CENTRE FOR ENVIRONMENT, FISHERIES AND
AQUACULTURE SCIENCE

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ARTICLES

FARMING THE NATIVE 'FLAT' OYSTER

Peter Hoare

In demand

Much has been written about the native European flat oyster, *Ostrea edulis.* The Romans prized them so much that during their occupation of these islands they transported them back to Rome (in brine!)

They have been the food of the poor and at one time 700 million were consumed annually in London alone and at the height of their popularity in Britain the industry employed over 200,000 people.

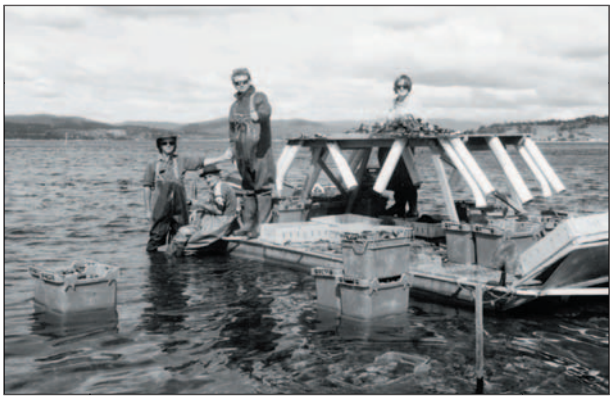
The early settlers to New Holland and Van Diemens land discovered Natives, *Ostrea angasi,* in abundance to supplement their meagre diet. Middens of shells showed the aborigines had been living off them for many thousands of years. There was a bigger oyster industry in Tasmania 150 years ago than there is today (30 million oyster meats were exported annually to London – in barrels of brine). In New Zealand the Bluff Oyster *Tiostrea chilensis* is greatly revered. The French, too, had a huge *edulis* industry

…….and then came ***BONAMIA*** *!!*

In Tasmania

My experience with 'flats', as we called them in Tasmania, gelled when the State fisheries department removed half a million 50-70 mm (No 4-3's) from my farm lease and dumped them on Hobart municipal tip. *Bonamia* had been unheard of before and I (my company) had been licensed to 'help myself' to 1 million flats from a wild bed annually for 5 years. They were removed the day after we had transported them from the harvest area to the farm lease (some 100 miles) by nameless humans in white coats, when a sample from the load tested positive for *Bonamia*. As it was and still is, an IOE notifiable disease the Tasmanian fisheries department ordered their destruction (like foot and mouth!).

In hindsight it was realised that *Bonamia* had only showed when the oysters had been moved and were under considerable stress. As there was no historical link to any movement and relaying of flat oysters around the state and as Pacific oysters didn't carry the parasite, the Department deemed that *Bonamia* was endemic to the wild *angasi* population.



Harvesting native flat oysters (Ostrea angasi) in Georges Bay, St. Helens, Tasmania

This incident girded me into proving to the Fisheries Dept that they could still be farmed, in spite of *Bonamia,* and as there were no 'flats' on the farm presumably there was no *Bonamia* either?

A *Bonamia* expert in New Zealand advised that as the parasite cannot jump from the brooders to the larvae in the hatchery you would therefore get *Bonamia*-free seed! To this end I got 20,000 *angasi* seed from the Shellfish Culture hatchery and my colleague and I designed and built sub surface growing systems. Most of the oysters grew to 70 – 90 mm within 2 years (at a density of 3 dozen per bag at 5 bags per unit system) and were sold to the Melbourne markets. Others I kept in the systems and at 4 years these were 100 – 120 mm. Throughout this time there were no significant mortalities and annually they tested negative for many diseases and pathogens including *Bonamia*.

Back in England

Having banged on about my success in Tasmania to the SAGB, after my return to this country, Clive Askew appeared on 25 April 2003 at the farm site in the Fleet in Dorset with 6,000 10 mm *edulis* seed from the Seasalter hatchery. These were put into four (small) mesh bags (1,500 each). Two were suspended and two sitting off the bottom. All were sub surface 99.9% of the time.

They were checked regularly and then sorted/graded for the first time in mid July. Results were good with 30%

grown to 15-25 mm and the remainder 10-15 mm with no significant mortalities.

The larger ones were put into another sub surface system and placed onto a trestle in a different location only accessible at very low water springs.

……..and then came ***BONAMIA*** *!!*

The author with an off-bottom oyster culture bag

I had chosen to look at them on what was probably the hottest day of the summer ~ 25th August. The Pacific oysters on the farm had spawned out long ago so if there were to be any show of mortalities it would be now. To my disappointment there were roughly 50% mortalities in all the systems. CEFAS Weymouth came the next day and took away samples for testing.
The oysters had *Bonamia* at an overall level of infection of 25-30% (slightly higher for the larger oysters) with moderate to high levels of infection in the individuals in which the parasite was detected. The different growing systems suffered the same percentage mortality and similarly the different locations. Over the next couple of months the dead shell was removed and the survivors thinned out and rehoused in clean systems. Monthly inspections since September have shown NO further mortalities.

The oysters were sorted in October, when the survivors were replaced in clean bags at lower densities. These have all survived over winter

Whether this has shot a hole in my theory I don't know, as the Fleet is a known *Bonamia* area. I am very surprised, however, the parasite so readily infect new (naïve!) seed stock. There are wild natives growing on trestle iron in close proximity to the trial site and on structures either floating on moorings or permanent fixtures. It could be there is a reservoir of infection at a low level that can lay in wait for along time. Or maybe there are other organisms in the mud that host *Bonamia*.

Having experienced *Bonamia* in Tasmania and New Zealand where there are no records of any transfers and movement of stock for relaying the question is asked how did it get there? Could it be endemic to the wild species as the southern hemisphere experts decreed? In historical records (in Tasmania) there is mention of huge mortalities of oyster stocks periodically occurring. Could this have been due to *Bonamia*?

The survivors in the Fleet to date are doing well (up to the end of March 2004). It is hoped to use other locations with greater depth before the warm weather arrives.

Further information

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SPAT COLLECTION IN NATIVE OYSTER PONDS

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[2] Rossmore Oysters Ltd.

Background to the experiments

Most native oyster farmers utilise spat from wild sources, but at Rossmore in Co. Cork, Ireland, oysters are bred in spatting ponds from selected, older oysters, which have survived the disease Bonamiasis for longer.

Bonamiasis is a disease of the European native oyster, *Ostrea edulis.* The spread of this disease throughout Europe, on top of over a century of over- harvesting and mismanagement of beds, is recognised as a serious threat to wild and cultivated native oyster stocks.

The disease does not cause significant mortality until oysters are mature enough to reproduce, resulting in very low selection pressure for resistance.

In previous experiments comparing Rossmore oysters with those exposed to the disease, but only subject to natural selection, Rossmore oysters showed significantly lower prevalence of infection, intensity of infection and mortality. It is hoped that Rossmore oysters could be used to repopulate many of the depleted areas around Europe.

A re-population of European beds

At present Rossmore oysters are settled onto mussel shells, resulting in attached spat far too bulky for practical transport around Europe. The aim of this project was to investigate the feasibility of producing cultchless spat by settling and then quickly removing spat from plastic sheets. Similar techniques are used in hatcheries, but not in the more environmentally variable pond system. It is also hoped that the ongrowing of these spat in the protection of bags on trestles might then reduce the very high losses of small spat spread on the seabed.

Ponds at Rossmore, Ireland

Each pond holds approximately a million litres of water pumped in from the Cork estuary and 300 – 700,000 mussel shells are spread against the sides and the bottom. Environmental parameters of the ponds, such as temperature, pH and visibility, vary considerably both between ponds and from day to day.

An aerial photograph of the breeding ponds

During the summer of 2003 spat were settled on to 1ft by 1ft square plastic sheets placed around the pond edges at the top of the water column. After settlement, the spat were scraped from the sheets with a razor blade to obtain cultchless spat, which were grown in an upwelling system, using pond water.

Increasing spatfall on the sheets

The following factors were investigated to increase levels of spatfall on the sheets.

Surface Roughness

It is widely recognised that spat settle preferentially on rough rather than smooth surfaces. The new sheets had a glossy finish, assumed to be a poor surface for settling, so many were sanded until the shine had been removed. Yet when this assumption was tested we were surprised to find a clear preference for the smooth sheets in white sheets, but with a preference for rough on grey sheets.

The effect of sheet texture on spatfall

Sheet colour	Average spat/ rough sheet	Average spat/ smooth sheet	Confidence level (t-test)
White	247	654	<0.01
Grey	116	61	<0.05

Sheet colours

Three different colours of sheet were used, black, white and grey. The literature has contradictory information on colour preferences for spat settlement. When tested at Rossmore, it was found that the black sheets attracted significantly fewer spat than the white or grey.

Fouling

Sheets left in a marine environment rapidly become covered in a layer of algae, bacteria and other substances. With use, sheets also accumulate spat scars. Fouling of sheets was expected to result in increased settlement, but the results from Rossmore were ambiguous. Fouling alone attracted insignificant spatfall, yet sheets that had been treated with oyster extract, but had not been fouled, also attracted insignificant settlement. This suggests that good spatfall requires both fouling and extract and while this assumption was not further tested, for all other experiments fouled and treated sheets were used. It is interesting that the benefits associated with fouling tolerate both scraping with a razor blade, and being dried in the sun for several hours.

Oyster extract

Oyster extract was prepared by dicing an adult oyster and putting it through a 1.5 mm mesh sieve before diluting with salt water. Dried sheets were dipped in a bucket of extract for a few seconds and then dried again before placing in the ponds. Sheets were re-treated after every scraping.

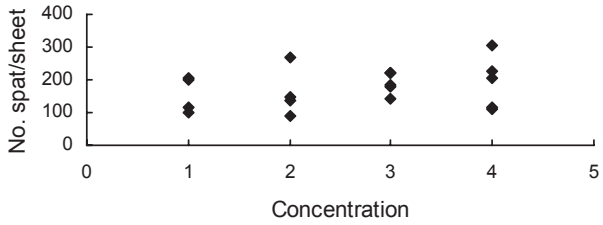
The extract was very useful in encouraging spatfall on sheets, and was used uniformly for all sheets.

The effect of extract on spatfall

Colour	Treated	Untreated	p-value
White	385	20	<0.001
Black	155	0	<0.05

Concentration of extract

The concentration of extract in salt water from 1:50 to 1:200 was varied, but no difference between any of the four concentrations was found, as the graph demonstrates.



The effect of extract concentration on spatfall. Dilution of extract: 1= 1:50; 2 = 1:100; 3 = 1:150; 4 = 1:200.

Top/underside of the sheet

A trapping effect had been previously noticed in spat settlement, with higher concentration of spatfall on the underside of mussel shells in the ponds. A similar effect has been found on the sheets, with a highly significant increase in settlement on the underside of the sheets. Two typical examples are shown in the table below.

The difference of settlement between the upper and lower surfaces of a sheet

Experiment testing:	No. spat /sheet (under side)	No. spat/ sheet (top side)	P-value
Concentration of extract	177	52	<0.001
Treated/untreated with extract	292	68	<0.001

Height in Pond

Previous research from Rossmore noted that the mussel shells nearer the pond surface attract significantly more spat than those lower down. The preferred height in the ponds was tested in 2 ways – examining sheets from other experiments to see where spat had settled, and comparing sheets held at the surface and 15 cm lower. Looking at data from other experiments there is a clear and highly significant difference between the top and bottom of the sheets, with almost double the spatfall on the top quarter of the sheet compared to the bottom quarter. The second experiment took place during the end of the settling season when the numbers of spat settling were very low and gave ambivalent results.

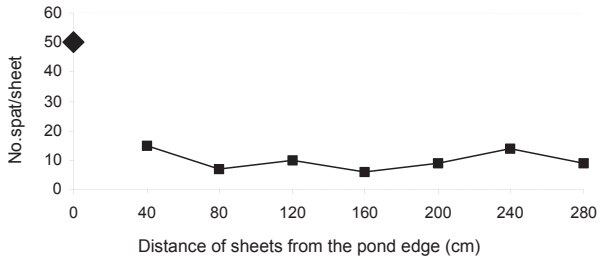
Location on the pond surface

To find the best position of the sheets on the surface of the pond to get greatest spatfall the sheets were suspended at intervals from a rope strung across the middle of the pond.

For a comparison, some sheets were also placed along the pond edge in the normal fashion, around where the rope left the pond side.

One of the ponds showing 4 tiles in the foreground against the pond edge, and then the beginning of a row of tiles leading into the pond centre, comparing settlement at different locations. The polystyrene was effective at keeping the tile angles approximately constant in the centre and along the pond edge. This picture also shows the mussel shell on the pond edge

The results in the graph show no trend with increasing distance from the pond edge. Instead it can be seen that sheets lying on the edge of the pond gave by far the greatest spatfall.



The effect on spatfall of distance of the sheet from the edge of the pond. The black diamond represents results from sheets placed along the edge of the pond

Growing the spat

Once the spat had been collected they were grown in an upwelling system designed on site. The initial flow rate through the system was too high and unfortunately resulted in the loss of a significant proportion of the spat. Growth rate and spat survival were low in the upwelling system. Mortality was estimated to be as high as 40%. This high mortality and low growth rate are likely to be due to the poor quality of the food supply. Next year, to increase both survival and growth rate, spat will be grown in an algal enriched airlift bin, where an ideal quality and quantity of food can be provided.

Project potential

An estimated half a million spat were collected. This was achieved using only three fifths of the available collecting time, and much time was taken with setting up the system and with the experiments. With the present knowledge gained and if work is focussed purely on collecting spat, there is little reason why this figure could not be increased by an order of magnitude.

Further information

There is a web site (www.bonamia.com) devoted to the latest research on bonamia, including progress reports on the EU-funded Project BOLCI (*Bonamia ostreae* life cycle investigations).

THE BST LONGLINE OYSTER SYSTEM

Vaughan Williams
Boddingtons Limited, Blackwater Trading Estate, The Causeway, Maldon, Essex CM9 4GG

A new system

The patented BST long-line oyster system was originally evolved to meet the needs of the oyster growing industry in the often rough and difficult waters of Southern Australia. It has proved so successful that it has largely replaced the traditional rack systems and there are now over 600 hectares of oyster cultivation using this method in Australia and Tasmania.

The heart of the system is the use of high tensile nylon line to support specially designed baskets, which are free to move in tidal currents and can easily be adjusted for height. This ability to easily raise and lower the lines of baskets enables the true farming of the oysters in a way that has not been possible before.

The movement of the baskets produces a 'rumbling' action which produces a clean strong shell and the ability to raise and lower the line means the grower can control the growth of the oyster and optimise the meat to shell ratio. The good quality of the oysters raised in this system has led to demand constantly outstripping supply in the Australian market

The patented BST long-line oyster system

The system is designed to minimise handling as the baskets can easily be unclipped directly onto a boat and taken directly to grading and rapidly returned to the lines with minimum manpower.

Growth trials

The oysters grow rapidly in the BST system and it is expected that they will reach market size in European conditions in no more than 18 months, which is half the time in the current bag and trestle system. In Australia, in relatively poor water, oysters have been grown to market size in 9 months. This offers obvious savings, which combined with the reduction in labour, have led to considerable interest.

Following the first trial systems in Donegal introduced by BIM in 2002 after visiting the growers in Australia, other sites are now in operation in Lough Swilly, and the Blackwater Estuary in the UK. The growth and quality of the oysters in these systems has been remarkable. Importantly two trials are planned in Brittany this year, which will be under the control of the Aquaculture Department of Caen University. These independent trials will monitor all aspects of the performance of the BST system compared to control oysters in the conventional bag and trestle system running alongside. It will cover growth, quality, cost and labour and it is hoped the results will be published early in 2005.

There are a number of additional sites in Ireland, Scotland and England where the system will be installed in 2004.

Further information

Boddingtons Ltd, who also manufactures the mesh bags, markets the patented BST system in Europe. Information on the system is on their website at www.boddingtons-ltd.com.

NON-NATIVE OYSTERS IN CHESAPEAKE BAY, USA

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Industry at the crossroads

The struggling oyster industry in Chesapeake Bay stands at the crossroads The native species *Crassostrea virginica* has been depleted to less than 1% of its original abundance through a combination of heavy fishing pressure, habitat deterioration, and recent high mortalities due to the spread of two parasites, *Haplosporidium nelsoni* and *Perkinsus marinus*, responsible for MSX and Dermo diseases, respectively. Consequently, survival of the oyster industry in Virginia and Maryland is at stake. To address this problem, Virginia has been exploring the option to introduce a non-native species, firstly unsuccessfully with the Pacific oyster, *C. gigas*, and more recently, with the Suminoe oyster *C. ariakensis*. Pilot studies have been carried out in open waters since 2000. These were described briefly in an earlier issue of Shellfish News (Number 15, May 2003, pp 20-21).

A study is commissioned

However, opinions on the likely risks and benefits of introducing a non-native oyster differ among States and Federal agencies that participate in regional agreements through the Chesapeake Bay Program. Rising awareness of potential ecological and economic problems associated with invasive non-native species has made resource managers highly cautious. Because

Chesapeake Bay is the largest estuary in the United States, having over 8000 miles of shoreline. It is almost 200 miles in length and up to 25 miles wide in places

of the high stakes associated with the decision to introduce such a species, the Chesapeake Bay Commission requested that the National Research Council (NRC) undertake a study of the pros and cons of introducing (or not) *C. ariakensis* as an infertile triploid for aquaculture purpose or as a reproductive diploid for stock enhancement.

A committee is formed

A committee, co-chaired by James Anderson (University of Rhode Island) and Dennis Hedgecock (University of California, Davis) and composed of nine other members, including the author of this article, was formed to address these issues. The remit for the committee is reproduced below:

"This study will examine the ecological and socio-economic risks and benefits of open water aquaculture or direct introduction of the non-native oyster, *Crassostrea ariakensis*, in the Chesapeake Bay. The committee will address how *C. ariakensis* might affect the ecology of the Bay, including effects on native species, water quality, habitat, and the spread of human and oyster diseases. Possible effects on recovery of the native oyster, *Crassostrea virginica*, will be considered. The potential range and effects of the introduced oyster will be explored, both within the Bay and in neighbouring coastal areas. The study will investigate the adequacy of existing regulatory and institutional frameworks to monitor and oversee these activities.

The committee will assess whether the breadth and quality of existing research, on oysters and on other introduced species, is sufficient to support risk assessments of three management options: 1) no use of non-native oysters, 2) open water aquaculture of triploid oysters, and 3) introduction of reproductive diploid oysters. Where current knowledge is inadequate, the committee will recommend additional research priorities."

The Suminoe oyster Crassostrea ariakensis (Photo: Jodi Dew)

The 15 month project, during which four committee meetings were held, was sponsored by the US Environmental Protection Agency, the National Oceanic and Atmospheric Administration, the US Fish and Wildlife Service, the National Fish and Wildlife Foundation, the Maryland Department of Natural Resources, the Virginia Sea Grant, the Virginia Department of Environmental Quality, the Maryland Sea Grant, and the Connecticut Sea Grant.

The final report

The NRC has now published the report of the committee, entitled "Nonnative oysters in Chesapeake Bay". It presents a landmark opportunity to identify concerns that should be addressed by decision makers when the introduction of a non-native species is under consideration. Moreover, this case study is of particular interest considering the present European strategy to develop aquaculture (EU-COM 2002 511 Final).

Recommendations

Since relatively little is known about the Suminoe oyster, one of the key questions to be addressed was how it might affect the ecology of Chesapeake Bay. Although strict application of the ICES protocols reduces the risk of co-introduction of undesirable organisms, including most pathogens and parasites, there remains some risk that serious ecological problems might emerge: compared to previous *C. gigas* introductions worldwide, it is difficult to predict whether or not a species will be invasive. *C. ariakensis* has environmental tolerances that make it well suited for growth and reproduction in Chesapeake Bay as well as in estuarine habitats along the Atlantic and Gulf coasts. Likely to compete with the native oyster, an overlap between both species distribution would probably occur if it became established. Therefore, further research on Suminoe oyster biology and ecological interactions has been recommended, including studies on genetics, larval dispersion modelling, reef building capacity, and response to environmental change and multiple stressors.

Economic and social impacts should also be considered. While both Maryland and Virginia states value the oyster fishery, policy differences relative to public and private leases will affect the way the industry adapts to hatchery products. Besides examining the present situation regarding public versus leased oyster beds, it is recommended to examine economic feasibility of alternative production systems and to develop models of community and household impacts for such alternative systems. Moreover, it is concluded that the existing regulatory and institutional framework is not adequate for regulating introduction of non-native species at the federal level.

