Australian Government
Department of Education, Science and Training

Recirculating Marine Aquaculture Systems

Report on an ISS Institute/ DEST Overseas Travel Fellowship

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1.0 The International Specialised Skills Institute (ISS Institute)

Since 1990, the ISS Institute, an independent, national, innovative organisation, has provided opportunities for Australian industry and commerce, learning institutions and public authorities to gain best-in-the-world skills and experience in traditional and leading-edge technology, design, innovation and management.

ISS Institute offers a broad array of services to upgrade Australia's capabilities in areas that lead to commercial and industrial capacity and, in turn, return direct benefits to Australia's metropolitan, rural and regional businesses and communities.

Our core service lines are identifying capabilities (knowledge, skills and insights) to fill skill gaps (skill deficiencies), which are not available in accredited university or TAFE courses; acquiring those capabilities from overseas (Overseas Skills Acquisition Plan - Fellowship Program); then placing those capabilities into firms, industry and commerce, learning institutions and public authorities through the ISS Research Institute.

Skill Deficiency

This is where a demand for labour has not been recognised and where accredited courses are not available through Australian higher education institutions. This demand is met where skills and knowledge are acquired on-the-job, gleaned from published material, or from working and/or study overseas. This is the key area targeted by ISS Institute.

Overseas Skills Acquisition Plan - Fellowship Program

Importantly, fellows must pass on what they have learnt through a report and ISS Institute education and training activities and events such as workshops, lectures, seminars, forums, demonstrations, showcases and conferences. The activities place these capabilities, plus insights (attitudinal change), into the minds and hands of those that use them - trades and professional people alike - the multiplier effect.

ISS Research Institute

At ISS Institute we have significant human capital resources. We draw upon our staff, industry partners, specialists in their field and Fellows, here and around the world.

Based on our experience and acute insights gained over the past fifteen years, we have demonstrated our capabilities in identifying and filling skill deficiencies and delivering practical solutions.

Our holistic approach takes us to working across occupations and industry sectors and building bridges along the way:

- Filling skill deficiencies and skill shortages.

- Valuing the trades as equal, but different to professional disciplines.
- Using 'design' as a critical factor in all aspects of work.
- Working in collaboration and enhancing communication (trades and professional).
- Learning from the past and other contemporary cultures, then transposing those skills, knowledge and insights, where appropriate, into today's businesses.

The result has been highly effective in the creation of new business, the development of existing business and the return of lost skills and knowledge to our workforce, thus creating jobs.

We have no vested interest other that to see Australian talent flourish and, in turn, business succeed in local and global markets.

Carolynne Bourne AM, ISS Institute's CEO formula is "skills + knowledge + good design + innovation + collaboration = competitive edge • good business".

Individuals gain; industry and business gain; the Australian community gains economically, educationally and culturally.

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2.0 Acknowledgments

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- Special thanks to the farmers and organisations that were willing and informative hosts to us on our trip. The information they freely provided was invaluable. Full contact details for the participating overseas organisations and individuals are detailed in Appendix 2.

- Our application for funding was greatly assisted by the following individuals and their organisations who were involved in advising and verifying skills and knowledge gaps.

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3.0 Summary of benefits to Australia and the Northern Territory

The subject of our fellowship was recirculating aquaculture with a special emphasis on ozone usage and waste removal from marine systems.

3.1 The Australian Context

Background:

Aquaculture is the fastest growing primary production sector in Australia. Valued at $743 million in 2002-03, aquaculture accounted for 32 per cent of the total gross value of production for Australia's fisheries for that year. In real terms, the gross value of Australian aquaculture production nearly trebled between 1991-92 and 2001-02. Worldwide, the volume and value of aquaculture production is also growing.

It has been suggested that the Australian aquaculture industry could triple production by 2010 if it can successfully exploit its competitive advantages to meet increasing domestic and global demand for Fisheries products[1].

Whilst the Aquaculture industry is experiencing this unprecedented period of growth it is important to ensure that the growth of the industry is aligned with the principles of Ecological Sustainable Development (ESD) to prevent environmental degradation and to provide for a profitable industry well into the future. There are many different types of aquaculture but one method that is being increasingly promoted as being compliant with ESD is recirculating aquaculture.

Recirculating Aquaculture refers to the process of re-using some (or all) of the water in a fish culture facility in order to better control the rearing environment, minimise water usage, achieve effective removal of waste products from the system and, in some cases, provide for a more efficient process for heating and cooling the water. Recirculating aquaculture systems are also generally more bio-secure than 'open' systems such as ponds or cages and they can be located in places that may not be suitable for the more extensive types of aquaculture, for example near major population centres and in areas with limited water resources.

Benefits to Australia

The Darwin Aquaculture Centre (DAC) is part of the Northern Territory Department of Primary Industry, Fisheries and Mines. The DAC and its staff fulfil many functions on behalf of the Northern Territory Government including research into new aquaculture species and techniques, commercial production of marine species (especially finfish) and extension support to the aquaculture industry.

The DAC's staff have a duty of care to industry clients to ensure that the information that is being extended to them is the latest and best available. The DAC has also been a 'development and testing ground' for new aquaculture methods, and techniques developed for hatchery culture of mud crabs and barramundi are considered to be among the best in the country. This has meant that the Centre is no longer just serving a local client base but

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[1] Source: Federal Department of Agriculture, Fisheries and Forestry, 2004.

is being sought out by interstate and overseas companies for advice and assistance with marine hatchery techniques.

With this background and in view of the responsibilities outlined above, this fellowship provided the perfect opportunity for Damon Gore and Glenn Schipp to improve their skills and knowledge and to then use the knowledge gained to further improve the operation of the DAC and, most importantly, pass this information on to industry. The DAC and its staff have an important role to play in helping to maintain strong industry growth and to attract more investment in aquaculture, particularly in the Northern Territory.

With the increasing importance of recirculating aquaculture to the growth of the aquaculture industry, it is important that skills and knowledge of the latest techniques in this field are acquired.

3.2 Organisations that have impacted and influenced our industry and those who will benefit from our findings.

The following organisations, companies and institutions are all potential beneficiaries of the information obtained through the current fellowship.

State Fisheries agencies, including: Western Australia Fisheries, South Australian Fisheries, Qld Department of Primary Industries and Fisheries; The National Aquaculture Council; the Australian Barramundi Farmers Association; The Fisheries Research and Development Corporation and Northern Territory aquaculture licensees.

3.3 Aim of the Fellowship

To undertake an overseas study program to gain an understanding of the use of ozone in sea water and to improve the operating efficiency of high-density marine recirculating aquaculture systems.

Including learning about:

- skills and knowledge required for the safe application of ozone to marine systems.
- methods for the collection and re-use of wastes in marine culture systems.
- information on improvements to the cost-effectiveness of the operation of marine recirculating fish farms.
- latest advances in recirculation equipment.
- progress with the domestication of new species for culture in recirculation systems
- improvements with Operational Health and Safety for staff working in intensive aquaculture production systems.
- methods for improving fish health and quarantine in closed system aquaculture.

3.4 The Skills and Knowledge Gaps

Design, planning and implementation of recirculation technology for marine aquaculture.

Industrial scale aquaculture is a relatively new industry and recirculating aquaculture even more so. Traditionally, recirculating aquaculture systems (or RAS) have focussed on the freshwater environment because this has enabled direct technology transfer from waste water treatment systems. RAS in the marine environment is a relatively new field of development and whilst some of the techniques that have been developed for fresh water systems can be directly applied to salt water, this is not always the case.

In the Northern Territory the Darwin Aquaculture Centre operates as a research and development facility to promote the development of marine aquaculture in the Top End. Over the past few years staff at the DAC have designed and constructed a number of small scale marine RAS for the culture of fish and zooplankton. With no formal training in the construction of these systems, the staff relied on their knowledge of aquarium systems and the information that could be gleaned from publications, the Internet and overseas advisors (principally from the US and France). We have not been able to locate, within Australia, a resource of reliable information with regard to marine RAS.

The lack of expertise in Marine RAS within Australia was especially apparent when a Northern Territory fish farmer recently approached NT Fisheries, through our extension service, for assistance with the design of a large scale system for the culture of juvenile marine fish. The scale of this project was outside of our experience or expertise (and beyond that of interstate agencies) and therefore it was necessary to employ a consultant from the United States to provide advice.

Even though it may always be possible to obtain advice from overseas consultants to assist with design input into marine RAS, it is an expensive process and one that doesn't really help to build the skill base of local people.

There are dozens of 'consultant' companies operating on the world market, each one advertising that their system and/ or equipment is the best. The best way of sorting the 'wheat from the chaff' is to visit operating farms in person to see exactly what is going on.
Operators of the facilities can be questioned in person about what works, and most importantly, what doesn't. This may help prevent Australian Industry from making expensive mistakes.

Waste removal and re-use from marine recirculating aquaculture systems.

One area of particular interest and need for the Darwin Aquaculture Centre is the development of a reliable, cost efficient and environmentally friendly way of collecting and disposing of fish wastes generated in marine systems. The DAC has an intensive marine nursery which generates in excess of 100 kg of wet waste per day when operating at full capacity. Rather than dump this waste in land-fill it would be preferable to find a beneficial use for it.

Fresh water recirculating systems can effectively re-use their collected wastes for fertilisation of horticultural crops. Wastes from marine systems, because of the high salt content, cannot usually be used in this way. The study tour itinerary was designed so that we could attend an international conference to hear presentations on advances in waste treatment and to visit facilities and researchers that were investigating novel ways of using wastes from marine systems.

This information can then be used to improve waste management at the Darwin Aquaculture Centre and through the Centre's extension service, be passed on to industry clients.

Use of ozone to improve the operating efficiency of high-density marine recirculating aquaculture systems.

Ozone is being increasingly used to help manage water quality in fresh water RAS. Its use in marine systems is also growing in popularity but indiscriminate usage can be problematic. Ozone is a chemical that must be used with extreme caution as it is highly toxic both to humans and to fish. Ozone generated by-products such as bromines can also have potentially toxic side effects when used in marine systems. Correct usage of ozone can lead to an increase in the reliability of production from hatchery systems.

The techniques for the application of ozone to marine systems are rapidly evolving and it was proposed for the overseas itinerary to include visits to large scale marine systems to view the way they apply ozone and to observe the safety procedures that they have in place.

Occupational Health and Safety is of paramount importance to the operation of the Darwin Aquaculture Centre and we need to ensure that ozone is applied in the most effective and safest manner.

4.0 Executive Summary

Between July 19 and August 11, 2006, Damon Gore and Glenn Schipp from the Darwin Aquaculture Centre undertook a study tour to investigate the latest developments and trends in recirculating aquaculture. The trip was jointly funded by the Department of Science, Education and Training, administered by the International Specialised Skills Institute, and the Northern Territory Department of Primary Industry, Fisheries and Mines.

We attended the 6th International conference on recirculating Aquaculture in Roanoke, Virginia, USA, on July 21 and 22. After the conference we travelled to Europe and Scandinavia to visit manufacturers and farmers involved in developing and using recirculating technology.

One of the main aims of the tour was to gain a better understanding of the best techniques for waste removal and re-use, and the safe and efficient use of ozone in intensive recirculating systems.

The tour also provided the opportunity to learn about other pieces of equipment, techniques and protocols that could increase the operational effectiveness of the Darwin Aquaculture Centre and which could also be of benefit to the Australian aquaculture industry.

The recommendations from the study tour are summarised in Table 1.

The information obtained from the tour ranged from the bizarre, such as the wolffish research being undertaken in Norway, where they are investigating the culture of this deep sea species at densities as incredible as 500 kg/ m^3, to the practical, such as using sponge based disinfection mats instead of messy footbaths.

Much of the valuable information centred on increasing biosecurity of aquaculture facilities. Only by maintaining rigorous disinfection and quarantine protocols can the health of the growing stock be safeguarded.

One thing that did surprise us was that very few facilities had embraced the technology of ozone disinfection. Reading the aquaculture based literature prior to leaving we could have been forgiven for believing most facilities were now using ozone. This proved to be far from the case. Reluctance to use ozone stems in part, from occupational health and safety concerns for the staff, and also in part from the uncertain effects the ozone has on water chemistry and fish health. Targeted use of ozone, such as the disinfection of fish eggs and disinfection of the larval rearing water for mud crabs is recommended. The paper, by Helge Liltved, appended to this report cautions that current ozone doses used for fish egg disinfection may not be high enough to be effective in controlling viruses and this is something we will investigate further at the Darwin Aquaculture Centre.

It was also clear from the manufacturers we visited that the cost effectiveness of the establishment and operation of intensive recirculating farms is improving all the time. However, whether or not their claims of being as cost efficient as sea cage farming are true, should become evident in the near future, given the number of farms currently being installed. Also, the continued tightening of environmental restrictions for aquaculture means that recirculated farms are going to become more desirable, not less.

The fact that recirculating farms are being accepted for their environmental credentials is being reflected in the simplification of the approvals and licensing process in the Netherlands and Denmark. To be able to get a farm fully licensed in less than six months is something that the Australian aquaculture industry can only dream about at this stage.

Another aspect of the tour that took us a bit by surprise was the growing interest in barramundi farming around the world. It appears that countries as diverse as the USA, Russia, Israel, the Netherlands, Denmark, the UK and Iran are either showing an interest in developing barramundi farms or are actually doing it. The word is out… barramundi is a great aquaculture fish!

Overall the tour was very enjoyable and educational and we look forward to implementing the recommendations and to passing on the knowledge we obtained on recirculating systems.

Table 1. List of recommendations. The priority ranking is 1: very important, 2: important, 3: good idea.

Topic	**Recommendation/ Information**	**Priority**	**Page Ref.**
Ozone			
O1	Investigate the suitability of using ozone for the larval rearing process of mud crabs.	**1**	**17**
O2	Develop Standard Operating Procedures for the use of ozone for the disinfection of marine fish eggs. Use of ozone has serious OH&S implications. Investigate whether or not the concentration of ozone for fish egg disinfection needs to be increased to make it more effective.	**1**	**76**
O3	Maintain a watching brief on the need/value of using ozone continuously in recirculating systems. There is not currently enough convincing information to say that its use is required or beneficial. There are serious concerns about the effects ozone can have on human and fish health and the benefits of using ozone needs to be balanced against OH&S.	**2**	
Waste removal			
W1	Drum filters are still the most efficient method for removing solid waste from aquaculture discharge water. The removal of solids can be further improved by treating the backwash water from the drum filter with an appropriate polymer, to flocculate the solids and then remove them using a belt filter. This semi-solid waste can be used for composting. Commercial polymer mixing tanks are available.	**1**	**23**
W2	Dissolved nutrients in waste water can be significantly reduced by the use of appropriate polymers and alum or by the culture of sea weeds. The sludge generated from the neutralisation of acid mine drainage can be used to reduce dissolved phosphorous.	**1-2**	**23, 24**
W3	InterAqua Advance are developing a new style of mechanical filter which are supposed to be very energy efficient. These 'contact filters' are being trialled successfully in high density rainbow trout farms. Follow up on the performance results for these filters.	**2**	**42**
W4	The AquaOptima designed centre drain, the 'EcoFlo' significantly reduces the amount of solid waste in a recirculating system and makes it possible to reduce mechanical filtration by 50%.	**2**	**57**
W5	Filter equipment needs to be chosen carefully and after sales service is a very important factor. 'Salsnes' brand filters have a very good reputation for after sales service.	**3**	**57**

Topic	**Recommendation/ Information**	**Priority**	**Page Ref.**
Waste Re-Use			
Wu1	Solid waste from marine aquaculture systems can also be used as fertiliser. The small amount of salt contained in the waste is not considered to be a problem, especially when the waste is used as fertiliser over a wide area. The waste can be stored in above or below ground tanks until required to be used.	**1**	**38**
Wu2	In Denmark the recirculating farms are required to re-use their own waste, and so the farms are normally associated with agricultural land.	**1**	**36**
General Recirculation. Equipment			
E1	The use of a trickle filter is still recommended by most European equipment manufacturers. The added advantage of a trickle filter is its degassing properties.	**2**	**28**
E2	Vacuum degassing is now a common treatment practice for the inlet water of marine hatcheries and nurseries.	**2**	**64**
E3	Clip lock oxygen tubing is cheaper and to easier to install than plastic tubing, t-junctions and hose clamps.	**2**	**64**
E4	It is recommended that all oxygen flow meters and controls should be centralised in one, easily accessed, panel near the oxygen monitoring computer.	**2**	**52, 56**
E5	Consider the use of 'Curler Advance' media in moving bed bioreactors because of its non-clogging capabilities.	**3**	**43**
E6	Automatic tank sweepers are commonly used in larval rearing and nursery tanks.	**3**	**52, 62**
E7	Plastic flexi tubing, as used at Hesy in the Netherlands, appears to be a more cost effective option instead of copper piping.	**3**	**30**
E8	If tank wall strikes by larvae are considered a problem during larval rearing then the use of 'donut' lids should be investigated as a solution.	**3**	**50**
E8	Trial the use of the plastic water flow gauges (seen at Fosen Aquacenter and the Institute of Marine Research in Norway) for controlling water flow in the larval rearing and rotifer areas at the Darwin Aquaculture Centre.	**3**	**51**
Biosecurity			
B1	Improved signage, designating quarantine areas and biosecurity protocols.	**1**	**16**
B2	Foot bath sponge mats to replace current trough system, and replace spray bottle hand disinfection with lever-action bottles.	**1**	**63**
B3	Further restrictions on visitation to the critical areas of the hatchery.	**1**	**51**
Other			
O1	An investigation of the applicability of 'pulse' or 'cyclic' feeding to control maturation and improve the FCR of farmed barramundi should be considered.	**3**	**53**
O2	Continue discussions with Dana Feed to explore options for collaborative projects.	**3**	**45**
03	Test the performance of the Dana Feed and Otohime diets on barramundi.	**2**	**45**
04	When designing nursery systems, give consideration to incorporating a centralised grading area.	**3**	**30**
O5	Move the lunch facility room at the DAC to give a better ocean view and upgrade the coffee machine ☺	**3**	

5.0 Introduction

In November 2005 Glenn Schipp and Damon Gore were awarded an International Specialised Skills Institute (ISSI) Overseas fellowship, sponsored by the Federal Department of Education, Science and Training. The fellowship helped to fund the travel for a study tour investigating the latest developments in recirculating aquaculture. First stop was the USA to attend the 6th International Conference on Recirculating Aquaculture in Roanoke, Virginia USA. After the conference concluded on Saturday July 22 we drove up to Washington DC to catch a train to New York. From New York it was an overnight flight to Amsterdam via London.

For the next two weeks we navigated around various recirculating aquaculture farms and manufacturers in the Netherlands, Denmark and Norway in a (thankfully) very economical Ford Focus. After more than 6000 km we ended up back in Amsterdam and left just in time to avoid the total chaos caused by the latest bombing scares – the joys of international travel.

The trip concluded back in Darwin on the morning of August 11.

This report summarises the information we obtained at our various ports of call during this very interesting and enjoyable study tour.

6.0 Report on the 6th International Conference on Recirculating Aquaculture, University of Virginia, Roanoke, Virginia, USA July 21-23, 2006.

6.1 Introduction

The 6th International Conference on Recirculating Aquaculture featured oral presentations and poster sessions from leading experts in recirculating aquaculture. In total, well over 80 papers were presented during the event. The conference was attended by more than 300 delegates from around the world and attendees included equipment manufacturers, company representatives, producers, researchers, regulators, and potential investors. The conference also included a small trade show.

Table 2. Summary of relevant information obtained from the conference.

