities that were using this had a clear efficiency and production advantage, with lower labour requirements, and lower capital infrastructure requirements. They also experienced greater returns from eyed larvae through to spat, with up to six times greater returns. This has significant implications for operating costs and production outputs, requiring significantly less larvae and algae to be produced to reach the same objectives.

Cultch settlement processes are typically quite simple in concept, but were being undertaken on an industrial scale. The typical cultch settlement system consists of a tank suitable to hold seawater. This may have supplemental heating to manage water temperatures. These tanks are usually established outdoors in close proximity to the seawater source. The cultch, whether it is shell or rope/mesh, is stacked or hung in the tanks. Oyster or clam shells are usually held in a mesh bag, similar to an open weave 'onion' bag, or stacked in purpose-built handling frames. These tanks have aeration installed, and the capacity for flow-through seawater.

Algae paste is often used for supplemental feeding, removing the need for the remote setting operator to also grow microalgae. Some operators had sites with sufficient natural levels of microalgae, so that no supplementary feeding was necessary though the settlement stage. "*Remote Setting of Oyster Larvae*" by Gordon and Bruce Jones is a thorough review of the process of remote settlement and available from their website (www.InnovativeAqua.com).

The recognition that cultch settlement systems represent a materials handling challenge was clear with the examples provided by the producers of oysters. They had developed sophisticated shell collection and cleaning systems, and then bagging/framing operations, that provided for a quicker turn around of the raw material (oyster shells), and automated cleaning and sorting processes reducing the number of people that are involved in this operation. The bulk handling of the shell post-settlement also saw innovations at some facilities with reusable frames being adopted, which removed the requirement for shell to be bagged into plastic mesh bags, and then the subsequent manual handling in moving this around, and final deployment of this onto the growing fields.

The deployment of this settled shell onto growing fields had a number of facilities demonstrating innovation in handling practices. Barges were loaded with settled cultch, and this was then distributed over the growing area using water jets from deck hoses. One facility

went so far as to incorporate their settlement tanks into a refurbished dredging barge, which enabled them to reduce the post-settlement handling, and simply ‘steam’ out to the growing fields, and open the floor of the barge, distributing the settled cultch.

The adoption of remote setting and supply of eyed larvae is a significant difference between the US oyster industry and that in Australia. This is partly because of the diversification of product that the American market caters for, with both an oyster meat market and a half shell market. This contrasts with the Australian industry, where oyster meats are not produced, except in some circumstances, within the Sydney Rock industry, with oyster meats being produced as a result of misshaped ‘stick’ cultured oysters not suitable for the half shell market.

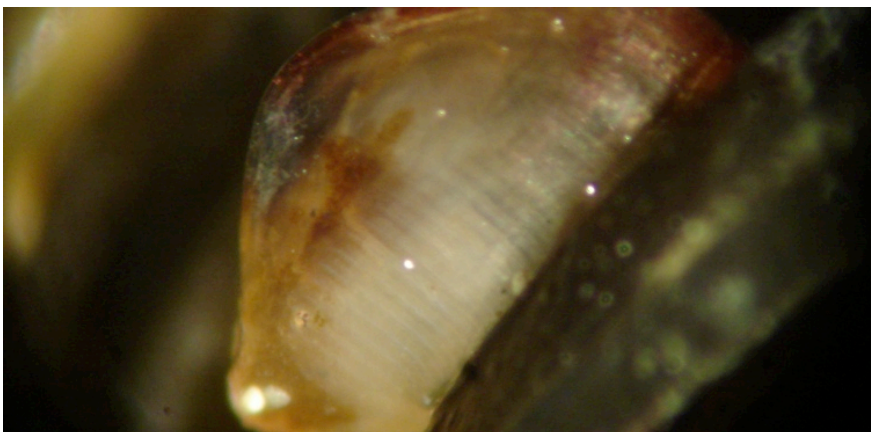
Eyed larvae were being supplied to growers directly for settlement onto cultch by the oyster growers, and to specialist growers that would also produce single seed oysters to then on sell.



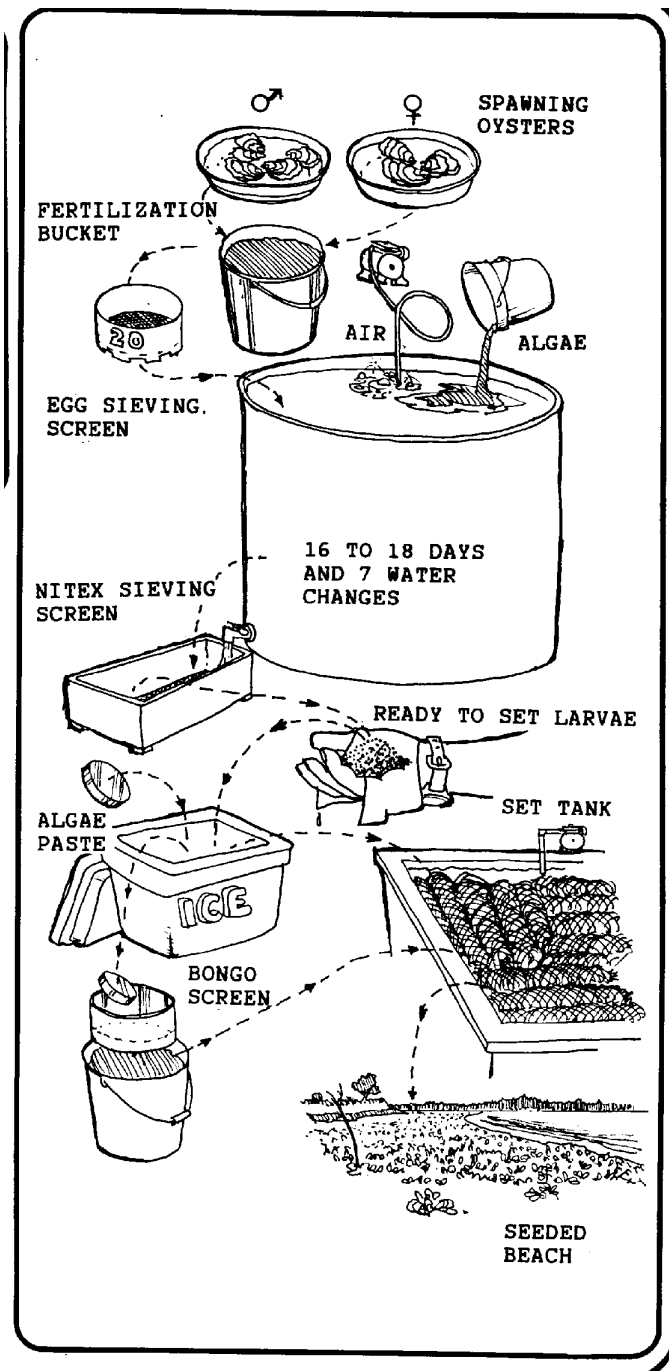
Eyed Larvae of Crassostrea virginica – Horn Point Hatchery (website)