Of the above three management options evaluated, the main risk identified under the first option (no use of non-native oysters) is a continued failure of native oyster restoration efforts, with continued decline of the oyster fishery and erosion of traditional economies and cultures of Chesapeake bay watermen. Such a situation would result in erosion of confidence in the ability of managers to address resource management issues. Illegal introductions might arise from frustration of the limited success of the current restoration efforts. Oyster pathogen or other pest species associated with such rogue releases might result in irreversible side effects.

It is not possible to predict if a controlled introduction of reproductive *C. ariakensis* will improve, further degrade or have no impact on the oyster fishery or the Chesapeake Bay ecology under option 3 (introduction of reproductive diploid oysters).

In contrast, contained aquaculture of triploid *C. ariakensis* (option 2) provides an opportunity to research the potential effects of extensive aquaculture or introduction of reproductive individuals on the Chesapeake Bay ecology, while offering economic opportunities. Therefore the committee has recommended this management option as a short term or interim measure, allowing more management flexibility in the future. However, stringent regulations are necessary to ensure that triploid culture does not result in the establishment of a self-reproducing population. Before any deployment, the Committee has recommended that a protocol is developed to minimize and monitor unintentional release of *C. ariakensis*, based upon Hazard Analysis Critical Control Point (HACCP) used to ensure food safety. Five items should be taken into account here: acceptable limits to prevent release of reproductive animals, disease and quarantine broodstock certification, confinement and accounting at all life stages, stability and sterility of triploids, population parameters of cultivated triploids.

Further information

A copy of the full report, which is 326 pages long, can be ordered from the National Academies Press (500 Fifth Street, NW, Lockbox 285, Washington, DC 20055, USA) website (www.nap.edu).

A BRIEF GUIDE TO AQUACULTURE GRANTS & FUNDING

Keith Jeffery
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Obtaining information on grant funding for aquaculture-related projects is not always easy for fish and shellfish farmers. The situation can vary considerably between different parts of England and Wales, as do the criteria used for determining whether projects are funded in different schemes. The aim of this article is to provide pointers to various sources of funding and advice to assist farmers in inquiring into their own areas of interest.

Financial Instrument for Fisheries Guidance (FIFG)

This is the EU structural fund for fisheries and aquaculture. Grants are available for expenditure under various headings, one of which is aquaculture. Grant rates and eligible expenditure vary between different areas of the UK, depending on local spending priorities and the funds available.

In Cornwall and the Isles of Scilly and parts of Wales, funding is available for investment in aquaculture facilities before the point of harvest. This could include the construction, modernisation or equipping of fish farms. Aid is also available for processing and marketing operations after the point of harvest, for example smoking or packing facilities. In the rest of England, grants for pre-harvest facilities are not available, although grants are available for investments in the processing and marketing of aquaculture products.

For more details on eligible expenditure and grant rates please see

www.defra.gov.uk/fish/grants.htm

or contact Defra,
Room 308, East Block, 10 Whitehall Place,
London, SW1A 2HH.
Tel (English Applications) 020 7270 8045; (Enquiries) Merseyside Applications 020 7270 8048;
Fax: 020 7270 8019; email fifg.grant@defra.gsi.gov.uk.

In Cornwall and the Isles of Scilly please see

www.swpesca.co.uk

or contact Ms Clare Leverton, South West PESCA Ltd,
Trevint House, Strangway Villas, Truro,
Cornwall TR1 2PA.
Tel: 01872 270333; Fax: 01872 242470;
email clare@swpesca.prestel.co.uk.

Farmers in Wales should be aware of Opportunity Wales who provide similar help for Objective 1 areas and can also provide information on the Princes Trust. See

www.opportunitywales.co.uk

or Tel 0845 8500 888.

Services

- Beta Technology Limited provides assistance to small and medium sized enterprises to obtain European funding for research and development projects, and by providing innovation services to companies to help them develop, promote and acquire new technologies. Beta is the designated UK National Contact Point (NCP) for SMEs for co-operative research within the EU's Sixth Framework Programme. Please see

  www.betatechnology.co.uk

  or contact Beta Technology Ltd.,
  Barclay Court, Doncaster Carr, Doncaster,
  South Yorkshire, DN4 5HZ.
  Tel: 01302 322633.

- Throughout Europe there is a network of European Information Centres that can provide fund-matching services that allows companies and other organisations to identify relevant funding opportunities. This is not restricted to specific organisations or sectors, but is open to all organisations and businesses in the relevant region that wish to explore funding opportunities. For information on all offices please see

  http://europa.eu.int/comm/enterprise/networks/eic/eic-geo_cover_en.html

  An example of one of these centres is the Southern Area European Information Centre at

  www.euro-info-centre.co.uk

  or contact Southern Area EIC,
  Northguild, Civic Centre, Southampton, SO14 7LW.
  Tel: 023 8083 2866.

- The Welsh European Funding Office also offers a similar match funding service at

  www.wefo.wales.gov.uk

  or Tel: 01443 471100;
  e-mail enquiries-wefo@wales.gsi.gov.uk.

- The Business Link service is often able to provide further information on funding at a local level and provide further business services. Examples of websites for these being

  www.businesslinkwessex.co.uk
  www.blinkdandc.com

- The Seafish Industry Authority (Seafish) has two aquaculture development officers who can provide help and assistance to fish farmers and prospective fish farmers. Please see

  www.seafish.org/sea/aquaculture.asp

  or consult the contact details in the back of this issue of Shellfish News.

- Additionally there is various information that can be found on-line that has been written by consultants and includes other areas such as Lottery funding.

SHELLFISH STUDIES AT C-MAR

Niall McDonough (Manager)
Centre for Marine Resources and Mariculture (C-Mar), Queen's University Marine Laboratory, 12 The Strand, Portaferry, Co. Down, BT22 1PF, Northern Ireland

The Centre for Marine Resources and Mariculture (C-Mar) at Queens University Belfast, is currently undertaking a number of bivalve shellfish studies as part of its brief "to facilitate and stimulate sustainable development of mariculture enterprise".

Mussel cultivation

Work began in 2003 on an investigation of the blue mussel (*Mytilus edulis*) seed resource in the North Irish Sea and on-growing strategies for the Northern Ireland seabed mussel cultivation industry. This study is funded by DARD Northern Ireland through the EU Programme for Building Sustainable Prosperity. The primary goal is to assess current and alternative seed supply and on-growing methodologies to maximise yield and return for mussel growers, per tonne of seed and per hectare of seabed. It is hoped that information derived from this study will contribute to the ongoing development of a sustainable seabed mussel sector in Belfast Lough and Carlingford Lough.

Seed mussel (Mytilus edulis) off the Ards Penninsula, Northern Ireland (Photograph by Dr Nuala McQuaid)

Native oysters (Ostrea edulis) in Strangford Lough, Northern Ireland (Photograph by Dr. Dai Roberts)

Native oyster restoration

Another study (funded in 2003 from the same source) is currently contributing to native oyster (*Ostrea edulis*) restoration in Strangford Lough. Following on from previous restocking programmes in the late 1990's, this project involves continued monitoring of distribution and recruitment of native oysters in the Lough. Also included is a genetic analysis of stocks of natives from within the Lough and further restocking with seed produced both at a commercial hatchery and C-Mar's hatchery at Portaferry, Co. Down. The project is being carried out in conjunction with the Strangford Lough Shellfishermen's Co-op.

Razor clam harvesting

A three-year project entitled, "Sustainable Harvesting of *Ensis* (Razor clams)" or "SHARE" has just been awarded funding of €0.86m by the Interreg IIIB Altalntic Area Programme. Lead by C-Mar, the project has partners in Spain (University of La Coruna and CIMA), Portugal (IPIMAR) and the Republic of Ireland (BIM). The project aims to take a "seed to market" approach to the development of sustainable production of razor clams (*Ensis siliqua* and *Ensis arcuatus*) in the Atlantic Area. The project partners hope to achieve this by developing protocols and recommendations for hatchery rearing, nursery production, ranching and reduced-impact harvesting. BIM will examine product development, quality indices, and optimal criteria for holding and transport to market.

Further information

Updates on the results of the above research will be submitted to future editions of Shellfish News. In the meantime, further information on these and the rest of C-Mar's projects can be obtained by contacting C-Mar at +44 28 42729648 or logging on to our website at www.qub.ac.uk/bb/cmar.

A VIRUS INFECTION IN EUROPEAN BROWN SHRIMPS (*CRANGON CRANGON*)

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The brown shrimp

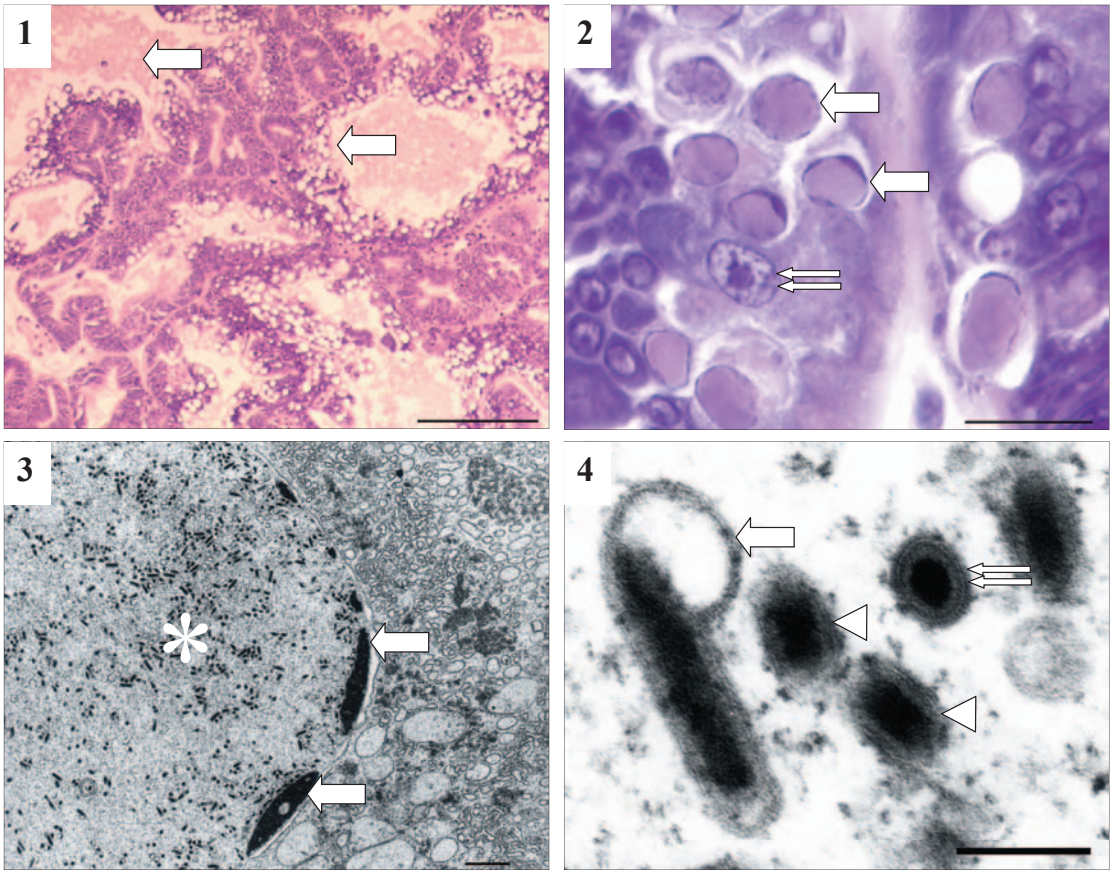
Crangon crangon supports an important fishery in Europe, with catches amounting to over 25,000 tonnes per annum (with a first sale value of more than 80 M Euro). The largest fishery for this species is located in the Wadden Sea (a nursery area for this species). Fishing fleets from Germany, the Netherlands, Denmark and France are responsible for the majority of landings. Due to its occurrence in large populations and its role as a predator and a prey organism, it is a key species involved in cycling of the benthic habitat. In this fishery, it has been stated that in addition to fishing mortality, environmental factors (such as salinity, depth and predator density) can affect the natural mortality rate. Recent studies on UK estuarine populations of *C. crangon* carried out in our laboratory have led to the discovery of a highly prevalent virus infection in the hepatopancreas. As such, due to the apparent pathogenic nature of this virus, we propose that this disease may also play a role in modulating populations of this important species.

A strange pathology in the hepatopancreas

C. crangon collected from sites in the Clyde, Mersey, Thames and Alde estuaries during sampling exercises arranged under the National Marine Monitoring Program (NMMP) were processed for histology and electron microscopy using standard methods.

Whilst there were no obvious signs of external disease in any of the shrimp collected, histological analysis revealed a characteristic pathology in 98% of the shrimp from the Clyde estuary and 95%, 72% and 70% of the shrimp from the Mersey, Alde and Thames estuaries respectively. Affected shrimp showed degeneration of the hepatopancreatic tubules, causing a loss of histological structure in the organ (Figure 1). At higher magnification, significant changes were seen in the nuclei of epithelial cells lining the hepatopancreatic tubules and of the epithelial cells lining the midgut (Figure 2). Cells containing such nuclei appeared singly or in clusters and the cytoplasm of intact affected cells appeared denser than unaffected cells, with an apparent loss of lipid storage inclusions. The percentage of hepatopancreatic epithelial cells containing aberrant nuclei and the relative degree of pathology differed between individual shrimps, suggesting that the condition had a progressive nature, leading eventually to the degeneration of the hepatopancreas.

Higher power views of affected cells, obtained using a transmission electron microscope revealed that the nuclei of these aberrant hepatopancreatic epithelial cells contained large numbers of rod-shaped, cylindrical, envelope-bound particles (Figure 3). The mean length of these particles was approximately 280 nm, with a mean diameter of 72 nm. The envelope surrounding each particle was approximately 5 nm in width (Figure 4). The structural nature of these particles is in accordance with previous virus infections described in invertebrates. They most closely resemble the intranuclear bacilliform virus (IBVs) described infecting other crab, crayfish and shrimp populations from around the world. This is the first report of an IBV in the Crangonidae family of shrimps and as such, we have termed this virus *Crangon crangon* bacilliform virus (CcBV).



Figures 1-4. CcBV-infected hepatopancreatic epithelium. (1) Low power light micrograph of tubular degeneration in mid-late infection (arrows). (2) Higher power light micrograph of infected cells showing aberrant nuclei (arrows). An apparently uninfected nucleus is also highlighted (double arrows). (3) Electron micrograph of infected cell showing periphery of nucleus containing masses of virions (asterisk). Note the margination of host genetic material (arrows). (4) High power electron micrograph of virions in lateral section (arrow), transverse section (double arrow) and elliptical section (arrow heads). Scale bar = 100 nm

Significance to brown shrimp stocks

Description of this highly prevalent and apparently pathologic IBV in *C. crangon* is particularly important due to the relative lack of information on such infections in wild (non-cultured) crustacean species and the importance of *C. crangon* as a fishery resource. Although pathogenesis trials have not yet been carried out, it is suggested that the pathology associated with CcBV infection is serious enough to lead to dysfunction of the hepatopancreas and ultimately to death of the host.

Since *C. crangon* supports an important fishery in Europe and due to its key position in many benthic habitats, it is recommended that further studies are carried out on the prevalence of this infection in important fisheries sites. If significant infection prevalence is detected on such fishing grounds, it is likely to create an additional loading on natural mortality in *C. crangon* stocks. In addition, since *C. crangon* is also regarded as cannibalistic (accounting for between 2 and 42% of mortalities in these fisheries) and that it is presumed that CcBV is transmitted horizontally (as for other IBVs), it is tempting to suggest that cannibalism may also lead to the very high prevalence of CcBV infection in *C. crangon* populations. However, the pathological manifestation of CcBV would also lead to liberation of infective stages (as loose virions and sloughed epithelial cells) in the faeces. In this way, transmission may occur by coprophagy and benthic scavenging by *C. crangon*. Further studies are required to assess whether CcBV has the ability to affect recruitment in *C. crangon* fisheries and to whether this virus is specific *to C. crangon* or can infect other benthic crustacean species inhabiting these environments.

Acknowledgements

The authors acknowledge the support of the Department of Environment, Food and Rural Affairs (Defra) under contract CDEP 84/5/312

Reference

Stentiford, G.D., Bateman, K., Feist, S.W. Pathology and ultrastructure of an intranuclear bacilliform virus (IBV) infecting brown shrimp *Crangon crangon* (Decapoda: Crangonidae). Diseases of Aquatic Organisms 58, 89-97.

TOOTHPASTE FROM SHRIMP SHELLS

It is reported that crab fishermen have healthy teeth, because they often chew on freshly cooked crabs.

A year ago a student in Germany told his father of a project in his school studying shrimp shells. The shell is made of chitin, from which chitosan can be obtained. Chitosan has antibacterial qualities and it was found that it could cover teeth with a fine film that protects them from bacteria.

The father of the student was the head of the company "B&F-Elektro" and together with the college, The Institute for Environmental Technology (Eutec), this gave him the idea to develop a toothpaste made from shrimp shells. The product, named "Chitodent", was introduced, with much publicity, to the German market on 1st March this year.

Initially the tubes are being filled manually at a small establishment, located next to the college, with production of only 2,000 tubes per month. Negotiations with companies that can produce greater amounts at a more competitive price are in progress. At the moment, this natural dental paste can be only acquired via the Internet (see http://www.chitodent.de/en/index.html), but the distributors are also already negotiating with some supermarket chains. Each tube of "Chitodent"

costs 6.48 euro. Despite this high cost orders are arriving at the rate of 10,000 per month, from all over Germany.

The toothpaste does not smell or taste of shrimp as chitosan is odourless and at the high degree of purity used is also non-allergenic. The bitter flavour from the additional products used to create the foam is disguised with mint, which is common to many types of toothpaste.

The main problem at present is in obtaining sufficient raw material necessary for the production of "Chitodent". Most of the shrimps fished locally are exported whole to Morocco, but the manufacturers hope to buy in chitin in a pulverized state, possibly from China.

SUPPLEMENTARY GUIDANCE FOR LABORATORIES UNDERTAKING THE TESTING OF LIVE BIVALVE MOLLUSCS

Committee of representatives from UK microbiological laboratories undertaking shellfish testing including the CEFAS Weymouth Laboratory (as the National Reference Laboratory), FRS Marine Laboratory Aberdeen, Health Protection Agency, Northern Ireland Public Health Laboratory and Hospital Trust Laboratories

Introduction

The UK standard method for E. coli in shellfish is described in the appendix to Donovan TD, et al (1998) [Modification of the standard UK method for the enumeration of *Escherichia coli* in live bivalve molluscs. Communicable Disease and Public Health **1,** 188-196]. Additional advice was published in November 2002 in Shellfish News Number 14 (pp 26-28, see http://www.cefas.co.uk/publications/shellfishnews/shellnews14.pdf). Further developments at the national and international level have necessitated this supplementary guidance document.

Receipt of Samples

Transporting a sample under appropriate conditions in a suitable container until commencement of testing is a vital part of any microbiological analysis. The quality of the result is dependent upon the quality of the sample. If the sample examined is not representative of the material under test, is contaminated during sampling or storage, or is incorrectly stored during transport, then the reported result may be misleading, no matter how reliable the test method is.

(a) Sample container
A sample must be received in an intact food grade plastic bag. The container/bag should be labelled with the sender's reference number and any other relevant information (e.g. species). Samples should not be examined if they are received unlabelled.

(b) Temperature on receipt
For samples taken as part of the harvesting area classification programme, the sampling officer should take the temperature of the surrounding seawater at the time of sampling and record this on the collection form. Shellfish samples properly packed in a cool-box should reach a temperature of less than 8°C within 4 hours and then maintain this for at least the maximum elapsed period specified in the sampling protocol. Such samples should not be received frozen, if they are, they should not be tested. On receipt, the internal air temperature of the cool-box should be checked and recorded.

Elapsed time between sample collection and receipt is 4 hours or more
If the internal air temperature exceeds 8°C then the between sample temperature should be checked. If this is at or below 8°C, the samples are satisfactory. If this is above 8°C, they are not satisfactory.

Elapsed time between sample collection and receipt is less than 4 hours
The air and contents will not necessarily have reached 8°C or less within this period but should be less than the temperature at the time of sampling.

(c) Condition of sample
A sample is also considered unsatisfactory on receipt when:
- The sample bag is received leaking such as to lead to potential contamination of that of other samples
- The shellfish are immersed in water or mud/sand

If samples are received in a state in which they are considered unsatisfactory, a note should be recorded to this effect and the sending authority should be informed that this may be a factor affecting the quality of the result.

Testing Live Bivalve Molluscs for *Salmonella*

The appendix to Donovan et al 1998, also includes a description of a method for the detection of Salmonella in bivalve molluscan shellfish. Since that was published, EN ISO 6579 "Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp." has been revised and the use of this method is now recommended. However, because the revised method has not been thoroughly validated with respect to *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C, laboratories testing samples specifically for these organisms may wish to consider supplementing EN ISO 6579 with the method for *S.* Typhi given in Donovan *et al*. 1998.