Topic	**Summary**
Biosecurity	This has always been important, but with the intensification of production that is involved with recirculating aquaculture, it becomes of paramount importance.
Waste removal-physical	The recommended waste collection/removal method for suspended wastes is for the discharge water to be filtered by a drum filter fitted with a 60-100 μm mesh screen. The back wash water from the drum filter can then be treated in a mixing chamber with a polymer to flocculate the waste material The flocculated waste is then removed as a 'semi dry' cake by a belt filter. The 'cake' can be used as fertiliser or for composting.
Waste removal-chemical	Three possible methods for reducing the concentration of dissolved nutrients in the discharge water from an aquaculture facility are: 1. Using neutralised 'acid mine' sludge to remove phosphorous; 2. re-using the water to grow sea weeds of commercial value and 3. treating the water with a combination of alum and polymers to reduce levels of nitrogen and phosphorous.
Larval rearing diets	Recommend trialling Otohime diet from Japan to see if it further improves production of fish from the Darwin Aquaculture Centre.
Future of Recirculating systems	As environmental regulations for aquaculture become more restrictive, more emphasis will be placed constructing recirculating systems. The future for these systems appears to be in the use of large volume tanks, operated with high exchange rates and low head pressure to make them cost-effective.
De-gassing systems	The optimum air: water ratio for de-gassing systems is 10:1.

6.2 Summary of oral presentations

6.2.1 Day One, July 21:

Opening Address:

Steven Summerfelt:

Like Australia, imports are the biggest threat to US aquaculture. The press is winning the battle of the negative image of aquaculture – Recirculating Aquaculture Systems (RAS) can be an answer to the negative perceptions but needs to be 'certified' so consumers can be certain of what they are getting.

There is a need to move towards zero discharge, lower densities and humane slaughter. Also need to improve production per unit investment. Basically RAS is still reserved for high value species, larval rearing and for nurseries.

RAS tanks need to be bigger. Tanks limited to 45' diameter, otherwise the velocity of the water gets too fast at the edge of the tank for the fish. Need a certain velocity in the middle of the tank for self cleaning and if the tank is too large then the velocity at the edge is too fast.

Other desirable design features – automatic mort removal from the centre and automatic grading using 'clam shell' grader. They also use a CO^2 gradient to move fish from one tank to another – creating undesirable CO^2 conditions in one tank to force the fish to swim towards a tank with better water quality.

Take home message:

There may be economies of scale by moving to large diameter, deep tanks. Tanks of 600 m^3 to one megalitre.

A CO^2 gradient can be used as a relatively stress free method to move fish.

There is a maximum diameter of 45' (13.7 metres) for tank design.

July 21, Presentation 1:

Implementation of biosecure practices in marine recirculating hatchery systems: a necessary step in disease prevention.
S. E. Laramore

Fairly standard fish health talk. Usual discussion about the interaction of environment, disease and fish. As fish production intensifies so to does the need for improved biosecurity.

Good wording for biosecurity signage –

Strict Biosecurity Measures in Effect

DO NOT ENTER
Unauthorised Entry Prohibited

Take home message:

For hatcheries that use fresh feeds for their breeding stock, they should consider irradiating the feed as a biosecurity measure.

The development and implementation of Standard Operation Procedures is critical to the efficient and effective operation of an aquaculture facility.

July 21, Presentation 2:
Recent advances in nutrition for better fish and shrimp health.
R. A. Bullis:

Fish meal prices have doubled in the past twelve months. The cost of fuel is causing many trawlers to stay home. Discussed options for fish meal and oil replacement.

Take home message:

Totally replaced fish meal and oil in shrimp diets and 80% in salmon diets.

July 21, Presentation 3:
Decentralisation of recirculating marine hatcheries using the air-lifted bead filters and moving bed biofilter reactors.
R. F. Malone:

Unfortunately this was not a well presented talk and it contained minimal useful information.

Take home message:

Visit www.polygeyser.com for designs specs for airlift systems.

July 21, Presentation 4:
*Use of ozone in the larval culture of spiny lobster (*Jasus edwardsii*) and striped trumpeter (*Latris lineata*) in Tasmania.*
G. G. Smith:

The use of antibiotics during lobster culture improves survival, just like it does for mud crabs. They change tanks for lobster larvae weekly to reduce biofilm effects. Surge tanks reduce phyllosoma interaction. They also use formalin to disinfect *Artemia*.

Ozone by-products (OBP) are used to control fouling on larvae. The half life of ozone in sea water is 5 seconds. OBP half life is in the region of an hour.

A selective ion probe is more accurate and more responsive for measuring disinfection levels in larval tanks than colorimetric methods.

ORP readings: 300 mV= no ORP, 400 mV = low, 500 mV = medium and 600 mV = high.

Bromide ions relate to ORP. 5 ppb = 300 mV, 15 ppb = 400 mV, 25 ppb = 500 mV and 35 ppb = 600 mV.

Ozone goes via venturi into a contactor and stays in contact chambers for 10 minutes then to larvae tanks. They believe there is no need for carbon in marine systems.

Showed great Scanning Electron Micrographs of lobster larvae in Bromine treated water and those not in Bromine treated water. Those not in Bromine treated water were covered in filamentous bacteria.

Take home message:

Information may be very applicable to mud crab larvae. Need to test the use of a selective ion probe and to investigate the effect of bromide residuals on survival of crab larvae.

July 21, Presentation 5:
*Nutritional studies related to hatchery technology development for black sea bass (*Centropristis striata*).*
C. A. Woolridge:

Standard talk on marine fish larval rearing. The main interest for Australia is that they used the Japanese diet, Otohime, for larval rearing and weaning. They claimed the use of Lansy diets increased deformity rates.

Take home message:

Otohime is a better micro diet that Lansy**.**

July 21, Presentation 6:
*Larvae culture and production of the black rockfish, (*Sebastes schelegeli*).*
J. –Y. Jo. Black Rockfish:

Rockfish culture started in Korea in 1989. Now there is more than 20 000 tonnes of production.

They use Chlorella paste (Korean made). Rotifers (*Brachionus rotundiformis*) are claimed to be cultured at 100 000 per ml on chlorella paste at 2.5-8 billion cells per ml. 200 ml of Chlorella per 100 million rotifers per day. If they want to increase their rotifers by 15 fold then they feed 1200 ml/100 million per day.

The DHA (essential marine oil) content of their chlorella is too low, so they boost. 6-20 ml of emulsion/ 100 million rotifers for 2-12 hrs. Add paste as green water to larvae at 3 x 10^5 cells per ml, reduce to 1 x 10^5 by day 20, reduce to zero after 40 days.

Light levels during larval rearing are about 700 lux at the water surface on a 16L:8D cycle which is changed to 14L:10D later in the culture cycle.

Take home message:

Good demonstration of what can be achieved when both the government and industry are committed to developing an aquaculture industry. The talk also raised several questions for the operation of the larval tanks at the Darwin Aquaculture Centre. The Japanese chlorella we use is claimed to be high in essential oils…this needs to be further verified. What effect would changing day length have on our larvae?

July 21, Presentation 7:
The low-head Mega-Flow air driven recirculating system – recent improvements and the construction of pilot systems in Israel.
Roly Haddas:

This talk was an update of a talk given in 2004. The Mega Flow system has been developed in Israel and involves large volumes of water being moved around the fish holding system by air. The developers claim it is extremely cost effective to operate. The South Australian Government is investing in a small system at the moment.

They claim that for the production of sea bream, Mega Flow uses 5.2 KWh per kg and traditional systems use 8.3 kWh. If power costs in Darwin are 16.3 cents/ KWh*, then the cost of producing a kilogram of fish is 85 cents and $1.41 for the Mega Flow and traditional systems respectively.

For tilapia the figures are 2.8 KWh and 45.64c for Mega Flow and 3.7 KWh and 60 cents for traditional.

*Note in Israel power costs are 8.7 cents/ kWh.

Take home message:

South Australia are finalising their purchase of a MegaFlow system and it will be interesting to see if it lives up to the manufacturer's claims.

July 21, Presentation 8:
Advances in cobia larviculture and production.
S. Craig:

Used Otohime with better results than other micro diets. Cobia limited by only being able to raise larvae at 1/ litre. Some progress made in the last two years with nutrition but much more to do.

Asked the author about deformity rate with Cobia and the use of Otohime. Jaw deformities still range from 9-30 per cent so Otohime is not really seen as a solution. He said that most hatcheries have jaw deformities but no-one talks about it. His response was if we solve the problem please let him know!

Take home message:

Try Otohime as a micro-diet… but it is not expected to solve the problem of deformities in juvenile fish.

July 21, Presentation 9:
Ozonation and UV irradiation provide synergy for inactivating bacteria in a coldwater recirculating system.
Mark J Sharrer:

Ozonation followed by UV irradiation is used to break down ozone and reduces the total heterotrophic bacteria counts and total coliform bacteria counts in water exiting the UV irradiation unit in fresh water.

Take home message:

If using ozone in fresh water in the future, Ultra-Violet light can be used for the destruction of residual ozone as well as improving water quality.

July 21, Presentation 10:
A low-head biofilter with degassing and oxygenation capabilities
Michael Couturier:

Discussed the hydrodynamics, nitrification, degassing and oxygenation of a horizontal moving-bed bio filter. Benefits were that it is not only used as a bio-filter but also for degassing and oxygenating treated water in a low head unit, with reduced pumping cost.

Take home message:

Could look into this further as an option at the Darwin Aquaculture Centre instead of using energy intensive fluidised bed biofilters.

July 21, Presentation 11:
Low-head, low-energy, recirculating technology
Jeffrey Koch:

The speaker has designed a four tank system run on a common filter and driven mainly by airlifts. It has a low running cost and maintenance requirement. This was very much a back yard operation but he claimed that it cost him $13 000/year to produce $20 000 profit.

Take home message:

Some of his construction ideas were good and could be passed on to hobby farmers. Would recommend a back up generator in case of power failures as fish species other than Tilapia probably would not survive for very long in this system if the power failed.

July 21, Presentation 12:
The semi-intensive floating tank system (SIFTS) – a new technology for commercial finfish production.
Bruce Ginbey:

Technology developed in Western Australia. The system runs on low-pressure air which efficiently delivers large volumes of oxygenated water to fish held in circular floating tanks

(McRoberts system). Air also drives a waste extraction unit which removes up to 95 per cent of the solid waste. This system was trialled in inland saline ponds with promising results of three fish species. Dissolved nutrients can cause algae blooms which are managed by adding zooplankton.

Take home message:

They are looking at developing a larger system based at the entrance of the Swan River in WA. System not suited to tropical waters with large tidal movement but has potential in ponds and sheltered waters.

6.2.2 Day Two, Saturday, July 22:

July 22, Presentation 1:

Controlling water velocity within large "Cornell-Type" dual drain culture tanks.
Steven Summerfelt:

Talk was about using larger tanks to decrease the production cost in producing finfish.

The example used was a 150 m^3 circular 'Cornell-type' culture tank. Tank dynamics were controlled by using an inlet manifold with nozzles. The top half of the nozzles are set at a 45 degree angle and the bottom half set facing the middle of the tank. The aim is to get a velocity of 6-10 cm/sec in the middle of the tank. At this velocity the solids move to the middle of the tank in approximately 3-6 mins before being sucked out and removed. This also gave a uniform dissolved oxygen reading throughout the tank.

Comparison test were done on salinities of 0-34 ppt and water temperatures of 15-25 ˚ C.

Take Home Message:

The removal rate of solids is independent of salinity or temperature and a large tank can be designed to be self cleaning.

July 22, Presentation 2:

Comparing carbon dioxide stripping column performance in freshwater and seawater.
Steven Summerfelt:

- CO^2 Stripping is easiest when water droplets fall through the air.
- CO^2 accumulates to toxic levels in systems with excess O^2 demands
- pH may drop in systems with high oxygen and low aeration

Trials were done using 1-1.5 metre high degassing columns, pH and alkalinity were tested.

Air / Water ratio was 10:1. It was found that 55-65 per cent of CO^2 was removed from fresh water at 15 C and only 40 per cent removed from salt water. Taller columns did not improve results.

Take Home Message:

The optimum air to water ratio for degassing columns is 10:1.

July 22, Presentation 3:

Comparative performance of CO^2 measuring methods.
Timothy J. Pfeiffer:

Traditionally the measurement of CO^2 has been done using a titration method which has limitations in salt water due to compounds that can interfere with the CO^2 determination, (including phosphates, silicates, sulphide, ammonia and nitrite). The titration method is also not applicable for samples that contain high dissolved solids.

Companies such as Point Four and CEA Instruments have recently produced CO^2 analysers.

Tests were conducted comparing various CO^2 meters from the above companies. All tests were done using samples with known CO2 concentrations. It was found that the probes needed to be left in position for up to 30 minutes to get an accurate reading.

Take Home Message:

CO^2 measurement has traditionally been problematic. This talk suggests that 'in situ' probes may be a valuable addition to larval rearing systems in the future.

July 22, Presentation 4:

The screening and evaluation of alum and polymer combinations as coagulation/ flocculation aids to treat intensive aquaculture effluents.
James M. Ebeling:

As the practice of aquaculture grows world wide the need to treat waste water is increasing. In the past both polymer and alum have been used separately for the treatment of waste water. This talk commented on trials that were conducted comparing the use of both of these products either singly or in combination with the view of improving both solid and chemical waste removal.

Polymers are great at removing solids when used at the correct dose, (polymers need to be chosen to suit the individual requirements of each site). Alum is mainly used to decrease phosphorus.

When both of these products were used in the trials, phosphorus was reduced by 92 to 99 per cent to as low as 0.07 mg/L P. Nitrogen levels were also reduced by between 64-87 per cent. The rates for BOD and COD were also significantly reduced, by an average value of 97.3 per cent and 96.4 per cent.

The effect of the use of polymers and alum is synergistic.

Take Home Message:

Waste removal from an aquaculture facility can be significantly improved by the use of a belt filter coupled with the use of an appropriate polymer and alum. There may be a relatively large set-up and on-going operational cost. There may also be a need for a lot of automation and trial work to find the right products and concentrations to use.

It really boils down to the amount of waste that is needed to be treated. One side effect of using alum is that it also decreases pH which needs to be managed if the collected waste is to be further used as fertiliser etc.

July 22, Presentation 5:

Performance evaluation of the Hydrotec belt filter in intensive recirculating aquaculture systems.
James M. Ebeling:

This work was done in conjunction to the work in the previous talk using polymers and alum for waste water treatment.

The belt filter had a 120um mesh and was used to remove waste that had settled out after being treated with a polymer/alum combination.
Slides of the waste showed a very concentrated waste with a moisture content of approximately 15 per cent.

Take Home Message:

The use of the belt filter in combination with the polymer/ alum water treatment appears to be an effective technique for improving the management of waste from intensive, land based aquaculture systems. All of the equipment required for this process would require to be housed in a shed with good ventilation.

The set up and running cost would be high.

July 22, Presentation 6:

Performance evaluation of geotextile tubes.
James M. Ebeling:

This talk was number three in his series.

Trials were conducted to see how long it would take to de-water aquaculture waste using geotextile bags. Without the use of polymers the dewatering process was not very efficient. If using long stranded polymers it was also not very efficient as the bags become blocked rapidly. The most efficient process was having the waste pumped into the bags with an appropriate polymer added in a vortex mixed with waste.

Bags took up to two weeks to drain leaving 18 per cent solids. The bags are capable of treating 58.7 l/day per m^3 of geotextile material with 93 per cent TSS removal. There were high phosphates in the recovered water.

The Darwin Aquaculture Centre has already tried using geotextile bags in the past and decided that this was not the appropriate method for treating our waste water.

The Darwin environment is very different to the one where Ebeling's tests were carried out. There was a large problem with flies laying eggs and subsequently a maggot problem, that's without mentioning the bad smell all of this created!

Take Home Message:

There may be some locations where the use of this technology may be appropriate, however it is not considered to be suitable in the tropics at this stage.

July 22, Presentation 7:

Nitrification performance of a moving bed biofilter utilising sinking media: effect of mixing times.

W. Johnson:

This was a very technical talk and was more to do with the mathematics of the operation of these systems than any actual practical discussion of the implications.

Take Home Message:

The advantage of sinking (solid) biofilter media is that there is no chance for the build-up of organic material in the centre of the media (which is a problem with some of the commercially available plastic media.

The nitrification rate of the biofilter is related to mixing time within the filter. The shorter the mixing time, the faster the nitrification rate.

July 22, Presentation 8:

Characterisation of ammonia uptake in 4 species of Chesapeake Bay macroalgae and performance in recirculating aquaculture effluents.

D. Terlizzi:

The use of seaweeds to lower nutrient levels in aquaculture effluents is an attractive option, particularly if the seaweeds have some commercial value themselves.

The criteria for selecting a suitable seaweed are: it must be local, have a high ammonia removal ability, ability to respond to sudden increases in nutrient load, be easy to cultivate and have economic value.

Their studies examined one red and three green seaweeds from the Bay. The green seaweeds are more efficient at removing ammonia than the red. Apparently the 'red' seaweeds that were part of the subject of this talk were unusual because they had the ability to remove nitrate as well as ammonia. Most seaweeds shut down their 'nitrate removal function' if ammonia is available.

Take Home Message:

The use of commercially valuable seaweeds to lower nutrient levels in the effluent channels and settlement ponds of aquaculture farms is worth further investigation.

July 22, Presentation 9:
Development and empirical assessment of a model describing NO^3 *removal from RAS using the activated sludge concept with intrinsic organic matter as the electron donor.*
O. Lahav:

The talk discussed using fish waste in a nitrate reduction reactor. The concept is that some of the effluent water from the farm is slowly percolated through a bed of fish waste to reduce Nitrates to Nitrogen gas and therefore lower the amount of nitrogen in the effluent.

This is all good in theory except that the retention time in the 'reactor' needs to be longer than 20 days to be effective!

Take Home Message:

This method is attractive because it removes the need for extra chemicals (eg alcohol) to be added to the waste to lower nitrogen levels. The downside is the long retention time, but this may not be a problem in a facility with a large water storage capacity or a small waste water flow.

It is more likely that some other form of bioremediation (eg constructed wet lands) is going to more practical in removing excess nutrients from the waste water on a large scale.

July 22, Presentation 10:
Phosphorous removal from aquacultural effluents with iron-based precipitates produced by the neutralisation of acid mine drainage.
P. L. Sibrell:

This was an interesting talk. Sludge from mines is often rich in iron hydroxide and aluminium hydroxide. If this 'acid mine drainage' is dried out and then the effluent from an aquaculture facility is run through it, it is very effective at removing phosphorous. The material apparently retains its shape and the contactors can be used for up to 100 days without recharge or regeneration.

Take Home Message:

This could be an interesting use of mine wastes in the future.

July 22, Presentation 11:
Use of nozzle deflectors for water dispersion in packed columns.
M. Smith:

This was a 'fill-in' talk as the scheduled speaker did not make it to the conference.

The presenter spoke about his company's own products that, in his words, produced better water dispersion in trickle filters, to eliminate dry spots and improve the efficiency of the filters. It was a reasonably convincing advertisement for the product.

Figure 6.1. Water dispersion nozzle recommended for use instead of dispersion plates.

Take Home Message:

Water dispersion deflectors should be considered when designing/ constructing trickle filters.

July 22, Presentation 12:
Modern recirculation facility at Richloam State Fish Hatchery/ Florida Bass Conservation Center.
T. L. Johnson:

This talk demonstrated an impressive new fish production facility in Florida. The construction and operation of the facility is funded entirely from sales of fish for recreational sport fishing. The new facility cost US $15 million to construct (Figure 6.1).

Take Home Message:

The power of the recreational fishing dollar is very evident in Florida.

6.3 Conclusions from the 6th International Conference on Recirculating Aquaculture.

The 6th International Conference on Recirculating Aquaculture was well run and generally well attended. Whilst some of the talks bordered on being too technical, with little in the way of demonstrated practical use, the variety of talks was impressive and there was something in it for everybody.

It is evident that recirculating aquaculture has a lot more development work to do to become cost effective compared to the less intensive forms of aquaculture. There was a heavy focus on systems that incorporated low head (and therefore lower pumping costs) designs and it is likely that this is the way the industry will go, unless power costs drop dramatically, and this is highly unlikely!