Mussel Spat settled on rope



Mussel Spat – Mytilus galloprovincialis



A simple view of hatchery operation and general systems flow for eyed larvae & cultch settlement (Jones & Jones, 1988).



Eyed Oyster Larvae being distributed onto 'cultch' – Horn Point Hatchery (website)



Oysters settled onto 'Cultch' & Single Seed Oysters



Single Seed Clam Spat - grown in upweller.

Chapter 6

Nursery Production - Technology.

The Nursery is the stage where the spat are on-grown, in preparation for transfer to the growout production stages. The process for both oysters and clams typically consists of shellfish retained within a container with a mesh base, and seawater flowing up through the container. These containers are described as ‘upwellers’, ‘pots’ and ‘bins’. Some facilities also had containers with seawater that flows up through the vessel, but the shellfish weren’t retained by a mesh screen, and the inflow had a ‘marble’ or ‘ball’ acting as a stopper on the entry orifice, with the shellfish typically being ‘fluidised’ but the ‘marble’ rolling back over the water inlet and retaining the shellfish if the water inflow stopped. This system was typically referred to as a ‘bottle’ system. Mussels and scallops can also be grown in upwellers, but were typically grown either on a substrate such as mesh or rope in the case of mussels, or attached to the bottom of tanks called ‘raceways’ (in the case of scallops) where the seawater is directed across the top of the shellfish.

Supplementing the natural seawater with microalgae was practised by some facilities, and the temperature of the water was also managed. Heating was achieved through a variety of means from propane, diesel, solar and electric, both direct and ‘heat-pump’. Heat recovery technology was being incorporated into the systems. Production timing was also managed to reduce the change in water temperature, with some facilities simply using the natural seawater, either directly from the ‘sea’ or on some occasions from ‘sand wells’. The seawater from the sand wells didn’t have supplementary algae, but did provide a consistent temperature which was not always available when drawing water direct from the sea.

The nursery stage of shellfish production oversees a rapid increase in biomass, and the shellfish typically require regular handling to maintain good husbandry conditions, to keep the shellfish free of fouling of the shell, and also (in the case of oysters) to help manage the shape of the shell. Grading is also an important consideration to maintain comparative size classes. Even though shellfish do not interact the same way that other, more mobile animals (such as fish) might, where the larger fish might ‘bully’ the smaller ones and therefore have a dominate relationship to the smaller sized fish, effectively stunting them through depriving them of food. The situation with shellfish is that the large shellfish can filter a greater amount of seawater, effectively getting a greater take of the available food, and this is further influenced

by the larger shellfish having a greater density than the small shellfish. This enables them to 'sit' lower in the upwellers, nearer the 'new' water. This is clearly demonstrated when the shellfish are fluidised and there is a clear distinction visible between the various size classes.

This requirement to handle the shellfish for both general husbandry, cleaning and for grading influences the style of nursery technology adopted. The facilities visited all adopted a nursery system that utilised upwellers throughout the nursery stage, taking advantage of the handling advantages this technology offers over the enclosed 'sea-trays' which are used predominately within Australia.

The factors influencing successful upweller nursery systems relate to three main points;

1. Ease of handling
2. Availability of food
3. Costs of pumping

The upwellers varied in size from containers carrying 500 grams of shellfish through to 250 kg. The handling also varying according to the scale of the unit utilised from simply manual lifting to use of gantry systems and cranes.

The availability of food to the shellfish is influenced by the concentration of algae within the water, and the flow of that water through the shellfish. Accordingly, if the algae density in the water is low, this can be countered somewhat by increasing the flow of seawater, thereby delivering more algae. Siting the nursery at locations with good natural microalgae production capacity was well practised. A number of facilities also undertook to grow, or enhance the available algae by providing for enclosures such as ponds that retained the seawater. This was also done in order to manage the risk of disease from the broader environment.