Additional Information

Additional information on the microbiological testing of shellfish with respect to the UK may be found on the Internet at: www.nrlcefas.org

SEWAGE DISCHARGE IMPROVEMENTS IN THE SOLENT AND THEIR EFFECT ON THE SHELLFISHERY

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Introduction

The Solent contains the largest native oyster (*Ostrea edulis*) fishery in Britain, with beds extending from Hurst Point (mainland) and Totland Bay (Isle of Wight) in the west to Spithead and Bembridge Ledge some 40 km or so to the east.

Shellfish harvesting areas are classified under the Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998, according to the extent of contamination shown by monitoring *E.coli* in shellfish flesh. The classification categories and associated processing requirements are shown in Table 1.

The Food Standards Agency (FSA) is the competent authority for these hygiene controls in the UK. CEFAS manages the microbiological monitoring programme on behalf of the FSA and also has a role in providing shellfish related water quality advice to Defra and the Environment Agency (EA). This advice is aimed at maximising the potential of water quality improvements under water company investment plans through pre-scheme discussions and negotiations with both EA regions and water companies. Liaison is also undertaken on water quality issues in shellfish harvesting areas with Government and industry in relation to protection of current status and investigation of contamination problems. Local Food Authorities (LAs) undertake the sampling and have enforcement responsibility for areas within their jurisdiction.

Bivalve molluscs obtain their nourishment from the water column by filtration of small particulate material. By this action they will concentrate many contaminants, including microbial pathogens if these are present. Sewage contamination appears to be the most significant cause of shellfish-associated illness and, in temperate developed countries, gastro-intestinal illness associated with viruses derived from human faecal contamination predominates (Rippey, 1994. Infectious diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Reviews*, 7, 419-425).

There are densely populated areas around the Solent and a large proportion of the sewage generated in these areas has historically been discharged directly into the Solent often, until quite recently, with minimal treatment.

Recent classification history of the Solent

In 1995 there were two areas designated as Prohibited for the collection of bivalves (Yarmouth and Pennington/Lymington Banks – see map) with the remainder of the Solent being split between class B and C areas.

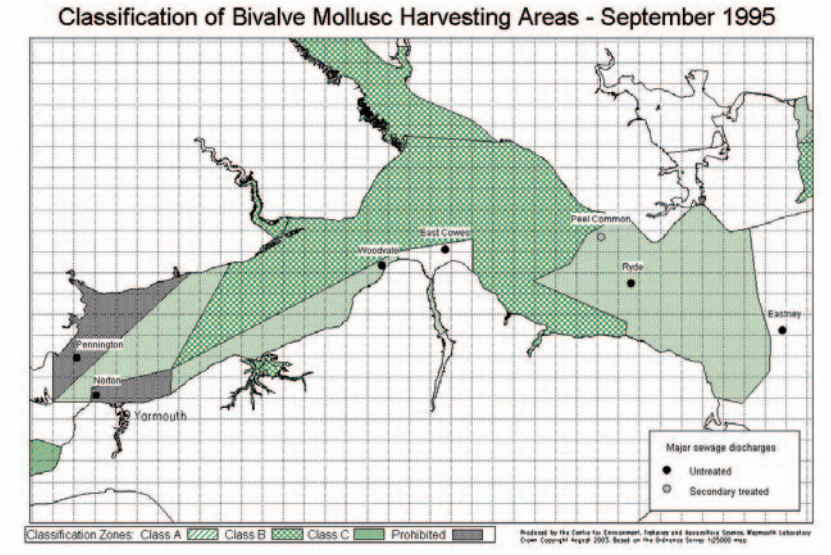
With sewage scheme improvements introduced under the Bathing Waters Directive, Urban Waste Water Treatment Directive and more recently the Shellfish Waters Directive drivers, the bivalve mollusc classification scenario has gradually improved in the Solent to the point that today all of the Solent oyster fishery is at least class B. For comparison, maps showing the classification status of the Solent in 1995 and 2003 together with the sewage discharge arrangements in place at the time are shown opposite.

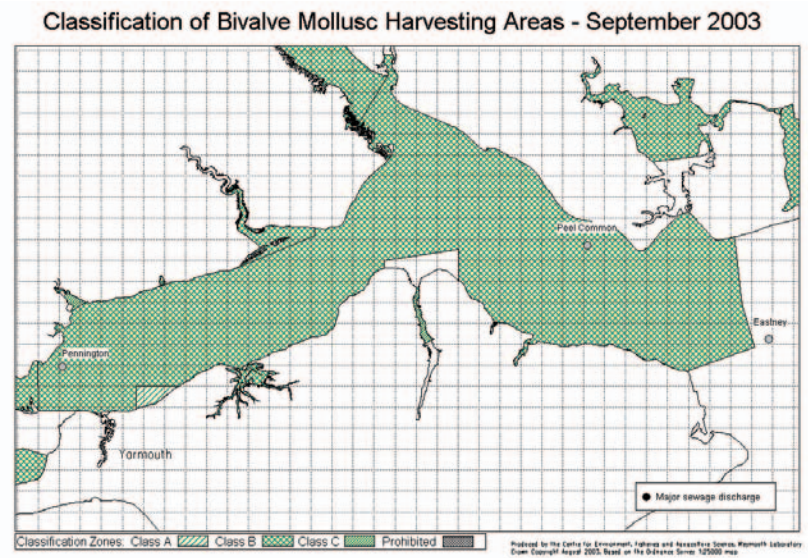
***Pennington area**:* The discharge from the waste water treatment works at Pennington was improved from crude to secondary treatment in April 1997 and following this change, a marked improvement in the shellfish results was noted from monitoring nearby.

At points adjacent to the outfall, the average level of contamination has been reduced by around a factor of 10. In addition, the spread of results has been reduced.

Table 1. Classification categories under the Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998

Class	Criteria	Requirements
A	<300 faecal coliforms or 230 *E. coli* per 100g	Can be collected for direct human consumption
B	90% compliance with 6,000 faecal coliforms or 4,600 *E. coli* per 100g	Must be purified or relayed to meet class A; may also be heat-treated by an approved method
C	<60,000 faecal coliforms or 46,000 *E. coli* per 100g	Must be relayed for a long period (at least 2 months) to meet class A or B; may also be heat-treated by an approved method





That is, results are generally more consistent. This may reflect improved storm water retention arrangements. There may now be fewer overflow events and/or more consistent continuous effluent quality. The improvement in shellfish results that the discharge improvements brought about in this case led to the classification being upgraded from Prohibited and eventually to class B.

Yarmouth area*:* More recently, Southern Waters' £200 million Seaclean Wight scheme (in response to Bathing Water and Urban Waste Water Treatment Directives) has brought more widespread improvements in quality to the Solent shellfishery. Key to the scheme was a new sewage treatment works at Sandown receiving flows diverted from the discharges in the Solent at Norton. Treated wastewater from the Sandown works is pumped out through a 3.2 km outfall off of Sandown itself on the south of the Island. As well as removal of continuous discharges, the improvements have also led to a reduced number of storm water spills to sea with the construction of a series of underground storage tanks built to intercept flows during heavy rain and pass them for treatment after the rainfall has stopped. . Of relevance to the shellfish at Yarmouth, Seaclean Wight resulted in the cessation of continuous discharges of untreated sewage from the long sea outfall at Norton. In April 2001 this effluent was diverted from Norton to Sandown. The number of intermittent discharges of contaminated storm water during wet weather was also reduced.

There appears to have been a reduction in the average level of the shellfish results by a factor of 100 at the sites here. As was seen at Pennington, the general spread of results also appears to have decreased with greater consistency now being achieved. The classification of the bed has improved from Prohibited to class B/A.

Portsmouth area: Another major scheme leading to significant improvements was the Portsmouth and Havant Environmental Improvement Scheme. Under this scheme, wastewater from Portsmouth was

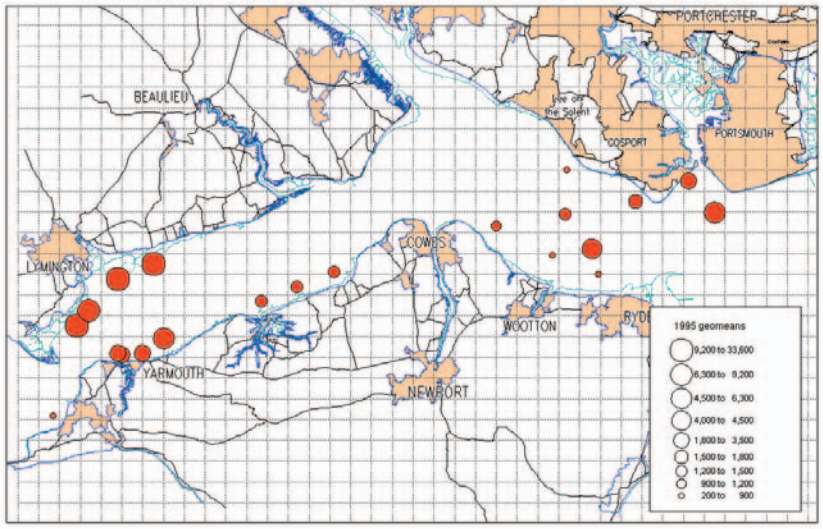
transferred to Budds Farm (Havant) treatment works for secondary treatment. Previously, flows were released into the Solent through a long sea outfall at Eastney after basic screening. However, the completion of a 7.8 km transfer tunnel, underneath Langstone Harbour, to Budds Farm, allowed the wastewater to be fully treated at the upgraded Havant site. The treated wastewater is then released 5 km out to sea at Eastney via a new pumping station. The old outfall into Langstone Harbour no longer discharges sewage continuously (although it retains an intermittent discharge capability) as the combined flows have been pumped back to Eastney and discharged down the existing 6 km outfall since October 2001. The continuous discharge of untreated sewage from Ryde long sea outfall ceased in April 2001.

Following these improvements, the last remaining class C areas in the Solent at the mouth of Portsmouth Harbour have now been upgraded to class B. The average level of contamination has been reduced by around a factor of 10 at the most impacted sites. Maps are shown to illustrate the change in average level of contamination between 1995 and 2002.

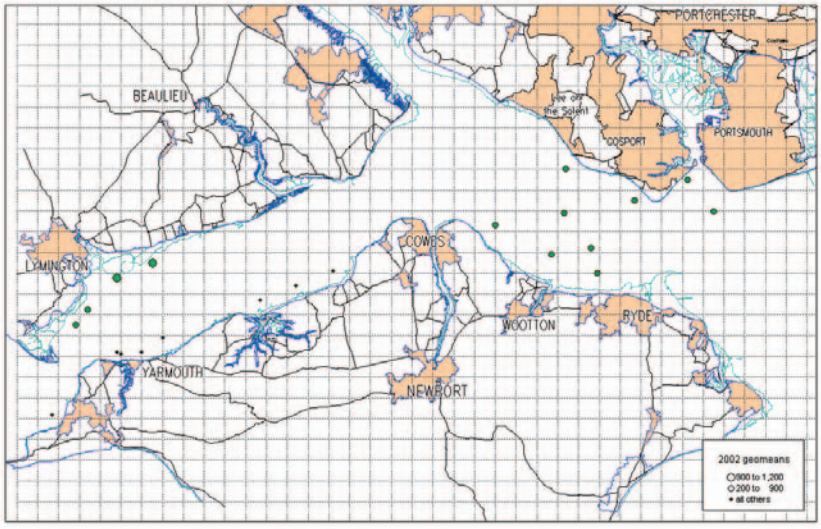
Benefits and future plans

The various discharge improvements that have taken place in the Solent have clearly had a significant beneficial effect in terms of the microbiological quality of the shellfishery. Any improvements in classification should of course bring benefits to the industry in terms of an improved price for the shellfish on the open market. The fact that one area (Yarmouth East) is now showing class A compliance when it was previously Prohibited demonstrates the degree of improvement that can be achieved. There remain, however, a number of intermittent discharges into some parts of the Solent and these will of course continue to have an impact from time to time. Planned improvements still to be delivered in Southern Water's current Asset Management Plan 3 ('AMP3') investment period from 2000 to 2005 include tertiary treatment (UV disinfection) for sewage works discharges to Chichester Harbour and into the Eastern Solent (Peel Common and Budds Farm Sewage Treatment Works) and reduction of spill events from a number sewer overflows to the Solent and its harbours programmed for completion by the end of March 2004 and end of March 2005.

Geometric mean E.coli results over 1995 by monitoring point



Geometric mean E.coli results over 2002 by monitoring point



There are no discharge improvement schemes targeted specifically at Solent shellfish waters currently planned in the next water companies' investment programme AMP4 (2005-2010). However, benefits of improvements to be delivered towards the end of AMP3 together with benefits accruing in AMP4 from schemes identified under other drivers e.g. Urban Waste Water Treatment Directive and Habitats Directive, should deliver a continued trend of improvement for shellfish waters in the Solent over this period.

Acknowledgements

The authors would like to acknowledge information provided on discharge improvements by Southern Water and the Environment Agency.

THE RELATIONSHIP BETWEEN PHYTOPLANKTON AND ALGAL TOXINS IN SHELLFISH, FAL ESTUARY UK

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Introduction

The technique of monitoring water samples for causative organisms of algal biotoxins is one of the most valuable techniques to provide early warning of potential harmful toxicity incidents in a shellfish harvesting area. In some cases, however, there does not appear to be a direct relationship between the presence of toxic algae and the amount of toxins in shellfish. This may be due to a number of factors including sampling methods, frequency of sample collection and local environmental conditions. Non-correlation of results may also arise due to the presence of non-toxic strains of a phytoplankton species. Toxic and non-toxic strains may be morphologically identical and therefore not distinguished by microscopy, yet genetically distinct. In these cases species identification can only be determined by molecular methods. One such example is *Alexandrium tamarense*, which has both toxic (containing PSP toxins) and non-toxic strains.

Outline of the study

This project was designed in order to investigate the relationship between the presence of toxic phytoplankton in the water and toxins in shellfish in the Fal Estuary and address the possible reasons for non-correlation of results. The Fal Estuary was chosen for this project due to the economically important bivalve shellfishery and the historic record of toxins occurring in shellfish in the Fal. Paralytic Shellfish Poisons (PSP), Diarrhoeic Shellfish Poisons (DSP) and Amnesic Shellfish Poisons (ASP) have all been recorded in shellfish from the Fal and the causative algal species (*Alexandrium*, *Dinophysis* and *Pseudo-nitzschia)* have also been present in water samples. This was one monitored site of a larger FSA funded project led by Eileen Bresnan at the FRS, Aberdeen. The objectives have included the identification of toxic algal species, analysis of shellfish toxins, comparison of sampling methods used and the comparison of the genotype versus toxicity of suspected toxic algal species.

Methods

The sampling programme ran from April 2001-September 2002. Water samples were collected by a variety of methods, including a tube sampler for an integrated water column sample (Figure 1). Live phytoplankton samples were collected for the

Figure 1. Use of 15 m (three 5 m sections) tube sampler in the Fal

establishment of laboratory cultures of potentially toxic species. High Performance Liquid Chromatography (HPLC) methods were used for analysis of toxins in shellfish and phytoplankton cultures.

Results

Phytoplankton analysis detected *Alexandrium* species at each of the monitored sites during the period of May–June of both years with maximum occurrence of 130,775 cells l^{-1} in early June 2001. Maximum PSP toxin concentration in mussels reached 63.98 µg STX/100g tissue in June 2001 and corresponded to the presence of *Alexandrium* cells in water samples. A number of *Alexandrium* cells were successfully cultured from this period and were positively identified as *Alexandrium minutum* by molecular sequencing and microscope analysis. PSP toxin profiles of the mussel samples were comparable to the *A. minutum* PSP toxin profiles and suggest that this dinoflagellate was the causative organism of PSP toxins in shellfish in the Fal during 2001. *Alexandrium* cell densities were much lower in 2002 and this was reflected in the low toxin concentrations seen in shellfish in that year.

A *Pseudo-nitzschia* (Figure 2) bloom was present in the Fal during June 2001 and travelled northwards up the estuary from the English Channel. Domoic acid was detected in shellfish when *Pseudo-nitzschia* spp. cell concentrations were above 500, 000 cells l^{-1}. A number of *Pseudo-nitzschia* have been successfully cultured and identified by Transmission Electron Microscopy (TEM) (Figure 3) and molecular sequencing. Toxicity analysis of cultures has shown the coexistence of non-toxic and toxic *Pseudo-nitzschia* species in the Fal. These results are a possible explanation of periods of high cell counts of *Pseudo-nitzschia* and low ASP concentrations detected in shellfish in the Fal Estuary.

Possible causative organisms of DSP, *Prorocentrum lima*, *Dinophysis acuminata*, *D. acuta*, *D. norvegica* and *D. rotunda* have also been found infrequently and sporadically at sites within the Fal and this has been reflected in shellfish samples, where DSP toxins have only been found infrequently and at very low concentrations.

Further work

Statistical analysis of results will provide information to compare the efficiency of the various sampling methods used in this project and help to provide information to improve the design of future water monitoring programmes.

Molecular techniques are being developed to monitor *Alexandrium* species in water samples to aid in the explanation of periods of non-consistent correlation between *Alexandrium* cell counts and levels of PSP toxins in shellfish which have been highlighted by two years of water and shellfish sampling in the Fal Estuary.

Further information

This work forms part of a PhD registered at the University of Westminster that is scheduled for completion in September 2004. This work was part-funded by FSA contract B04005 and CEFAS Seedcorn. Contact: Linda Percy, email l.a.percy@cefas.co.uk.

Acknowledgments

Dr Eileen Bresnan, FRS Aberdeen (B04005 contract leader). At CEFAS Weymouth; Dr. Steven Morrris (toxin analysis), Dr. David Stone (molecular biology) and Kelly Bateman (electron microscopy). For sample collection; Mike Tuffrey (*Patrice II*) and Falmouth and Truro Port Health Authority Officers Colin Bate and Shaun Walker.

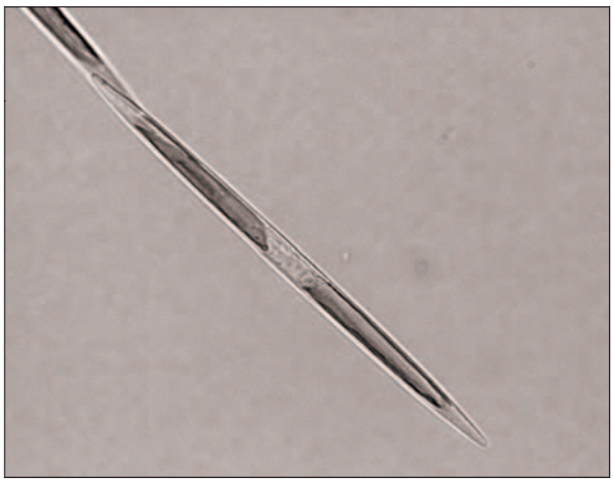

Figure 2. Light micrograph of Pseudo-nitzschia fraudulenta (culture). Cell length = 80 µm

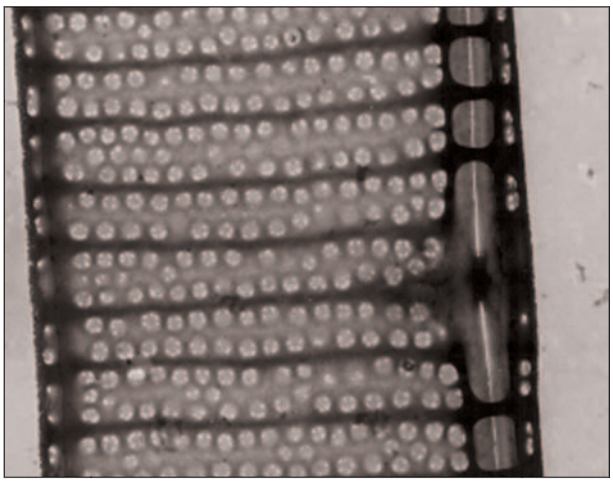

Figure 3. Transmission electron micrograph of Pseudo-nitzschia fraudulenta (culture). Scale Bar = 200 nm

ASP ELISA – A VALIDATED RAPID ASSAY FOR THE DETERMINATION OF ASP LEVELS IN SHELLFISH

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Background

To protect consumer health and increase confidence in food safety with regards to shellfish, the European Union has implemented quality assurance programs and value limits for marine biotoxins. Live shellfish must be analyzed prior to harvesting, and processed shellfish must meet the end product standards related to algal toxins according to Directive 91/492/EC, and Decisions 225/2002/EC and 226/2002/EC.

