AQUACULTURE HEALTH INTERNATIONAL

ISSUE 4 FEBRUARY 2006 NZ$10.00

CONTENTS

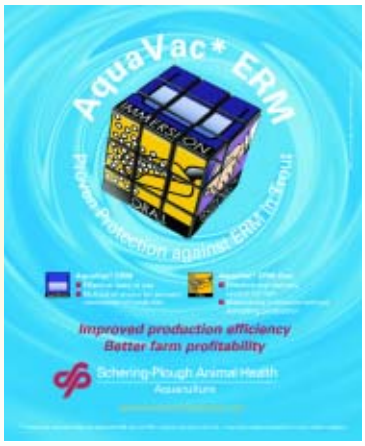
ISSUE 4, FEBRUARY 2006

FROM THE COVER

Schering-Plough Aquaculture
Aquaculture Centre, 24-26 Gold Street
Saffron Walden, Essex CB10 1EJ, UK
Telephone 44 (0) 1799 528167
Facsimile 44 (0) 1799 525546

Schering-Plough
Animal Health Corporation
PO Box 3182, Union
New Jersey 07083 1982, USA
Telephone +1 908 629 3344
Facsimile +1 908 629 3365

www. spaquaculture.com



AQUACULTURE HEALTH INTERNATIONAL

ISSN 1176-86330 ISSN (web) 1176-8649

An informative journal for the aquaculture health professional

Published by:
VIP PUBLICATIONS LTD
4 Prince Regent Drive
Half Moon Bay, Auckland 1706
New Zealand
Ph +64 9 533 4336, Fax +64 9 533 4337
Email keith@aquaculturehealth.com
www.aquaculturehealth.com

EDITORIAL DIRECTOR: Dr Scott Peddie
PUBLISHER: Keith Ingram
MANAGER: Vivienne Ingram
ASSISTANT EDITOR: Mark Barratt-Boyes
CONTRIBUTORS:
Julie Alexander, Dr Eugene Burreson, Dr Allison Carrington, Dr Duncan Colquhoun
Ian Carr, Dr Joyce Evans, Dr Erik Johnson
Dr Phillip Klesius, Silvia Murcia, Jarle Mikalsen
Dr Barbara Nowak, Dr Anne-Berit Olsen
Emiliano Rabinovich, Amy Rose
Dr Mark Sheppard, Dr Craig Shoemaker
Dr Simon Wadsworth, John Whitehead
DESIGNER: Rachel Walker
PRE PRESS/CTP: BPG Digital
PRINTERS: Business Print Group
WEBSITE: Web4U

CAN PARTNERSHIPS BE POSITIVE?

Partnerships between fish farm critics and fish farmers – do they have positive implications for fish health research?

DR SCOTT PEDDIE, EDITORIAL DIRECTOR

The recent announcement of closer research cooperation by Marine Harvest and the Coastal Alliance for Aquaculture Reform in British Columbia, Canada is an interesting one from a fish health perspective.

The debate in west coast Canada surrounding the environmental impacts and sustainability of net-pen salmonid culture has been highly polarised for some time. The inevitable result of this has been what some would view as an unhelpful politicisation of scientific enquiry, involving claim and counter-claim from the main protagonists.

The debate centering on the issue of the potential link between sea lice infection in farmed salmon and reduced performance in wild juvenile pacific salmon has raged on in the fish farming press and wider media for several years. Against such a backdrop it is difficult to see how any rational progress can be made in resolving either perceived or real issues in this area.

Thankfully, both parties have realised this and have jointly produced a Framework for Dialogue. Although the framework still allows both Marine Harvest and the alliance to publicly state their respective cases and respond to issues as and when they arise, it provides the basis for a more constructive relationship. It should therefore be welcomed as an important step in the right direction.

The issues raised in the document that are up for discussion appear at first glance to be very broad indeed, ranging from aboriginal rights to technology and socio-economic issues. As I read it, the question that immediately popped into my mind was, "Does this wide-ranging approach run the risk of unravelling for want of a clear focus?"

It seems that this has been mitigated against, at least to some degree, by the mutual identification and documentation of immediate research priorities as part of the framework.

It is perhaps not surprising that the focal areas are the interaction between wild and farmed salmon and sea lice, migration corridors, and the economic evaluation of commercial-scale closed containment systems. It is a reminder, if any were needed, that fish health issues are of interest to a wide audience, not just readers of this magazine!

For me, the key point in this document is that such research will be undertaken on a collaborative basis, reducing the likelihood of any bias, intentional or unintentional, creeping into the process. Nevertheless, as the saying goes, the proof of the pudding is in the eating. Given that the results of such research could potentially have far-reaching consequences for all parties involved, it remains to be seen how it will play out at a practical level.