The costs of pumping water, and the availability nursery facilities were also addressed by the adoption of FLUPSY's (Floating Upweller Systems) by many operators. The advantage of these units is that they have little or no frictional head loss associated with delivering the seawater up through the shellfish. The variety of types ranged from small floating dock styles, which were operated by solar powered 'de-icing' impeller motors. These were operated by growers purchasing small, single seed spat, from hatcheries through to large industrial FLUPSYs that had paddlewheels moving over 30,000 L/min with around 1 hp motors. These

large units were being utilised by hatcheries or large growers that may also supply juvenile stock to other producers.

The alternatives to these FLUPSY's were shore-based upweller nurseries and the pumping of water was achieved via 'pool' pumps in small systems, with 500 L/minute through to axial flow pumps that lift 30,000 L/minute for around 5 kWh (KiloWatt Hours) of energy.



Guernsey Sea Farms – FLUPSY and hatchery (Aerial Photo – GSF website) – Oysters & FLUPSY in background.



assostrea virginica - Mike Consgrove – Oyster Seed Holdings

Chapter 7

Algae Production - Technology.

Algae production is important as it underpins the shellfish production process providing the food required by the shellfish at all stages from the broodstock and larval rearing through to settlement and spat within the nursery stage.

The production of microalgae to support the culture of shellfish is an essential part of the hatchery process and it represents a significant cost. Infrastructure requirements for algae production can be substantial and operating costs can represent as much as 33% of the hatcheries production costs.

The composition of algae species was similar across all facilities. Axenic cultures aren't readily available to support industry with difficulty in getting quality stocks, some arriving with both bacteria and even ciliates.

The management priority for starter cultures varied across facilities with some employing laminar flow cabinets and axenic transfer procedures. Many simply applied 'clean' transfer methodology on bench tops, over heat. The quality control procedures with respect to microbiological monitoring of transferred stocks were generally lacking, and at a number of facilities were only something that was undertaken if a problem was suspected.

Starter cultures from test-tube and small flasks were typically autoclaved, with some using microwaves. Culture vessels from 10 L and greater often relied on chlorination/dechlorination procedures, with some facilities utilising UV lights, or pasteurisation.

The utilisation of batch culture algae production was common, varying in vessel type and volume from carboys of 20L through to plastic bags at 200-500L through to 16,000 – 125,000L fibreglass or concrete tanks. This production process typically delivers lower density algae concentration, (1,500 cells/ μ L vs. 3,000 – 20,000 cells/ μ L) and has a greater risk of bacterial contamination than the semi-continuous and continuous algae production systems employed within Australia. However the technology supporting it is very simple, and although it is labour intensive, compared to alternate approaches, the skill required by the labour is lower, and new cultures are only ever a few days away if there has been a problem. A number of places utilised both continuous 'high-tech' culture systems and the batch

systems and saw the batch system as having inherent safety, with it isolating the ‘batch’, so that a loss can occur without the risk of taking a whole system down. The typical approach to the batch systems was a scaling up in culture volume, progressing every two to three days to keep the cultures ‘fresh’ in the exponential growth stage. The initial capital expenditure is reduced using batch culture technology, but the operational footprint is greater as is the on-going labour requirements for the equivalent daily algal cell harvest.

The continuous algae systems adopted were all adaptations of John Bayes’ SEACAP system, utilising pasteurised seawater and a variety of plastic bags. The New Zealand facilities utilised the ‘skinny’ hanging bags on a continuous harvest. Many European facilities were using these bags or similar, but were still using them in batch culture. One facility also operated a ‘BioFence’ that is a continuous algae culture system utilising chlorination/dechlorination and small diameter acrylic tubing. The densities that this system is able to attain are quite remarkable, but the range of species that this system can be used successfully to culture is reduced, due to the physical turbulence that exists within the system. It was recognised by many that the skill and experience of the staff contributed greatly to the successful operation of these continuous algae culturing systems.

Production of microalgae requires nutrients to supplement the natural levels available within the seawater. A variety of nutrient formulas were utilised, ranging from F₂ to Walnes, to some that were custom formulas. Many facilities took advantage of the commercially available formulas, either as a dry mix, or pre-mixed liquid solution. These pre-mix formulas were seen to provide an advantage in saving staff time and reducing human error in the preparation of the nutrient solutions.

Supplemental carbon dioxide was a regularly used to enhance algae cultures, as was supplemental lighting. The lighting typically consisted of fluorescent tubes and metal halide lamps. Many facilities utilised greenhouse structures to take advantage of naturally available sunlight. Where cultures were grown indoors the effect of the light on ‘heating’ the culture rooms was identified as a consistent problem. This problem with excess heat was addressed through use of air-conditioners, evaporative cooling panels, and directing freshwater onto the external surface of bag cultures, occasionally as a misting system. The electrical costs associated with supplementary lighting, and then the costs to manage temperature were recognised as being significant within the hatchery production process, so there was considerable interest in developing LED lighting solutions, with savings in direct electrical costs, maintenance, and reduced heat. All these were seen as advantages in adopting this

technology. Limited progress was seen in adoption of this technology as further development is necessary to provide a commercial 'product'. This presents an opportunity to develop a product that delivers the required wavelengths for microalgae and is suited to a seawater environment potentially even submersible.

Chapter 8

Triploid & Tetraploid Shellfish Production.

Triploid and tetraploid refers to the number of chromosomes that the shellfish contain within somatic cells. Typically shellfish are diploid - that is they have two sets of chromosomes, one each from the male and female parent. Mitosis then underpins growth with cell division and DNA replication. Gametes, the eggs and sperm responsible for reproduction, recombine their chromosomes, and then lose one of their sets of chromosomes and become haploid (single set of chromosomes), this is called meiosis. Sexual reproduction can be described as meiotic recombination.