The analysis of shellfish for toxins causing amnesic shellfish poisoning (ASP) is currently being performed by the reference method high-performance liquid chromatography (HPLC) with UV-detection, or in combination with mass spectrometry (LC-MS). Although HPLC analysis is accurate and provides high-resolution profiles, this method requires expensive instrumentation with high maintenance costs, highly skilled operators, and a well-established laboratory infrastructure. Furthermore, HPLC has a low daily sample-throughput, and requires the shipment of samples from the shellfish farming facility to a centralized laboratory, adding a significant delay due to logistics. Given current concerns on marine biotoxins there is a requirement for alternative rapid assays, which allow real-time monitoring on-site, in accordance with the HACCP concept (Hazard Analysis at Critical Control Point).

The marine biotoxin program at Biosense Laboratories aims to present reliable, validated rapid assays that can accurately quantify algal toxins in shellfish, and meet the method performance standards set by regulatory authorities.

The ASP ELISA kit

The ASP ELISA kit is a rapid assay designed to accurately measure the ASP toxin component domoic acid (DA) in shellfish to comply with the regulatory limit at 20 µg DA/g shellfish. The kit has also proved suitable for the analysis of DA in other matrices, like algal extracts and seawater. Each ASP ELISA kit contains all the key components sufficient to accurately quantify 36 individual samples. The assay analysis time to complete one kit is about 1.5 h.

The assay is based on the detection of DA by specific antibodies in a direct competition format, where free DA in the sample competes for binding to the specific antibodies with DA-conjugated protein coated on the plastic wells. Samples are mixed with the antibody in a DA-coated microwell, and after incubation and subsequent washing, a colour reaction indicates the level of DA present in the sample (Figure 2). The colour intensity is read using a standard microplate absorbance reader. Prior to analysis, the sample preparation for the ASP ELISA involves only a 50% MeOH extraction of the shellfish homogenate.

Figure 1. The ASP ELISA kit contains a sealed pre-coated 96-microwell plate, and all the key components required to accurately measure DA levels in 36 individual samples

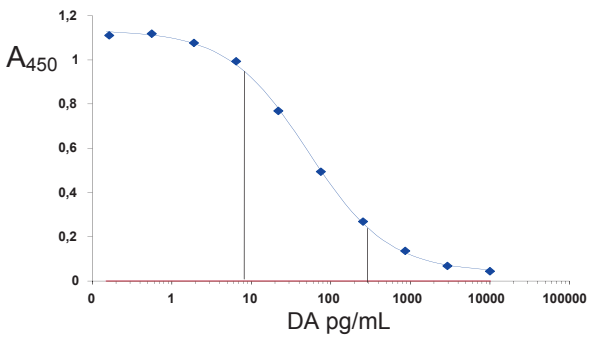

Figure 2. The ASP ELISA kit employs a 10-point calibration curve. The calibration range is defined by the kit software for each assay run, and samples are diluted to fall within this range (10-260 pg/mL)

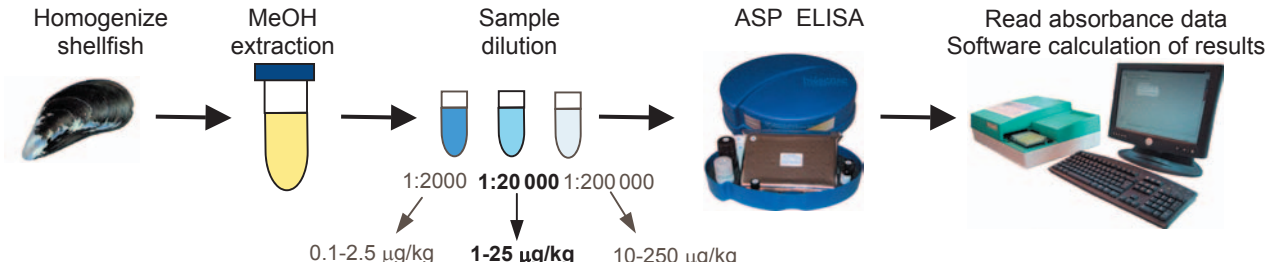

Figure 3. Performing the ASP ELISA involves a simple sample preparation, dilution of the extract according to the concentration range of interest, running the direct immunoassay (1.5 h), reading the 96-microwell plate on a platereader, and run the absorbance data through user-friendly software that automatically quality controls the analysis and calculates the sample results

In contrast to the laborious preparation of MeOH-extracts before HPLC analysis, which involves time-consuming pre-clean up and pre-concentration steps, the MeOH-extract can be analyzed directly by the ASP ELISA without any further treatment. Due to the high sensitivity of the assay, the MeOH-extracts are routinely diluted 1:20 000 with buffer to fall within the calibration curve (Figure 2) and quantify samples from 1 to 25 µg/g (Figure 3). The ASP ELISA can accurately determine low levels of DA in shellfish, equal to 20 000 times below the regulatory limit (limit of quantification 0,01 µg DA/g shellfish), and the working range has shown to be linear for naturally contaminated samples up to 250 µg/g.

The specific polyclonal antibodies were developed from immunized sheep by AgResearch Ltd (Hamilton, New Zealand), and show no unspecific cross-reactivity to non-toxic, structural analogues to DA or compounds normally interfering with LC-MS analysis. A 1:200 dilution of toxin-free MeOH-extracts of both raw and pre-cooked blue mussels, king scallop (whole scallops and muscle plus gonads) and pacific oysters overcomes the matrix interferences observed at lower dilutions. For the direct analysis of toxin-free seawater, pre-filtration and 1:30 dilution was sufficient to eliminate any unspecific assay response, providing a calculated detection limit at 300 ng free DA/L seawater. The ASP ELISA has proved useful for environmental monitoring, as the high assay specificity allows the direct analysis of body fluids from mammals intoxicated by DA.

Free software to ease sample calculation

To ensure that data handling and calculation of the sample concentrations is performed consistently and reliably, user-friendly software is designed which is provided together with the ASP ELISA kit. The calibration curve for each assay run is automatically and quality controlled against specified parameters, to ensure that the given calibration is valid. Furthermore, the data for each individual sample calculation is also automatically checked for any errors during the sample analysis. The results are given directly as µg DA/g shellfish, or as ng/mL for samples in solution.

Internal validation of the ASP ELISA kit

The assay performance parameters of the ASP ELISA kit were evaluated by an internal single-laboratory validation, performed according to AOAC guidelines. The sensitivity and working range were confirmed by the analysis of blank shellfish samples (blue mussels, king scallops and pacific oysters) that were spiked with certified reference material containing DA. The within-laboratory recovery at 106% and repeatability at 9.4% (n=172), proved the ASP ELISA to be accurate and reliable for DA analysis.

Inter-laboratory validation and method comparison with HPLC and LC-MS

To obtain inter-laboratory validation data on the precision and accuracy, a collaborative study was performed in accordance with AOAC guidelines. The study was coordinated in collaboration with external technical assessors at the Cawthron Institute (Nelson, New Zealand). A total of 16 laboratories from 10 countries (Europe, USA, Chile, New Zealand) participated in the study. All the participants were analyzing marine biotoxins on a routine basis, but few had any previous experience with the ELISA technique. Five of the participants analyzed all samples with their accredited in-house HPLC or LC-MS method in parallel to the ASP ELISA. From over 300 spiked samples distributed and analyzed in total, the mean inter-laboratory recovery was 104%, the repeatability was 15% and the overall inter-laboratory reproducibility (%CV) was 23%. The HORRAT value was 1.7, according to the AOAC requirements.

The correlation between the ASP ELISA against the expected spike values was excellent (r^2=0.99) with a slope at 1.015, but the HPCL and LC-MS values tended to underestimate the expected spike values with a slope at 0.889 (r^2=0.99). Due to this underestimation, the ASP ELISA correlation to the instrumental methods was 0.98 (slope 1.29).

Conclusion

Through comprehensive validation studies, we have shown that the ASP ELISA is suitable for the routine monitoring of ASP toxins in shellfish. The inter-laboratory study has shown that the ASP ELISA is reliable, and that the accuracy is excellent when correlated to expected spike values. The methodology gives a significantly higher sample throughput capacity, and is therefore a good alternative to the HPLC method. The ASP ELISA kit will allow the rapid analysis of ASP in regional laboratories in accordance with the HACCP concept, without the costly and time-consuming shipment of samples to HPLC laboratories.

Biosense Laboratories aims to obtain method accreditation for the ASP ELISA within the near future, with the intention to have the method accepted by European regulatory authorities as an alternative to the HPLC method. Similar rapid assays for other marine biotoxins are under development, and will be validated similar to the present study on the ASP ELISA kit.

Further information

The results presented in this article are based on Biosense Laboratories Reports 2003:1/HK and 2003:6/HK. Manuscripts are currently being prepared for submission to peer-reviewed journals.

For any further information, please contact Dr. Hans Kleivdal, Tel: +47 5554 3967; e-mail: hans.kleivdal@biosense.com or visit the biosense web site (www.biosense.com).

ANNOUNCEMENTS

NEW SHELLFISH WATER DESIGNATIONS ANNOUNCED

The Government has designated five new shellfish waters and extended five existing designations in England under the EC Shellfish Waters Directive (79/923/EEC). The announcement was made in the Secretary of State's Principal Guidance on the 2004 periodic review of water price limits published on the 11 March.

Following the designation of 93 English shellfish waters in 1999, the Government pledged to keep the number of shellfish water designations in England under review. A review of designations has now been completed and five new shellfish water designations have been made in the following areas:

Humber
Stour Estuary (Kent)
Weymouth Bay
Start Bay
Fowey (Wiseman's Reach)

The Government has also extended the existing shellfish water designations listed below:

Osea Island
Percuil
Camel
Lune (Wyre Estuary)
Morecambe Bay East

Defra has now served a Notice on the Environment Agency to officially designate the new areas as shellfish waters within the scope of the Directive. The Notice came into effect in April 2004.

The UK is committed to maintaining a broad match between designated shellfish waters and shellfish harvesting areas and the new designations and extensions will help maintain this match. Future designations may also be made, if necessary, to maintain the broad match.

PUBLICATION OF STRATEGY UNIT REPORT "NET BENEFITS: A SUSTAINABLE AND PROFITABLE FUTURE FOR UK FISHING"

The Prime Minister's Strategy Unit have just concluded their year-long investigation into the future of the UK fishing industry. The project was set up in March 2003 following a meeting between the Prime Minister, the Fisheries Ministers and representatives of the UK fishing industry. The Strategy Unit report "Net Benefits" sets out a package of modernisation and reform for joint implementation by the UK fish catching industry and government. The report is available online from the Strategy Unit website

www.strategy.gov.uk

and from the following address: UK Fisheries Project, 4th Floor, Admiralty Arch, London, SW1A 2WH.

The report is a report 'to' government and is not a statement of government policy. It is, however, regarded by many with an interest in this sector as a catalyst for change and will provide the basis for active dialogue with stakeholders over the coming months. A new unit has been set up in Defra to engage with stakeholders, develop a plan of implementation and form the official government response.

Key recommendations include:

- Promoting a new package of rights and responsibilties. The Strategy Unit recommends introducing ITQs (individual transferable quotas) to provide greater clarity about ownership of access rights, progressive cost recovery of the costs of management and clearly defined advisory roles for the fishing industry and other stakeholders within regional and inshore management structures
- Reforming the inshore fisheries management regime, a process that is being taken forward in the light of this report and the review of enforcement activities in England and Wales
- Developing shared commercial strategies between industry and government
- Creating inshore/shellfish managers for England, Scotland, N Ireland and Wales where these do not already exist
- Initiating a 'light-touch' data collection system covering the under 10s and shellfish sector to assess the true value of the sector
- Launching a feasibility study to develop a community quota scheme for vulnerable, fishing dependent communities compatible with EU state aid rules

The Strategy Unit will be hosting public meetings in England, Scotland, N Ireland and Wales to discuss the report in more detail. Fisheries departments will also attend to explain how the process is being taken forward. Details are available from the Strategy Unit website (at the address given above).

LANTRA NEWSLETTER

Lantra is the Sector Skills Council for the Environmental and Land-based Sector. Lantra is licensed by the UK Government to drive forward the new skills, training and business agenda for the sector. Lantra represents the interests of over 400,000 businesses and 1.5 million workers in the environmental and landbased industries in the UK, including animal care. They have recently issued a Newsletter for the aquaculture industry. The following items are selected from this publication.

Introducing the aquaculture industry group

To ensure that Lantra's work is industry-led and effectively meets the true needs of the aquaculture industry, an Industry Group meets and communicates regularly to steer priorities and actions on skills, training and business development issues.

"Lantra plays a critical role in embedding a training culture throughout the aquaculture industry and in promoting the self evident philosophy that training is an investment, not a cost." *Doug McLeod, Industry Group Chair*

Industry Action Plan

An industry-specific action plan is helping the aquaculture industry meet its skills and workforce development needs. The Industry Action Plan, developed following consultation with the industry group and the wider network, identifies priority issues currently impacting on the industry, and provides a well-needed focus for addressing these challenges. Key priorities identified in the Action Plan include:

- Tactical research to provide clear and robust information on future needs, current skills gaps and the necessary learning supply for aquaculture

- Coherent qualification routes and career pathways
- Greater interaction between Higher Education and the industry
- Supporting the industry's awareness and understanding of changing skills and learning needs, in light of constant changes in legislation, markets and technology
- Addressing the current image of industry to ensure successful recruitment and a sustainable industry
- Promoting all levels of training and educational opportunities both within and outwith the industry

Progress to date against the action plan has been significant. Feedback is currently being sought on how the plan should be developed to build on progress.

Scottish Modern Apprenticeship approved for Aquaculture

A Scottish Modern Apprenticeship in Aquaculture has been developed and approved, following significant interest from aquaculture employers - providing a tailored solution to the industry's need for employees who can combine technical knowledge with practical skills.

Modern Apprenticeships are a route by which people can begin a career in aquaculture, learn practical skills and achieve nationally recognised qualifications. They are government backed training schemes aimed primarily at young people aged between 16-24, however in certain regions funding is available for older people.

Candidates enrolling on the Aquaculture Scottish Modern Apprenticeship will gain the nationally recognised Aquaculture Scottish Vocational Qualification (SVQ), in addition to the Core Skills of communication, working with others, problem solving, IT and numeracy. Apprentices also gain industry-specific certificates appropriate to their own circumstances and the business in which they work, such as Sea Survival, a Fish Vaccination Course, or Emergency First Aid. For more information about Modern Apprenticeships, contact Karen Lawlor on 02476 858432 or email karen.lawlor@lantra.co.uk.

FREE SkillChecks in Wales

Lantra is offering free training needs assessments to aquaculture businesses in Wales. Any training needs identified by the 'SkillCheck' could be funded by up to 50% and could include a range of courses from business management and IT to practical skills courses and online learning. For more information, contact Philippa Davies on 07867 908188 or email philippa.davies@lantra.co.uk.

Scottish Progression Awards

If you work in the Aquaculture industry, or are thinking about a career in Aquaculture, you might be interested in the Scottish Progression Award (SPA) in Aquaculture. This new qualification is suitable for anyone who would like an introductory course in Aquaculture. You could be working part-time in the industry and want a qualification, or perhaps at school or college and want to see if the industry suits your needs? The SPA will equip you with the basic skills, knowledge and understanding to work in the industry. It will help you progress to more advanced qualifications, such as the Scottish Vocational Qualifications (SVQs) at Level 2 and 3 in Aquaculture, or eventually to a HNC. For more information about the Scottish Progression Award in Aquaculture, visit www.lantra.co.uk or contact Billy Sweeney on 07867 908184 or email billy.sweeney@lantra.co.uk.

Lantra's New Look Website

Lantra has launched a new and improved version of its website, including a section dedicated to Lantra's work for the aquaculture industry. At www.lantra.co.uk/aquaculture, you can sign up to receive Lantra's latest news, find out how to access work-based and online training, download Lantra's Action Plan for Aquaculture and view the latest National Occupational Standards, research findings, strategic documents and much more!

Further information

If you would like to get involved in Lantra's activities, please contact Tricia Bloomfield on Tel: 01620 822633 or email tricia.bloomfield@lantra.co.uk.

SEAFOODplus - SEAFOOD FOR HEALTH AND WELL-BEING

The EU is sponsoring an integrated seafood research project that for its form and scale is unique throughout Europe. The main focus of SEAFOODplus is consumers and their desire for healthy products. It will be concerned with tailor-made products, better utilization of by-products, ethically acceptable fish farming, and more besides. The Project started on 1 January 2004.

Professor Torger Børresen, a Danish fisheries researcher, has assumed the overall leadership and responsibility for this key project. A council consisting of 12 experts, who together cover all the project areas, will support him in his work. One of their tasks will be the coordination and timing of the numerous individual activities, because a lot of the projects build one upon another.

This is the biggest research project that the EU has ever sponsored in the seafood sector - not only with regard to the total budget sum of about €26 m, of which the European Commission contributes €14.4 m, but also with regard to the content and complexity of the research programme. It takes into account all the different stages within the value-adding chain and hardly any aspect will be omitted from the investigations. The programme will focus on consumers' demands for healthy, safe products that have been produced using sustainable, environment-friendly methods and processed using state-of-the-art techniques.

Over 70 partners involved

SEAFOODplus has not only high scientific standards but also a broad networking of sub-projects. About 70 partners from 16 European states, among them both research institutes and small and middle-sized companies, are co-operating in sub-projects. Even outside Europe there has been an interest in participating. So, the Canadian enterprise 'Aquanet' is also a partner in the project. Altogether, there are 20 sub-projects embedded in the Integrated Research Project SEAFOODplus.

Research projects divided into five areas

SEAFOODplus is subdivided into five strategic clusters. These will constitute the pillars upon which the overall project rests. Each of the individual projects deals with an exactly defined topic and can be allocated to at least one of these pillars. A sixth important topic area is devoted to problems of traceability. The SEAFOODplus researchers named this concept 'fork to farm'.

Commercial companies integrated within the project

Special attention will be devoted to co-operation with small and middle-sized companies as a lot of the research projects should ultimately deliver results that can be put to commercial use. In contrast to earlier projects, where the utilization of the results was only possible some time after completion of the research,

Seafood producers are invited to join this project

important results are to be made immediately available to the public, even while the programme is still ongoing. This applies in particular to new technologies that will create economic benefits for their users.

Fast distribution of results

There will be a special working group whose job it will be to distribute the research results. This group will use the full spectrum of modern communication options to make politicians, consumers and companies in Europe familiar with the important results from the projects: via, for example, specialist publications, the Internet, leaflets, presence at conferences and trade fairs, interviews and press releases.

Company applications still possible

Even after the start of the project, for example, partners can be changed or new partners added. And small and middle-sized companies still have the chance today to work with SEAFOODplus. This particularly applies to innovative European companies that are interested in the implementation of new research results or technologies. They can contact the responsible parties directly via the SEAFOODplus website to apply for participation in individual projects.

Further information/web site

http://www.seafoodplus.org/index_start.htm

NEW ORGANISATION TO SUPPORT CORNISH SEAFOOD

The whole Cornish community is to benefit from a new organisation set up to support the local seafood industry, thanks to a funding package of more than £½ million.

'Seafood Cornwall' will work with local individuals and businesses to develop and enhance the competitive performance, quality practices, and global position of the Cornish seafood industry. This in turn will have a positive impact on the local economy.

"The seafood industry is an important sector of the Cornish economy. Through Seafood Cornwall we will help to sustain job opportunities in communities throughout Cornwall" says Paul Trebilcock, spokesman for Seafood Cornwall.

The venture has been made possible with joint funding from Seafish, European Objective One Fisheries Programme (FIFG) and Cornwall County Council.

Seafish Development Director Jon Harman said: "We are delighted to be supporting such a worthwhile initiative. Seafood Cornwall is a great example of how our industry is working together to become a more confident and forward-looking industry which works in harmony with the environment, producing a high quality end product which is not only healthy but positively beneficial to eat."

Over the next three years, Seafood Cornwall will carry out a number of programmes to support the local industry. These include:

- A marketing programme - promoting local seafood produce.
- Various quality and hygiene programmes – improving quality practices from the net to the plate.
- An electronic information programme, which will look at innovative ways of recording information at sea, so that buyers can trace where the product has come from.
- A product and species development programme to encourage Cornish seafood producers to use a wider variety of seafood species and produce more innovative products.

The former west Cornwall MP David Harris is a well known and respected advocate for the fishing industry and he has been elected as Chairman and an interim management committee has now been set up to take the initiative forward as a co-operative.

If you work in the Cornish seafood industry and would like to get involved, contact Clare Leverton at Seafood Cornwall, Trevint House, Strangways Villas, Truro TR1 2PA
Tel: 01872 270333
email: admin@swpesca.co.uk.