The fact that removal of nutrients and wastes from aquaculture effluents was the topic of 15 of the presentations indicates that the industry is taking this topic seriously and that there is also merit in re-using some of this waste nutrient to 'value-add' such as producing seaweed products or using it as a fertiliser.

We were able to obtain a significant amount of useful information from the conference to help support the aims of our fellowship. Of particular interest was the suggestion of targeted application of ozone for the larval rearing of crustaceans such as mud crabs. We were also able to walk away from the conference with a configuration for the effective and efficient concentration and re-use of wastes from intensive systems that we could recommend to our industry.

With increasing restrictions on the environmental performance of aquaculture systems it is almost inevitable that more emphasis will be placed on developing cost-effective, recirculating systems. Apart from the advantages conveyed by being able to collect and treat all wastes, there are also the significant advantages of being able to efficiently and effectively manage biosecurity and production parameters for a wide range of fish and shellfish species.

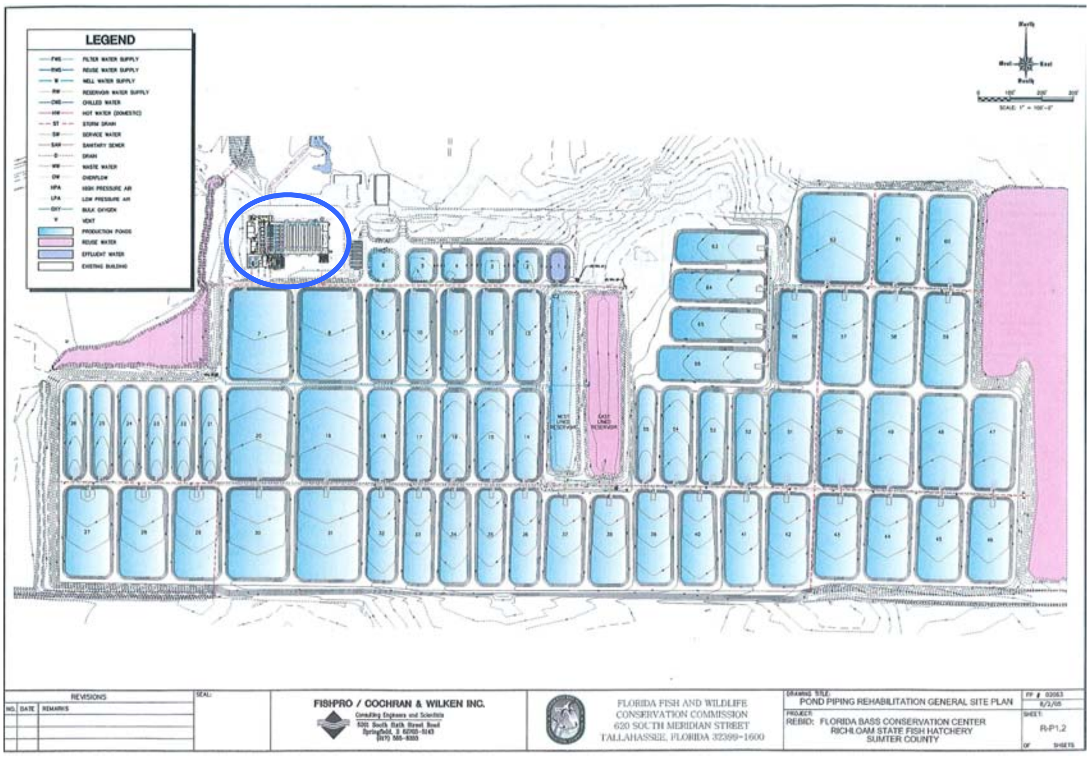

Figure 6.2. Overview of the Florida Bass Conservation Center. The important thing to note is the extensive area of ponds. To give an idea of scale, the hatchery building (circled) is about 100 metres long x 30 metres wide…more than three times the size of the Darwin Aquaculture Centre. The building also includes a public display area which explains the operation of the facility and the restocking program it supports.

7.0 The Netherlands

7.1 Introduction

Marine aquaculture in the Netherlands consists of extensive culture of mussels and oysters, predominantly carried out on the bottom of leased grounds. These grounds, or 'plots', are located in the Wadden Sea and in the Dutch delta area. There is at present no culture of marine fish in Dutch coastal waters. Only one company operates a land-based turbot farm. The inland aquaculture sector is small but growing. Inland aquaculture generally takes place in closed recirculation systems with eel and African catfish being the main cultivated species.

The Netherlands have always been famous all around the world because of their water. As long as people can remember, there has been a struggle against flooding in the Netherlands, as one third of the country lies below sea level. If there were no dikes or dunes, 66 per cent of the Netherlands would be flooded on a regular basis. The country has learned many lessons along the way. A lot of knowledge and experience has been gathered over the time in dealing with fresh water of the lakes and rivers, as well as with brackish and salt water of estuaries, tidal areas, coast and seas.

7.2 Hesy Aquaculture BV

Address: Bovendijk 35-2 City, Rv Kwintsheul, Ambachtstraat 16-B 2861, Netherlands

Hesy Aquaculture is a private company with over 20 years of experience in the design and operation of intensive recirculating fish farms. They have developed systems in over ten countries. The smallest system designed produces two tonnes/year, the biggest greater than 1000 tonnes/year. Their systems are designed for a variety of species such as:

Barramundi	*Lates calcarifer*
Murray cod	*Maccullochella peelii*
Eels	*Anguilla anguilla*
White sturgeon	*Acipenser transmontanus*
Trout	*Oncorhynchus mykiss*
Turbot	*Hippoglossus hippoglossus*
Salmon smolts	*Salmo salar*
Pike Perch	*Sander lucioperca*
Sea bream	*Sparus aurata*
European carp	*Cyprinus carpio*

and various catfish, tilapia and grunter.

They offer training to all customers, which includes start up of new systems, intake of fish, grading, after sales service, technical service and biological service. They also provide operation manuals for specific systems as well as other on-going support as required.

Hesy are strong believers in having trickle filters at the core of their recirculating systems. These type of filters have value not only for treatment of nitrogenous wastes but also an important role in degassing $C0^2$ from the water.

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Map 1.0 Map of the Netherlands showing the places visited. **1.** Hesy Aquaculture BV, head office, Kwintsheul; **2.** Hesy Aquaculture BV, farm under construction in Urk; **3.** Cell Aquaculture Systems Europe BV, in Tolbert.

7.2.1 Summary of Discussions

We met with the Manager of Hesy Aquaculture, Arie de Bondt at their new facilities in Kwintsheul, near The Hague, south eastern Netherlands. The facility consists of a workshop of 250 m^2 where tanks and other fish farm equipment are either fabricated or assembled. The office is 100 m^2 and is where all of the design work for individual systems is undertaken.

At the time of our meeting Hesy were involved in projects in Russia, Israel, Greece and Chile as well as constructing a facility in Urk (on the Zuiderzee – the Netherlands inland sea) in the north. We discussed some of their system designs such as waste removal from the centre of culture tanks using a double drain configuration and concentration of waste using polymers and a belt filter. Hesy are already employing the polymer technology that was discussed at the US conference to concentrate farm wastes and produce sludge with 15 per cent moisture content.

Hesy systems are designed based on the kilograms of feed used per day. For example a system fed 100 kg of feed per day will have a recirculation rate of 200 000 litres per hour and use 200 litres of new water per day per kg of feed.

Hesy are now confident that recirculating systems are cost-effective compared to cage farming. They do not support the use of ozone in marine systems but believe it may be useful in some freshwater farms.

Examples of their system construction costs (not including shed costs):

Eels:	5.5 Euro (AUD 9.00)	/ kg of fish produced
Tilapia :	1-2 Euro/ kg (AUD 1.60 – 3.30)	/ kg of fish produced
Sea Bream	7-8 Euro/ kg (AUD 11.50-13.20)	/ kg of fish produced

7.2.2 Hesy system in Urk

This facility was being constructed for ex-commercial fishermen with financial support of the European Union of up to one million Euros. The Dutch government is encouraging commercial fishermen to move into aquaculture to reduce the pressure on the wild fish stocks, particularly in the Zuiderzee.

Julian de Bondt from Hesy showed us around the facility under construction in Urk. The building, 1800 m^2 under roof and infrastructure, cost €360 000 (AUD 600 000) and the equipment cost a further €400 000 (AUD 660 000). The facility is designed to produce 120 tonnes of pike perch *Sander lucioperca,* which is a fresh water species. Estimated product cost per kilogram is €3.89 with sale price of around €8.00.

As seen in the photos all drainage is cemented in place. The main drain goes to the water treatment area of the shed and the minor drain to a centralised grading tank. Most of the equipment for the recirculating system was being unpackaged at the time of our visit and tanks made out of high density polyethylene were under construction (HDPE).

One piece of equipment that was of particular interest to us was the special flexible oxygen pipe that was being installed. To fit the farm with copper piping would have cost more than €15 000 (AUD 24 700) but the flexible piping was installed for €500 (AUD 825).

Another impressive aspect, and something that is a major advantage of recirculation systems, is that it takes less than six months to get all the necessary government permits to operate the farm.

The whole shed is operated on less than 80 kW/h of electricity. If the farm was operated at maximum capacity this would give an annual electricity cost of around $125 000 based on Darwin electricity prices.

Figure 7.1 The Hesy built recirculating farm for pike perch in Urk, the Netherlands.

Figure 7.2 The Hesy company logo

Figure 7.3 Inside the fully insulated shed

Figure 7.4 Stack of pre-fabricated 'double drains' ready to be installed.

Figure 7.5 The 'skeleton' of the biological trickle filter. The sump in the foreground is designed to hold the drum filter.

Figure 7.6 The flexible piping used to reticulate oxygen around the shed. Much more cost effective than copper.

Figure 7.7 The polymer mixing tank. Effluent water from the drum filter is pumped into this tank, mixed and then the flocculant goes to the belt filter.

Figure 7.8 The belt filter, just out of the box and ready to be installed.

7.3 Cell Aquaculture Systems Europe B.V

Address: De Holm 10a, 9356 VB Tolbert, The Netherlands.

Cell Aquaculture is an Australian company which started back in 1999 in Western Australia. Over the past seven years they have researched and developed the EcoCell^{tm} "Hatch to Dispatch" recirculating system. This is designed to be used as a low cost modular system that can be placed close to large population centres. They are currently targeting both American and European markets.

The site visited in Tolbert was set up as a display farm and received its first barramundi fingerlings in March 2006. The fish originated from the Darwin Aquaculture Centre. It consists of 16 modular systems located inside a large shed that was a chicken farm in a previous life. The system is capable of producing 66 tonnes of barramundi, which they believe is the minimum amount required to make barramundi production economically viable. Systems are design to handle stocking densities of up to 75 kg/ m^3 but at the time of our visit they were only running at 2 kg/ m^3.

7.3.1 Summary of discussions

We met with the company's local manager. He was brought in from Australia to set up the demonstration farm. He escorted us on a farm tour and explained the details of the company's modular 'cell' system for barramundi production.

Each modular system comprises:

- 2 x 10 000 litre tanks and 1 x 4000 litre HDPE tank
- A mechanical filter, which is a homemade belt filter fitted with a 63 µm screen. Screens are attached by Velcro and are removed and cleaned daily.
- A moving bed reactor biofilter.
- Oxygen stones, supplied from an oxygen generator. Oxygen controlled manually to all tanks – no automatic control.
- 2 x 1 Hp pumps

Other features:

- They are going to use ozone to maintain an oxygen redux potential (ORP) of 120-200mV.
- All feeding is done by hand.
- The farm is designed to be run by 2-3 people.
- A nursery area is located at the back of the shed, but it is not physically isolated from the grower tanks.
- The production cycle consists of keeping the fish for two months in the nursery, two months in the 4000 litre tank and then two months in the 10 000 litre tanks.
- They had no special plans for waste treatment, other than collecting it and trucking it off site.

Figure 7.9 Cell Aquaculture's demonstration farm, near Tolbert, the Netherlands.

Figure 7.10 Inside the shed. The former chicken shed has been refitted as a fish farm. The tanks are all high density HDPE and there are 16 'cells' each containing 2 x 10 000 litre and 1 x 4000 litre tanks. The shed is dimly lit to less than 150 lux.

Figure 7.11 Examining the dedicated nursery area at the back of the shed.

Figure 7.12 Oxygen generator attached to a large air dryer (to the right).

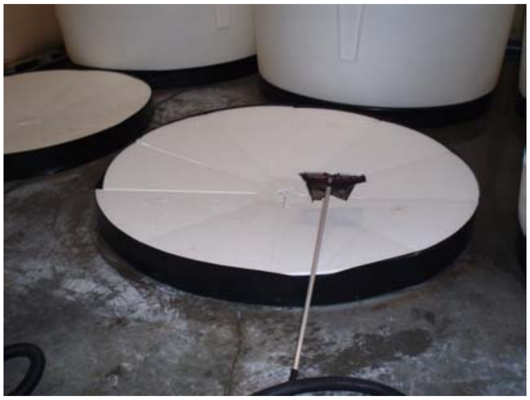


Figure 7.13 The bases of the culture tanks are made from wedge shaped pieces of polystyrene.

Figure 7.14 Cell Aquaculture intend using ozone in their system and the ozone generator was just being installed when we visited.

7.4 The Netherlands - summary

- The Hesy system is a proven technology. The company has been involved in aquaculture production for many years and has a number of farms successfully using their system.
- The Hesy design is reasonably simple and relies on the construction of a large trickling biofilter as the primary source of biofiltration.
- The claimed construction and operating costs of a Hesy system are reasonable compared with the value of the fish produced.
- Hesy's grower tanks, made from HDPE welded on-site is a cost effective method of construction.
- Cell Aquaculture's system may be attractive for some investors, in that it can be easily retrofitted into existing sheds without the need to cut concrete etc.
- The Cell Aquaculture system would benefit from the addition of more automation as the system observed in the Netherlands appeared to be very labour intensive. Particularly, automation of the oxygen system would seem to be required as the manual adjustment of 48 tanks on a daily basis could become a safety issue for the fish…ie incorrect adjustment or an unexpected change of oxygen conditions in the tank could be disastrous.
- Two of the clear advantages of recirculating systems in the Netherlands are the existence of substantial European Union subsidies to encourage fishermen to take on aquaculture and also the efficient licensing system which can see farms up and running in a matter of months.

8.0 Denmark

8.1 Introduction

Ranking sixth in the world's leading exporters of fish products, Denmark has a strong position in fish production and aquaculture has a long and well established tradition in the country. The main product produced is rainbow trout from freshwater ponds and mariculture units, the latter also producing roe as an important by-product. Eels are farmed in recirculated freshwater tank systems; mussels and oysters are produced in minor quantities and turbot fry are exported for further on-growing. A variety of other species are raised primarily for restocking for recreational fishing which represents an increasing share of total turnover.

In 2003 total annual aquaculture production in Denmark was around 36 000 tonnes, or 3.3 per cent of the total fish production in Denmark, but it was worth 20 per cent of the total value of fish produced. Earnings from the aquaculture sector were about US $114 million, making it worth more than the economically important Danish cod fisheries; about 90 per cent of production goes for export.

Danish aquaculture is strictly regulated by environmental rules, with the exception of full recirculation eel farms, all Danish fish farms have to be officially approved in accordance with the Danish Environmental Protection Act. A fixed feed quota is assigned to each individual farm in addition to specific requirements including feed conversion ratios, water use and treatment, effluents, removal of waste and offal, etc. Fresh water farms are required to use their waste as fertiliser.

Eel farming

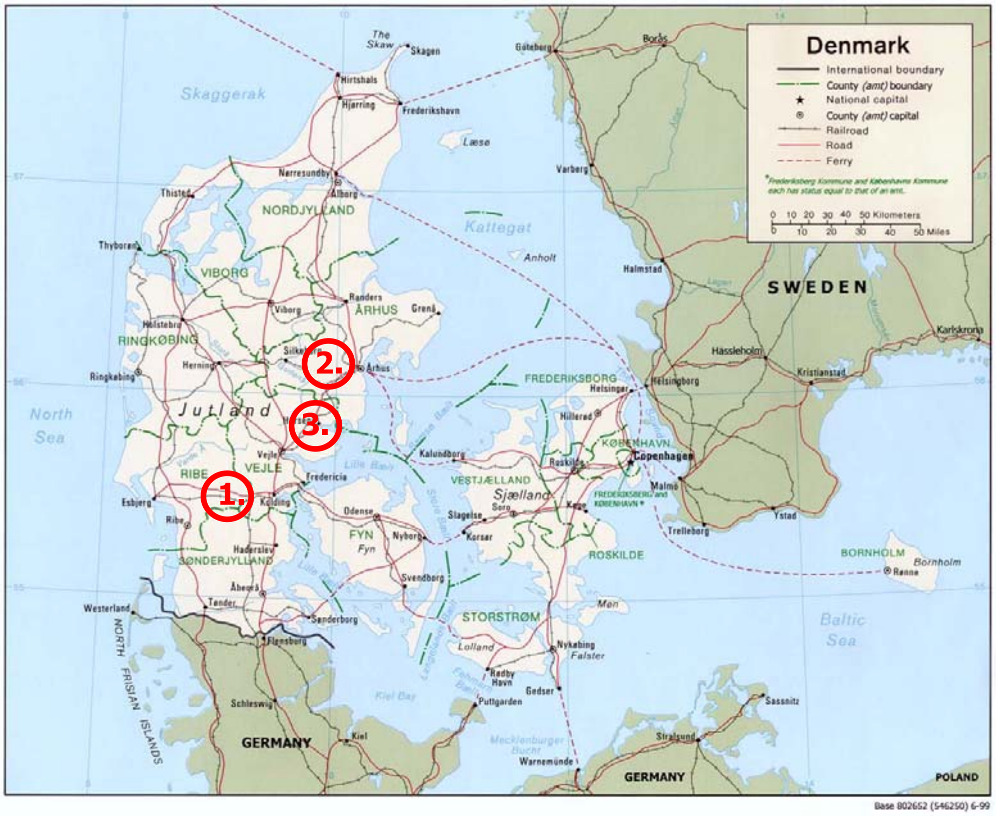
In Denmark, eel (*Anguilla anguilla*) farming is still considered a relatively new activity, having been in existence for about the last 25 years. The recirculation technology used to farm the eels is now well established and is also suitable for a number of other species. Denmark currently has 11 eel farms.

Despite many years of research it is not yet possible to produce eel fry in captivity and successfully breeding eels remains as one of the 'Holy Grails' of aquaculture.

Fry (glass eels) caught from natural waters are the raw material for eel farming. A proportion of the production is sold as fingerlings for restocking purposes; however, the majority are on-grown and exported for human consumption at about 100–200 grams each. Smaller amounts are grown to a size of 300–800 grams.

Production from Danish eel farming peaked in the late 1990s at about 3000 tonnes a year from a total of 30 farms. Since then, many of the farms have closed and the 2003 production dropped to 2000 tonnes, worth US $17 million from 11 farms. However, Danish eel aquaculture as an industrial sector is sustaining a developing industry for recirculation technology that has an important export market.

With recirculation technology requiring strict waste management, Danish eel farming has had no difficulty in complying with environmental regulations. Danish eel farming technology is of a high standard and there is a considerable level of export of this technology and know-how.



Map 2.0 Map of Denmark showing the places visited. **1.** Billund Aquaculture Service ApS (head office and eel farm, Billund; **2.** InterAqua Advance ApS, Hornslet; **3:** Dana Feed ApS, Horsens.

8.2 Billund Aquaculture Service ApS

Address: Kløvermarken 27, DK-7190 Billund, Denmark

Billund Aquaculture is a private company with considerable experience in the design and operation of intensive fish farms. According to their website they promote themselves as the leading company in the world in respect of design and management of modern fish farms.

The company's experience in recirculation system design and operation has been gained in co-operation with Danish research institutes and experts in water treatment systems. The practical know-how has been obtained through their own production facilities from which they have been successfully producing eels for more than 22 years.