The loss of the chromosomes to become haploid occurs through a process described as meiosis I and II. In each of these stages, 'polar bodies' (packaged chromosomes) are extruded. This process occurs in many shellfish such as oysters, clams, mussels and scallops after fertilisation has occurred, with many other animals it typically occurs before fertilisation has been completed.

It is because of this occurrence of meiosis after fertilisation that the opportunity to modify the 'ploidy' of shellfish is available to producers. This is typically achieved through the blocking of the release of polar bodies at either meiosis I or meiosis II. The blocking of either of these polar body releases results in the shellfish becoming a triploid (three sets of chromosomes). The cells of a triploid can undertake mitosis in the usual way, however it infers a level of sterility as three sets of chromosomes cannot successfully recombine during meiosis.

This use of triploids presents an opportunity to shellfish producers, as triploids have larger cells due to the additional chromosomes, and this infers a level of growth advantage over diploids. The sterility of triploids also provides an advantage, with resources not being put into the development of gametes, which can then be released and lost through spawning,

causing up to a 70% loss in meat yield. The development of gametes also changes the texture of the shellfish significantly, and some markets prefer the winter 'meaty' texture to that of a 'creamy' summer shellfish. In France triploid oysters are referred to as 'Four Seasons', implying that they are suitable for consumption any time of the year. The development of triploids has had significant producer and market acceptance and is seen as one of the advantages to using hatcheries for shellfish production.

The production of triploid shellfish is achieved by two different methods. The first involves the blocking of either the first or second polar body through the use of chemicals, temperature or pressure. This allows direct induction, but shows some variability of success rates, and the use of the chemicals is facing resistance, due to the toxicity of the material and OH&S considerations. The number of shellfish that are successfully produced is also reduced, due to the stress of the induction on early egg development.

The second process involves the use of tetraploids, which have four sets of chromosomes. Inhibiting the polar body release of triploid eggs that have been fertilised by a diploid male typically produces tetraploids. The tetraploid female shellfish are crossed with a diploid males and the result is 100% triploid offspring.

Dr Stan Allen pioneered this development of triploid production in shellfish, with the tetraploid production being patented and the company 4C's Breeding Technologies Inc. was established to manage the licensing and transfer of the technology. This company, managed by Tom Rossi, has been successful in striking commercial relationships with a number of hatchery suppliers globally. The French industry, with the support of IFREMER (French Research Institute for Exploration of the Sea), fought the patent and reached a settlement with 4C's that allowed them to utilise the technology. The patent on this technology is ending in 2013, and Tom Rossi is reporting that the interest in the technology has actually increased, with a number of approaches from businesses that require assistance in incorporating the processes into their production of shellfish.

IFREMER is producing tetraploid oysters, and controls their use by selling the males to the hatcheries on the condition that the males are returned, either alive or their empty shells, demonstrating they have been used for the single spawning. IFREMER is claiming a process of direct production of tetraploid shellfish, without the need to inhibit polar body release on a triploid x diploid cross.

The end of the 4C's Breeding Technologies Inc. patent will usher in a new age of tetraploid production to enable triploid production. There is considerable research to be undertaken to

enhance their ability to be incorporated into selective breeding programs, with particular importance to managing disease resistance. The inheritance of specific traits, and the time taken to incorporate tetraploids into breeding programs are areas of great importance.

There is an opportunity for companies such as 4C's to bring their specific knowledge together with a variety of global shellfish hatcheries to drive further advancement in this technology. (For further explanation and description of the polyploidy process the 4C's website is: www.4cshellfish.com.)

Chapter 9

Disease – Shellfish Production.

There are a number of diseases influencing the production of shellfish around the world, ranging from protozoans, bacterial and viral pathogens. The disease that has the greatest consequence for the Australian oyster industry is OsHV-1, referred to locally as Pacific Oyster Mortality Syndrome or POMS. This disease is wide spread throughout Northern Europe, extending from Morocco, to France, England and Ireland. POMS has also devastated the New Zealand industry (which is largely reliant upon wild catch), taking it from a \$30 million industry down to \$5 million. The French industry experienced 80-90% losses, and hatchery production has grown exponentially to make up for the wild catch reduction. Farmers are buying extra stock in anticipation of having significant losses.

The disease managed to spread over such a large geographic distribution due to the 'Highway' farming techniques employed by oyster growers who move oysters and equipment around at different stages of the production cycle. Another contributing factor was that the cause of the mortalities occurring was not easily determined, so the presence and role of the virus was not known from the outset. For most of the year, with water temperatures below 16°C, the oysters were not dying and this in effect 'hid' the fact that oysters were carrying the virus, and allowed oysters that weren't symptomatic to be moved, which contributed to the spread of the disease.

The diagnostics of the virus has been an area of significant research and development, with detection moving from histopathology and electron microscopy through to PCR (polymerase chain reaction) and live PCR detection platforms. Continued development of effective and economical diagnostics is continuing and is a high priority for the countries already facing

infected populations, as well as for neighbouring nations that want to provide some level of security to their existing industry.

Australia has identified POMS within selected NSW growing regions, and has an urgent need to understand the factors influencing the spread and infectivity of the disease. The opportunity exists for the Australian industry to work collaboratively with the international oyster industry in developing a greater understanding of the disease, and establish robust diagnostic and management protocols to minimise the spread and the effect of the disease. Collaboration can also contribute to the control of the impact the disease has upon the producers through identifying husbandry and alternative strategies of production that avoid or minimise the onset of the mortality of the disease.

Selective breeding is also being vigorously pursued by both the New Zealand and French industries, with the French hatcheries collaborating to develop family lines that infer a resistance to their progeny. This breeding collaboration is occurring despite the very competitive market condition of the French oyster hatchery suppliers.