UK MICROBIOLOGICAL LABORATORIES UNDERTAKING SHELLFISH TESTING

Resolutions of Seventh Meeting: October 7th 2003

A periodic meeting of laboratories undertaking microbiological testing of bivalve shellfish was held at Whitehall Place, London on October 7th 2003. The group comprised representatives from CEFAS Weymouth (the National Reference Laboratory), the Health Protection Agency, Hospital Trust Laboratories in England, the FRS Marine Laboratory Aberdeen and the Northern Ireland Public Health Laboratory.

The remit of the group is:

1. To provide, with reference to Council Decision 1999/313, a UK technical forum for discussion of issues relating to microbiological testing of shellfish.

2. To agree, where possible, common methods and approaches relating to shellfish testing for use throughout the UK and their quality assurance.

3. To advise the central UK competent authority, and the developed administrations, of the views of testing laboratories as outlined above.

4. To enable CEFAS Weymouth, as the UK National Reference Laboratory (NRL), to represent the views of UK testing laboratories in the European Laboratory Framework specified in Council decisions 1999/313 and to co-ordinate with UK laboratories initiatives arising at the European level.

The group agreed the following regulations:

1. To progress to completion a current draft document giving additional recommendations to UK testing labs on the state of shellfish on receipt.

2. To recommend the adoption of ISO 6579 for the testing of *Salmonella* in shellfish with the proviso that laboratories specifically testing for *S.* Typhi or *S.* Paratyphi should consider supplementing the ISO method with the present selenite method recommended for *S.* Typhi in shellfish.

3. To recommend that CEFAS include *Salmonella* in any further shellfish homogenate ring trial and to request that laboratories compare the use of Selenite and MKTTn enrichment in order to demonstrate ability of the latter to recover *Salmonella* from shellfish.

CROWN ESTATE TO FUND SHELLFISH MONITORING IN SHETLAND

A £23,037 funding package has been secured from The Crown Estate to establish a shellfish biotoxin monitoring service at Shetland Seafood Quality Control Ltd, based at the North Atlantic Fisheries College in Scalloway.

Seafood Shetland chief executive Ruth Henderson welcomed this announcement. She explained that it meant that levels of shellfish toxins in farmed mussels could be monitored in Shetland and growers could act quickly to recall product and protect consumer confidence if there was a potential problem.

Mussel production in Shetland now represents a significant proportion (almost 40%) of the total Scottish supply, making it essential that any harmful toxins, which could lead to shellfish poisoning, can be detected swiftly and effectively. A locally based service, using expertise already available at Shetland Seafood Quality Control, will eliminate any delays that might be encountered through the current need to send samples to mainland Scotland for analysis.

The funding will allow for setting up the service and establishing running costs for a two year-period, after which it is expected that the service will be self-sustainable.

NEWS FROM SEAFISH

SEAFISH

Seafish announce industry awards

Seafish has announced a major new pan-sector awards venture aimed at raising industry standards and recognising excellence throughout the seafood sector.

The awards called *The Seafood Industry Awards* will encompass the whole industry with around ten awards including *Catching for the Market*, *Fish Processor of the Year*, *Best Fishmonger, Best Multiple* and *Best Seafood Restaurant*.

Andrew Dewar-Durie, Seafish Chairman said: "We are delighted to announce this new event which will further highlight the importance of seafood and roles that each part of the industry plays in bringing quality seafood to the consumer's plate."

Entry information for *The Seafood Industry Awards* can be obtained from Grant Collier, Divisional Marketing and Events Manager, Highbury Business on 01322 660070 or by contacting Alison.Levick@nexusmedia.com.

Seafood Week 2004

Seafood Week 2004 kicks off on Friday, 1 October this year – providing the perfect opportunity to celebrate the diversity and excellence of the 100 or so varieties of seafood currently available in the UK.

Organisers Seafish will be encouraging thousands of companies to once again take part in this annual seafood extravaganza. Sectors of the industry, including supermarket chains, hotels, restaurants, fishmongers and fish and chip shops, will all be organising special seafood events and promotions throughout the week, in venues throughout nationwide.

Mandy Queen, Head of Communications at Seafish said: "More than 6,000 separate organisations were involved last year, resulting in a massive boost to seafood sales across the country. We have ambitious plans to continue this success by making this year's event even bigger and better than before."

To register your interest or find out more abut what's happening in your local area email seafoodweek@seafish.co.uk

Seafood Week starts on October 1st in 2004

New FIFG development officer at Seafish

Stephen Bailey has been appointed FIFG Development Officer at Seafish.

Steve Bailey, new FIFG officer at Seafish

In this role, which is fully funded by Defra and FIFG (Financial Instrument for Fisheries Guidance), he will be responsible for considering areas of the seafood industry's activity for which access to the EC Fisheries Grant Aid, FIFG would produce the greatest benefit. He will also be helping Seafish staff, as well as other businesses and individuals, prepare project proposals and applications for grants.

For more information contact Stephen on 0787 6035 051 or email him at: s_bailey@seafish.co.uk

Aquaculture Update

The first issue of a new Seafish newsletter *Aquaculture Update* was published in January. If you have not yet received a copy and would like to, or would like to be added to our mailing list for future editions then please contact Anne Kennedy with your mailing details on 0131 524 8658 or a_kennedy@seafish.co.uk.

Issue number two of the newsletter is due out in July, and articles of interest to the shellfish sector will include an update on research co-sponsored by Seafish, market trends and FIFG in non-objective 1 areas.

Dispelling the cholesterol myth about prawns

A drive to dispel myths about high levels of cholesterol in prawns and highlight their health benefits has been launched by Seafish.

Many consumers are confused by the message that prawns contain high levels of cholesterol and as a result limit their intake of shellfish. Now Seafish is working to reassure consumers that cholesterol levels are affected more by the amount and type of fat in the diet, than the amount of dietary cholesterol and prawns are naturally low in saturated fat. They also have a very low calorific content compared to other proteins and contain a number of essential nutrients such as magnesium and zinc.

For more information, go to www.seafish.org.uk

Seafish makes shellfish safer

Seafish, in collaboration with Aberdeen University and Moredun Research Institute, has been funded by the Food Standards Agency to carry out a survey to investigate the occurrence of the microorganisms *E. coli* O157 (VTEC O157) in UK commercial bivalve shellfish, and investigate the depuration of *V. parahaemolyticus* and *C. parvum*.

The survey covered 14 major commercial shellfish sites looking at the main molluscan bivalve species - common mussel, native oyster, Pacific oyster and common cockle. The 12-month sampling programme aimed to take account of seasonal variation and provide an even geographical spread over England, Wales and Northern Ireland. *E. coli* O157 was not identified in any bivalve species sampled at any of the locations, suggesting that bivalve molluscan shellfisheries in England, Wales and Northern Ireland do not pose a significant human health risk from *E. coli* O157contamination.

Depuration trials were conducted at FRS Marine Laboratory and Moredun Research Institute. The trials investigated the ability of current depuration technology to remove the micro-organisms *V. parahaemolyticus* and *C. parvum* from mussels, oysters and scallops. The results showed that under specific trial conditions of time, temperature, salinity and handling regimes, levels of these micro-organisms can be reduced considerably.

Further research is required on each shellfish species to determine the optimum depuration conditions and Seafish is actively seeking funds to carry out the work.

This article is based on a more detailed report summarising the survey and depuration results. The report is available from The Food Standards Agency library on 020 7276 8181. Alternatively contact Seafish on 01482 327 837 (seafish@seafish.co.uk).

NEWS FROM THE TRADE ASSOCIATIONS

SHELLFISH ASSOCIATION OF GREAT BRITAIN (SAGB)

INDUSTRY MOVES TO TAKE MORE RESPONSIBILITY FOR PUBLIC HEALTH

A beneficial diet

In these days of almost unbroken public discussion of health issues related to food, the shellfish industry in Britain is even more sensitive to health concerns than other sectors of industry. There are a number of angles to the debate. The benefits of a diet that includes a significant amount of seafood are clear. Both crustacean and molluscan shellfish are low in fat, as they do not store energy reserves as fat. Being low in the marine food chain, they do contain beneficial essential fatty acids, which are only produced by marine algae. The omega-3 fatty acids, which are the main basis for the healthy-eating claims for oily fish, are only synthesised in nature by plants. Although shorter chain omega-3 fatty acids such as alpha-linoleic acid (ALA) are present in flaxseed and linseed the essential longer chain ones such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are only produced in quantity by marine algae. In the sea they are produced by algae in the phytoplankton, which are eaten by crustacean zooplankton, in turn eaten by fish. The bivalve shellfish also feed directly on the phytoplankton and so acquire the same essential fatty acids. They do not concentrate them in the way that oily fish do, however, because molluscs have predominantly carbohydrate reserves, not oil or fat.

Although the crustaceans contain relatively high levels of cholesterol, the intake of ready-made cholesterol from food is unimportant, as little is taken up by the body. Cholesterol is a problem when it is generated within the body from saturated fatty acids, but shellfish are extremely low in these.

Then there are the various trace elements, such as zinc, which is beneficial, and the neuro-active substances that may account for the reputed aphrodisiac qualities of some, most famously oysters high in dopamine. Finally, the real benefits of the ‘feel good factor’ through the way in which this acts on the serotonin function of the brain are now being recognised and again there is a link with the essential fatty acid EPA. Shellfish is usually associated with good times and something a little out of the ordinary. Beneficial claims for bivalves have been validated, notably the anti-inflammatory properties of green lipped mussels.

Industry responsibilities

Against all these benefits, the potential for shellfish to cause illness, from natural algal toxins, bacteria and viruses or simply allergy is recognised and as far as possible minimised. Even in the highly regulated climate in which shellfish producers operate, the responsibilities placed on industry by the demands of due diligence mean that higher standards are required wherever operators become aware of particular problems.

Whilst the bacterial problems are mainly well understood and have been largely eliminated, and algal toxins are mainly regulated in the UK, as in the rest of Europe, by the official monitoring programmes, there are moves for industry to take more of the responsibility in reducing viral risks, and in end-product testing for toxins, as convenient and low cost methods become available.

In Scotland the rope-grown mussel industry has led the way in having chemical testing (phosphatase inhibition) carried out routinely as an end-product test to ensure freedom from the predominant Type1 DSP toxins (Okadaic acid, DTX 1,2 and 3). This was partly to avoid late product recalls, some of which have occurred when the official monitoring results come in at the end of a week, when product has already been harvested, packed and even consigned to the customer. Chemical testing methods also have the benefit of being quantitative; so can give a better picture of developing problems when the levels may still be below the regulatory limit. In Spain the mussel industry carries out mouse-bioassay, but in the UK the industry cannot do this and has no desire to do so. SAGB's view is that mouse testing should be phased out as a matter of urgency.

The scallop industry has also been at the forefront driven by a need to confirm that it is meeting the very stringent levels for ASP toxin, domoic acid. This is a simpler toxin to deal with, as testing is entirely by chemical measurement (HPLC). Simple test-kits are also available for ASP and for PSP, though these are non-quantitative. A quantitative PSP test kit (Ridascreen) has also been developed by Rhone Diagnostics in Scotland, though this needs to be used in a laboratory and cannot serve as a simple pre-harvest test.

The CIVIT proposal

One outcome of the 'atypical DSP' affair has been the proposal by cockle producers for an industry led quality assurance scheme titled CIVIT (Cockle Industry Voluntary Initiative on Toxin Testing). This recognises the potential problems that algal toxins in cockles could cause and also that the responsibility to ensure that cockles are free of algal toxins rests with the industry. The proposal is that ASP (domoic acid) would be determined as now by HPLC reported within 48 hours, PSP by an initial weekly screening by Ridascreen and DSP Group 1 toxins by phosphatase inhibition assay, which directly measures the effects of the toxins on a crucial enzyme system (PP2A). Confirmatory LC-MS is proposed if any Group 1 DSP toxins are encountered. Other potential toxins, such as Group 4 DSP (the azaspiracids) would be monitored on a monthly basis by LC-MS, with the frequency increased if they were found. The proposed scheme would involve batch testing if any toxins are present, even below regulatory limits, or if PSP were above 10µg/100g and agreed cessation of fishing by participating companies if trigger levels are exceeded.

CIVIT aims to provide state of the art consumer protection that will comfortably exceed that provided by national monitoring. It will provide industry-wide standards that will put the UK cockle industry in the lead in consumer safety within Europe. It will be responsive to advances in testing technology and the possible emergence of new toxins to ensure that it remains at the cutting edge of food safety. To help gain acceptance for this approach both industry and regulators should work together to produce acceptable validation of those methods that do not already have full validation.

SEAFOODplus

On the virus front, SAGB is a partner, along with CEFAS and French and Spanish institutions, in the major SEAFOODplus project, part of which is aimed at reducing viral risks. One aim is to assess the use of simple measures such as salinity downstream from likely polluting discharges and on the shellfish beds in an estuary to identify times of high risk. The aim would be to alert producers to times when either greater depuration might be recommended, or in extreme cases voluntary suspension of harvesting. In the past SAGB has alerted members to high risk times of the year, usually in February, when there have been many reports of gastro-enteritis outbreaks in the press (unrelated to shellfish) and heavy rainfall. This does seem to have been effective and the system now envisaged would be a more formal alert mechanism based on similar considerations.

It is clear that a large scale and successful shellfish industry certainly cannot risk making its customers ill and increasingly needs to minimise costly product recalls, wasting scarce products. Improved quality assurance methods, better communications and improved testing methods will achieve both objectives.

Further information

The Shellfish Association of Great Britain, Fishmonger's Hall, London Bridge, London, EC4R 9EL (Tel. 020 7283 8305) (Fax. 020 7929 1389) (email: SAGB@shellfish.org.uk)

ASSOCIATION OF SCOTTISH SHELLFISH GROWERS (ASSG)

VIEWS FROM THE ASSG

Extended depuration

The consolidation of much of European food legislation proceeds apace, and after an ASSG Conference that focused on some of these important issues, the theme has continued through recent meetings of the European association, 'European Mollusc Producers Association' (EMPA). The issue of extended depuration at higher temperatures continues to be a major concern to all the individual country associations represented on EMPA, and representations against this development are continuing in Brussels as well as at national level.

Scallops and biotoxins

Readers may recall another aspect of European legislation designed to safeguard consumers against biotoxin contamination while enabling farming and capture fishery harvesting to continue, namely the 'tiered marketing regime' for scallops afflicted with ASP. Well, after much debate and sometimes heated discussion, and the contribution of much time and effort from many individuals in the industry, agencies and governments, the Scottish Executive has finally decided not to introduce this system of fishery management into Scottish inshore waters.

Ecosystem-based management

However, looking to the future, a novel way of managing the industry is appearing on the horizon, the so-called 'ecosystem-based approach'. Whilst the ASSG accepts and indeed endorses many of the ideals and aspirations stated in the recent JNCC consultation document, there is, in our view, a need for a stronger recognition of the economic and social dimensions of 'sustainable development' rather than the current emphasis on nature conservation objectives. In particular we would seek an overt recognition and declaration of support for the future development and expansion of the environmentally benign activities of the shellfish cultivation sector.
In considering possible ecosystem based management systems it is important to integrate the concept with other facets of inshore management, including the impact of the extension of Local Authority planning controls on aquaculture developments to these waters, development of industry Codes of Practice, the relationship with organic and sustainable certifications for fishery products, the implementation of the Water Framework Directive and the impact of government supported initiatives such as the 'Strategy for Scotland's Coast and Inshore Waters' (Scottish Coastal

Forum) and the 'Strategic Framework for Scottish Aquaculture' (SEERAD *et al*).

The worst possible outcome for the development of an ecosystem based management regime would be if it were constructed in a vacuum, devoid of connection with these other realities and in isolation from the legitimate aspirations of the shellfish cultivation sector. In this context, we believe it is essential that research into the ecosystem approach is encouraged. In the interim, it is equally important that industry is not overly constrained by blanket application of 'the precautionary principle'.

Funding

Meanwhile, the impact of the European Commission's 'Strategy for the sustainable development of European aquaculture', with its welcome emphasis on environmental concerns and support for the shellfish sector, has become apparent in proposed amendments to the FIFG financial support scheme. The proposal for financial compensation for shellfish farmers in the event of prolonged closure due to algal blooms, is clearly a major positive element, although the restriction to a single claim through 2006 appears unnecessarily harsh, given the potential for extensive closures. In the view of the ASSG such compensation (income loss) should be extended to cover recurrent seasonal suspensions and not limited to closures in excess of six months.

The definition of and extension of FIFG funding to pilot projects focused on applied research is also to be welcomed, as well as the proposal to encourage/support statistical, economic and market related activities.

The raising of the financial support for improvement of 'traditional aquaculture activities such as mollusc farming' to a high priority is similarly strongly supported by the ASSG, which views this as a formal acknowledgement of the environmentally benign nature of our operations.

The emphasis on reducing environmental impacts whilst supporting the expansion of shellfish cultivation is in alignment with the ASSG's strategic views for the future development of the sector in Scotland. This 'steer' from the Commission, via FIFG funding, should, in our view, be reflected in policy initiatives and practical support for shellfish entrepreneurs seeking to establish, extend and modernise their operations – we would include facilitating the granting of Several and Regulating Orders under this strategic 'steer'.

In light of these positive amendments, the ASSG is therefore inevitably concerned at the implied SEERAD constraint on additional support indicated by a comment in the penultimate paragraph of the consultation letter of 3 March 2004: "there may be other competing priorities for the existing funding allocation in respect of the additional measures proposed for this sector".

Nevertheless, we must hope that the positive view from Brussels is reflected in implementation realities in Edinburgh, particularly in light of the new Seafish programme of research funding, which may similarly encourage grower focused research projects to flourish over the next few years.

AGM

This year's ASSG AGM, which is open to all, not just ASSG members, has been scheduled for the afternoon of Thursday 20 May, during the 'Aquaculture International 2004' exhibition being held in the SECC, Glasgow, 19 – 21 May. As many of you will be aware, each year we invite a distinguished non-member Keynote Speaker, with a tangential or adjacent involvement in the industry, to give an outsider's perspective on our sector. This year I am pleased to continue our tradition of expert and articulate speakers (a tradition which has included Simon Pepper, CEO WWF Scotland, Rhoda Grant, MSP, Lord Sewel, Scottish Office Fisheries Minister, Jim Wallace, QC, MP, MSP, Donal Maguire, BIM, and Peter Hunt, SAGB) by welcoming John Rutherford, Chief Executive of the Sea Fish Industry Authority, to present his view of Scottish shellfish cultivation and to explore the relationship between Seafish and the sector.

Further information

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MONITORING REPORTS

THE MARINE BIOTOXIN MONITORING PROGRAMMES FOR SCOTLAND: APRIL 2003 – MARCH 2004

Joyce Petrie and Eileen Bresnan
Fisheries Research Services, Marine Laboratory, Aberdeen

Introduction

Fisheries Research Services (FRS) has conducted an extensive monitoring and surveillance programme for marine biotoxins in bivalve mollusc flesh in Scotland since 1991, and for the causative phytoplankton since 1995. The monitoring programmes are operated to comply with the requirements of the Shellfish Hygiene Directive, 91/492/EEC, and with the current UK implementing legislation, The Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998, as amended. The programmes are undertaken on behalf of the Food Standards Agency (Scotland) (FSAS).

Marine biotoxins are produced by certain species of phytoplankton and can accumulate in the tissues of filter feeding bivalve molluscs. The toxins pose a health hazard to human consumers, and the monitoring programmes are designed to ensure that no potentially hazardous shellfish are placed on the market for human consumption.

Monitoring is carried out on samples of bivalve molluscs collected from classified harvesting areas, and from offshore scallop fishing grounds. Water from selected sites around the Scottish coast and offshore areas are monitored for the presence of potential toxin producing phytoplankton cells. Sampling is undertaken throughout the year. The programme is based upon a shellfish sampling frequency of monthly during the period October to March, and fortnightly or monthly during the period April to September. The sampling and testing frequency is based on a risk assessment of each site and fishing area, and takes account of the shellfish production and the historical occurrence of toxins. Sampling frequency may be increased to weekly if toxins are detected, and species other than bivalve molluscs can be tested. Water samples are requested to be sent in monthly from November - March and weekly from April to October.

During the period 1 April 2003 to 31 March 2004, 714 water samples were analysed from 26 inshore sites around Scotland, and from 74 offshore areas. A total of 5400 shellfish samples were analysed, of which, 1573 were analysed for Paralytic Shellfish Poisons (PSP), 1179 for Diarrhetic Shellfish Poisons (DSP), and 2648 for Amnesic Shellfish Poisons (ASP).