Billund's intensive fish farms are based on a modular concept and they tailor their designs to individual and specific requirements. The modular basis for construction means that expansion of the farm is possible without causing major disruption to ongoing production. It also ensures a high degree of production safety. The modules are isolated from each other which helps biosecurity.

8.2.1 Summary of Discussions

We met with the company's Managing Director, Christian Sørensen. Christian explained some of the company's history and gave a brief outline of current projects. One project near Australia they have recently finished (within the last three year's) is a large recirculation system at the Gondol Research Institute of Mariculture (GRIM) in Bali.

Billund manufacture some of their equipment on site as well as coordinating purchases from other manufacturers. Like Hesy they are also involved in system development in Chile.

Christian Sørensen estimated that the Billund system suitable for barramundi production can be constructed for between 6000 Euro (~AUD 9900) to 8000 Euro (AUD 13 500) per 1000 kg of production depending on the level of intensification of production.

Christian believed that the salt content in the fish waste collected from a marine system would not present significant problems, particularly if it was spread over a wide area of farmland and mixed with fertilisers of other origin.

As an approximate guide the claimed construction and operational costs for the Billund system are as follows:

High intensive:

This design is used for species like eel and tilapia and also hatchery and fingerling units for both freshwater and sea-water fish. The system has a very low exchange of new water (approx. 100 - 200 litres / kg feed/ day).

The required investment level is approximately 8000 euro per tonne production. A complete 100 ton unit, including equipment and buildings would cost 800 000 Euro or around AUD 1.35 million. Electricity consumption is estimated at 5 kW/ kg feed. Based on Northern Territory electricity prices a plant producing 100 tonnes of fish per year would cost about $115 000* to run (assuming a food conversion ratio of 1.3:1 and an electricity tariff of 17.5 cents per kW hour).

*Calculations: 100 000 kg average standing stock x 1.3 = 130 000 kg of feed x 5 kW per kg feed = 650 000 kW per year x 17.5 cents = $113 750.

Intensive:

This system is suitable for species such as barramundi, sea bass, salmon smolt, trout and sturgeon. A higher exchange of new water is required at a rate of around 600 - 800 litres / kg feed/ day.

The required investment level is approximately 6.000 euro per tonne production. A complete 100 tonne unit (including equipment and building) would cost 600 000 Euro or AUD 990 000.

The estimated electrical consumption is 2.5 kW / kg feed. Based on Northern Territory electricity prices a plant producing 100 tonnes of fish per year would cost about $57 000* to run (assuming a food conversion ratio of 1.3:1 and an electricity tariff of 17.5 cents per kW hour).

*Calculations: 100 000 kg average standing stock x 1.3 = 130 000 kg of feed x 2.5 kW per kg feed = 325 000 kW per year x 17.5 cents = $56 875.

The company estimates that investment required for a 600 tonne farm would be 5-10 per cent lower per tonne of production than for a 100 tonne farm.

Approvals for fresh water recirculating fish farms in Denmark are relatively straightforward and can be obtained within a few months of application. One of the requirements is that all waste produced by the farm must be re-used as fertilizer.

Denmark has very restrictive environmental regulations for aquaculture development and when these were introduced several years ago they were seen as causing the stagnation of the country's aquaculture industry.

8.2.2 Billund Aquaculture Eel Farm

As indicated in the introduction to Billund Aquaculture, the eel farm has been operating successfully for over 22 years. The farm is located in a series of sheds on a 100 hectare agricultural farm about 20 minutes drive from the township of Billund.

The information obtained from the farm visit is listed below:

Production information:

- The farm produces 250 tonnes of market sized (150g) and 4 million fingerling size, eels per annum.
- Glass eels are sourced from all over Europe and then either grown to fingerling size (2-3 grams) for sale to other eel farmers or on-grown to edible size, average 160 grams.
- Eels can be stocked at 60 kg/ m^3 for the smaller sizes and up to 250 kg/ m^3 for final growout.
- It takes up to 20 months for the eels to reach 160 grams.
- The eels cost €6.0 to 6.5/ kg to produce, and sell for €8.5 to 9.0/ kg.
- 1250 kg of feed per day at an average Food Conversion Ratio (FCR) of 1: 1.
- Five staff operate the farm.

Water quality and filtration system:

- Water from tanks is gravity fed to a sump and then passes through a large Hydrotec disc filter.
- Biological filtration consists of a trickle filter, moving bed reactor, and a submerged filter.
- After the biofilters the water is pumped from another sump to oxygen saturators and back to the tanks.
- All tanks are automatically monitored and controlled for DO and pH.
- A large exhaust fan is positioned above the biofilters to remove CO^2.
- The juvenile tanks have a water exchange rate of two times per hour and the larger tanks are between one and two times per hour.
- The farm does not use ozone but does treat ⅓ of the recirculated water with UV.
- 250 000 litres of new water are used per day on the farm. The new water comes from a bore at 8-10° C.

Waste Removal:

- A secondary, small drum filter is positioned after the disc filters to further concentrate the solids in the waste water.
- The discs on the filter are pressure washed manually every two weeks in addition to the normal automatic backwash.
- The submerged biofilter is backwashed every two weeks.
- After the small drum filter the waste goes to a 600 000 litre settling tank and the overflow water from this tank goes to earthen reservoir.
- The settling tank is cleaned out once per year. Water from the reservoir is used for irrigation.
- All tanks are fitted with a double drain system (see Figures 8.5 and 8.6). Some of the screens on the double drains are fitted with a self cleaning system.

Oxygenation:

- Liquid oxygen is used to supply conical saturators. The saturators are located below floor level adjacent to the main biofiltration areas.

Biosecurity:

- No footbaths or hand wash stations were evident however visitation is strictly controlled.
- The water quality appeared to be of a high standard and the facility was clean and well maintained.
- Production units were housed in three physically separated sheds.

Other:

- The facilities are designed for forklift access between the tanks for the transport of feeds and eels.
- An automatic feeding system is used throughout the farm.
- All tanks can be discharged into a centralized grading area.

Figure 8.1 Inside Billund Aquaculture's eel farm. These 6000 litre tanks hold the glass eels and juveniles.

Figure 8.2 The 30 000 litre grower tanks.

Figure 8.3 These are juvenile eels showing they are quite adept at climbing walls!

Figure 8.4 A tank full of hungry eels almost ready for the market.

Figure 8.5 A double drain on one of the juvenile tanks. Water from the centre of the tank, upwells to the left of this dish and then passes through the round, self-cleaning, screen and away to the biolfilters.

Figure 8.6 Same concept as Figure 8.5, except this is a double drain on a grower tank. It has the added feature of a slot cut into it which serves as a surface skimmer.

Figure 8.7 The recirculating system attached to the juvenile tanks. The blue 'box' at the back is the trickle filter and the oxygen cones are to the left.

Figure 8.8 The disc filter attached to the main growout system. The discs are removed and pressure cleaned every two weeks.

Figure 8.9 The small drum filter attached to the backwash stream of the disc filter. This filter further concentrates the solid waste before it goes outside to a settling tank.

Figure 8.10 The 600 m^3 outdoor settling tank.

Figure 8.11 The overflow from the settling tank goes to a storage dam (in the distance) and from there it is used for irrigation.

Figure 8.12 The nitrate concentration of the recirculated water is reduced by passing some of the flow through a denitrification tank, which is supplied with alcohol to drive the process.

8.3 Inter Aqua Advance ApS

Address: Rosenholm Udviklingspark, Sortevej 40, DK-8543 Hornslet, Denmark

According the Inter Aqua Advance web site the company was established in 1978 and was one of the first companies in the world to offer recirculation water treatment systems. Ever since Inter Aqua Advance claims to have been at the forefront of research and development in this area. Through the years they have focused on the highest possible water quality, low energy consumption and user-friendly designs. Their system design is now up to its third generation and they state that it is currently unsurpassed by any other concept. They have a continuing development program and aim toward keeping the systems at a level that is competitive – and cheaper in production cost and management than conventional systems.

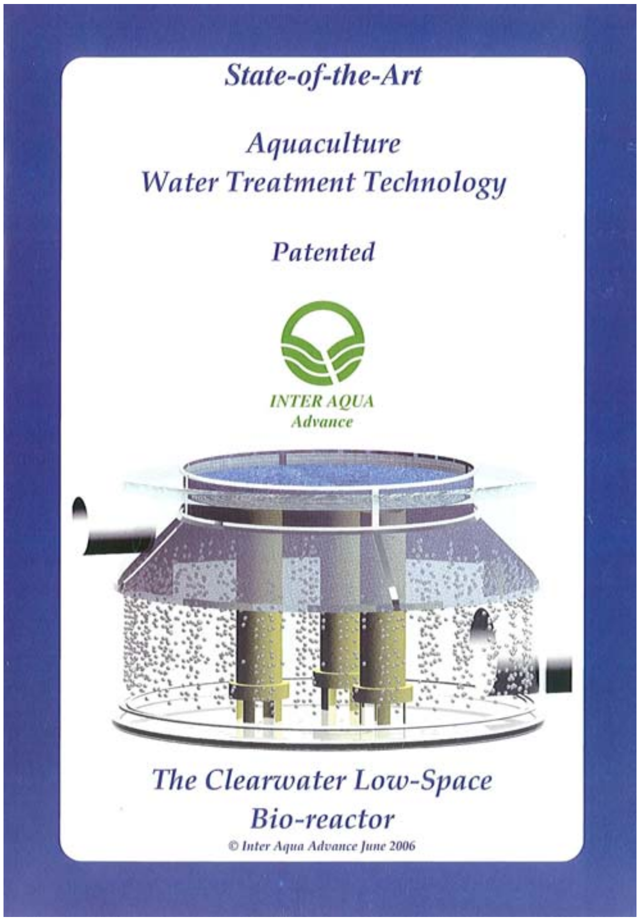
8.3.1 Summary of discussions

One of the key design components of the InterAqua system is that it operates with a low pumping head. While most contemporary system have a pumping head (the distance between the water level of the tanks and the top of the biofiltration towers of at least 2.5 metres, InterAqua systems have a head height of around 1.5 metres. The higher the head the higher the pumping cost, therefore InterAqua systems cost less to operate per volume of water moved. Having a low pumping head means that it makes it cost effective to have an increased water flow through the fish tanks which makes for better water quality.

Features of the InterAqua Advance system:

- Low head system ~1.5 m vs 2.5 m
- High recirculation rate of 3000 litres/ second!
- The company has developed a patented low head oxygenation system.
- The biomedia developed by InterAqua, 'curler advance' is claimed to be superior to other plastic media because of its open design which prevents clogging and gives improved nitrification performance.
- Curler advance biomedia is used in the company's Clearwater low-space bioreactor. This bioreactor is designed with an internal airlift system to maintain oxygen, off-gas CO^2 and to keep the biomedia moving and operating with a thin, healthy biofilm for improved nitrification performance.
- Recently InterAqua has developed a new, non-mechanical filtration system. Termed 'contact filters' they consist of long raceways filled with sinking plastic media. The media slows the flow of the recirculated water, causing solids to fall out of suspension. The raceways are flushed periodically to remove accumulated wastes.
- Contact filters are already operating successfully in large trout farms in Denmark.
- Simple cost estimate for a plant producing 600 tonnes of fish per year:
  - €2.9 million for the plant (AUD 4.8 million)
  - €0.4 million for the shed (AUD 660 000)
  - €1.9 million production cost (AUD 3.2 million)
    - Feed AUD 1.5 million
    - Electricity AUD 300 000
    - Labour AUD 700 000
    - Stock AUD 480 000
    - Maintenance, insurance, ancillary costs etc AUD 200 000
- Income estimate from 600 tonnes x AUD 8.00/ kg = AUD 4.8 million

In recent years the company established one of their systems for yellowtail kingfish in New Zealand. The farm was not successful apparently because of communication difficulties between Denmark and the farm's local managers. InterAqua said that they always back up their sales with quality service, but in this instance their recommendations were not implemented by the farm managers.



State-of-the-Art

Aquaculture
Water Treatment Technology

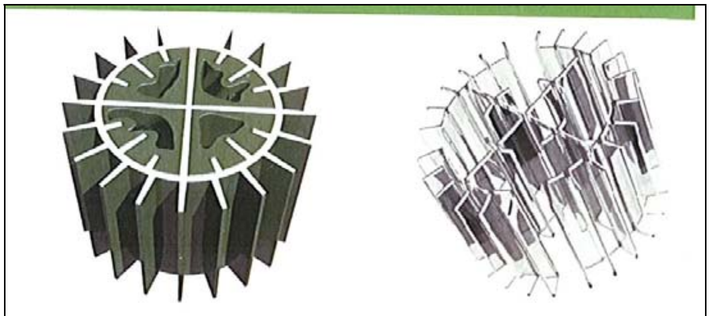
Patented

INTER AQUA
Advance

The Clearwater Low-Space
Bio-reactor

© Inter Aqua Advance June 2006

Figure 8.13 The cover of the brochure for InterAqua Advance's patented biofilter design.



Graphical presentation of a clogged filter element.

Graphical presentation of a clean filter element.

Figure 8.14 InterAqua Advance's patented biomedia and its anti-clogging design.

8.4 Dana Feed ApS

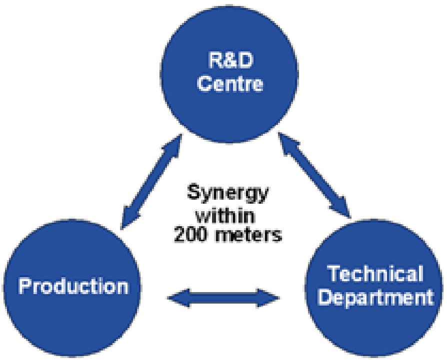
Address: Havnen 13 DK-8700, Horsens, Denmark

The Dana Feed company has specialised in the production of aquaculture feeds since 1997. The company has developed a reputation for innovation and producing feeds with improved environmental performance (less wastage). In 2001 the company opened a dedicated R&D facility to help it further improve its aquaculture feed products.

DANA FEED Research Centre

The Dana Feed research centre is a modern well-equipped building situated on the waterfront of the harbour of Horsens – only a few minutes walk away from the Dana Feed factory.

The company claims that its research activities are precise and applied, meaning that they are aiming at creating new, improved production processes and feed products. The research activities take place in a dynamic process within the technical and production departments of the company. The company believes this ensures interplay and synergy.



The objects of the Research Centre

- Optimization of existing mixed feedings.
- Test of new mixed feedings.
- Test of new ingredients.
- Test of technical qualities in fish feed.
- Development and optimization of feed programmes.
- Documentation and comparative analyses of competing feed products.
- Establishment of a scientific basis for the research work performed in cooperation with customers and suppliers.
- An attractive place to visit – with the purpose of exchanging information.

8.4.1 Summary of discussions

We met with two of Dana Feed's R&D 'engineers', Troels Samuelsen and Bjarne Vestbö.

The Dana Feed R&D centre houses three separate recirculating systems. One is designed and built by Hesy, one by Billund and one built in-house. Troels said both systems performed well but that he had a slight preference for the Billund system and he believed it was a bit more robust.

The purpose of the three recirculation systems was to conduct R&D on feed development. The systems could be operated as either fresh or salt water and they had been used for a variety of different species. At the time of our visit they were used to house trout, sturgeon and turbot.

Part of our reason for visiting Dana Feed was to discuss the use of their larval and broodstock diets. Many of the farms we visited during this European study tour were using Dana Feed products and were very complimentary of the products.

The Dana Feed researchers were interested in further discussions of possible research collaboration and we are interested in obtaining their broodstock and larval diets to trial with barramundi.

Figure 8.15 The entrance to Dana Feed's R&D Centre at Horsens.

Figure 8.16 Overview of the Centre's Billund Aquaculture tank system.

Figure 8.17 This system was built 'in-house' for flat fish experiments.

Figure 8.18 Overview of the Centre's Hesy Aquaculture system

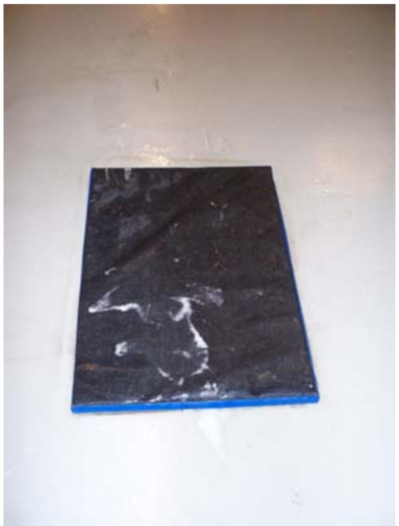


Figure 8.19 Biosecurity was implemented by the use of footbath mats, hand washes and overcoats.

Figure 8.20 The sign says it all.

Figure 8.21 They were conducting feed research on sturgeon. Very placid and enchanting fish.

Figure 8.22 All the tanks in the centre were mapped on the computer based oxygen monitoring system.

Figure 8.23 Foam fractionation in their homemade system. The horizontal approach.

Figure 8.24 The view from their lunch room window was as equally impressive as the rest of the facility.

8.5 Denmark Summary

- Billund Aquaculture's recirculating systems have been operating successfully for more than 22 years.
- The company uses a combination of biofiltration methods within its system design.
- Recirculating systems are particularly cost-effective for species that can be grown at extremely high densities, such as tilapia and eels.
- InterAqua's low head system design is a good concept but needs to be proven on a full commercial scale for marine systems.
- InterAqua's contact filter design appears to be a very cost effective solution to improving filtration in recirculating systems.
- Dana Feed's larval rearing and broodstock diets should be assessed for their performance with barramundi.
- Development of collaborative projects with Dana Feed should be investigated.
- Like the Netherlands, Denmark has a simplified approvals process for the establishment of recirculated aquaculture systems.

9.0 Norway

9.1 Introduction

The history of Norwegian marine farming during the last forty years is a history of the development of an expansive and dynamic export industry. It is one of Norway's most international industries, exporting to over 100 countries, that is characterised by its natural localisation along most of the coastline.

Norway manages some of the world's largest and most productive coastal and sea areas. A long protected coastline, accessible areas and clean sea water with a high water replacement rate and good water quality provide good biological prerequisites for aquaculture production.

The Norwegian aquaculture industry exports about 90-95 per cent of its production. Atlantic salmon and rainbow trout represent by far the largest part of the Norwegian aquaculture industry, and the export in 2005 was about 513 000 tonnes representing a value of NOK 14.5 billion (AUD 2.9 billion). The European Union, Japan and Russia are the most important markets for farmed Norwegian salmon and trout. Species like cod and blue mussels are new species in the Norwegian industry. The production of farmed cod has expanded from 170 tonnes in 2001 to 3168 tonnes in 2004, and the production is estimated to reach about 40 000 tonnes in 2010.

9.2 Institute of Marine Research, Austevoll Research Station

Address: Austevoll, N-5392, Storebø, Norway

The research station is located on the island of Austevoll 36 kilometres South of Bergen.

Institute of Marine Research Austevoll is one of the largest, most advanced research facilities of its type in Europe. Since its modest start with a full-time staff of two in 1978, the station has developed into a major research centre.

The Institute operates a number of experimental research facilities and laboratories with a range of highly specialised analytical instrumentation. It focuses primarily on the cultivation of marine species and employs 30 staff. At the time of our visit they were conducting research mainly on Halibut, breeding cleaner wrasse and some work on cod.

Figure 9.1. Aerial photograph of the Austevoll Research Station.

Map 3.0 Map of Norway showing the places visited. **1.** Bergen area - Austevoll Research Station; Aqua Niva; Marine Harvest Cod Hatchery (CCN); **2.** Fjord Halibut, Otrøya; **3.** Trondheim locations: AquaOptima Norway AS head office, Trondheim and Fosen Aquacenter, Fosen.

9.2.1 Site information

Incoming water is sourced from 50 metres below sea level and passed through sand filters before being used. Some of the systems in use are straight flow through with no water treatment whilst some are full recirculating designs.