Chapter 10

Selective breeding in shellfish production.

Shellfish hatcheries provide the opportunity to manage breeding, so that positive traits can be introduced or maintained in the shellfish being commercially produced. The focus of this breeding can extend to maintaining genetic diversity, through to traits that deliver a direct commercial advantage such as growth, yield and survivability. Other characteristics that provide for product differentiation, such as shape and shell colour, can also be selected.

Most facilities managed selection of broodstock to some level, even if just at the superficial selection of observable physical traits of the broodstock. There were a number of industry managed, selective breeding programs. The US West Coast industry has a program called the Molluscan Broodstock Program that focussed on the improvement of the Pacific oyster, even to the extent of bringing new Pacific oyster strains in from Japan. This program was supported by Federal Government funding, however this funding is not going to be renewed. A continuation of the program for the whole of this industry was sought but it appears that its continuation will be dependent upon individual hatchery businesses. The US East Coast has a well-established breeding program for *Crassostrea virginica* that is interesting, as the program

started before the commercial cultivation, and was in fact critical to providing disease resistant lines to support a cultivated oyster industry. The Virginia Institute of Marine Science (VIMS) calls its program the Aquaculture Genetics and Breeding Technology Centre (ABC). The selectively bred oyster broodstock available from this has underpinned the establishment of the commercial cultivation of *C. virginica* and has provided resistance to diseases. This program is also responsible for providing tetraploid oysters through an agreement with 4Cs, which has enabled the industry to fully utilise triploid oysters. Management of the breeding program to also allow the incorporation of tetraploid oysters is an area of further development, with work to understand the mechanisms of inheritance within a polyploidy oyster.

ABC also investigated the introduction of alternative species of oysters that would have used the tetraploid technology to maintain a sterile population being introduced into the Chesapeake Bay. This was overruled on the basis of the precautionary principle, and unknown changes to the natural ecosystem if an introduced species took hold.

The French Pacific oyster industry has also collaborated to develop a breeding program that was established solely to develop disease resistance to the POMS.

The New Zealand mussel industry has a number of large producers that have collaborated on the establishment of a selective breeding program, while at the same time developing hatchery production processes that can be adopted into the future. This industry recognises the vulnerability it faces, being reliant upon wild settlement and the variability of supply this presents. With an increased level of value-adding and automated processing of the mussels, the consistency of characteristics throughout the growing cycle, and then at harvest, has been recognised as a distinct advantage of selectively bred hatchery produced mussels.

The opportunity for selective breeding programs to work together on the design, data management and methodologies of in-field testing to support the selection process exists. Benefits would exist for international collaboration in developing efficient technologies and processes to produce large numbers of lines through the hatchery and nursery, delivering them to in-field assessment. This would support the international trade in genetics, much the same as exists across many other agricultural industries now.

Chapter 11

Education & Training.

The focus on education and training within the shellfish industries in countries visited was refreshing. There are many University and industry collaborations building capacity in core and advanced research capacity to both existing and new entrants to the industry.

The industry was also taking a leading role with a number of facilities offering internships, providing a range of 'jobs' throughout the business. This gave the intern a very good overview of the activities required to produce shellfish through the hatchery, nursery, growout and processing, through to sales and marketing. This strategic approach to training was developing well-rounded employees that had a fuller understanding of their eventual role in the business, and enabled them to draw upon the skills and relationships they built upon during the internship.

Structured internships based around the activities of the selective breeding program were also on offer, including semester work assisting hatchery production and research work. Hatchery-specific training programs were also available, teaching algae, larvae and settlement processes while utilising the students as labour for production outcomes.

Extension programs and officers to assist existing and emerging industry were also well established, with technology development and transfer given a high priority. The outcomes of these programs were significant industry development and growth, particularly around the re-training offered to former 'watermen' and fishermen that wanted to build sustainable industries and businesses that empower them to still live and work on and around the water.



Stan Allen (Director - red shirt) & Anu Frank-Lawale (Geneticists - blue jacket) – Virginian Institute of Marine Science (VIMS) with Oyster Aquaculture Training (OAT) Program participants. Shellfish Hatchery in Background

Chapter 12

The future of Shellfish Aquaculture

My general impressions across all these countries are that the future for shellfish aquaculture is bright.

The challenges that it faces include:

- Environmental conditions, such as ‘ocean acidification’, pollution, ‘ocean up-welling’
- Social licence, urban encroachment, conflict/competition for space.
- Disease, including existing and new and emerging types.
- Shortage of skilled, knowledgeable ‘Shellfish Technicians’.

The reason I see the future as bright is that oysters in many parts of the world have a historical cultural importance, and are in short supply to the consumer. Oysters and other bivalves are being sought out not just as a source of protein, or nutrition, but because of the ‘good-times, or experience’ that they represent to the consumer. As a colleague of mine is fond of saying, *“Oysters are HAPPY food”*. This attitude, combined with the clear environmental advantages that shellfish production represents in today’s world of ‘carbon footprints’ and environmental responsibility, leaves the industry with greater opportunities than negatives.

The questions of disease and people development are being addressed by leading scientists and motivated educators that have a passion for the humble shellfish. The challenge the industry faces is how to best attract and retain these bright young people, and provide an opportunity for them to participate in a meaningful way within the industry.