Results of the 2003 – 2004 Monitoring Programmes

Phytoplankton

Two distinct blooms of the PSP producing species *Alexandrium* were observed during the year, mainly concentrated in the Orkney and Shetland area. The first occurred in late March/April while a second was observed during July/August. The maximum number of *Alexandrium* spp. cells observed was 18,860 cells. l^{-1} from Sandsound, Shetland in July 2003 (the highest number of *Alexandrium* spp. cells observed by the Scottish monitoring programme to date). High numbers of non-toxic dinoflagellates were also observed in the water column during this period.

On the West Coast, high numbers of *Alexandrium* were observed in Loch Roag (5,700 cells.l^{-1} in June 2003) and Loch Striven (1,400 cells.l^{-1} in May 2003) during the year.

Moderate numbers of the potential DSP producer *Dinophysis* spp. (<5,000 cells.l^{-1}) were recorded this year. The highest numbers were observed from June – September on both the East and West coasts. The maximum value recorded was 4,960 cells.l^{-1} from Loch Ewe during June 2003.

Two blooms of the potential ASP producer *Pseudo-nitzchia* spp. were also observed during the year. High numbers of *Pseudo-nitzschia* 'delicatissima type' species (diameter < 5µm) were recorded during March/April, while high numbers of *Pseudo-nitzschia* 'seriata type' (diameter >5µm) were observed from July –September. The maximum value observed was 1,400,000 cells.l^{-1} observed at scallop box M10 (offshore of the North East Coast) in April 2003.

Pseudo-nitzschia cells from 10 samples were analysed using Transmission Electron Microscopy to identify the cells present to species level. Seven species were observed six of which (underlined), are potential toxin producers *P. australis, P. fraudulenta, P. pungens, P. cf. seriata, P. delicatissima, P. pseudodelicatissima* and *P. cf. heimii*.

Shellfish

Inshore Harvesting Areas

The incidence of PSP was the lowest recorded since the monitoring programme began. Low levels were detected at Loch Scridain, Loch Greshornish, Loch Harport, Loch na Keal, Loch Stockinish, Seilebost, and at various sites in Shetland. The occurrence of PSP was first noted in early May when toxins were detected in mussels from Loch Striven at 112µg Saxitoxin equivalents per 100g tissue (STX.100g^{-1}). By late May, toxins had been detected at other sites in Skye, the Western Isles, Mull and at several sites in Shetland. Levels in Loch Striven (May) and in two Shetland sites, Riskaness in Vaila Sound (July) and Baltasound (August) exceeded permitted levels and Voluntary Closure Agreements (VCAs) were introduced. VCAs were in place for no more than one month at each site and by early September levels had dropped in all areas.

DSP toxins were detected in many areas from April through to December. DSP was first detected in April in mussels from Ronas Voe, Shetland. By mid-May DSP had been detected in other mussel sites at Scarvar Ayre, Basta Voe and Gruting/Seli Voe in Shetland, at Loch Roag and Loch Leurbost in the Western Isles and from Isay in Skye. DSP was also detected in May in queen scallops (*Chlamys opercularis*) from Ura Firth in Shetland and from Loch Fyne. In June, DSP toxins were detected at Olna Firth, Browland Voe and East Burra Firth in Shetland and at Loch Ailort, Loch Nevis, Loch Striven, Lamlash Bay, Lunderston Bay and Forth Estuary. During July, DSP was detected in a large number of additional areas including Loch Inchard, Loch Laxford, Loch Eishort, and at Riskaness in Shetland. Other sites affected later in the year included St Abbs, Loch Stockinish, Arisaig, Loch Ailort, Loch na Keal and further sites in Shetland. The main species affected was mussels but DSP was detected in queens (two areas) and Pacific oysters (two areas). The FSAS implemented harvesting restrictions and VCAs where appropriate. In some instances, due mainly to the lack of provision of samples by the industry, these remained in force for several months.

At inshore aquaculture sites, ASP resulted in voluntary closures at one mussel site in September (Loch Spelve - 22µg domoic acid (DA).g $^{-1}$ shellfish flesh). Levels fell quickly and this closure was in place for only two weeks. ASP levels above the closure limit were also detected in gonad at five scallop sites from June to September (Loch Ewe, Badicaul, Loch Crinan, Loch Caolisport and Scalpay). Few samples have been provided from all but Loch Ewe and levels at these sites have been greater than 20µg DA.g $^{-1}$ until February 2004. Loch Crinan was opened in December, and Lochs Ewe and Caolisport were opened in early March. Levels of DA reached 65µg.g $^{-1}$ in gonad samples and were greater than 250µg.g^{-1} in whole scallop samples. These levels of toxicity have remained in whole scallops at most of these sites, and were still prevalent at the end of March. Harvesting restrictions were imposed as necessary, but in cases where whole scallops were affected, if gonad tissue levels were below the regulatory limit of 20µg.g $^{-1}$ tissue, FSAS allowed shucked and processed material to be placed on the market.

Offshore Scallop Grounds

In the course of the year, monitoring detected all three of the toxin groups in scallops from offshore fishing areas.

In April PSP toxins were detected in whole scallops from grounds in the Moray Firth and in Shetland at levels of 41 and 34µg saxitoxin (STX).100g^{-1} tissue respectively. During the summer toxins were more widespread, also affecting East Coast scallop grounds, and Scapa Flow. Levels in whole scallops in the Moray Firth grounds were found up to 95µg STX.100g^{-1} tissue, and in Shetland up to 138 µg STX.100g^{-1}, however elsewhere levels were below the regulatory limit of 80µg STX.100g^{-1} flesh. By September, levels had fallen in most areas to below the regulatory limit, however isolated occurrences above this were still detectable.

Positive DSP results from whole scallops were found in most areas from April to August when tested by bioassay. However, subsequent chemical assays found little or no trace of DSP toxins. When chemical assays were used routinely, no DSP was detected above trace levels.

ASP toxins continued to cause problems in scallop fisheries, and were continually detected throughout 2003. The toxins affected all major scallop fishing grounds during the course of the year. Scallop samples were obtained on a regular basis throughout the year from all grounds by fishing vessels specifically chartered for this task by the FSAS.

Of the scallop samples analysed, the highest level detected from scallop gonad tissue was 117µg DA.g $^{-1}$ from the Jura area and, in whole scallops from July to the end of 2003, levels were routinely greater than 250µg DA.g $^{-1}$. Levels had fallen in most areas by mid March 2004 but were still generally greater than 100µg DA.g $^{-1}$ in whole scallop samples.

FSAS imposed fishery closures, under the Food and Environment Protection Act (FEPA) 1985, in areas where ASP toxins in scallop gonad tissue exceeded the permitted level of 20µg DA.g $^{-1}$ tissue. In areas where ASP toxins in gonad tissue were below this level, but the total toxin loading in the whole animal was above it, a requirement was imposed that scallops harvested from such areas must be processed before being placed on the market for human consumption. These restrictions were revoked whenever total levels fell below the permitted limit. At the end of the monitoring year, April - March, eight FEPA Orders were in force with processing restrictions on most other boxes.

Acknowledgements

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Further Information

The Food Standards Agency (Scotland) will publish a summary of the annual reports for the Shellfish and Phytoplankton Monitoring Programmes in Scotland on their website later in 2004, and full reports will also be available on request from FSAS for a small administration fee.

THE 2003 UK *BONAMIA* AND *MARTEILIA* SAMPLING PROGRAMME

Ian Laing, CEFAS Weymouth Laboratory

Introduction

The two most serious diseases of native oysters in Europe are Marteiliosis and Bonamiasis. In 2002 the UK achieved Approved Zone status in respect of these two diseases (Commission Decision 2002/300/EC). Approved Zone status recognises that it has been demonstrated, by regular and targeted sampling and testing, that the oysters in the specified areas are free from the causative organisms of these diseases. For *Marteilia refringens*, the whole coastline is approved and for *Bonamia ostreae* the whole coastline, except for three restricted areas, where the disease is found, is approved. These three areas are (1) from the Lizard to Start Point; (2) from Portland Bill to Selsey Bill and (3) from Shoeburyness to Felixstowe.

Approved zone status enables us to operate import controls aimed at preventing the introduction of these diseases from elsewhere in the EU, where they are known to occur, or where no sampling and testing is carried out. Movements within the UK are also controlled according to the health status of these areas. Anyone wishing to deposit or relay any molluscan shellfish taken from the controlled (restricted) areas listed above must apply for permission to the Fish Health Inspectorate at the CEFAS Weymouth Laboratory (for England and Wales) or the Fisheries Research Services at the Marine Laboratory, Aberdeen (in Scotland). Addresses are at the back of this issue of Shellfish News.

Sampling results for England and Wales in 2003

For *Bonamia*, Table 1 gives a summary of the results for all sites from which samples of native oysters (*Ostrea edulis*) were taken in autumn 2003. The usual sample size at each site was 30 oysters. Sites in the unrestricted areas (i.e. those free from *Bonamia* and *Marteilia*) were also sampled in spring 2001. No cases of *Bonamia* were detected in these samples.

All samples were also examined for *Marteilia*. This was not detected in any samples. In addition, all oysters were routinely examined for evidence of any other clinical disease.

Table 1. Summary of results of native oyster sampling in England and Wales for Bonamia 1999-2003

Year	**Restricted Area 1** The Lizard to Start Point		**Restricted Area 2** Portland Bill to Selsey Bill		**Restricted Area 3** Shoeburyness to Landguard Point		**Unrestricted Areas**	
	Sites	% infected (range)	Sites	% infected (range)	Sites	% infected (range)	Sites	% infected (range)
1999	5	6-13	21	0-16	16	0-34	8	0
2000	8	0-13	24	0-27	14	0-50	6	0
2001	11	0-30	22	0-26	15	0-60	5	0
2002	11	0-10	23	0-29	12	0-46	6	0
2003	11	0-25	23	0-33	15	0-62	6	0

In Table 1 the results for 2003 are compared with those for the previous 4 years. The level of *Bonamia* infection in 2003 appeared to be slightly higher than in previous years. The average for all the sampled farm sites was 21.86% of oysters infected, compared with 12.8% the previous year and a ten-year average of 17.9%. The average for all the fishery sites sampled was 4.38%, compared with 2.90% the previous year and a ten-year average of 4.15%. The disease has not spread outside of the restricted areas in which it has been recorded in previous years.

Sampling results for Scotland in 2003

Approved Zone status for the notifiable diseases Bonamiasis and Marteiliosis was maintained in 2003. Testing confirmed the absence of these diseases in samples taken from all eight farm sites holding native oysters in Scotland. Two tests (spring and autumn) were carried out, with a sample of 30 oysters examined in each case.

Further information

Shellfish farmers should note that if they have a mortality problem with their stock then they are legally obliged to report it to the appropriate Fish Health Inspectorate (Weymouth or Aberdeen) for investigation. The Inspectorate will then identify the causes and where appropriate take any action to limit the spread of disease and minimise economic losses to the industry.

RESEARCH NEWS

Research News includes abstracts of recent work that may be of interest to the shellfish industries. These abstracts can be taken both from papers published in international scientific journals and from project work undertaken by students at Universities and Research Laboratories. Results from the latter are usually not widely available and ***supervisors of student projects are encouraged to submit abstracts to Shellfish News as a means of publishing this information***.

1. Cultivation of dulse for abalone feeding

Pilot scale studies were carried out in France to develop a method for cultivation of fresh young fronds of dulse (*Palmaria palmata*) to supply abalone hatcheries. This is the first report of cultivation of *P. palmata* in land-based tanks using isolated spores as seed stocks. Spores were released from field-collected tetrasporphytic fronds. These are fertile during the winter along the coast of northern France. Techniques for the induction of sporulation, for spore settlement and for incubation of germlings in 1.5-m-diameter tanks in semi-controlled conditions were developed.

Tetrasporophytic fronds were selected, cleaned of sediments and epiphytes with absorbent paper and cut into 5 square cm pieces that were left overnight on a nylon mesh basket at a temperature of 4 °C in darkness. After desiccation, fertile pieces were transferred into a large 22-l flask filled with 1 µm filtered seawater. Spores were released over a period of 1 h by bubbling air in the flask. The yield of released spores was in the range of 5,000 to 25,000 per g of fertile sections.

Spores in solution were counted, filtered through a 50 µm mesh filter to retain residues and transferred into a 1.5-m-diameter polyethylene tank filled with 100 l of 1 µm filtered seawater. Spores were settled on roughened white polystyrene plates (35 × 20 cm, 3 mm thick), which were maintained on the bottom of tanks by a ballast plate made of stainless steel. Stainless steel was used in order to minimise leaching of trace metals into the seawater. These plates inoculated with spores were subsequently directly used as a substrate for abalone juveniles and so they were perforated with holes to allow the abalone to cross from one side of the plate to the other.

Three days after settlement, when spores were firmly attached to the substrate, the water level in the tank was progressively increased to 250 l. Light was provided, with a photoperiod of 12:12 h light: dark. Tanks were maintained at a temperature of 10 °C. Bubbling was initiated 2 days after spore settlement. Mean spore density on the substrate placed in the hatching tanks was 500 spores per square cm. Spore germination occurred 3 days after inoculation and at this stage survival rate was high (80%). A few days after spore inoculation, both male and female gametophytes had germinated and a male to female ratio of approximately 1:1 was observed. Female gametophytic discs remained microscopic whereas males developed gametophytic fronds. Two water exchange rates, weekly and twice per month, were tested. At each change tanks and cultivation substrates

were cleaned with a low-pressure jet of seawater to remove diatoms and other contaminants. Growth in frond length was two-fold higher when supplemented seawater was changed once a week rather than twice a month. Growth was stimulated by the addition of nutrient supplements to the seawater. The addition of 1 ml per litre of medium supplement, once a week, was the most suitable treatment to promote growth of the *P. palmata* plantlets. In comparison with the control culture (seawater only), this treatment increased the development in length of plantlets more than 10-fold. Higher concentrations of medium additions did not give better results. In the control culture, germlings survived but became colourless with very few developing an erect frond.

After 3 months of cultivation in tanks, survival rate was 35% and some plantlets measured more than 2 cm. At this stage, substrates covered with soft young fronds were transferred to an abalone hatchery to feed abalone juveniles.

Reference

Le Gall, L., Pien, S., Rusig, A.M., 2004. Cultivation of *Palmaria palmata* (Palmariales, Rhodophyta) from isolated spores in semi-controlled conditions. Aquaculture Vol 229, pp 181-191.

2. Abalone settlement

An experimental culture system was developed to test various alternatives to diatom biofilms for settling larval abalone (*Haliotis discus discus*) and on-growing the resulting post-larvae. The system was designed to allow artificial micro-particulate food to be sprayed onto hard corrugated plastic plates, using agar as a fixative. Two trials were conducted to test substances that could be utilized to improve the larval settlement and metamorphosis rate when using the above experimental system.

In trial 1, the plates were sprayed with a mixture containing 1% agar and one of the following: natural diatom powder, *Spirulina* powder, *Chlorella* powder and two different concentrations of gamma-aminobutyric acid (GABA), each with and without antibiotics. The negative control was a clean plastic plate without any additives and the positive control was plastic plates covered with a living natural diatom biofilm. There was no significant difference in the final metamorphosis rate of larvae induced to settle on the three micro-algal powders and the positive control (25.8–35.8%). However, these treatments had a significantly higher larval metamorphosis rate when compared to the negative control and the GABA treatments (1.7–7.5%).

In trial 2, the effect of using both algal powders and mucus, obtained when juvenile abalones were allowed to graze the plastic plates for 4 days, was tested. Combining the effects of mucus and micro-algal powders or the positive control and mucus resulted in a final larval settlement rate of 80.8–89.2% and metamorphosis rate of 73.3–87.5%. This was significantly higher than the treatments that only incorporated micro-algal powder or the positive control, having an attachment rate of 20.8–41.7% and a total metamorphosis rate of only 3.3–28.3%.

The results from this study show that there is potential to use micro-algal powder as a substitute to living diatoms, to act as a settlement cue for larval *H. discus discus*. Abalone mucus should also be used in addition to micro-algal powders to increase the settlement rate of larvae and also ensure a high rate of metamorphosis.

Reference

Stott, A.E., Takeuchi, T., Koike, Y., 2004. Testing various substances that have been bound to plastic plates with agar to induce larval settlement and metamorphosis of abalone *Haliotis discus discus* (Reeve). Aquaculture Vol 231, pp 547-557.

3. Are your oysters in good shape?

Shell samples of Pacific oysters were taken from three different U.S. West coast farms. Industry experts described each shell sampled as being either desirable (good) or undesirable (bad). There were slight differences in the categorization of good and bad oysters among farms, but common trends were evident.

The ratio of greatest shell depth to greatest shell length (D/L) was found to be the most effective indicator for discriminating between good and bad shell shapes. Good oysters had a mean D/L of 0.316, whereas the 'bad' oysters had a significantly lower mean of 0.219.

Using a threshold value of D/L of greater than 0.25 for good oysters, 85.6% of all sampled oysters could be correctly assigned to good and bad categories, as defined by industry participants. The use of D/L and greatest shell width to greatest shell length (W/L) may be beneficial in distinguishing shell shape quality and allow for the rapid assessment of many sampled oysters. These findings have implications in the development of industry standards for shell shape; furthermore, such standards would be useful in designing oyster breeding programs to improve shell shape.

Reference

Brake, J., Evans, F., Langdon, C. (chris.langdon@oregonstate.edu), 2003. Is beauty in the eye of the beholder? Development of a simple method to describe desirable shell shape for the Pacific oyster industry. Journal of Shellfish Research Vol 22, pp 767-771.

4. Selective breeding of Pacific oysters to improve yield

Parental families (Go) in three lines of Pacific oysters were selected based on live weight and meat yields at harvest. The average live weight yield of progeny (G_1) from crossing Go-selected lines in seven trials was 9.5% greater than that of non-selected control families and live weight yields were significantly greater in four out of seven trials. The response to selection was often greater if G_1 families were tested at the same site as that from which their parents came. A significant genotype x environment interaction affected yields in two cohorts. In addition, correlation between the yields of the same families planted at both inter-tidal and sub-tidal sites was positive but weak, indicating that selection for high yield in one environment would likely result in a low correlated response in a different environment. Nonetheless, it was possible to identify six families in cohort 5 and four families in cohort 7 that were among the top 10 families at both sites. Further evaluation of families across a wider range of environments is needed to determine if it is possible to substantially improve yields by selecting "generalist" families that perform well along the whole Pacific coast, or whether it will be necessary to select lines that are suited to particular sites.

Reference

LANGDON, C., EVANS, F., JACOBSON, D., BLOUIN, M., 2003. Improved family yields of Pacific oysters *Crassostrea gigas* (Thunberg) derived from selected parents. Aquaculture Vol 220, pp 227–244.

5. Inbreeding in Pacific oysters

Understanding the effects of inbreeding is critical to the long-term viability of shellfish breeding programs, especially as hatchery operators attempt to develop selected lines with small effective population sizes. Inbreeding depression in shellfish is well documented for the offspring of selfed individuals and full-sib crosses. This study was conducted to determine if crossing more distantly related parents would result in measurable inbreeding depression of performance traits in adult Pacific oysters raised in a commercial inter-tidal growing environment.

All families of oysters for this study originated from a naturalized population. These wild individuals, referred to as G_0 animals, were pair-spawned to create the G_1 families. Subsequent crosses used to produce the G_2 and G_3 families were conducted by mating one male of a particular family with four females of another family. No inbreeding, relative to the G_0 base population, occurred until the G_3 population, which consisted of 19 crosses of unrelated parents, 13 crosses of first cousins and 9 crosses of siblings. The families thus created had, respectively, an expected 0%, 6.25% and 20.3% of their total genome identical by descent (IBD). This can be expressed as inbreeding coefficients (F) of 0, 0.0625, and 0.203 respectively. Average family yield, individual growth rate, and survival were recorded after the first and second growing seasons.

Significant inbreeding depression in yield, individual growth rate and survival was observed after the first growing season in families with F=0.203, but not in families with F=0.0625. After two growing seasons, significant inbreeding depression of yield and individual growth rate was observed in both these families. Significant depression of survival at harvest was observed only in families with F=0.203. These results emphasize the importance of maintaining pedigree records in shellfish breeding programs to help avoid the deleterious effects of inbreeding depression, even among crosses of distantly related parents.

Reference

EVANS, F., MATSON, S., BRAKE, J., LANGDON, C., 2004. The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*). Aquaculture Vol 230, pp 89-98.

6. Growth and survival of Pacific oysters

In order to study individual growth variability and its relationship with survival in juvenile Pacific oysters, parental oysters were sampled at four sites located along the French Atlantic coast and bred under controlled hatchery conditions. Four groups of larvae were obtained by crossing five males and five females from each of the four sites, and a fifth group by crossing these 20 males and 20 females together in a pool. Larvae were reared under conditions allowing the maintenance of a maximum variability of size and gave five experimental groups. Oysters were individually monitored for growth and survival from 3 to 10 months after fertilization. The individual growth performances were relatively stable over time and no noticeable compensation for growth occurred. Early growth rate was a very good predictor of size later in life: 66% of variation in the live weight at 10 months could be explained by variation in the initial growth rate calculated between 3 and 4 months. A significant group effect was observed on survival and on growth rate. Mortality mostly occurred between 3 and 5 months and appeared to affect the slower-growing animals more.