The Centre was subjected to strict biosecurity protocols with plenty of quarantine stations fitted out with footbaths and hand wash facilities. Signage pointing out the quarantine policy was evident throughout.

We were shown around the facility by Dr Anders Mangor-Jensen, senior scientist, environmental physiology.

9.2.2 Cleaner wrasse

The first section visited was where they were undertaking research into the culture of ballan wrasse, *Labrus bergylta*, a species of 'cleaner' wrasse. Ballan wrasse are being cultured for use as a control method for sea lice in the salmon net pens. Sea lice *Lepeophtheirus* *salmonis* have been recognised as a health problem in Norwegian fish farms for many years and Norway has so far been the leading country in the practical use of cleaner wrasse to control these parasites on commercial salmon farms. One of the issues with the use of cleaner wrasse is that the salmon often eat them. It is hoped that the use of the larger, ballan wrasse that this will be less of an issue, especially when the wrasse are stocked at a similar size to the salmon. Ballan wrasse grow to a maximum size of 60 cm.

Ballan wrasses are found naturally from Trondheim in the north of Norway, down along the European and British coasts through to the Canary Islands. A research project involving a PhD student from New Zealand, Simon Muncaster, has been set up with the aim to produce large numbers of wrasse. He has been able to induce the adults to lay fertilised eggs on spawning mats within the tanks and he has successfully taken some of the larvae though to juveniles.

Ballan wrasses also have a reputation as being a great addition to fish soups around Western Norway. Simon indicated that he had spoken to several people that would like to try and market it as a table fish. He has heard unconfirmed reports that at the moment it can be worth up to AUD 16.00 per kilogram. This could prove to be an added bonus to salmon farmers in the future.

9.2.3 Halibut

At the time of our visit there was a production run of halibut underway in one of the Centre's research areas. Unfortunately these fish had just been diagnosed with a nodavirus infection. This meant that, due to the Research Station's biosecurity protocols, we were unable to visit any other sections after the halibut.

The juvenile halibut were housed in 2000 litre tanks in an insulated room. The tanks had self cleaning arms run off a centralised motor located underneath the tank. The cleaning arms were made out of long windscreen wipers that rotated around 1-1.5 times an hour sweeping the tank debris into one easily cleaned location.

Tank strikes (where larvae hit the wall of a tank and damage themselves) can be a problem with halibut during the larval rearing phase. To overcome this, the larval rearing tanks were fitted with 'donut' lids that shielded the tanks edges in darkness and concentrated the light into the centre of the tanks. The positively phototactic larvae were thus attracted away from the tank walls into the centre.

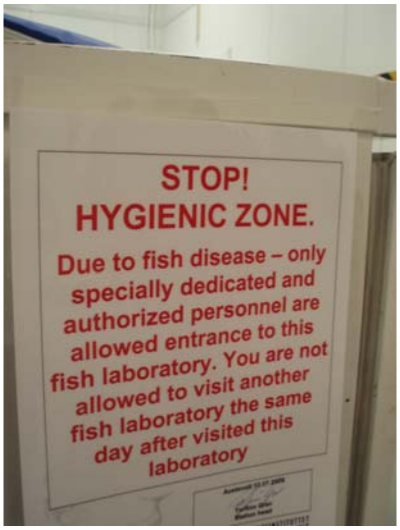
Figure 9.2 One of the broodstock tanks for the Ballan cleaner wrasses. The green spawning mat can be seen on the bottom near the fish.

Figure 9.3 Much of the cleaner wrasse research is being undertaken inside an old fish tank…good recycling!

Figure 9.4 Part of the halibut larval rearing system.

Figure 9.5 Jaw deformities (lower larva) can be a major problem with halibut.

Figure 9.6 Plastic flow gauges were in use through out the facility and appeared to be more robust than the plastic gauges with metal flow indicators that have previously been on offer in Australia.



STOP!
HYGIENIC ZONE.
Due to fish disease – only specially dedicated and authorized personnel are allowed entrance to this fish laboratory. You are not allowed to visit another fish laboratory the same day after visited this laboratory

Figure 9.7 The sign says it all.

Figure 9.8 The hygiene station at the entrance to the facility.

Figure 9.9 Old broodstock never die…they just end up on your wall.

Figure 9.10 Part of the automatic tank cleaning system for the larval rearing tanks. The wiper blade attaches to a central rotating shaft. See 9.11

Figure 9.11 The inside of a halibut culture tank. The rod in the centre of the tank is the shaft to which the cleaning blade attaches. It is driven by a motor positioned underneath the tank.

Figure 9.12 All oxygen controls for the larval rearing system are in one, easily accessed panel.

Figure 9.13 Austevoll also has a small cage system so they can conduct research into netpen culture of cod and halibut.

9.3 Norwegian Institute of Water Research (NIVA)

Address: Regional Office Bergen, Nordnesboder 5, NO-5005 Bergen, Norway

NIVA was formed in 1958 and is now Norway's leading multidisciplinary research institute in the field of use and protection of water bodies and water quality, in both fresh and salt water. It consists of the foundation of the Norwegian Institute for Water Research and its two subsidiaries Akvaplan-NIVA AS, of which NIVA owns 93 per cent and NIVA Tech AS, of which NIVA is the sole shareholder.

NIVA has a research section dedicated to farmed and wild fish, with a particular focus on connections between water quality and fish health. It also undertakes consequence analyses and coastal zone management. This section is also involved in projects in several developing countries. Typical areas of work involve water quality requirements for fish, counselling for the fish-farming industry, effects on the environment from fish farming, impact analyses and localisation studies, fish studies and migration and remediation measures, as well as investigation and follow up on acute fish deaths.

At the NIVA office in Bergen we met with the branch manager of western Norway, Dr Åse Åtland, Dr Erik Vikingstad, aquaculture consultant and Dr Atle Foss, senior aquaculture consultant.

9.3.1 Summary of discussions

- The groundwaters in the western part of Norway often have high levels of Aluminium and iron and consequently also have pH problems. Ten per cent of farms experience iron levels above 200 µg/ litre. NIVA is investing in research to remediate these waters to make them suitable for aquaculture. The addition of sodium silicate to the water supply helps to restore the water to a usable quality, as does the addition of sea water to help remove the excess iron.
- They have found a correlation between high CO^2 levels in the hatchery/ nursery phase and slower growth later on.
- Slow growth has also been found in fish subjected to NH_3 levels above 2 mg/litre.
- They are trialling a 'cyclic' feeding program control the onset of maturation of halibut. Maturation is a problem with many farmed fish species and farmers prefer that their fish put energy into somatic (muscle) growth instead of reproductive development.
- Halibut can go for up to one year without feed. The trials have tested a feed for five weeks, starve for five weeks cycle. The trials have been going for two years and so far indicate a better food conversion ratio because of compensatory growth and it looks like maturation is being controlled.
- They are also investigating the use of high intensity culture of spotted wolffish *Anarhichas minor*, in shallow raceways. These fish are being cultured at an impressive density of 600 kg/ m^3. Other marine fish are cultured at 100 kg/ m^3.
- Some other facts and figures about wolffish are that they hatch after 1000 day/degrees and immediately start feeding on dry diets. Going straight onto a formulated diet is very unusual for a marine fish. The fish are cultured at a temperature of 5 ^{o}C and it takes them three years to reach a market size of 5 kg. Fillet yield is just over 50%.
- NIVA are currently the only producer of wolffish fry in the world.
- We were supplied with a copy of a paper on the use of ozone in aquaculture written by Helge Liltved. This is attached as Appendix 4.

Figure 9.14 NIVA is housed inside an old herring storehouse. You can still smell the herring in the wood!

Figure 9.15 Apart from the interesting discussions we had with the NIVA staff, they also had the flashest coffee machine we saw on tour!

9.4 Marine Harvest Cod Hatchery

Address: Øygarden, Sandviksboder 78, PO Boks 4102, Dreggen 5835, Bergen, Norway

Marine Harvest is the world's largest producer of finfish with operations in 80 countries over five continents employing 6000 people. They work with a variety of species such as:

Atlantic salmon	*Salmo salar*
Halibut	*Hippoglossus hippoglossus*
Atlantic Cod	*Gadus morhua*
Barramundi	*Lates calcarifer*
Tilapia	*Oreochromis niloticus*
White Sturgeon	*Acipenser transmontanus*
Japanese Yellowtail	*Seriola quinqueradiata*

We visited Marine Harvest's cod, *Gadhus morhu*, hatchery, Cod Culture Norway (CCN) in Øygarden about 45 minutes drive North of Bergen, and were shown around by Thor Magne Jonassen. The facility is situated within a large insulated shed and employs nine staff. It was built to produce eight million 2 gram fish. Last year they produced 2 million x 10 gram fish over four batches at a cost of AUD 1.42 per fish.

9.4.1 Summary of discussions

- Incoming water is pumped from 160 metres below sea level and is treated by sand filtration before being utilised.
- Netting is placed over broodstock tanks to prevent stock from jumping out and plastic curtains are placed around individual tanks providing biosecurity and photoperiod control.
- Broodstock weigh in at around 20 kilograms and are fed twice a week. Their diet consists of a mixture of prepared pellets and attractants rolled into gelatinous balls as well as trash fish.
- All tanks have oxygen monitoring equipment attached, including dissolved oxygen (DO) probes placed in the outlets of each tank and electronic flow gauges. All collected data is fed to a centralised computer.
- Ideal growing temperature for cod at this hatchery is 12-13 °C.
- We were told that cod can tolerate a temperature range from 4-20 °C but start to stress at 16 °C and over.
- Thor suggested that the cod can also tolerate brackish water, ~20ppt, as they are found in the fjords.
- Spawning in broodstock tanks occurs naturally with eggs collected externally from the holding tank.
- Hatching tanks are stocked with two million eggs per 250-260 litres. These tanks have an up welling water flow with no surface skimmers and are kept at 9 °C.
- The incubation period is 12 days with newly hatched larvae about 3 mm in length. Mouths open two to three days later and first feeding commences between days 7-8.
- Juvenile grow out consists of 10 x 9 tonne tanks which are stocked at 20-30 kg/m^3.
- Fish are moved into nursery cages on farms at 10 grams. Fish reach 4-6 kilograms within 18 to 24 months.
- These tanks are run off a French designed recirculated system comprising of a French designed (Faivre) drum filter, protein skimmer, UV treatment and sand filtration.
- Tanks are self cleaning. Cleaning arms are run from a small motor and gear box located centrally on top of the tank and rotate 1.5 times per hour. This differs from the

'underneath' motors we saw at Austevoll and seemed to be more practical in terms of servicing the motors.

- Rotifers are produced in 10 x 5 tonne batch culture tanks and are fed a variety of diets such as Selco, Algamac and live algae. They had previously used the same Japanese chlorella as the DAC to grow rotifers, but stopped (couldn't tell us why) and now use chlorella paste produced in Germany.
- They have a continuous algae culture system consisting of a series of clear glass tubes filled with small plastic balls to keep the tubing clean. Artificial lighting is produced by both halogen and fluorescent lights (Fig. 9.20).
- Rotifers are rinsed when required and then placed into cold storage and enriched for 24 hours.
- No *Artemia* are used as larvae move straight from Rotifers to artificial food.
- Biosecurity was enforced with strict hygiene employed at the site. Visitors are made to wash hands with disinfectant, shoe coverings and disposable over coats were also issued.

Take away comments:

- Centralised oxygen monitoring equipment is a practical and user friendly way of setting up a system. Could look at implementing this in the future at DAC
- The level of biosecurity employed on this site sets a good example.
- Cleaning arms in tanks looked like a good idea in a low flow system.
- Chlorella paste produced in Germany may be an option for us if we find there are problems with the Japanese paste.

Figure 9.16 Biosecurity was a key feature of CCN. A hand wash station was located at the entrance to each functional area

Figure 9.17 Protective coats and boots must also be worn.

Figure 9.18 Cod broodstock are housed in controlled environment tanks and are spawned throughout the year.

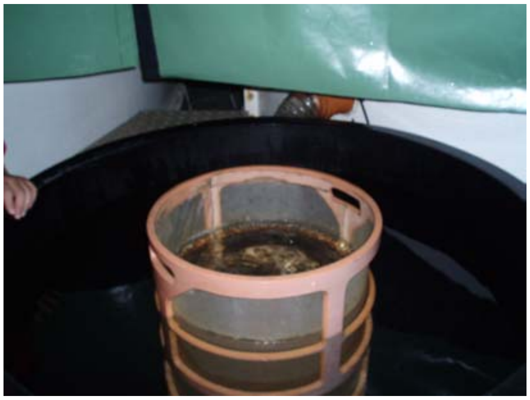


Figure 9.19 Cod egg collector. On the day we visited they obtained 24 litres (12 million) eggs.

Figure 9.20 Part of their continuous culture system for microalgae.

Figure 9.21 This farm also used a centralised oxygen distribution and monitoring station.

Figure 9.22 A vacuum degassing column was part of the recirculation system for the nursery tanks.

Figure 9.23 They had recently purchased a new Vaki brand grader. Although efficient we have doubts on how practical it is to operate. It appears to be very 'top heavy' and potentially unstable.

Figure 9.24 The main sump, sand filters and degassing area for the recirculation system. This was considered to be 'old' technology.

Figure 9.25 Part of the cod nursery. Each tank was serviced by a small Arvo Tek automatic feeder.

9.5 AquaOptima Norway AS

Address: Kjøpmannsgt. 35, 7011, Trondheim, Norway

AquaOptima started back in 1993. It has delivered components and recirculating systems in over 16 countries offering complete onshore hatcheries of both recirculating and flow through design. It caters for cold and warm water species such as Rainbow trout, Arctic Char, Tilapia, European Sea bass, sea bream, halibut, Atlantic cod, Japanese flounder (Hirame), tiger puffer and more recently barramundi.

Our visit was originally arranged with the Managing Director, Idar Schei who unfortunately was unable to be present. We met instead with Øyvind Aaleskjaer their Marine Biologist. Øyvind had previously visited us in Darwin and so was a familiar face. He provided us with an overview of facilities and equipment that they have designed and installed. Two of these facilities, Fjord Halibut farm and Fosen Aquacenter are located in Norway and we were able to visit both these hatcheries.

One stand out piece of equipment that is designed and produced by AquaOptima is the patented Eco-trap. The trap is a modified centre drain for a fish tank that is designed to remove up to 90 per cent solids from the tank using a small amount of water. The EcoTrap comes in a variety of sizes ranging from 110 to 400 mm but can be designed larger if required. Waste collected by the Eco Trap is diverted to an 'Eco-Sludge' collector, located on the side of the tank. The 'Eco-Sludge' is a small swirl separator. The installation of the Eco-Trap system is claimed to allow for a 50 per cent reduction in the size of mechanical filtration in a system. Its other advantages are that it is a passive, non-mechanical system with little to no chance of failure.

Recently AquaOptima have assisted with the installation of a large recirculation system for barramundi in the UK. The Aqua Bella farm located in New Forest, England was constructed in 2004 and is designed to produce 400 tonnes of barramundi per year. Harvesting from the facility commenced in March 2006. It is a fully recirculated system comprising of 48 tanks maintaining water at 28 ^{o}C whilst treating three million litres of water a day. New water is added at the rate of 5 per cent per day. AquaOptima's management were very interested in developing more projects with barramundi.

AquaOptima have recently developed a simple method for the construction of large octagonal tanks. The use of large plastic formed, lock in place panels that can be core filled with concrete offers an easily transportable and cost effective solution to tank construction.

AquaOptima recommend the Salsnes belt filter over the Hydrotec brand. They said that even though the Salsnes brand was more expensive, the after sales service was second to none.

Figure 9.26 AquaOptima's Eco-Trap drain.

Figure 9.27 The adjustable swirl separators that attach to the Eco-Trap drain.

Figure 9.28 Detail of the junction plates between two Eco-Tanks

Figure 9.29 AquaOptima's oxygen saturator.

Figure 9.30 These plastic tubes are used for distributing air beneath trickle filters (see 9.31).

Figure 9.31 Plastic air diffusers assembled in series.

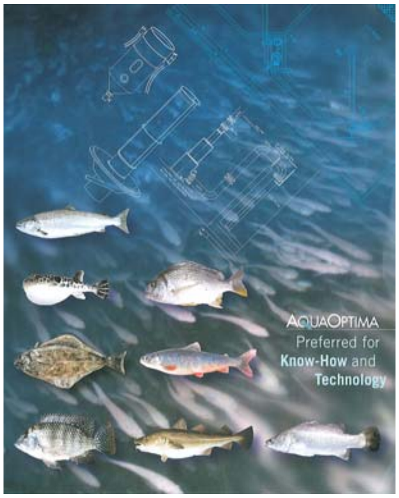


Figure 9.32 Cover of the AquaOptima brochure. Note the barramundi, bottom right.

Figure 9.33 AquaOptima also have a nice view from their office window!

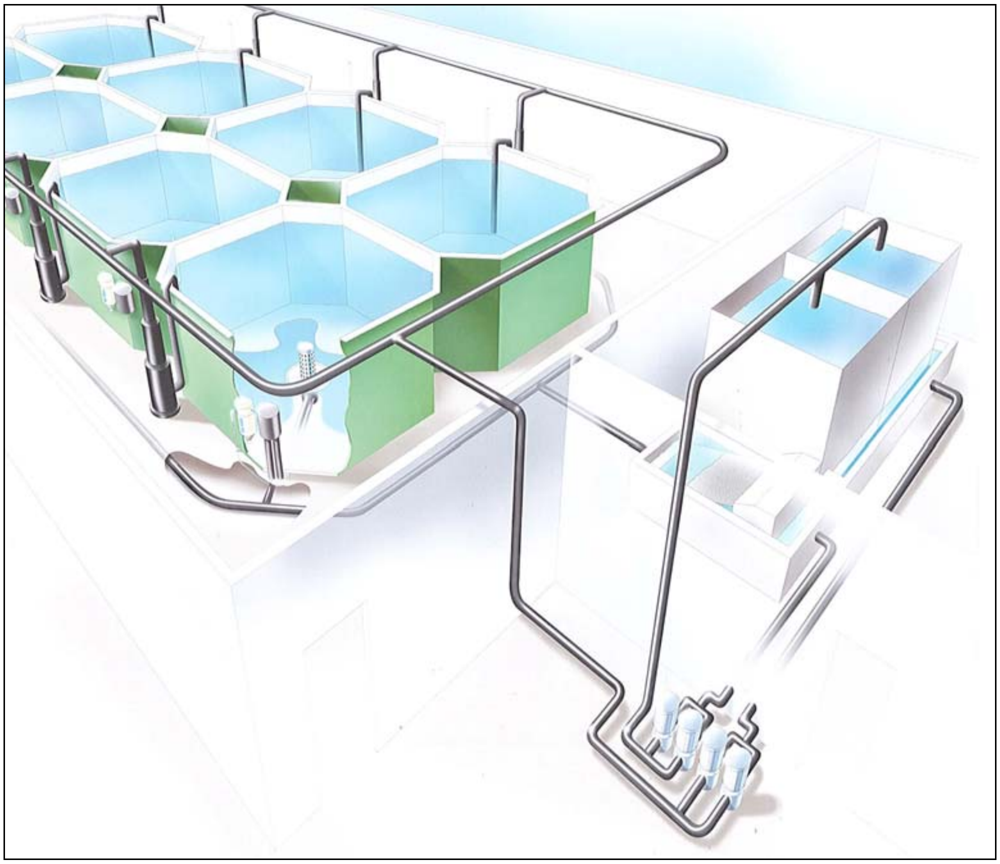


Figure 9.34 Schematic of an AquaOptima tank system.

9.6 Fjord Halibut Farm, Otrøya

Otrøya is an island about 20 km from the town of Molde on the west coast of Norway and has a total population of 2000 people. Olav, the Hatchery Manager, was our tour guide.

9.6.1 Summary of discussions

The farm was started back in 1984-85 by a couple of guys with only a few tanks and it was one of the first private hatcheries in Norway. They installed an AquaOptima system in their hatchery two years ago.