The opportunities that exist are perhaps best demonstrated by the oyster industry on the east coast of America, around the Chesapeake Bay. The Chesapeake Bay fishery once produced more oysters than all of China or France does today. The current production is a sliver of what it once produced and has been influenced by environmental conditions, overfishing and disease. The good news is that hatchery production has been able to combat the disease issue successfully, with improved survivability available through selective breeding. There is a new enthusiasm amongst ‘watermen’ pioneering the cultivation of oysters, developing innovative nursery and grow-out technology and processes to suit the environment of the Chesapeake Bay. There is also a passionate movement towards restoration of ‘natural’ beds of oysters. And all this ties into the story of oysters and shellfish being good for the water quality of the bay, and opens a new era of oystering into the future.

Recommendations

The Australian Shellfish industry would benefit by implementing and developing the following recommendations in addition to those outlined in the Executive Summary. Though coordinating our ‘collective intelligence’ as an national and international industry we will be in a position to continue to adapt and innovate meeting the challenges we face today and into the future.

- Coordinate the establishment of an international shellfish hatchery network, to promote communication and relationship building.
- Identify areas of significance for development, across technological development, capacity building of people.
- Prepare a ‘Dummies Guide’ to algae culture, larvae culture, genetics, water chemistry, system design, water pumps, nursery operations etc., supported by simple fact sheets.
- Nationally identify the factors that have influenced and allowed for growth internationally. Assess the risk and opportunities that this presents for the Australian industries.
- Coordinate International research and development across areas of ‘common’ interest for the shellfish hatchery industries.
- Build relationships between international producers and investigate the KPI’s that influence profitability, and investigate the economics of the various production strategies. This would be supported by benchmarking and forming discussion groups and forums, which would help prioritise the areas that require further investigation and development.

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Horn Point Oyster Hatchery - www.hpl.umces.edu/hatchery/, Donald W. Meritt
Hatchery Program Director, Phone (410) 221-8475, E-mail: dmeritt@umces.edu

Technology development for a reliable supply of high quality seed in Blue mussel farming, Blue Seed (Project no. 017729)

The state of World fisheries and aquaculture, FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United, Rome, 2012

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Plain English Compendium Summary

Project Title: Global Perspective of bivalve hatchery processes.

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Objectives

Identify the opportunities and challenges that the Australian Shellfish Hatchery industry faces, by investigating current technology and knowledge, that exist both in Australia and around the world.

Background

The need to study Shellfish Hatchery businesses from around the world, and identify “World Best Practice” and the future trends in shellfish production is important for the future growth of the Australian Shellfish industry. **Internationally there are significant disruptions to supply due to environmental and disease impacts. Identifying these and their likely solutions and consequences for the Australian Industry is important.**

Research

I travelled extensively during this Nuffield scholarship, visiting eleven countries specifically to investigate shellfish aquaculture. These included; United States of America, Norway, Sweden, Portugal, Spain, New Zealand, Canada, France, Guernsey, England and Scotland.

Outcomes

Collaboration has been essential for the European and American producers, who experienced greater than 50% reduction due to disease and environmental factors. Lessons for the Australian Industry are:

- Build strong on-going relationships with key staff, as they are the fundamental to your business success.
- Develop a network of international shellfish hatchery producers, providing for opportunities to interact, not just at the managerial level, but also between technicians, encouraging discussion of issues influencing “success”.
- Recognise the advantages of using ‘Collective Intelligence’ between hatcheries where they collaborate to find solutions to common problems, such as disease management, selective breeding, polyploidy and water quality.
- Encourage staff exchanges, secondments between national and international colleagues within the shellfish industry, where issues of direct competitive pressures aren’t significant.

Implications

Species and product diversification should be adopted by shellfish businesses to mitigate risks and optimise human and capital resources. Vertical integration was also successfully demonstrated, as were synergistic ‘joint-venture’ relationships between shellfish hatcheries/nurseries and grow-out producers who share similar values and attitudes. The opportunity to build a clam aquaculture industry exists with current hatchery technology existing in Australia for its immediate development.

Appendix 1

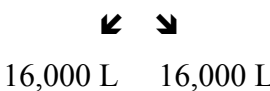
Generalised impressions across industries Visited.

Having visited so many operations, and respecting the privacy of the individual businesses who opened their doors to me, I have prepared the points below to provide for general impression of the distinguishing features of each countries' industry that I visited.

USA

- Large vertically integrated shellfish businesses, joint venture, supply arrangements with smaller producers, leveraging large businesses investments in hatcheries, processing, distribution etc.
- Geographic diversity, hatchery sites on West Coast & Hawaii.
- Family and individually owned – although considerable scale
- Strong relationship between key staff and owners, 15-33 years employment durations, allow employees to hold shellfish business interests outside of the employers business
- Knowledgeable, experienced and committed staff
- In house training, and career advancement opportunities
- West Coast Industry Breeding Program – being continued privately, due to lack of whole industry support.
- Institutional support of breeding program on the East Coast, supporting and building capacity within the industry. Many of the former employees of the program have moved onto positions of responsibility within the private sector.
- East Coast – moving to re-train/skill-up existing ‘watermen’ to the new industry of aquaculture. Government grants and assistance were available.
- Research focus, i.e. employ technical staff to understand issues such as water quality
- Around 50 billion eyed oysters produced each year (West Coast)
- Demand for growth, with current hatchery production seen as limiting industries development.
- Water heating uses diesel or propane – electricity is considered too expensive.
- Heat recovery of water – starting to adopt, as the pressure from power costs increasing
- Diploid and triploid production (both Tetraploid x Diploid), and direct induction (non-chemical)
- Oysters, clams and mussels produced at all hatcheries (West Coast)
- Larvae batch sizes in the vicinity of 150-250 million eyed larvae