Reference

BOUDRY, P (pboudry@ifremer.fr), COLLET, B., MCCOMBIE, H., ERNANDE, B., MORAND, B., HEURTEBISE, S., GERARD, A., 2003. Individual growth variation and its relationship with survival in juvenile Pacific oysters, *Crassostrea gigas* (Thunberg). Aquaculture International Vol 11, pp 429-448.

7. Red oysters

During a 4-week experiment on oyster grazing in coastal ponds (claires) near La Rochelle, France, a red coloration was observed in the digestive gland of oysters. This pigmentation was attributed to an intensive grazing on cryptophyte nanoflagellates. Three different techniques led to this conclusion, describing for the first time free-living cryptophytes as responsible for such a phenomenon. Spectrofluorometry was used to demonstrate that the red coloration was due to the presence of a phycoerythrin, characteristic of cryptophytes. Microscopic observations and accessory pigments analyses in water ponds confirmed that the source of this pigment was of cryptomonad origin. Oyster grazing on these algae is shown by large differences in alloxanthin concentrations and flagellate abundances between the two experimental ponds. The winter occurrence of this phenomenon is of importance as it may affect sales of oysters. Short-term depuration is suggested to remove the undesirable pigmentation.

Reference

Pastoureaud, A. (apastour@ifremer.fr), Dupuy, C., Chretiennot-Dinet, M.J., Lantoine, F., Loret, P., 2003. Red coloration of oysters along the French Atlantic coast during the 1998 winter season: implication of nanoplanktonic cryptophytes. Aquaculture Vol 228, pp 225-235.

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8. Effects of PCBs on oysters

Because of their resistance to environmental degradation, polychlorinated biphenyls (PCBs) are among the most widespread environmental contaminants. PCBs have high bioaccumulation potential and may affect a number of biological/ physiological processes including disruption of the endocrine system function, lipid metabolism and reproduction. The objective of this study was to test whether conditioning sexually immature oysters with PCB-contaminated algal diets affects their subsequent reproductive success.

Sexually immature oysters were conditioned in individual containers and fed daily with 0.7 g algal paste containing 0, 0.35 or 3.5 µg PCBs for up to 76 days. The impact of suspended sediment load on PCB accumulation in oysters was also tested by exposure of a subset of oysters to clay particles. Oysters in different treatments were sampled 56 days after conditioning with PCB-contaminated algal diets to determine uptake and distribution of PCBs in gonad, digestive gland, mantle, gill and muscle, and the presence of gametes. Tissues from oysters exposed to PCBs alone for 56 days were also analyzed for lipid and fatty acid composition. Following 61 and 76 days of PCB exposure, remaining oysters from all treatments were induced to spawn via thermal stimulation. Non-spawned oysters were stripped to determine if sexual products were present. Oysters exposed to PCBs alone and PCBs plus clay particles showed similar trends in PCB accumulation, but concentrations were generally lower in the latter. PCB accumulation in oysters increased with an increase in PCB concentrations in the algae paste fed. The accumulation also varied with organ types and was correlated with lipid content. The highest PCB concentration was in the gonad and the lowest in gill and muscle.

PCB exposure appeared to impair both lipid metabolism and reproductive success. Although PCB exposure produced only slight changes in the lipid class composition in the oysters, decreases in phospholipids were observed in gonad, muscle and mantle of oysters exposed to 3.5 µg PCBs daily for 56 days. No significant difference was noted in reproductive success (production of spawned females and males) between sediment-treated and non-treated groups after 76 days of PCB exposure compared to controls, PCB-exposed oysters produced fewer spawned females, but no dose-dependent relationship was observed. No well-developed mature eggs were observed in any of the non-spawning oysters examined for the presence of sexual products.

Reference

Chu, F.L.E. (chu@vims.edu), Soudant, P., Hale, R.C., 2003. Relationship between PCB accumulation and reproductive output in conditioned oysters *Crassostrea virginica* fed a contaminated algal diet. Aquatic Toxicology Vol 65, pp 293-307.

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9. How toxic are trace metals?

This study evaluated the risk posed by trace metals to the culture of bivalves along the Galician coast (NW Iberian Peninsula). Cultivation in this area utilises both natural and hatchery-reared seed.

Toxicity tests were carried out with embryos of the commercial bivalves *Ruditapes decussatus* and *Mytilus galloprovincialis*. The toxicity of mercury, copper, zinc, cadmium and lead was quantified in terms of median effective concentration (EC50) and toxicity threshold. The EC50 values ranged from 4 to 10 µg/l for mercury and copper, from 100 to 300 µg/l for zinc and lead, and from 400 to 2000 µg/l for cadmium. The toxicity of copper and zinc combinations could be predicted using a strictly additive model. The above effective concentrations were compared to measured metal concentrations in the Galician Rias, and to local and international seawater quality criteria, in an attempt to evaluate the risk posed by metals to these commercial species. Zinc and especially copper pollution were found to represent a serious threat in certain areas. Furthermore, local seawater quality criteria recently implemented are, in the case of copper and zinc, two orders of magnitude too high to offer any protection for these sensitive early life stages of bivalves.

Reference

Beiras, R., Albentosa, M. (marina.albentosa@co.ieo.es), 2004. Inhibition of embryo development of the commercial bivalves *Ruditapes decussatus* and *Mytilus galloprovincialis* by trace metals; implications for the implementation of seawater quality criteria. Aquaculture Vol 230, pp 205-213.

10. Bonamiasis in Spain

The prevalence of the parasite *Bonamia ostreae* in flat oysters has been studied in both natural beds and on cultivated stocks (rafts and inter-tidal beds) throughout the coast of Galicia from 1992 to 2000.

Overall, there appeared to be a reduction in levels of infection over the period from 1992 to 2000. However, while infection levels in cultivated oysters both on rafts and on inter-tidal beds appeared to be reducing, prevalence of *Bonamia* on the natural beds, although much lower initially, increased slightly, from 1994 to 2000. Oysters cultivated on inter-tidal beds were more affected by the disease than those on rafts, although this may be due to less than ideal environmental conditions at the specific sites. The results also suggest that some locations are better adapted than others for oyster culture, although the parasite is present almost everywhere. The authors do not recommend the use of oysters from natural settlement as a source of seed for culture, as these oysters were apparently significantly more parasitized.

Reference

Conchas, R.F., Santamarina, J., Lama, A., Longa, M.A., Montes, J., 2003. Evolution of Bonamiosis in Galicia (NW Spain). Bulletin of The European Association of Fish Pathologists Vol 23, pp 265-272.

11. Manila clams and *Perkinsus* infection

Manila clams are an important aquaculture species on the west coast of North America and are also cultured in Europe, Asia, and elsewhere. Clams cultured on the west coast of North America are free of *Perkinsus* sp. infections, while clams from certain Asian and European sources are infected. Infection in Korean Manila clams is reportedly associated with high morbidity and mortality. We evaluated the health status of Manila clam juveniles from Korea, following a proposal to import them into Mexican waters for fattening, prior to shipping into the United States, either to market or to receiving waters.

A high prevalence of a *Perkinsus* sp. was found in the juvenile Korean Manila clams, causing significant tissue damage. Taxonomic verification of the parasite was made using a genus-*Perkinsus* SSUrRNA gene-specific DNA probe for in-situ hybridization. The use of this probe is validated and reported for the first time. As a result of this finding, no importation of this clam stock took place, in order to prevent the introduction and spread of this disease into the clam stocks of the west coast of North and Central America.

Reference

Elston, R.A. (aquatech@olypen.com), Dungan, C.F., Meyers, T.R., Reece, K.S., 2003. *Perkinsus* sp infection risk for manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) on the Pacific coast of North and Central America. Journal of Shellfish Research Vol 22, pp 661-665.

12. Brown ring disease

The occurrence of brown ring disease (BRD) in farmed Manila clams is seasonal. Development of the disease is believed to require the presence of the infective agent *Vibrio tapetis* and particular environmental conditions. This paper studies the effect of salinity (20 to 40 parts per thousand (ppt)) on measurable immune parameters of Manila clams, and the progression of BRD in experimentally infected individuals.

At 20 ppt, the total haemocyte count was reduced and disease prevalence was highest. At 40 ppt significantly fewer clams showed signs of BRD, and this was correlated with increases in the measured immune parameters. Inoculation of clams with *V. tapetis* did not have a significant effect on the immune parameters measured.

Reference

Reid, H.I., Soudant, P., Lambert, C., Paillard, C., Birkbeck, T.H. (h.birkbeck@bio.gla.ac.uk), 2003. Salinity effects on immune parameters of *Ruditapes philippinarum* challenged with *Vibrio tapetis*. Diseases of Aquatic Organisms Vol 56, pp 249-258.

13. Salinity tolerance of Manila clams

A series of experiments was conducted to determine under what conditions winter mortalities of the Manila clams might be the result of excessive exposure to low salinity. Clams were exposed to various concentrations of salinity to determine their physiological response, the duration they could withstand lethal or marginal low salinities through the mechanism of shell closure, and diagnostic structural changes in tissues indicative of low salinity exposure.

Salinities of less than or equal to 10 parts per thousand (ppt) were not tolerated in long-term exposures of 13 groups of clams. A salinity of 12.5 ppt was considered marginal, while no significant mortality occurred at greater than or equal to 15 ppt.

Clams could withstand lethal low salinities of 5 ppt and 10 ppt for between 6 and 8 days, but all populations

exposed to lethal low salinities for 14 days and then placed at high ambient salinity (31 ppt) showed a high cumulative mortality. Clams may not die until several days after exposure to lethal low salinity followed by placement in a recovery tank at the normally tolerated high salinity. We found no significant difference in the responses of several groups of clams to the marginal salinity of 12.5 ppt when exposed at temperatures of 6°C, 12°C, and 18°C.

Reference

ELSTON, R.A. (aquatech@olypen.com), CHENEY, D.P., MACDONALD, B.F., SUHRBIER, A.D., 2003. Tolerance and response of Manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) to low salinity. Journal of Shellfish Research Vol 22, pp 667-674.

14. Controlling sea squirts

A sudden increase in the population of the solitary ascidian *Ciona intestinalis* (L.), or common sea squirt, is causing serious bio-fouling problems for shellfish growers on the Atlantic coast of Nova Scotia, Canada. This study was therefore undertaken to monitor the growth, spawning, and recruitment patterns of this species, and to develop strategies to minimize its impact on the culture of European oysters at two locations in Lunenburg Bay, Nova Scotia.

Profiles of condition index, which may be indicative of spawning activity, suggested that the *Ciona* population at the Bayport site spawned from mid-May to June, whereas the population at Mason's Beach spawned from mid-July to mid-August. Histological assessment of reproductive status indicated a period of gametogenesis in March-April, when temperatures were greater than 3°C, followed by spawning from mid-May to mid-August, at temperatures of 8°C and above. Although mature eggs were observed in the ovary in July-August, spawning trials suggested a decline in the fecundity of the Bayport population during this period. Two main recruitment events were observed at Mason's Beach (June and August), but only one at Bayport (June). From the data on fecundity and settlement rates, it was estimated that a 100-mm long *C. intestinalis* (0.6 g dry weight) may produce 12,000 eggs in a season and that recruitment intensity may reach 3,000 individuals per square metre.

Laboratory experiments showed that natural predation rates by green crabs were likely to be low. In a series of chemical eradication trials, exposure to 5% acetic acid was found to be the most effective strategy for eliminating *C. intestinalis*. This treatment was better than hydrated lime, saturated brine, or hypochlorite solution. Total mortality was observed following exposure to 5% acetic acid for 15 to 30 seconds, with no corresponding mortality in the control mussels or oysters. Initial field trials indicated that spraying with acetic acid might prove to be an effective means of eliminating *C. intestinalis* under commercial conditions.

Reference

CARVER, C.E., CHISHOLM, A., MALLET, A.L. (amallet@ns.sympatico.ca), 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. Journal of Shellfish Research Vol 22, pp 621-631.

15. Is mussel fouling a problem?

The effects of fouling organisms are a cause for concern among mussel growers. On Prince Edward Island, Canada, most of the fouling organisms are sedentary filter feeders, and therefore might be competitors with cultured mussels for available resources. This could translate into a reduction in meat yield in mussels.

Laboratory experiments were carried out in July, September, and December to determine the relative impact of fouling organisms on the uptake and release of nutrients. The uptake of chlorophyll a, and the production of ammonium, phosphate, nitrate, nitrite, and organic matter were investigated.

More chlorophyll a was taken up by mussel/fouler units than by mussels alone. Foulers increased the amount of ammonia production before water temperatures dropped in the autumn (end of September-early October). Mussel/fouler units consumed nitrate, however, there was no significant difference when foulers were absent. There was a significant consumption of organic matter by foulers in summer only.

In summary it was concluded that fouling organisms have the potential to prolong phytoplankton blooms by increasing the production of inorganic nutrients, especially ammonia. This study shows that the effect of fouling organisms on mussels may not be as great as previously thought, and it may not be profitable to invest time and money in trying to reduce them.

Reference

LEBLANC, A.R., LANDRY, T., MIRON, G. (mirong@umoncton.ca), 2003. Fouling organisms of the blue mussel *Mytilus edulis*: their effect on nutrient uptake and release. Journal of Shellfish Research Vol 22, pp 633-638.

16. Effect of suspended mussel cultivation on sea stars

The potential indirect effects of long-line mussel (*Perna canaliculus*) farms on an important benthic predator, the sea star *Coscinasterias muricata* were investigated. Surveys on the distribution and abundance

of sea stars and other benthic consumers beneath four active farms, an abandoned farm and three unfarmed areas of seabed in Pelorus Sound, New Zealand were undertaken. The data were used to calibrate a model that simulated the reproductive success of sea star populations in farmed and unfarmed areas of the bays.

Deposits of living mussels and mussel shells covered up to 55% of the seafloor beneath farm sites, but were absent from soft sediments at unfarmed sites. Mean densities of sea stars were up to 39 times larger at active farm sites than in unfarmed areas and were correlated with the abundance of living mussels on the seafloor. Within individual farms, the distribution of sea stars was highly aggregated, with most (63%) individuals occurring within 2 m of the nearest neighbour. In unfarmed areas, sea stars were much more widely dispersed. Our simulations indicate that, because of the extremely clumped distributions of sea stars, spawning individuals at farm sites would on average have substantially greater reproductive success than those foraging in areas where farms are absent, and total zygote production could be as much as 1,500 times greater than in unfarmed areas.

Farm development could therefore result in occasional outbreaks of starfish populations. Without appropriate monitoring such events might be dismissed as rare, natural phenomena rather than a consequence of shellfish culture.

Reference

INGLIS, G.J. (g.inglis@niwa.cri.nz), GUST, N., 2003. Potential indirect effects of shellfish culture on the reproductive success of benthic predators. Journal of Applied Ecology Vol 40, pp 1077-1089.

17. Managing a non-native oyster fishery

Culture of a non-native species, such as the Suminoe oyster (*Crassostrea ariakensis*), could offset the declining harvest of the native eastern oyster (*Crassostrea virginica*) fishery in Chesapeake Bay. Because of possible ecological impacts from introducing a fertile non-native species, introduction of sterile triploid oysters has been proposed. However, recent data show that a small percentage of triploid individuals progressively revert to a diploid status, introducing the possibility that Suminoe oysters might establish self-sustaining populations.

To assess the risk of Suminoe oyster populations becoming established in Chesapeake Bay, a demographic population model was developed. Parameters modelled were salinity, stocking density, reversion rate, reproductive potential, natural and harvest-induced mortality, growth rates, and effects of various management strategies, including harvest strategies. The probability of a Suminoe oyster population becoming self-sustaining decreased in the model when oysters are grown at low salinity sites, certainty of harvest is high, minimum shell length-at-harvest is small, and stocking density is low. From the results of this model, we suggest adopting the proposed management strategies shown by the model to decrease the probability of a Suminoe oyster population becoming self-sustaining.

Reference

DEW, J.R., BERKSON, J. (iberkson@vt.edu), HALLERMAN, E.M., ALLEN, S.K., 2003. A model for assessing the likelihood of self-sustaining populations resulting from commercial production of triploid Suminoe oysters (*Crassostrea ariakensis*) in Chesapeake Bay. Fishery Bulletin Vol 101, 758-768.

18. How cockles settle

Some newly-settled bivalve molluscs can experience a second dispersal stage in the water column and colonize areas distant from the initial settlement zone ('secondary settlement'). To document mechanisms involved in such a process, experiments were conducted in a re-circulating 13-m flume, using small juvenile cockles *Cerastoderma edule* of shell length between 0.8 and 5.7 mm.

After 4 h under current surface velocities of 10, 20 and 24 cm per second, all juveniles left a Plexiglas substratum (the site of initial introduction) and 42.4, 58.6 and 76.2% of them, respectively, were retrieved from a downstream sand area that represented only 7.7% of the total flume surface. Naked-eye observations showed that smaller individuals were borne within the water column, whereas larger individuals tended to roll or slip on the substratum. Byssus threads produced by the juveniles were often seen, sometimes covered in sand grains. These observations were confirmed by finding larger juveniles in the upstream part of the sand area. When sand was replaced by mud, a reduced proportion of cockles were retrieved in the experimental substratum (18.4, 20.0 and 16.4% at the above current speeds, respectively). Observations showed that juveniles rarely succeeded in anchoring themselves in mud. When initially introduced on a favourable substratum (medium sand), more than 87% of juveniles were retrieved at all flow rates. This study shows that secondary settlement occurs for juvenile cockles up to 5.7 mm in shell length and depends not only on flow velocity but also on substratum type.

Reference

DE MONTAUDOUIN, X. (x.de-montaudouin@epoc.u-bordeaux1.fr), BACHELET, G., SAURIAU, P.G., 2003. Secondary settlement of cockles *Cerastoderma edule* as a function of current velocity and substratum: a flume study with benthic juveniles. Hydrobiologia Vol 503, pp 103-116.

19. Natural cockle diet

Filtration of cockles was studied for large particles (60 to 500 µm diameter). They readily ingested particles up to a maximum size of about 500 µm, and calculations of clearance for sizes between 60 and 300 µm were in the range of 0.5 to 3 1itres per hour per dry weight, which is close to rates measured for micro-flagellates. Field studies on large particles in the near-bed layer (0 to 10 cm) show that this fraction contributes significantly to total seston. It is concluded that large particles like plant detritus, benthic diatoms and invertebrate larvae may accumulate in the near-bed layer and form a significant food source for benthic bivalves.

Reference

KARLSSON, O. (orjan.karlsson@tmbl.gu.se), JONSSON, P.R., LARSSON, A.I., 2003. Do large seston particles contribute to the diet of the bivalve *Cerastoderma edule*?. Marine Ecology-Progress Series Vol 261, pp 161-173.

20. Effects of suction dredging for cockles

Suction dredging for cockles removes large cockles from tidal flats but may also cause mortality of non-target fauna and make the habitat less suitable for some species. This study examined whether suction dredging for cockles on tidal flats of the Dutch Wadden Sea had affected densities of non-target fauna, directly after fishing and one year later.

Densities of non-target fauna in two randomly chosen undredged locations were compared to densities at the surrounding heavily commercially dredged area. Cockle dredging was associated with reduced densities of 0-group Baltic Tellin (*Macoma balthica*) and this effect persisted one year after dredging. The dredged area also appeared to be less suitable for settlement of mussels *Mytilus edulis*. No significant effects of dredging on the mud snail *Hydrobia ulvae* and on 0 and 1-group cockles were found. For the mobile young *Macoma balthica* it seems unlikely that the effect found after one year was still due to the mortality caused by dredging and this suggests that the habitat was less suitable as a consequence of dredging. Thus, even in the highly dynamic ecosystem of the Wadden Sea, some effects of bottom disturbance by cockle dredging may persist after one year.

Reference

HIDDINK, J.G. (J.Hiddink@bangor.ac.uk), 2003. Effects of suction-dredging for cockles on non-target fauna in the Wadden Sea. Journal of Sea Research Vol 50, pp 315-323.

21. Identifying scallop larvae in the plankton

An immunological technique has been developed, which discriminates *Pecten maximus* larvae from all other species present in plankton samples. This tool was used on various plankton samples over two years (between summer 1997 and summer 1998) to determine when scallop larvae are present in the water column. The least abundant period for larvae was expected to be from April to July 1998 and this was confirmed by this technique. Multi micro-cohort larvae were present principally from April to July 1998 probably corresponding with as many spawning events.