The hatchery now employs nine staff and is now owned by a Trucking magnate. It was designed to produce two million x two gram fish but we were told they would be happy to produce 300 000 fish. Apparently they need to produce at least 130 000 fish to break even.

Supply water to the hatchery is sourced from 230 meters below sea level and comes in at 8°C all year round with little variation. Water is pumped through a Hydrotec drum filter before entering a header tank. Water is then pressurised before being passed through sand filters and heat exchangers. Once this is completed the sea water passes through both fan forced and vacuum driven degassing columns before being used in the hatchery.

As halibut are a deep sea species, broodstock are housed in large 40 000 litre tanks which are totally enclosed in plastic curtains keeping them in darkness. Males on site range in size from 6-10 kgs and don't grow much bigger than 14 kg. The females kept for breeding are up to 60 kg but can grow as big as 200 kg. To handle these large fish the tanks are fitted with a moveable shelf system. When the broodstock need to be examined they are manoeuvred onto the shelf which is then winched out of the water.

Broodstock can spawn naturally in tanks and are also milked when mature. Females produce up to half of their body weight in eggs. One litre is the equivalent of 40-50 000 eggs.

We couldn't recommend one of their broodstock tank designs. As seen in figure 9.41 its only access door is located on the side of the tank with limited clearance. For these reasons the tank cannot be totally filled and it makes moving fish into and out of the tank extremely difficult.

Other points of interest were:

- Incubation of eggs takes 14 days.
- Non viable eggs are removed in the hatching tanks by using a salinity gradient to settle waste to the bottom.
- Takes 40 days for the larvae to absorb their yolk sac.
- The larvae start feeding on newly hatched and one day old enriched Artemia and continue on these for a period of 30 days.
- Various enrichment diets are used including combinations of Algamac and Selco products.
- Initially juvenile halibut are kept in shallow tanks (30 cm deep), however they start jumping when they are about 10 grams and then have to moved to deeper (1.5 metres) tanks covered with netting.
- Halibut are benthic (bottom dwelling) fish and to improve the utilisation of the water volume in the deeper tanks the surface area is increased by adding shelving.
- Deformity rates vary between 10-80 per cent. The majority of these include disfigured mouths, poor migration of the eyes and skin pigmentation.
- This farm has not had Nodavirus detected on site to date but tests were only conducted once a year.

Waste water treatment equipment is carried out by the following means:

- Waste is concentrated from tanks by the use of AquaOptima Eco-Traps. Olav was not impressed with the performance of the Eco-Traps but AquaOptima told us it was simply a matter of getting the correct adjustment.
- All the recirculating tanks drained to a belt filter located in a centralised sump. Note that the belt filter was not installed correctly and there was dead space between the filter and the incoming water; this allowed waste to settle and become anaerobic.
- Nearly 50 per cent of the water is passed through a protein skimmer with ozone treatment added and maintained at 350 mV. Note that accurate readings of ozone were hard to obtain.
- The other 50 per cent passes through a trickle filter which also forms a degassing column. Degassing is carried out via the use of large amounts of aeration beneath the filter.

Fjord Halibut has just been through a period of upgrading its equipment, including the following :

- Oxygen generator
- Vacuum degassing
- Ozone generator
- Protein Skimmer
- Back up pumps
- Large generator, big enough to be used to top up the local power grid!

Biosecurity at this site was employed with the use of footbaths and hand washes.

Figure 9.35 Fjord Halibut is located on the island of Otrøya in Midsund Kommune.

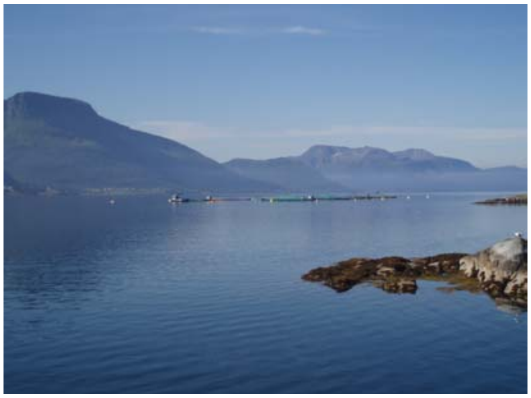


Figure 9.36 Part of the pristine Norwegian coastline which surrounds Otrøya.

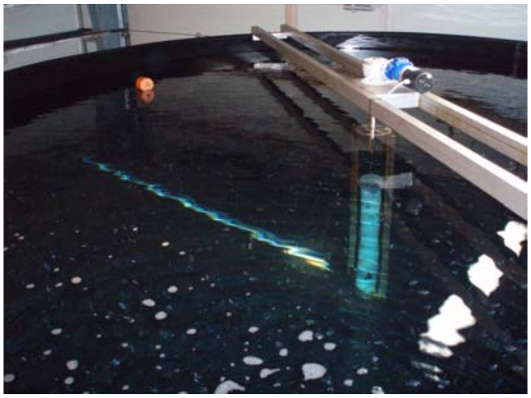


Figure 9.37 Juvenile halibut tank. The self cleaning bar can be seen through the water on the bottom of the tank. The small motor on top turns the brushes once per hour.

Figure 9.38 The brushes for the self cleaning tank system.

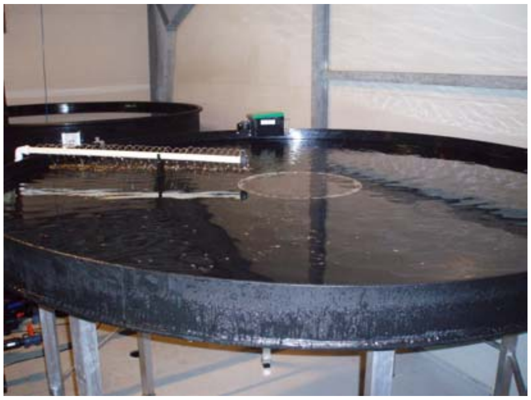


Figure 9.39 One of the advantages of working with flat fish…flat tanks.

Figure 9.40 Juvenile halibut ready to be transported to a growout farm.

Figure 9.41 Don’t try this at home! Not a very practical design for a broodstock tank.

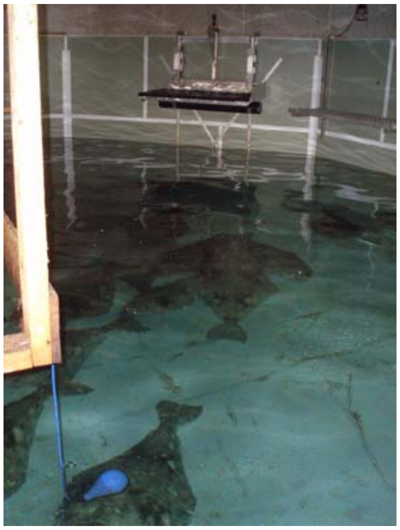


Figure 9.42 Some of the large female broodstock inside their broodstock house. The lifting shelf for the fish can be seen in the background.

Figure 9.43 Tank surface area is increased by the addition of small shelves.

Figure 9.44 Sponge mat style footbaths were used for disinfection. This type of mat has now been installed at the Darwin Aquaculture Centre.

Figure 9.45 Some of the equipment in the Fjord Halibut plant room. L to R, main recirculation pumps; oxygen generator; ozone generator. The problem of not having a floor drain in the bottom of the, below ground level, plant room has recently been overcome.

Figure 9.46 'Clip-lock' connectors and plastic oxygen tubing were in use in many of the facilities visited.

Figure 9.47 Vacuum degassing towers used to treat new sea water.

9.7 Fosen Aquacenter A/S Norway

Fosen Aquacenter is a cod hatchery and growout farm in Fosen Kommune, near Trondheim in the Center of the Norway's west coast. The farm uses all AquaOptima equipment.

9.7.1 Summary of discussions

When we visited the farm was going through its annual shut down and clean out phase. Some of the juvenile tanks still had fish in them but the main growout system was empty.

One interesting piece of information we obtained from this farm is that they rarely use their recirculation equipment, instead preferring to use a flow through operation. Given the high quality of Norway's coastal waters this is perhaps understandable. It is cheaper to simply pump the water in and out, rather than go to the trouble of treating the water and then recirculating. This does however raise the question of managing biosecurity and we guess that until the farm has disease problems they will continue to go for the simple option.

Because they were on a flow through system, collection and re-use of waste was not being practiced...it all went out with the effluent.

The octagonal AquaOptima tanks appeared to operate well and seemed to be a better option than space inefficient round tanks, while still maintaining good self-cleaning properties.

We did observe a number of (what we thought were) occupational health and safety issues with this farm, particularly with regard to electrical outlets near to water in the tanks and awkward positioning of tanks and equipment which would make operation potentially unsafe.

Figure 9.48 Outside Fosen Aquacenter.

Figure 9.49 Biosecurity was very evident here.

Figure 9.50 The AquaOptima designed bio towers and recirculation system

Figure 9.51 The juvenile cod tanks in action

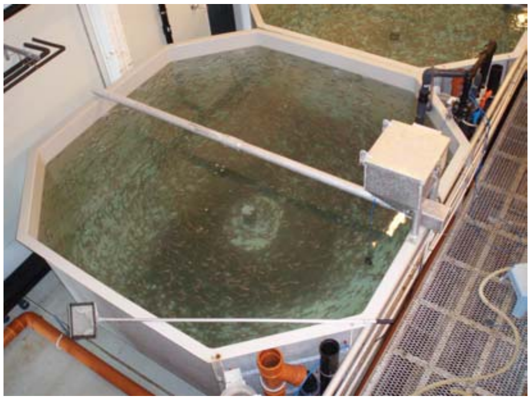


Figure 9.52 Overview of a juvenile cod tank.

Figure 9.53 One of the large cod growout tanks that had been drained for seasonal maintenance. The Eco-Trap drain is shown disassembled.

Figure 9.54 One of the rotifer production tanks. The proximity of the electrical outlet (top left) to the tank (less than 500 mm) is of some concern.

Figure 9.55 Walkway between the tanks in the growout area of the farm. One of the problems of retrofitting an old building as a fish farm can be seen in this photo…note the overhead beams and lack of head clearance.

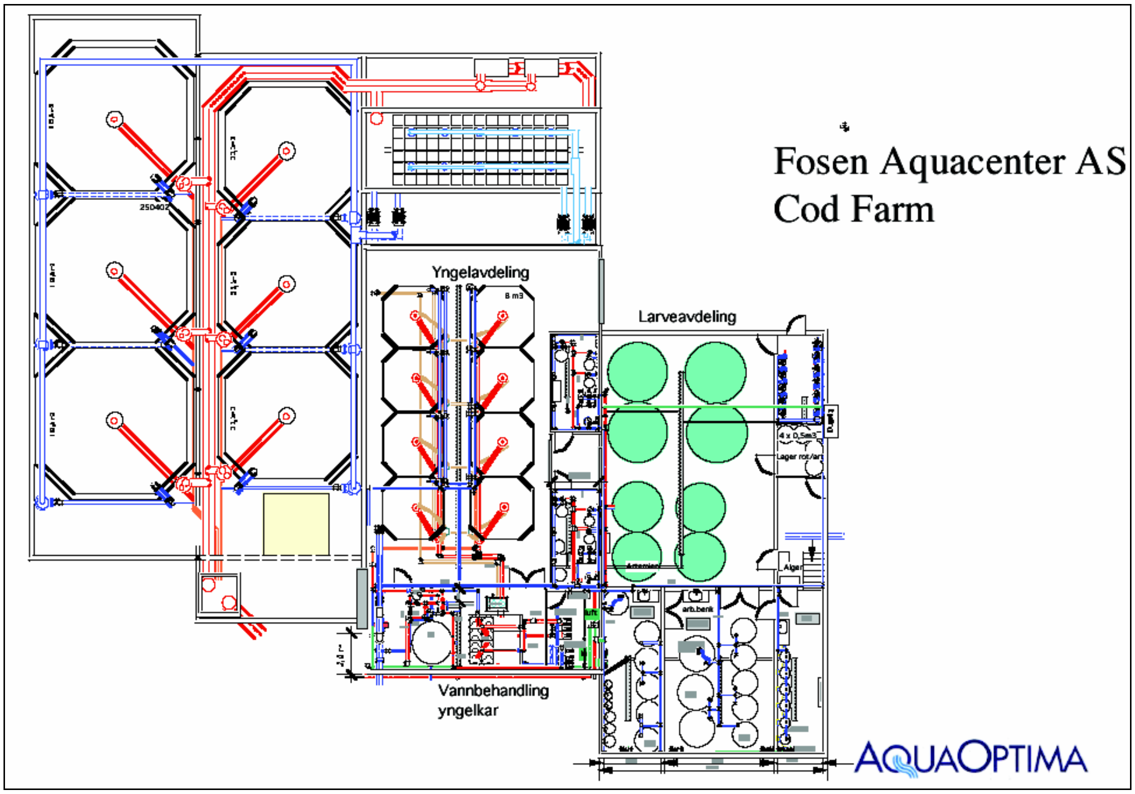


Figure 9.56 Plan drawing of the Fosen Aquacenter cod farm.

9.8 Norway – Summary

Norwegian aquaculture continues to boom. Most of this growth is being driven by the salmon industry which makes use of the extensive Norwegian coastline and its pristine water to grow the fish in sea cages. Recently the high prices being obtained for farmed salmon have meant many farms are back to making healthy profits. The other reason that sea cages are still popular in Norway is that country has not seen the need to impose the same overly restrictive environmental controls as Denmark. This difference has seen Norway surge ahead and Denmark struggle.

Despite this 'sea cage' focus, Norway is continuing to investigate and refine its technology for recirculating fish farms. It was however interesting to note that, by comparison with the sea cage industry the recirculating farms still appeared to struggling financially.

In fairness to some of the farms we visited, the timing was probably not the best for them as it coincided with their annual shut down and clean out. However our discussions with the local managers confirmed that it was not necessarily an easy way to make money. One farm that is apparently doing very well using AquaOptima technology is the Tiger puffer farm in Japan. We originally hoped to visit this farm on the return leg to Australia but were unable to due to time restrictions. Tiger puffer are a high valued delicacy much favoured in Japan.

If we refer back to the plenary session of the conference in Roanoke, it was claimed that recirculation technology was still mainly the preserve of high value species, hatcheries and intensive nurseries. The farms we visited in Norway appear to support this statement.

The take home points from Norway are:

- Barramundi appeared to be the 'flavour of the month'. Like Denmark and the Netherlands, some of our discussions in Norway revolved around the suitability of barramundi to recirculating aquaculture. AquaOptima are already heavily involved with this species at the Aqua Bella farm in the UK.
- Vacuum degassing is considered to be an essential treatment for the inlet water of marine hatcheries. Vacuum degassing lowers dissolved nitrogen levels whereas pressure degassing lowers CO^2. This may be of special importance in Norway where the pumps inlets are often more than 100 metres below sea level. Super saturation of gases can be a problem when this water is brought up to the surface.
- The Eco-Trap centre drain system is an efficient, non-mechanical means of reducing waste in recirculating systems.
- Pre-fabricated, plastic panels are available for the easy construction of large, octagonal tanks for the AquaOptima system.
- NIVA are doing some interesting research on new fish species. What particularly caught our eye was the 'cyclic feeding of halibut to control maturation and improve FCR's and the fact that wolffish can be grown at densities above 500 kg/m^3.
- Ozone is still not routinely used.
- The Salsnes brand filters may be a better option than Hydrotec because of better after sales service.
- Even the best designed recirculating equipment is no use without good management and technical ability.

10.0 Recommendations

The specific recommendations from each stage of the study tour are contained within Table 1. Specific recommendations that apply to government, industry and training are detailed below.

10.1 Government

One of the main recommendations we would make to government agencies is that re-circulating aquaculture needs to be embraced at the government level. What is required is a simplified approvals process that acknowledges the benefits, in terms of reduced environmental risks, of this type of culture. The target timeframe for the approvals and licensing process should be modeled on the Netherlands and Denmark, with both countries claiming to achieve this inside of six months.

The use of recirculating system wastes as fertilisers is an accepted practice in the Netherlands and is in fact a mandatory procedure in Denmark. The Australian industry should be strongly encouraged to adopt similar practices.

10.2 Industry

It was obvious from this tour that recirculating aquaculture still has some way to go to prove that it is a cost effective production method compared to large scale pond and cage culture methods. The recommendation we would make is for industry to keep a watching brief on re-circulating technology and most importantly to look at those companies who are already investing in this area, to see if there are any significant developments that improve its cost effectiveness. Despite the claims of the manufacturers, it is still difficult to find many companies successfully operating large scale, high tonnage, recirculating production systems. The exception to this is fresh water aquaculture of eels and tilapia, two species that tolerate extreme crowding, and the culture of high value marine species (eg tiger puffer in Japan) or the high value, early life stages (hatchery and nursery) of both fresh and salt water species.

At this stage we would hesitate to recommend the routine use of ozone in recirculating marine systems, instead suggesting that targeted use of ozone in larval rearing and for system disinfection is more appropriate.

One of the main messages we would have for the Australian industry is to adopt many of the strict biosecurity measures we saw in operation during the tour. Appropriate signage and detailed biosecurity protocols, including control of visitation and staff movement on-site, physically separate quarantine areas and thorough disinfection methods, should all be adopted.

10.3 Education and training

The design and operation of recirculating aquaculture systems should be an integral part of aquaculture based courses in Australia. It is up to the course coordinators to ensure that the material being presented to students is the most current available. Incorporating the use of specialist overseas lecturers should be considered to ensure that Australian students are given the best available information.

Another recommendation is that Australian engineering based courses (TAFE's/ Universities etc) should consider including aquaculture subjects such as recirculating system design, into their curricula.

10.4 Further Skill Gaps

It is difficult to identify further skill gaps as the technology for recirculating systems is continually evolving. We were unable to obtain definitive information from our trip on the effective application of ozone to marine recirculation systems. The main concerns expressed to us were the risks to staff health from the use of ozone in enclosed environments. We recommend that a watching brief be maintained on ozone usage to see if it emerges that there are clear, unequivocal benefits from the use of ozone and that staff well being can be protected.