- Big Larvae Rearing Tanks; 20-48,000 litre used predominately, both as static and flow through, relatively low larvae densities.
- Small Larvae Rearing Tanks; 200 litre run as flow through cultures with high larvae densities
- Algae culture; mix of species cultured, not as strong an emphasis on maintaining axenic cultures as Australian hatcheries, predominately production is from static batch cultures up to 125,000 litres e.g. 1 L → 24 L → 4,000 L



↙ ↘

16,000 L 16,000 L
- Continuous algae culture; adapted from John Bayes – SeaCap is used, but not as the primary production process
- Hatchery production problems have been attributed to Ocean Acidification, Ocean upwelling and *Vibrio* sp. Wild recruitment and hatchery production of Pacific oysters has been impacted with up to a 50% reduction. Other shellfish species haven't been impacted as significantly.
- Collaborative work is being undertaken to investigate water quality chemistry issues associated with Ocean Acidification and Ocean Upwelling's

Canada

- Limited development of hatchery capacity, large dependence upon American suppliers.
- Diversity of production, with a variety of shellfish species being produced.
- Similar production strategies to the American culture protocols.
- Recognition of the importance of the shellfish industry, with the establishment of the Vancouver Island University Centre for Shellfish Research.
- Innovative producers, finding niches within the industry, with speciality species production or arrangements, and diversity of products i.e. Algae pastes.

New Zealand

- Emerging hatchery production, for mussels and oysters, building upon many years of research and development into the hatchery and nursery production of mussels and oysters and to some degree clams.
- No 'commercial' hatchery producers currently operate (Cawthron Institute- research trust, is currently providing oyster spat to industry at commercial rates, but would like to divest themselves from commercial production).
- Oyster Selective Breeding Program started to address POMS threat (60 families).
- Plans for industry collaboration for the production of Greenshell Mussels through a hatchery.
- Production processes being adopted are a combination of existing global shellfish hatchery technologies with an emphasis upon the high density larval and algae production methodologies.
- Oyster nursery production systems are a hybrid of French and UK systems.
- Building human capacity, with support for education programs.
- Amazing opportunity for shellfish aquaculture exists now and into the future, with sub-tidal oyster cultivation now developing.

Norway & Sweden

- Hatcheries production is limited, but follows considerable investment into infrastructure and research and development.
- Strong support within the University sector for research and human capacity building.
- There is a shortage of technical experienced personnel, but no shortage of determination and passion.
- Larval rearing and algae production tends to follow the small static and flow-thru tanks, and small volume chlorination/de-chlorination processes.
- The opportunity to fill a niche' production need, and market accordingly exists.

Portugal & Spain

- Have a very strong shellfish sector, with community 'heritage'.
- Hatcheries had diversity of production across a variety of species.
- Larval rearing was typically small static tanks, occasionally flow-thru.
- Algae production was a mix of static and flow-thru, but typically avoided the large batch tanks systems of the USA.
- Education and Research and Development were very well supported, with specific courses being taught for shellfish aquaculture.
- Hatchery production has the opportunity to grow, supplementing or replacing the wild catch shellfish industry sectors.

Guernsey & United Kingdom

- Has a strong history in shellfish hatchery development, responsible for many of the innovations in algae, larvae and nursery production systems that have been adapted around the world.
- Strong relationships exist between businesses with many operators having worked together at some stage in their career.
- Emphasis on cost savings, in energy and labour inputs.
- Continuous algae production and larval rearing systems.
- Nursery systems utilise old 'quarries' and water impoundment to provide a level of protection from the sea and the capacity to enhance algae production as a food for nursery shellfish.
- Great depth of knowledge and experience.
- Participate in the international market for shellfish spat.



John Bayes – Seasalter Shellfish, pioneering hatchery producer, developed continuous algae systems, and intensive larval and nursery production systems.

France

- Observations of the French hatchery industry are difficult as I was 'locked-out', as the level of mistrust and competition are immense.
- Well-supported and politically strong support from IFREMER and industry.
- Division within industry between "Traditional" oyster growers that don't support hatchery production, and blame it for the current disease issues. As wild settlement continues to fail, demand for hatchery production has grown.
- POMS has a mortality of between 60-90% and this in conjunction with the reduction in wild settlement has seen a threefold+ increase in demand for oyster hatchery spat. With farmers putting additional oysters onto farm with expectations of having substantial losses.
- Growth in the hatchery sector is supported through European Union and French Government support.
- Nursery systems are typically shore based and have supplementary algae production.
- IFREMER and industry have strong collaboration in developing disease diagnostic capabilities.

Appendix 2

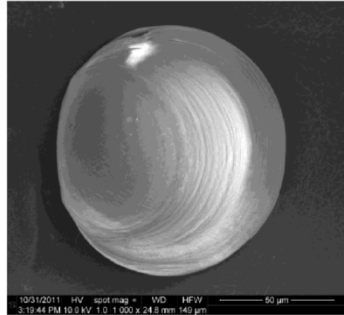
Ocean Upwelling and Acidification.

Ocean 'Upwellings' which happen to occur on the west coast of the USA, and influences the carbon chemistry of the seawater is a process where deep 'old' ocean seawater comes to the surface. This 'old' seawater has spent many years without mixing, resulting in a low oxygen concentration from aerobic and anaerobic respiration, and a high carbon concentration and resulting low pH. Carbon dioxide when dissolved in water forms carbonic acid, and this lowers the pH. This has an impact on shellfish, because as the name suggests they have a shell, and this is made mostly of Calcium Carbonate (CaCO_3). Calcium Carbonate can be present in variety of 'forms' and in adult oysters these are composed mainly of calcite; the shells of larvae are primarily aragonite. Following fertilisation of the eggs, and initial development in the first 1-3 days, the shells contain a significant amount of amorphous calcium carbonate, and this is quite sensitive to reduced pH. See Figure: *Larval Oysters are impacted by Ocean Acidification*.