Reference

PAUGAM, A., LE PENNEC, M. (Marcel.Lepennec@univ-brest.fr), MARHIC, A., ANDRE-FONTAINE, G., 2003. Immunological *in situ* determination of *Pecten maximus* larvae and their temporal distribution. Journal of The Marine Biological Association of The United Kingdom Vol 83, pp 1083-1093.

22. Low temperature tolerance of king scallops

The mortality of juvenile scallops (*Pecten maximus* L.) cultured at two densities at 8- and 15-m depth was investigated at 17 trial locations along the western Norwegian coast between latitudes 58 and 65 degrees N. During the winter of 1995/1996, scallops suffered high mortality at many sites. The results show a clear relationship between minimum temperature recorded on the sites and scallop mortality. All the scallops died at sites where minimum temperatures were lower than 2°C, while a mortality rate below 30% was only obtained at locations where the minimum temperature was 4-5°C. At temperatures between 2 and 4°C, mortality was intermediate, ranging from 52% to 100%. There were no mortality differences between culture densities, while a significantly higher mortality at 8- than at 15-m depth was shown for two of the four sites with a total mean mortality lower than 30%.

Reference

STRAND, O. (oivind.strand@imr.no), BRYNJELDSEN, E., 2003. On the relationship between low winter temperatures and mortality of juvenile scallops, *Pecten maximus* L., cultured in western Norway. Aquaculture Research Vol 34, pp 1417-1422.

23. A safe size to seed scallops

Handling time, persistence time and shell-breaking techniques by the crab *Cancer pagurus* (13-15 cm carapace width) offered juvenile cultured king scallops, *Pecten maximus*, was studied. Three shell height groups were used: 50-55, 60-65 and 70-75 mm, corresponding to the size ranges at which scallops might be seeded onto the seabed.

Upon capture, the crabs' handle the scallop and either reject or consume it. Persistence time was defined as the period from capture until the crab rejected the scallop by dropping it from its claws. Handling time was defined as the length of time from capture to the end of consumption.

The results showed that the crabs managed to open scallops from all the three size groups. The median handling time in the 50-55 mm group (788 seconds) was significantly shorter than the median handling time of the 60-65 mm (2482 seconds) and 70-75 turn (2980 seconds) groups. The median persistence time increased significantly with each scallop size, from 89 seconds in the 50-55 mm group to 97 seconds in the 60-65 mm and 125 seconds in the 70-75 mm group. The rate of consumption tended to decrease as the scallop size increased and a change in the shell-breaking technique was observed, from a dominance of smashed scallops in the 50-55 mm group to more punched and chipped scallops in the 60-65 and 70-75 mm groups.

Reference

Grefsrud, E.S. (ellen.sofie.grefsrud@imr.no), Strand, O., Haugum, G.A., 2003. Handling time and predation behaviour by the crab, *Cancer pagurus*, preying on cultured scallop, *Pecten maximus*. Aquaculture Research Vol 34, pp 1191-1200.

24. Controlling fouling on scallop nets

Fouling of scallop shells and cultivation nets by living organisms is costly to remove and can reduce scallop growth. Here we investigate biological control of fouling in suspended king scallop (*Pecten maximus*) cultivation.

In preliminary trials in the Irish Sea, off the Isle of Man, sea urchins (*Echinus esculentus* and *Psammechinus miliaris*) and hermit crabs (*Pagurus* spp.) removed fouling from nets more efficiently than did a range of other invertebrates. Sea urchins and hermit crabs were subsequently deployed from August 2000 to January 2001 at various densities (hermit crabs and *E. esculentus* at 1, 2, or 3 per net and *P. miliaris* at 3, 5 or 7 per net) in pearl nets containing scallops. After 6 months, the survival of biological control organisms and their effect on scallop growth and mortality, and fouling of nets and scallop shells, were assessed.

Only *P. miliaris* was associated with increased scallop shell growth, but no biological control organism reduced scallop growth or survival. All three biological control organisms significantly reduced the weight of fouling on nets (often by as much as 50%) and fouling of scallop shells; results were largely independent of biological control organism density. Sea urchins were most effective, removing hydroids and solitary tunicates efficiently; they could thus be commercially exploited alongside scallops in a form of polyculture. These results suggest that biological control could be an efficient and environmentally sound method of addressing the problem of fouling in scallop cultivation.

Reference

Ross, K.A., Thorpe, J.P., Brand, A.R., 2004. Biological control of fouling in suspended scallop cultivation. Aquaculture Vol 229, pp 99-116.

25. High Pressure processing destroys Vibrios

Multiple strains of *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* non-O1 were tested in phosphate-buffered saline for their sensitivity to high-pressure processing. Variability in sensitivity among strains was observed for all species; this variability decreased at higher pressures. *V. vulnificus* was the most sensitive and *V. cholerae* the most resistant to treatment. The O3:K6 serotype of *V. parahaemolyticus* was more resistant to pressure than other serotypes of *V. parahaemolyticus*. The results of studies involving *V. vulnificus* naturally occurring in oysters revealed that a pressure treatment of 250 MPa for 120 seconds achieved a greater than 5-log reduction in the levels of this bacterium. *V. parahaemolyticus* serotype O3:K6 in oysters required a pressure of 300 MPa for 180 seconds for a comparable reduction. When properly applied, High pressure Processing can thus be effective in improving the safety of shellfish with respect to *Vibrio* spp.

Reference

Cook, D.W. (docjoc@cableone.net), 2003. Sensitivity of *Vibrio* species in phosphate-buffered saline and in oysters to high-pressure processing. Journal of Food Protection Vol 66, pp 2276-2282.

26. Viral indicators

This study aimed to determine whether the concentrations of somatic coliphages, infectious enteroviruses or the detection of enterovirus genomes were associated with the detection of human pathogenic viruses in surface water.

Four French rivers were sampled monthly or fortnightly for the quantitative detection of somatic coliphages,

infectious enteroviruses and the qualitative RT-PCR detection of enterovirus, hepatitis A virus, Norwalk I viruses, Norwalk II viruses, astrovirus and rotavirus genomes over 12 months. All the 68 water samples tested were positive for the quantitative detection of somatic coliphages. Infectious enteroviruses were isolated by a cell culture system in only two (3%) of the 68 concentrated water samples tested, whereas enterovirus genomes were detectable in 60 (88%) of the same samples.

Positive RT-PCR detection of the genome was found for (number and percentage of positive samples in brackets) hepatitis A virus (1, 1.5%), Norwalk-like virus genogroup II (1, 1.5%) and astrovirus (2, 3%). Genomes of rotavirus and Norwalk-like virus genogroup I were not detected in the 68 concentrated water samples tested. All of the above four positive water samples were also positive for the detection of enterovirus genomes, whereas only one of them was positive for the isolation of enteroviruses on cell culture. Moreover, the genomic detection of human pathogenic viruses appeared not to be statistically associated with the concentration levels of somatic coliphages.

Taken together, our findings indicate that the quantitative detection of somatic coliphages and the isolation of enteroviruses on cell culture are not suitable parameters for the control of the viral contamination in surface water, whereas the detection of enterovirus genomes may be useful for predicting the presence of waterborne viruses.

Reference

Hot, D., Legeay, O., Jacques, J., Gantzer, C., Caudrelier, Y., Guyard, K., Lange, M., Andreoletti, L. (landreoletti@chu-reims.fr), 2003. Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. Water Research Vol 37, pp 4703-4710.

27. Estimating Norwalk virus in an outbreak

Gastroenteritis outbreaks linked to shellfish consumption are numerous and Norwalk-like viruses (NLVs) are frequently the responsible causative agents. However, molecular data linking shellfish and clinical samples are still rare despite the availability of diagnostic methods.

In a recent outbreak we found the same NLV sequence in stool and shellfish samples, supporting the epidemiological data implicating the shellfish as the source of infection. A semi quantitative approach using most-probable-number-RT-PCR demonstrated the presence of a hundred of RT-PCR units per oyster. Follow-up of the oysters in the harvest area, for approximately 2 months, showed persistence of NLV contamination of the shellfish at levels up to a thousand RT-PCR units per oyster prior to depuration of the shellfish. This finding is useful in beginning to understand shellfish contamination and depuration for use in future hazard analyses.

Reference

Le Guyader, F.S. (sleguyad@ifremer.fr), Neill, F.H., Dubois, E., Bon, F., Loisy, F., Kohli, E., Pommepuy, M., Atmar, R.L., 2003. A semi quantitative approach to estimate Norwalk-like virus contamination of oysters implicated in an outbreak. International Journal of Food Microbiology Vol 87, pp 107-112.

28. Hepatitis A virus in Italian mussels

The frequency of hepatitis A virus (HAV) contamination in mussels sold in the south of Italy, where a high incidence of HAV infection both in residents and visitors is reported every year, was investigated during a three-year period. Mussels, collected from the markets of five big cities, were analysed by RT-nested-PCR to detect RNA-HAV and by an integrated method (cell culture-RT-PCR) to confirm the presence of infectious virus.

In a total of 180 samples, 15.6% were contaminated by infectious HAV. This high percentage of mussels, which is potentially dangerous for consumers, together with the tendency to consume these shellfish raw or slightly cooked will contribute to the endemic status of HAV infection in some areas of the south of Italy.

Reference

Croci, L. (luciana.croci@iss.it), De Medici, D., Ciccozzi, M., Di Pasquale, S., Suffredini, E., Toti, L., 2003. Contamination of mussels by hepatitis A virus: a public-health problem in southern Italy. Food Control Vol 14, pp 559-563.

29. Correlating DSP toxins in seawater, algae and mussels

Diarrhetic shellfish toxicity is caused by the accumulation of okadaic acid and its derivatives, which are produced by particular species of the dinoflagellates *Dinophysis* and *Prorocentrum*. In the German Bight (North Sea) around the island of Helgoland, four toxic *Dinophysis* species occur, of which two exhibited successive biomass maxima in summer 2000 (*D. norvegica* ~ 400 cells per litre and *D. acuminata* ~ over 4000 cells per litre). In contrast to findings in some other marine areas, toxicity of mussels could be clearly attributed to the observed increases in cell abundances of both species. In several mussel samples toxin concentrations (max. 460 ng diarrhetic shellfish poisoning [DSP] toxins per g of hepatopancreas) were in a range that is considered dangerous for human consumption. While mussel toxicity coincided with

concentration increases of both *Dinophysis* species, toxicity in the water (maximum of 26 ng per litre) could be detected only when *D. acuminata* cell concentrations were at their highest. Mussel toxicity lasted 3 weeks after the end of the *D. acuminata* bloom, and this is attributed to toxicity remaining in decaying *Dinophysis* cells and detritus. Elevated summer temperatures and low silicate concentrations evidently supported the development of high concentrations of *D. acuminata*.

Reference

Klopper, S. (sascha.kloepper@zmt-bremen.de), Scharek, R., Gerdts, G., 2003. Diarrhetic shellfish toxicity in relation to the abundance of *Dinophysis* spp. in the German Bight near Helgoland. Marine Ecology-Progress Series Vol 259, pp 93-102.

30. Food species as invaders

A global market in seafood disperses many live organisms to distant locations. These organisms can be released into environments of the new locations, where they can establish reproductive populations. The risks of such introductions remain poorly understood.

A survey of bivalves (oysters, mussels, and clams) that are commercially available as seafood in the western United States was undertaken. This showed that twenty-four of the 37 available marine and estuarine bivalve species are non indigenous. Eleven of these 24 non-indigenous species have established, self-sustaining populations in northeast Pacific environments. Three of the remaining 13 non-indigenous species have been introduced outside their natural ranges elsewhere in the world.

We estimated the risks of some of these species being introduced by performing binomial probability analyses on these data. Our results suggest that there is a significant risk of introducing live seafood species into northeast Pacific ecosystems. Efforts to warn distributors and consumers to screen imported seafood species for invasiveness, to monitor estuaries and coastal ecosystems for early detection, and to develop rapid-response plans for containing new invaders are warranted.

Reference

Chapman, J.W. (john.chapman@oregonstate.edu), Miller, T.W., Coan, E.V., 2003. Live seafood species as recipes for invasion. Conservation Biology Vol 17, pp 1386-1395.

Shellfish in the Press

The following pages contain clippings from various newspapers and periodicals of items of interest to the shellfish farmer and harvester.

Because of copyright requirements the review of press cuttings is not available in this web edition

INFORMATION FILE

WHERE CAN I GET HELP OR ADVICE?

Policy Matters

Department for the Environment, Food and Rural Affairs, Nobel House, 17 Smith Square, London SW1P 3JR
(Switchboard tel. 020 7238 3000)
(General fax. 020 7238 6591)

Several and Regulating Orders, shellfish farming - Fisheries Division II, Room 110 10 Whitehall Place East, (Tel. 020 7270 8227) (Fax. 020 7270 8827)

Shellfish Health - Fisheries Division II, Room 106, 10 Whitehall Place East, (Tel. 020 7270 8826) (Fax. 020 7270 8827)

Public shellfisheries, excluding Regulating Orders - Fisheries Division III, Room 112, 10 Whitehall Place East (Tel. 020 7270 8256) (Fax. 020 7270 8310)

Shellfish Licensing Scheme - Fisheries Division IV, Room 314, 10 Whitehall Place East, (Tel. 020 7270 8128) (Fax. 020 7270 8146)

Grant Aid - Fisheries Division 1B, Room 328, 10 Whitehall Place East, (Tel. 020 7270 8041) (Fax. 020 7270 8019)

Marine Environment Protection and Pollution - Marine Policy Branch, Rural and Marine Environment Division, Room 150 Nobel House (Tel. 020 7238 5880) (Fax. 020 7238 5881)

Monitoring of fishing activities, licensing - Sea Fisheries Inspectorate, Room 13, 10 Whitehall Place East (Tel. 020 7270 8326/8160/8328) (Fax. 020 7270 8345)

Research and Development Programmes - Science Directorate, Cromwell House, Dean Stanley Street, London, SW1P 3JH (Tel. 020 7238 3000) (Fax. 020 7238 1590)

You can also visit the Defra website at http://www.defra.gov.uk

Welsh Assembly Government, Agricultural and Rural Affairs Department,
New Crown Buildings, Cathays Park, Cardiff CF1 3NQ
(Tel. 029 2082 3567) (Fax. 029 2082 3562)
(*http://www.wales.gov.uk*)

Scottish Executive Environment and Rural Affairs Department,
Pentland House, 47 Robbs Loan, Edinburgh EHG14 1TW
(Tel. 0131 244 6224) (Fax. 0131 244 6313)
(*http://www.scotland.gov.uk/who/dept_rural.asp*)

Department of Agriculture and Rural Development for Northern Ireland, Fisheries Division, Annexe 5, Castle Grounds, Stormont, Belfast, BT4 3PW
(Tel. 028 9052 3431) (Fax. 028 9052 2394)
(*http://www.dardni.gov.uk*)

Shellfish Hygiene

England - Food Standards Agency
Aviation House, 125 Kingsway, London, WC2B 6NH
(Tel. 020 7276 8000) (*http://www.food.gov.uk*)

Food Standards Agency (Scotland),
St Magnus House, 25 Guild Street, Aberdeen AB11 6NJ
(Tel 01224 285100);

Food Standards Agency (Wales),
Southgate House, Wood Street, Cardiff CF10 1EW
(Tel 029 20 678918);

Food Standards Agency (Northern Ireland), 1
0C Clarendon Road, Belfast BT1 3BG
(Tel 02890 417711)

Scientific and technical advice

CEFAS Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB (Tel 01305 206600) (Fax 01305 206601) - Cultivation techniques; health regulations; disease control; shellfish hygiene classifications and purification plant approvals; shellfish water quality and effluent discharges (microbiology) (England & Wales)

CEFAS Lowestoft Laboratory,
Pakefield Road, Lowestoft, Suffolk, NR33 0HT
(Tel 01502 562244) (Fax 01502 513865) -
Shellfish stocks (England & Wales)

CEFAS Burnham Laboratory,
CEFAS Laboratory, Remembrance Avenue,
Burnham-On-Crouch, Essex, CMO 8HA
(Tel. 01621-787200) (Fax 01621 784989) -
Pollutants (contaminants) and their effects

You can also visit the CEFAS website at http://www.cefas.co.uk

Fisheries Research Services, Marine Laboratory,
PO Box 101, Victoria Road, Aberdeen AB9 8DB
(Tel. 01224 876544) (Fax. 01224 295511)
(*http://www.marlab.ac.uk*) -
Shellfish stocks, cultivation, hygiene, and disease control (Scotland)

SEAFISH - Aquaculture Development Officers:
For Scotland and Northern Ireland: Craig Burton, PO Box 3, Acharacle, Argyll. PH36 4YF
(Tel/Fax: 01967 431 573; Mobile: 078 760 35771)
(email: c_burton@seafish.co.uk)

For England and Wales: Martin Syvret
c/o 62 Harrington Lane, Pinhoe, Exeter, EX4 8NS
(Tel/Fax. 01392 202043; Mobile: 078 760 35746)
(e-mail: m_syvret@seafish.co.uk)

SEAFISH Technology, Seafish House,
St. Andrew's Dock, Hull, HU3 4QE
(Tel 01482 327837) (Fax 01482 223310)

You can also visit the SEAFISH website at http://www.seafish.co.uk

Advice on commercial activities

The Shellfish Association of Great Britain,
Fishmonger's Hall, London Bridge, London, EC4R 9EL
(Tel. 020 7283 8305) (Fax. 020 7929 1389)
(*http://www.shellfish.org.uk*)

The Association of Scottish Shellfish Growers,
Mountview, Ardvasar, Isle of Skye, IV45 8RU
(Tel/Fax: 01471 844324)

Wildlife conservation and status of on-growing sites

Joint Nature Conservation Committee, Monkstone House, City Road, Peterborough PE1 1JY
(Tel. 01733 562626) (Fax. 01733 555948)
(*http://www.jncc.gov.uk*)

English Nature,
Northminster House, Peterborough, PE1 1UA
(Tel. 01733 455000) (Fax. 01733 568834)
(*http://www.english-nature.org.uk*)

Countryside Council for Wales,
Ffordd Penrhos, Bangor, LL57 2LQ
(Tel. 01248 385500) (Fax. 01248 355782)
(*http://www.ccw.gov.uk*)

Scottish Natural Heritage,
12 Hope Terrace, Edinburgh, Scotland, EH9 2AS
(Tel. 0131 447 4784) (Fax. 0131 446 2277)
(*http://www.snh.org.uk*)

Other Useful Numbers

Crown Estate Commissioners,
Crown Estate Office, Marine Estates Division,
16 Carlton House Terrace, London SW1Y 5AH
(Tel. 020 7210 4322, Dr Tony Murray)
(Fax. 020 7839 7847)
(*http://www.crownestates.co.uk*)

Central contact for local Sea Fisheries Committees - The Association of Sea Fisheries Committees of England and Wales,
24, Wykeham Village, Scarborough, North Yorkshire, YO13 9QP (Telephone and Fax: 01723 863169).

Co-ordinator for Defra - CARD R&D –
Dr. Mark James, Fisheries Resource Management Ltd., Coillie Bhrochain, Bonskeid, Pitlochry, Perthshire, PH16 5NP. (Tel./Fax: 01796 474473).
(*http://www.frmltd.com*).

USEFUL PUBLICATIONS

CEFAS

A variety of booklets and leaflets are available, including:

- A Guide to Shellfish Health Controls
- The Fish Health Inspectorate and You - Service Standards and Code of Practice for Enforcement
- Bivalve cultivation: criteria for selecting a site
- Scallop cultivation in the UK: a guide to site selection
- Storage and care of live lobsters

The above may be obtained from the CEFAS Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, DT4 8UB, (Tel no: 01305 206600; Fax no: 01305 206601)

A catalogue of CEFAS publications is available from the CEFAS Lowestoft Laboratory, Pakefield Road, Lowestoft, Suffolk, NR33 0HT, (Tel no: 01502 562244; Fax no: 01502 513865). Electronic copies of many of these publications can be found on the CEFAS web site at *http://www.cefas.co.uk/publications/default.htm*
Back copies of issues 6-16 of *Shellfish News* can also be viewed and/or downloaded as .pdf files from the CEFAS web site (*http://www.cefas.co.uk/publications/shellfish_news.htm*). Many of the illustrations are in full colour in the web edition.

Seafish Aquaculture

Detailed information on the technical and economic aspects of cultivation for individual shellfish species is available from Seafish Aquaculture. They publish a series of 'hyper-books' on CD-ROM that covers all aspects of cultivation. Economic models are also available.
For further information contact the Aquaculture Development Officer for your area (see above for contact details, or *http://www.seafish.co.uk/aquaculture/development.htm* for further information).
A full list of Seafish publications can be found on the Seafish web site at *http://www.seafish.co.uk/publications/publications.htm*