11.0 Appendices

APPENDIX 1. *Trip Itinerary*

MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
		July 19 Dep. Darwin: 00:30 Arrive Syd: 06:10 Depart Syd: 07:55 Arrive LA: 07:00 Depart LA: 09:05 Arrive. Wash: 17:00 Dep Wash: 21:40 Arrive: Roa: 22:37	**July 20** Recovery!	**July 21** 6th International Recirculating Aquaculture Conference. Roanoke, Virginia USA.	**July 22** 6th International Recirculating Aquaculture Conference. Roanoke, Virginia USA.	**July 23** Hire car and drive to Washington. Dep. By train at13:25 Arrive Newark: 16:19 Dep: 18:00 to London
July 24 Arr. London: 06:20 Dep. London:07:45 Arr. Amster. 10:00 Pick Up Hire Car	**July 25** Hire car Visit Arie De Bondt (Hesy). 09:00 Visit Hesy, Urk. 12:00 Afternoon Travel to Denmark via Germany. O/nite in Hamburg	**July 26** Hire car Arrive Billund in the afternoon	**July 27** Hire car Visit Billund Aquaculture 09:00 Travel to Hornslet in the afternoon.	**July 28** Hire car Inter Aqua Advance Hornslet 09:00	**July 29** Hire car Travel to Hirtshals to catch ferry	**July 30** Hire car/ ferry Depart Hirtshals 10:00 Travel o/night to Norway
July 31 Hire Car Arrive Bergen 08:00 Institute of Marine Research, Austevoll.	**August 1** Hire Car Norwegian Institute of Water Research (NIVA). Visit Marine Harvest cod hatchery 12:00	**August 2** Hire Car Travel to Molde	**August 3** Hire Car Molde – Fjord Halibut farm 09:00 Travel to Trondheim	**August 4** Hire Car Trondheim Fosen Aquacenter 09:00	**August 5** Hire Car Drive to Oslo and on to Larvik	**August 6** Hire Car Depart Larvik via ferry: 09:00, arrive Hirtshals 15:30
August 7 Hire Car En route to Amsterdam	**August 8** Hire Car Cell Aquaculture Tolbert 09:00	**August 9** Drop off hire car 10:00 am. Dep. Amst. 14:00 Dep. London 22:05	**August 10** Arrive Changi 17:55 Dep. Changi 22:15	**August 11** Arrive Darwin 04:15	August 12	August 13

APPENDIX 2. *List of people visited*

Contact	Company/ Institution	Location	Phone / Fax	Date/time for visit	email address
Netherlands					
Arie de Bondt	Hesy Aquaculture BV	Bovendijk 35-Z 2295 RV Kwintsheul The Netherlands	+31 (174) 22 01 40 (T)	July 25, 09:00	hesy@planet.nl
Julian de Bondt	Hesy Aquaculture BV	Urk		July 25, 12:00	hesy@planet.nl
	Cell Aquaculture Systems Europe B.V	De Holm 10a, 9356 VB Tolbert, The Netherlands.	+31 594549939 (T) +31 594520033 (F)	August 8, 09:00	h.rutgers@cellaqua.nl
Denmark					
Christian Sørensen	Billund Aquaculture Service ApS	Kløvermarken 27, DK-7190 Billund, Denmark	+45 75 33 87 20 (T) +45 75 35 35 27 (F)	July 27, 09:00	christian@billund-aqua.dk
Jens Ole Olsen	Interaqua Advance APS	Rosenholm Udviklingspark Sortevej 40 DK-8543 Hornslet Denmark	+45 88 80 99 98 (T) +45 88 80 99 88 (F)	July 28, 09:00	joo@interaqua.dk
Norway					
Anders Mangor-Jensen	Institute of Marine Research	Institute of Marine Research P.O. Box 1870 Nordnes 5817 Bergen, Norway	+47 56 18 22 63 (T) +47 56 18 22 22 (F)	July 31, 13:00	anders.mangor.jensen@imr.no

APPENDIX 2. *List of people visited*

Contact	**Company/ Institution**	**Location**	**Phone / Fax**	**Date/time for visit**	**email address**
Norway (cont.)					
Åse Åtland	Norwegian Institute of Water Research (NIVA)	NIVA Branch Office West Nordnesboder 5, 5005 Bergen	+47 55 30 22 50 (T) +47 55 30 22 51 (F)	Aug 1, 09:00	aase.aatland@niva.no
Thor Magne Jonassen	Marine Harvest	Øygarden (Sandviksboder 78 PO Boks 4102 Dreggen 5835 Bergen Norway)	+47 55 54 72 62 (T) +47 55 54 72 90 (F)	Aug 1, 12:00	Thor.Magne.Jonassen@marineharvest.com
Idar Schei/	Fjord Halibut, Molde	Kjøpmannsgt 35, 7011, Trondheim, Norway	+47 73 56 11 30 (T) +47 73 56 11 39 (F)	Aug 3, 09:00	idar.schei@aquaoptima.com
Idar Schei/ Øyvind Aaleskjaer	AquaOptima/ Trondheim	Kjøpmannsgt 35, 7011, Trondheim, Norway	+47 73 56 11 30 (T) +47 73 56 11 39 (F)	Aug 4, 09:00	idar.schei@aquaoptima.com
Idar Schei/ Øyvind Aaleskjaer	Fosen Aquacenter	Kjøpmannsgt 35, 7011, Trondheim, Norway	+47 73 56 11 30 (T) +47 73 56 11 39 (F)	Aug 4, 09:00	idar.schei@aquaoptima.com

APPENDIX 3. *List of people who assisted with the itinerary.*

Contact	Country	Company	Date contacted	email address	Result of contact
Clive Talbot	Europe/ Japan	Marine Harvest	22-Dec-05	clive.talbot@marineharvest.com	Passed on information to Thor Magne Jonassen
Ep Eding	Netherlands		22-Dec-05	ep.eding@wur.nl	The annual Dutch holidays prevented a visit.
Idar Schei	Norway	AquaOptima	22-Dec-05	idar.schei@aquaoptima.com	Ok to visit, gave details for farm near Molde, farm near Trondheim and another in Japan.
Arie de Bondt	Netherlands	Hesy	22-Feb-06	hesy@planet.nl	OK To visit
Jaap Van Rijn	Israel		22-Dec-05	vanrijn@agri.huji.ac.il	OK to visit, but we declined due to safety concerns
Tom Losordo	USA	NC State University	29-Nov-05	tlosordo@ncsu.edu	gave details for contacts in Israel and Netherlands
Thor Magne Jonassen	Norway	Marine Harvest	23-Dec-06	Thor.Magne.Jonassen@marineharvest.com	cod/ halibut hatchery- Ok to visit
Anders Mangor-Jensen	Norway	Institute of Marine Research	10-Feb-06	anders.mangor.jensen@imr.no	Ok to visit
Jens Ole Olsen	Netherlands	Interaqua Advance	6-Feb-06	joo@interaqua.dk	Ok to visit
Tony Rumbold	New Zealand	Scanz marketing	25-Jan-06		Gave contact details for Jens Ole Olesen
Christian Sørensen	Denmark	Billund Aqua	10-Feb-06	christian@billund-aqua.dk	OK to visit
Andries Kamstra	Netherlands	Solea	8-Feb-06	andries.kamstra@soleabv.nl	No response received
Kees Kloet	Netherlands	Zeelandvis	8-Feb-06	fishfarmyerseke@planet.nl	No response received
Helge Liltved	Norway	Norwegian Institute of Water Research	10-Feb-06	helge.liltved@niva.no	OK to visit – but visit with Aase Aatland in Bergen.

APPENDIX 4. *Ozone reference*

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High resistance of fish pathogenic viruses to UV irradiation and ozonated seawater

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Received 9 May 2005; accepted 10 May 2005

Abstract

Important fish pathogenic viruses, including infectious pancreatic necrosis virus (IPNV), Atlantic halibut nodavirus (AHNV) and infectious salmon anaemia virus (ISAV), were exposed to ultraviolet (UV) irradiation or ozonated seawater in a laboratory model system. Inactivation curves were obtained, and the occurrence of oxidants and ozonation by-products (OBP), including their removal by granular activated carbon (GAC) filtration, was studied. In ISAV, 99.9% inactivation was obtained by an UV dose of 7.7 mJ/cm^2. The corresponding figures for AHNV and IPNV were 105 and 246 mJ/cm^2, respectively. In ozonated seawater, total residual oxidants (TRO) were assayed by the DPD colorimetric method. The CT value (the product of TRO concentration and contact time) was calculated from the average TRO concentration (as mg Cl_2/l) during the respective contact time (s). 90% reductions in virus titers were obtained by CT values of 1.4 (mg s)/l for ISAV, 1000 (mg s)/l for AHNV and 1944 (mg s)/l for IPNV. The results of this study demonstrate a wide span in UV and TRO resistance among the viruses tested. ISAV was sensitive to both methods, while high resistance in AHNV and IPNV was experienced. The TRO resistance in IPNV and AHNV contradict earlier published results and suggests reconsideration of existing ozonation practise to inactivate these viruses in seawater. Considerably higher CT values than previously reported seem to be required. These discrepancies in results between studies clearly demonstrate the need for development of a standard procedure to conduct inactivation experiments and to develop improved analytical techniques to determine individual oxidants in seawater. Of the detected carcinogenic OBPs, bromate was found in concentrations of 50 and 70 μg/l after a contact time of 20 and 80 min, respectively, at a TRO concentration of 0.9 mg/l. Bromoform was the only trihalomethane (THM) found in significant amount. The bromoform concentration of 16.4 μg/l measured after 80 min contact time was reduced to 1.6 μg/l by GAC filtration with an empty bed contact time (EBCT) of 4.2 min. TRO and bromate was reduced below the detection limit after GAC filtration. However, long-term filtration experiments were not conducted. Such experiments should be conducted to determine the adsorptive capacity of GAC for bromate- and THM-removal in ozonated seawater.

Keywords: Viruses; Disinfection; Ozone; UV; By-products; Seawater

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doi:10.1016/j.aquaeng.2005.05.002

1. Introduction

Viral infections are one of the major problems in land-based aquacultural systems. Viral infections have been identified as a cause of setback in the commercialisation of several new marine species. In farming of Atlantic halibut (*Hippoglossus hippoglossus*), mortality caused by nodavirus is a significant problem in production of juveniles. In reports and strategic plans on farming of marine species, the requirements of hygienic barriers on all levels in the production (hatcheries, first-feeding and on-growing systems) is pointed out (Brock and Bullis, 2001; The Norwegian Research Council, 2001). It is important to gain knowledge on reliable methods to minimize the presence of viral pathogens in inlet water and recirculating water, and the potential impacts of the applied methods on the chemical water quality.

Ozonation and ultraviolet (UV) irradiation are the most frequently used methods for viral control in land-based aquacultural systems. These two methods can be used to eliminate pathogens in inlet water, effluent water and in recirculating water. Disinfection by ozonation and UV irradiation are also used in other aquacultural applications, for instance in reducing or eliminating potential pathogens associated with live prey such as rotifers in marine larval production systems and surface disinfection of fish eggs (Theisen et al., 1998; Munro et al., 1999; Grotmol and Totland, 2000).

1.1. Ozonation

Ozone is applied in most hatcheries, first-feeding and on-growing systems for marine species (flow-through or recirculating systems). In addition of being a powerful disinfectant, ozone is valued for its ability to improve general water quality if used correctly. Besides, the direct germicidal action fish diseases can be reduced by improving water quality due to reduction in environmental stress (Bullock et al., 1997). Application of low concentration of ozone in combination with flotation (ozone skimming) is widely used in seawater systems to remove particles and proteins. Several advantages of ozonation in recirculating systems have been reported (Bullock et al., 1997; Summerfelt et al., 1997; Krumins et al., 2001; Liltved, 2001; Tango and Gagnon, 2003; Summerfelt, 2003), including:

(1) reduction in total heterotrophic bacterial count;
(2) improved removal of colloids and smaller particles by filtration, sedimentation or flotation. Ozone may coagulate colloids and smaller particles into larger, more removable particles;
(3) oxidation of nitrite to nitrate;
(4) increase in biodegradability of organic compounds by creating smaller and more biodegradable molecules and thereby improved performance of biological filters;
(5) increase in oxygen content.

In freshwater, ozone decomposes rapidly to oxygen after application. By introducing ozone in aquacultural seawater systems, a series of redox-reactions take place and several reactive intermediates are formed. The halogen ions in seawater are oxidized by ozone to halo-oxy anions. The specific formation potential is highest for iodine and bromine and somewhat lower for chlorine species. Although the iodide ion may be completely oxidized, the low iodide concentration in seawater (<1 mg/l) makes the succeeding oxidation products less important. Typical bromide ion concentrations of 60–70 mg/l in seawater give a high formation potential of bromo-oxides, even at a common oxidation ratio of 10%. Correspondingly, chloro-oxy anions may be formed, but these are limited by a higher oxidation–reduction-potential barrier (Grguric et al., 1994).

The following reactions of ozone with bromide ion (Br^-) and hypobromite ion (OBr^-) have been proposed:

$$O_3 + Br^- \rightarrow O_2 + OBr^-, \quad k_1 = 160\,M^{-1}\,s^{-1}\,(20\,°C) \qquad (1)$$

$$O_3 + OBr^- \rightarrow 2O_2 + Br^-, \quad k_2 = 330\,M^{-1}\,s^{-1}\,(20\,°C) \qquad (2)$$

$$2O_3 + OBr^- \rightarrow 2O_2 + BrO_3^-, \quad k_3 = M^{-1}\,s^{-1}\,(20\,°C) \qquad (3)$$

The first reaction shows that ozone will oxidize the bromide ion in seawater to hypobromite ion. The hypobromite ion will hydrolyze into hypobromous acid (HOBr), which is a weak acid with a pK_a of 8.8 at 20 °C ($HOBr \leftrightarrow OBr^- + H^+$). The sum of HOBr and OBr^- is the biocidal bromine. In seawater with a typical pH of 8, hypobromous acid will predominate and be the most important disinfectant with a half-life of hours to days dependent on light conditions and water quality characteristics. Due to the acute toxicity of bromine to fish (LC_{50} of 0.068 mg/l BrO^- in rainbow trout) (Fisher et al., 1999), a treatment unit for removal of residual bromine has to be included when seawater is ozonated in aquacultural systems. Activated carbon filtration, addition of a reducing agent or passing through a sandfilter or biofilter will reduce or eliminate residual bromine (Ozawa et al., 1991).

The hypobromite ion can either be reduced back to bromide ion (2), or be further oxidized to bromate ion (BrO_3^-) (3). The bromate ion cannot be further oxidized and will be the final product of the oxidation of bromide ion in seawater. Bromate ion is a stable compound and not acutely toxic to aquatic animals. For fish early lifestages, a 96 h LC_{50} of 31 mg BrO_3/l of newly hatched larvae has been reported (Hutchinson et al., 1997). However, bromate is a known animal carcinogen (Kurokawa et al., 1986), listed as a probable human carcinogen by USEPA (2004) and has raised concern about possible bioaccumulation and chronic impact on fish health (Regli et al., 1992; Tango and Gagnon, 2003). The compound is regulated in ozonated drinking water. The European Drinking Water Directive sets the maximum contaminant level of bromate at 10 μg/l.

Natural and recirculating aquacultural seawater will contain organic substances that may be oxidized to halogenated organic compounds, such as trihalomethanes (THM). THMs in low concentration (4 μg/l) have been detected in recirculating aquacultural systems that use ozone (Liltved et al., 2002). The dominating THM in seawater is bromoform, which is a probable human carcinogen (USEPA, 2004), known to bioaccumulate in aquatic animals. Trials conducted by Gibson et al. (1980) showed that the bromoform concentration in aquatic animals was up to 50 times higher than in the seawater. The bioaccumulating properties of bromoform raise questions about human health risks associated with consumption of fish grown in ozonated seawater. Bromoform is highly persistent in the environment.

Use of ozone has been demonstrated to efficiently reduce or eliminate fish pathogenic bacteria and viruses in freshwater. Wedemeyer et al. (1978) found that a concentration as low as 0.01 mg/l inactivated infectious pancreatic necrosis virus (IPNV) within 1 min in phosphate-buffered distilled water, while elevated dosages were required in natural lake water due to ozone demand of water constituents. Of the few studies conducted to investigate the response of fish pathogenic viruses in ozonated seawater, Liltved et al. (1995) reported that TRO concentrations in the range of 0.10–0.20 mg/l as O_3 inactivated 99.99% of IPNV within 1 min, while Arimoto et al. (1996) reported that dosages of 0.1 mg/l for 2.5 min and 0.5 mg/l for 0.5 min eliminated the striped jack necrosis virus (SJNNV) (a member of the family Nodaviridae). It has also been revealed that by washing halibut eggs contaminated with nodaviruses in ozonated seawater, the development of viral encephalopathy and retinopathy (VER) in Atlantic halibut (*H. hippoglossus*) larvae was prevented (Grotmol and Totland, 2000). Residual oxidants concentrations of 0.3–10 mg/l as O_3 and exposure times of 0.5–10 min were used. Low levels of TRO (0.06–0.10 mg/l) have also been reported to inactivate bacterial fish pathogens in seawater (Sugita et al., 1992).

1.2. UV irradiation

Ultraviolet irradiation in the UV–C spectral region has become the first choice for disinfection of supply water and is included in most recirculating systems. The method is valued due to its effectiveness to inactivate fish pathogenic bacteria and some viruses (Liltved et al., 1995; Øye and Rimstad, 2001) and by not leaving toxic residues behind (Oliver and Carey, 1976; de Veer et al., 1994). However, indications of increased frequency of cataract of the eye in juvenile cod raised in UV treated seawater have been found (Björnsson, 2004). The effect was believed to be indirect, caused by chemical photoproducts from the UV irradiation.

The principal effect of UV–C irradiation in microorganisms is damage of the DNA or RNA caused by photo-induced dimerisation of opposite pyrimidine in the nucleic acid strand. Once the pyrimidine residues are covalently bound together, replication of the nucleic acid is blocked or results in mutant daughter cells unable to multiply (Stover et al., 1986). However, previous work has shown that UV–C inactivation of common fish pathogenic bacteria, such as *Aeromonas salmonicida, Vibrio anguillarum* and *Yersinia ruckeri*, can be temporary, due to repair mechanisms (Liltved and Landfald, 1996). Such repair should be taken into account when assessing the necessary dose for efficient UV disinfection of aquacultural waters. Repair has not been reported in fish pathogenic viruses, due to lack of essential enzymes, but high UV resistance has been demonstrated in IPNV. Required UV doses of 100–200 mJ/cm^2 for 2–3 log$_{10}$ units inactivation have been reported (Sako and Sorimachi, 1985; Liltved et al., 1995; Øye and Rimstad, 2001). High UV resistance has also been indicated for nodavirus. Frerichs et al. (2000) used an UV dose of 211 mJ/cm^2 for 99.9% inactivation of the sea bass (*Dicentrarchus labrax*) neuropathy nodavirus.

Due to the few published data on virus inactivation in seawater, the present study was initiated to supply fish farmers, consultants and manufacturers with more precise dose requirements of ozone and UV irradiation to accomplish inactivation of viruses important in aquacultural systems. Such data are crucial to establishing a firm basis for the design of disinfection systems and for better operation and control of existing installations. The virus studied were IPNV, Atlantic halibut nodavirus (AHNV) and infectious salmon anaemia virus (ISAV). To highlight some of the questions related to the complicated chemistry of ozonated seawater, the formation of ozonation by-products (OBP) and their removal by activated carbon filtration was studied.

2. Material and methods

2.1. Virus propagation and quantisation

Nodavirus (AHNV 692/9/98) was isolated from Atlantic halibut juveniles during an outbreak of VER in a Norwegian fish farm using the SSN-1 cell line (Dannevig et al., 2000). A fourth passage virus supernatant was used in the inactivation experiments. Quantization of virus was performed by end-point titration on monolayers of SSN-1 cells grown in 96-well microtiter plates using six wells per dilution (10-fold serial dilution). After 10 days incubation at 20 °C, the cultures were examined for infection by AHNV using an indirect immunofluorescence technique (IFAT) essentially as described by Grove et al. (2003) for detection of infected cells. An antiserum against AHNV (K 672) was used as the primary antibody and fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG (Southern Biotechnology Associates Inc., Birmingham, AL, USA) as secondary antibody. To facilitate observation in the fluorescence microscope, nuclei were stained by addition of 10 μg/ml propidium iodide (Sigma, St. Louis, MO, USA). TCID$_{50}$ /ml (tissue culture infectious dose infecting 50% of inoculated cultures) was calculated according to the method of Kärber (1931).

IPNV isolated in BF-2 cells (Lorenzen et al., 1999) from tissue samples from a disease outbreak was used (first or second passage of virus). Virus titration was performed essentially as described above using BF-2 cells, but virus infected cells were identified by development of cytopathic effect (CPE) after 7 days incubation at 15 °C.

ISAV strain Glesvaer/2/90 was propagated in SHK-1 cells (Dannevig et al., 1995) and a third passage virus supernatant was used in the experiments. The titration was performed on SHK-1 cells using five-fold serial dilution of the samples and virus infected cells were identified by IFAT using a monoclonal antibody against ISAV (Falk et al., 1998) as the primary antibody and FITC-labeled goat anti-mouse IgG as secondary antibody.

2.2. Water qualities used

Seawater used in the study was high-quality influent water to an aquacultural installation at the coast of Norway, pumped from 60 m depth. The water for the inactivation experiments was sterile filtered through a 0.45 μm Millipore filter before use. The water had the following characteristics: pH, 7.9; salinity, 33‰; dissolved organic carbon, 1.2 mg/l. The freshwater used was of drinking water quality, low in turbidity and organic carbon (<0.2 FNU and <2 mg C/l, respectively).