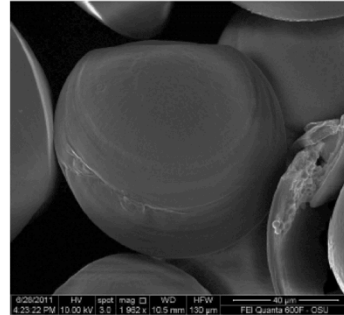
Ocean acidification refers to the observed decrease in the pH of the oceans that is caused by the uptake of carbon dioxide from the atmosphere. Recent industrialisation, burning of fossil fuels releasing carbon dioxide into the atmosphere, along with reduction in forests and other vegetation which acts as a sink for carbon has been reported to increase the levels of carbon dioxide into the atmosphere, with 33% of this 'new' carbon dioxide believed to have been absorbed into the oceans, forming carbonic acid and resulting in a measured reduction in the pH, which increases the hydrogen ion concentration making the oceans less alkaline. This changes the availability carbonate ions, which shellfish use for growing their shells. This leaves them vulnerable to degradation of their existing shells, and places metabolic 'stress' upon them in the building of new shell. This threatens the larvae and young shellfish spat, directly and makes them prone to disease.

Larval oysters are impacted by ocean acidification

Larval bivalves like these 9-day old Pacific Oysters from Whiskey Creek Shellfish Hatchery in Netarts Oregon are negatively impacted by corrosive seawater caused by ocean acidification. High levels of CO₂ in water are correlated with developmental abnormalities, reduced fertilization success, slowed growth, and the precipitation of weaker thinner shells. Scientists at Oregon State University are trying to understand the mechanisms behind these deleterious effects.



Normal larval shell



Abnormal larval shell

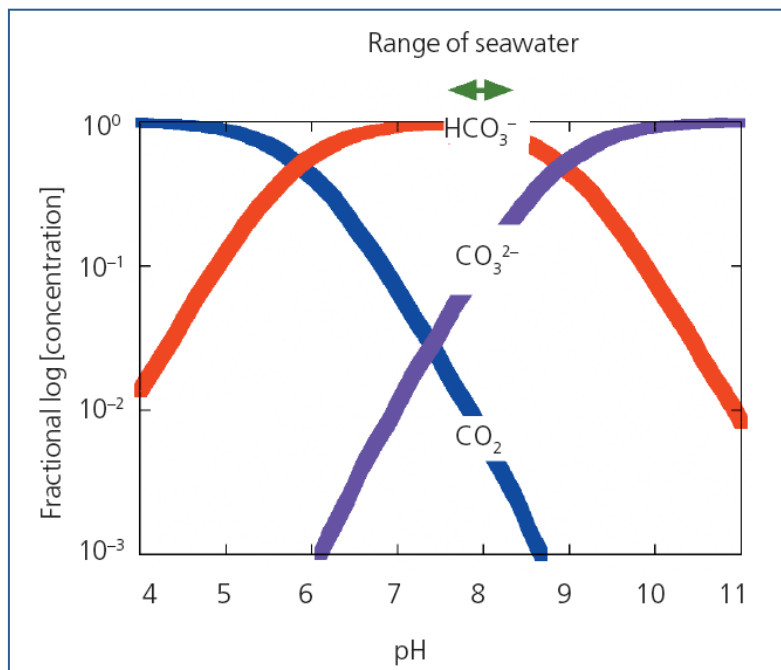
Photographs taken at Oregon State University by E.L. Brunner

PI: G.G. Waldbusser – NSF-Climate Research Initiative/Ocean Acidification #1041267



Larval Oysters are impacted by Ocean Acidification – Alan Barton, Whiskey Creek Shellfish

Oyster shell is made mostly of Calcium Carbonate (CaCO₃)



Effect of pH on the availability of Calcium Carbonate species. – Alan Barton, Whiskey Creek Shellfish Hatchery

Saturation State- the 'Magic Number' for dissolving shell

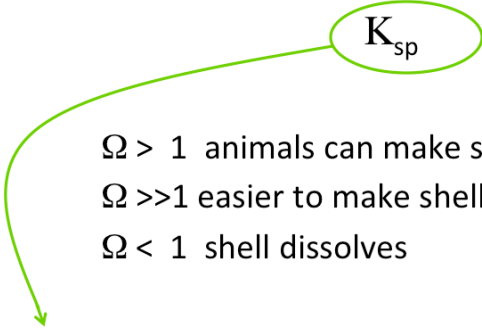
$$\Omega = [\text{Ca}^{2+}][\text{CO}_3^{2-}]$$

K_{sp}

$\Omega > 1$ animals can make shell

$\Omega \gg 1$ easier to make shell (Langdon & Atkinson, 2005)

$\Omega < 1$ shell dissolves



Adult oyster shell	-	calcite	small K_{sp}	harder to dissolve
Larval oyster shell	-	aragonite	bigger K_{sp}	easier to dissolve
Young oyster larvae	-	ACC	really big K_{sp}	<u>really</u> easy to dissolve

(Carriker & Palmer, 1979 Weiss et al 2002)

Saturation State of various 'species' of Calcium Carbonate (ACC=Amorphous Calcium Carbonate) – Alan Barton, Whiskey Creek Shellfish Hatchery