2.3. UV irradiation

Fifty ml seawater samples at 5 °C were added the viral suspension and kept on slow stirring in Petri dishes (53 cm^2 surface area, 1 cm water depth) during UV irradiation. Two-millilitre samples were withdrawn by a sterile pipette at given time intervals. The UV lamp used was a 15 W (3.5 W of 254 nm UV output) low-pressure germicidal lamp (Philips Ltd., Einhoven, The Netherlands) mounted in an apparatus, which provided a collimated beam (Qualls and Johnson, 1983). The light intensity (mW/cm^2) at 254 nm at the liquid surface (I_0) was measured by a calibrated UVX-25 sensor coupled to a UVX radiometer (Ultraviolet Products Inc., San Gabriel, CA, USA). The average intensity (I) in the suspension was calculated by the following equation (Morowitz, 1950):

$$I = \frac{I_0(1 - e^{-AL})}{AL}$$

where A is the absorbance of the suspension per centimetre and L is the path length of light in water (cm). Absorbance at 254 nm was measured with a spectrophotometer. The UV dose, defined as the product of average intensity across the Petri dish and the exposure time, was varied by varying the exposure time.

2.4. Ozonation

For the viral dose/response studies, seawater was preozonated by a laboratory model electrical discharge ozone generator (Ozonia CE Triogen, Ozotech, Norway) to predetermined concentrations. Fifty millilitres ozonated water samples in brown 100-ml glass bottles with screw caps were added to pure virus cultures to a ratio of 100:1 or 1000:1 and incubated at 5 °C. Two-millilitre samples were withdrawn at given time intervals, neutralized by sodiumtiosulphate and assayed for viable viruses. The total residual oxidant (TRO) concentration was measured immediately before and after the virus suspension was added and immediately after each sample withdrawal. The $C\,T$ value (the product of concentration and contact time) in each case was calculated from the average TRO concentration (mg Cl_2/l) during the respective contact time (s).

For the studies of the removal of oxidants and ozonation by-products by granular activated carbon (GAC), water volumes of 80 l were ozonated in a plastic container with diameter 55 cm and a water depth of 34 cm at 9.5 °C. The ozone was supplied through a micro pore diffuser. The decomposition of oxidants was studied, with and without aeration. Air was supplied through a micro pore diffuser at a rate of 17 l/min, corresponding to 0.2 l/(min l) of water. A 15 cm diameter GAC column applying different flow rates and empty bed contact times (EBCT) was used to remove the residual oxidants and the ozonation by-products. The filter bed consisted of 20 cm GAC layer with a grain size of 1.00–3.55 mm (Pool-Aktivkohle W 1–3, Permakem A/S, Oslo, Norway) on a sand and gravel support.

2.5. Analysis

Residual oxidants were measured by the colorimetric DPD method (American Public Health Association, 1989), which is the method recommended for measurement of TRO in ozonated seawater (Buchan et al., 2005). The method is based on the oxidation of *N,N*-diethyl-*p*-phenylendiamin (DPD), which turns to a pink Wurster-cation in the presence of strong oxidants. The intensity of the colour is proportional to the TRO concentration. The colour intensity was measured by a Hach DR/2000 spectrophotometer (Hach Company, Loveland, CO, USA). The method and the instrument give the results as total residual oxidants as mg Cl_2/l. THMs were extracted in pentane and measured by the use of a gas chromatograph (American Public Health Association, 1989). Bromate was measured by ion chromatography.

3. Results

3.1. UV irradiation

Inactivation curves for the UV irradiation experiments are presented in Fig. 1. The inactivation rate fitted first-order kinetics reasonably well. Reductions by 99.9% in viability were obtained when doses of 7.5 mJ/cm^2 for ISAV, 104 mJ/cm^2 for AHNV and 246 mJ/cm^2 for IPNV were applied (Table 1).

3.2. Ozonation

The depletion of residual oxidants in ozonated fresh- and seawater is presented in Fig. 2. Without any

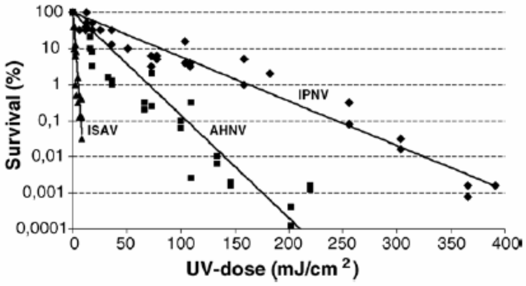

Fig. 1. Dose–survival curves of UV irradiated viruses. *Symbols:* (▲) infectious salmon anaemia virus (ISAV); (■) Atlantic halibut nodavirus (AHNV); (◆) infectious pancreatic necrosis virus (IPNV).

Table 1
Required UV doses for various degrees of inactivation of infectious pancreatic necrosis virus (IPNV), Atlantic halibut nodavirus (AHNV) and infectious salmon anaemia virus (ISAV) in seawater

Virus	Required UV doses (mJ/cm^2) for various degrees of inactivation		
	90.0%	99.0%	99.9%
IPNV	82.0	165	246
AHNV	35.0	70.0	104
ISAV	2.5	5.0	7.5

aeration the half-life of oxidants in freshwater was about 18 min, which decreased to less than 10 min when aerated with 0.2 l/(min l) water. In seawater, no reduction in oxidant concentration was detected within 50 min, neither with nor without aeration.

In Table 2, the concentrations of persistent and carcinogenic by-products in ozonated seawater and the efficiency of GAC filtration for their removal are shown. At the TRO concentration used in these experiments (0.9 mg/l), bromate was formed in concentrations of 50 and 70 μg/l after 20 and 80 min contact time, respectively. Among the THMs, only bromoform was formed in substantial amount. The bromoform concentration of 16 μg/l measured after 80 min contact was reduced to 1.6 μg/l by GAC filtration with an empty bed contact time (EBCT) of 4.2 min. Bromoform was the only THM detected after GAC filtration, while the TRO and bromate concentration was reduced below the detection limit.

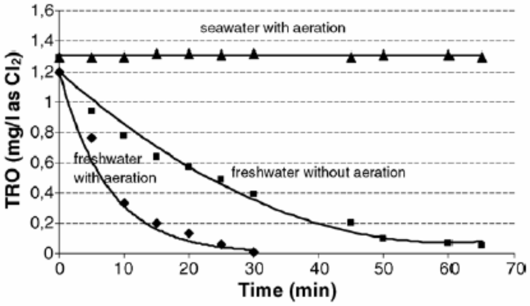

Fig. 2. Depletion of residual oxidants in ozonated fresh- and seawater at 9.5 °C. *Symbols:* (▲) seawater with aeration; (■) freshwater without aeration; (◆) freshwater with aeration.

Plots of virus survival versus CT values are presented in Fig. 3. Due to long initial lag-phases of IPNV and AHNV, these plots were made on a log–log scale and did not fit first-order kinetics. 90% reductions in virus titers were obtained by 1.4 (mg s)/l for ISAV, 1000 (mg s)/l for AHNV and 1944 (mg s)/l for IPNV. In Table 3, different combinations of concentrations and contacts times for various degrees of inactivation are given.

4. Discussion

The results obtained in the present study demonstrate large variation in UV and TRO resistance among fish pathogenic viruses and also significant deviations compared to results obtained by other investigators in some of the experiments. For the UV study, the results support the results of others that IPNV is among the most UV resistant viruses reported in scientific literature, probably partly as a result of its double-stranded RNA. The required dose for 3 $\log_{10}$ units reduction in virus titer was 246 mJ/cm^2, which is slightly higher than previously reported (Sako and Sorimachi, 1985; Liltved et al., 1995; Øye and Rimstad, 2001). On the other hand, the UV dose requirement for 3 $\log_{10}$ reduction in AHNV was 104 mJ/cm^2, which was only half the dose of 211 mJ/cm^2 used by Frerichs et al. (2000) for the same inactivation. Differences in experimental design and UV resistance amongst the two different nodaviruses applied may explain the deviation.

The UV resistance found in IPNV was significantly higher than in any studied human pathogenic viruses, by which numerous UV dose/response studies have been conducted. It has been revealed that human

Table 2
Concentration of TRO, bromate and seven trihalomethanes in ozonated seawater after 20 and 80 min contact, and after GAC filtration

	After 20 min contact	After 80 min contact	After GAC filtration
TRO (mg/l)	0.9	0.9	0.0
Redox-potential (Mv)	752	752	270
Bromate (BrO_3^-) (μg/l)	50	70	<10
Trihalomethanes			
Tetrachloromethane (μg/l)	<0.03	<0.03	<0.03
Bromoform (μg/l)	7.70	16.00	1.60
Chloroform (μg/l)	<0.30	<0.30	<0.03
Dibromochloromethane (μg/l)	0.10	0.22	<0.05
Dichlorobromomethane (μg/l)	<0.05	<0.05	<0.05
Tetrachloroethane (μg/l)	<0.03	<0.03	<0.03
Trichloroethane (μg/l)	0.12	0.21	<0.05
Total trihalomethanes (μg/l)	8.22	16.43	1.60

The initial TRO concentration was 0.9 mg/l. Seawater quality parameters: temperature, 9.5 °C; salinity, 33.9‰; pH 7.9, organic carbon content, 1.2 mg/l.

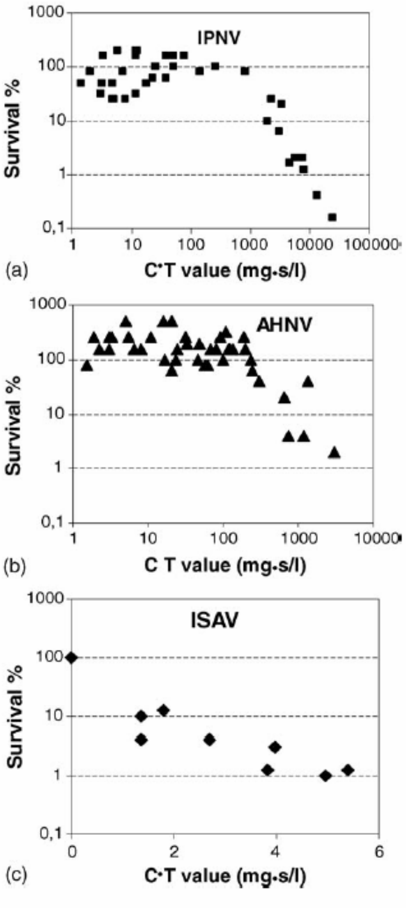

Fig. 3. Dose–survival plots of infectious pancreatic necrosis virus (IPNV) (A), Atlantic halibut nodavirus (AHNV) (B) and infectious salmon anaemia virus (ISAV) (C) in ozonated seawater. *C T* values are calculated from the average TRO concentration (as mg Cl_2/l) and the respective contact time (s).

rotaviruses and reoviruses have some resistance, requiring 42 and 45 mJ/cm^2 for 99.9% inactivation, respectively (Harris et al., 1987; Battigelli et al., 1993). Several investigations indicate that adenoviruses (double-stranded DNA viruses) are among the most resistant. Meng and Gerba (1996) and Gerba et al. (2002) found the required dose for 99.9% reduction to be 90–120 mJ/cm^2, which is still substantially lower than experienced for IPNV and in the same order of magnitude as for AHNV.

The variability in resistance to UV light among viruses has been attributed to the size and nature of the genome. It has been experienced that viruses with double-stranded genome, like IPNV and AHNV, exhibit higher UV resistance than single-stranded viruses. It has been claimed that these viruses are able to use the host cell enzymes, including enzymes present in the cell lines used for quantification of viable viral particles after irradiation, to repair UV imposed damages in the genome (Gerba et al., 2002). This also implies that different cell lines may provide different results, i.e. the greater availability of repair enzymes may result in a greater number of viral particles surviving UV treatment.

Based on previous studies, the general opinion among fish farmers and scientists is that IPNV and

Table 3
Combinations of TRO concentrations and contact times for various percentage inactivations of infectious pancreatic necrosis virus (IPNV), Atlantic halibut nodavirus (AHNV) and infectious salmon anaemia virus (ISAV) in seawater

Virus	TRO concentration (mg Cl_2/l)	Contact-time (min)	*C T* value ((mg s)/l)	Inactivation (%)
IPNV	8.1	4.0	1944	90.0
	2.8	18.3	3066	93.7
	6.7	14.0	5628	98.0
	2.5	31.0	4650	98.4
	7.9	17.0	8058	98.7
AHNV	0.9	12.2	643	80.0
	2.0	6.3	752	96.0
	1.8	10.8	1166	96.0
	1.6	31.5	3043	98.0
ISAV	0.09	0.25	1.4	90.0
	0.09	0.50	2.7	96.0
	0.33	0.25	5.0	99.0

nodaviruses are sensitive to ozone. The results from the present study suggest reconsideration of this opinion and existing ozonation practice and recommend the use of considerably higher TRO concentrations and *C T* values than previously applied for inactivation of IPNV and nodavirus in ozonated seawater.

The higher ozone dosages required in the current study compared to earlier freshwater studies can partly be explained by the fact that the oxidants formed in seawater (mainly bromine) are weaker disinfectants than gaseous ozone (the active substance in freshwater). To explain the deviations in resistance between the present study and former seawater studies, the use of different inactivation procedures and the lack of a standard analytical method for measurement of oxidants in ozonated seawater may have influenced the results. Several different sum methods have been applied to assay the TRO level and different units have been used to report TRO concentrations. In the former IPNV study of Liltved et al. (1995), the indigo colorimetric method (American Public Health Association, 1989) was applied. More recent unpublished studies have indicated that the method is useful for ozonated freshwater, but may not be suitable for seawater, because it may not include bromine, thereby producing too low readings. In the study by Arimoto et al. (1996), an iodometric method was used (Shechter, 1973). According to Buchan et al. (2005), this method gives too low TRO values (approximately one-fourth of the values measured by the DPD method) and was not recommended for ozonated seawater.

The problems associated with different sum methods used to measure TRO in seawater illustrate the need for the establishment of a standard analytical method. The DPD method used in the present study has been suggested and is probably the best available method today (Buchan et al., 2005). However, effort should be made to develop direct methods to measure the most important individual oxidants in seawater. Precise measurements of the individual oxidants will significantly increase the knowledge of the complicated chemistry of ozonated seawater and be a useful tool in scientific work and for practical operation of ozonation units. In addition to the errors introduced by the lack of standard procedures and standard analytical methods, variations in resistance between different viral strains may contribute to the variations in results among studies.

The difference in residual oxidant stability in ozonated freshwater and seawater was demonstrated in the present study. While the oxidant level was rapidly reduced in ozonated freshwater and further accelerated by aeration, no reduction was detectable in ozonated seawater with or without aeration (Fig. 2). The higher redox-potential and higher reactivity of the dissolved gaseous ozone in freshwater compared to that of the oxidants produced in seawater explain the more rapid depletion in freshwater due to higher losses in redox-reactions with organic and inorganic compounds. Stripping of gaseous ozone to the atmosphere will also contribute to depletion of ozone in freshwater, especially if aeration is applied. Due to the lower redox-potential and limited loss to the atmosphere of bromine and other oxidants formed in seawater, these compounds are much more persistent than gaseous ozone. Their relatively high persistence, combined with their high toxicity to aquatic animals, makes control of these oxidants important to avoid chronic or acute effects on the cultured fish species. As demonstrated, aeration will not be an efficient method to remove the residual oxidants formed in seawater, however, GAC filtration was demonstrated to remove TRO to very low effluent concentrations (Table 2).

In addition to TRO, the obtained results show that other persistent and carcinogenic by-products may be formed when the inlet seawater in land-based aquacultural installations is ozonated. The TRO concentration used was in the same range as normally applied for disinfection of eggs, slightly higher than for inlet water disinfection and high compared to concentrations used in ozone skimmers in recirculating aquacultural systems. The resulting bromate concentrations (50–70 μg/l) were higher than those reported from recirculation systems (10–25 μg/l) (Liltved et al., 2002; Tango and Gagnon, 2003) and substantially higher than the maximum contaminant level established in the EU Drinking Water Directive (10 μg/l). In recirculating systems, the presence of ammonia can inhibit the formation of bromate by reacting with bromine to form bromamines (Hofmann and Andrews, 2001). The presence of ammonia, in combination with the low ozone dosages applied in such systems, may explain the lower bromate concentration reported from recirculating systems. The ability of bromate to bioaccumulate is not known, but is probably limited due to its high water solubility.

The potential of bromoform formation in ozonated seawater demonstrated in the present study and previous evidence of low levels of bromoform occurrence in recirculating aquacultural systems (Liltved et al., 2002), is of serious concern due to the carcinogenicity of these compounds and their ability to accumulate in fish tissue. Assays should be performed to determine the level of bromoform and other THMs in fish tissue in aquacultural systems, where ozonation is a part of the water treatment.

GAC filtration in the present short-time study was shown to efficiently reduce the levels of bromate, THMs and TRO to very low effluent concentrations (Table 2). Long-term filtration experiments with ozonated seawater conducted by other investigators also show that GAC filtration can reduce TRO to acceptable levels (Ozawa et al., 1991). Information from the literature on THM- and bromate-removal by GAC filtration in seawater has not been found. Experience from freshwater systems indicates that THMs is easier to remove than bromate, but high salt content may reduce the adsorption of THMs in seawater. GAC filtration for removal of bromate in natural freshwater has not been successful. Kirisits et al. (2000) observed rapid breakthrough of bromate in the filters due to competition of adsorption sites from natural organic matter and anions. In a study, which also included other water treatment techniques, Kruithof and Schippers (1993) concluded that no conventional techniques are available to reduce or remove bromate. The advice was to prevent or minimize the formation of bromate rather than trying to remove it by water treatment after formation.

Acknowledgements

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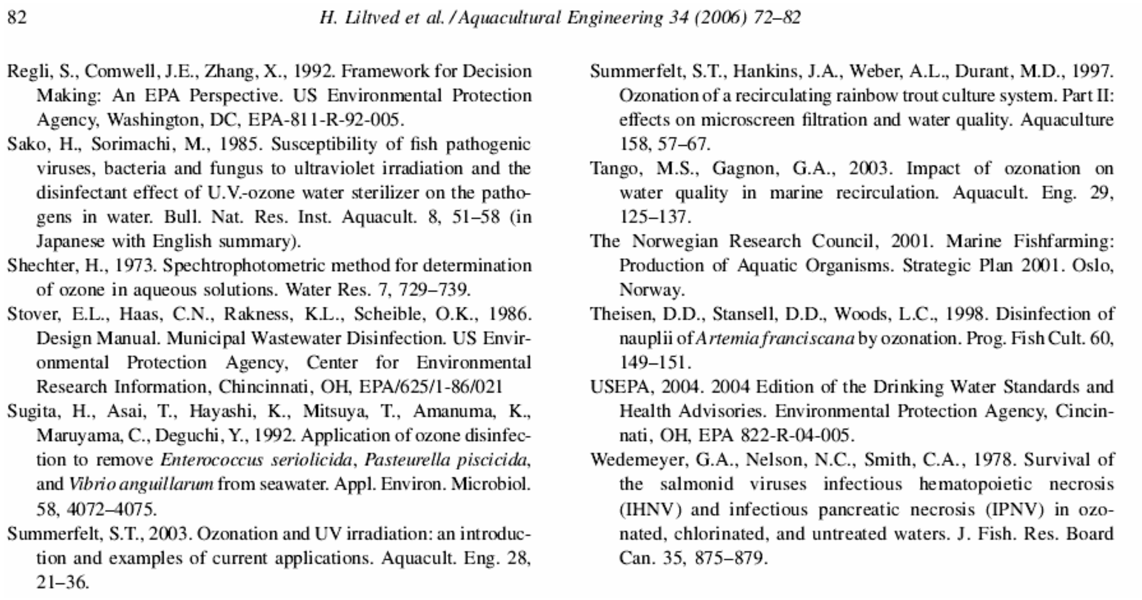
APPENDIX 4. *Ozone reference*



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