Notwithstanding the potential pitfalls that could befall such a venture as it beds down and evolves, it is certainly worth watching. And who knows, maybe it could even become a template for conflict resolution in other areas of the world where similar heated debates take place? ■

THE EXCELLENCE OF VIRGINIA'S SHELLFISH PATHOLOGY CENTRE

BY DR EUGENE BURRESON (SHELLFISH PATHOLOGY LABORATORY, VIRGINIA INSTITUTE OF MARINE SCIENCES, USA)

VIMS SPL STAFF FROM LEFT TO RIGHT: NANCY STOKES, DR RYAN CARNEGIE, SUSAN DENNY, KRISTI HILL, DR KIM REECE, RITA CROCKET, DR GENE BURRESON (BACK), AND DR CORINNE AUDEMARD

The Virginia Institute of Marine Science (VIMS) was established in 1940 as an agency to provide scientific advice to the state of Virginia as it related to the Chesapeake Bay and coastal waters.

Since its earliest beginnings, VIMS has had a long history of research in bivalve mollusc diseases. Early researchers Dr Jay Andrews and Dr Frank Perkins provided fundamental knowledge on the morphology and biology of the important oyster pathogens *Perkinsus marinus, Haplosporidium nelsoni* and *Haplosporidium costale.*

Both scientists also worked closely with the Virginia Marine Resources Commission, the local management agency, to develop methods to assist the oyster industry in managing around the diseases. Dr Andrews initiated a disease-monitoring programme in 1960 that continues to this day, and has provided fundamental knowledge on climate impacts on pathogen abundance and allowed a predictive capability of annual disease severity.

Today, the VIMS Shellfish Pathology Laboratory (SPL) maintains a broad research programme in parasitology, which encompasses *P marinus* and *Haplosporidium* species, *Bonamia* and *Marteilia* species and the clam (*Mercenaria mercenaria*) parasite QPX. The programme is fundamentally directed toward health management in molluscan populations in both aquaculture and restoration contexts.

VIMS has always had an academic affiliation with the College of William and Mary; in 1979 the institute was disbanded as a state agency and fully merged with the college, while retaining the title and advisory mandates of VIMS. The academic programme at VIMS is the School of Marine Science of the College of William and Mary, and is strictly a graduate programme.

FACILITIES

The VIMS SPL, housed within the Department of Environmental and Aquatic Animal Health, has a necropsy laboratory, a fully equipped histopathology centre with an automated tissue processor and slide stainer, and a fully functional molecular laboratory with platforms for both standard and real-time PCR as well as DNA sequencing. Microscope resources include multi-headed microscopes for consultation and teaching, an epifluorescent microscope for fluorescent in situ hybridisation, and a high-resolution imaging system for image capture and analysis. The VIMS SPL also has access to both scanning and transmission electron microscopes and running seawater laboratories for experimental work.

REGIONAL DIAGNOSTIC SERVICES

Because of the lack of commercial marine finfish or crustacean aquaculture in the region, diagnostic services have long focused on diseases of oysters *(Crassostrea virginica)* and hard clams *(Mercenaria mercenaria)*.

VIMS SPL provides diagnostic support services and identification confirmation to researchers and governments worldwide

The SPL provides diagnostic services to the local oyster and hard clam culture industries and to the Virginia Marine Resources Commission. Four important protistan pathogens are routinely diagnosed. QPX disease in *M mercenaria*, and *H nelsoni* and *H costale* in *C virginica* are diagnosed by histopathology; *P marinus* in *C virginica* is diagnosed by Ray's fluid thioglycollate culture medium (RFTM).

This work is partially subsidised by the state of Virginia. The SPL has also been active in developing and applying molecular diagnostic tools such as standard and real-time PCR and in situ hybridisation, which it applies toward these species as well as toward Bonamia parasites (*Bonamia ostreae*, and two undescribed species) that are also present in the region.

OIE REFERENCE LABORATORY

In 2000 the VIMS SPL was designated as the sole Office International des Epizooties (OIE) reference laboratory for the diseases Haplosporidiosis and Perkinsosis of bivalve molluscs. This designation recognised the laboratory's development of molecular diagnostic tools for both groups of oyster pathogens. With the new OIE focus on specific parasites (eg *P marinus*) rather than broad categories of disease (eg Perkinsosis), the SPL is currently the reference laboratory for *P marinus*, *P olseni*, *H nelsoni* and *H costale*. The SPL continues nonetheless to take a broader view of its role within the international aquatic animal health community, and serves as a resource for scientists and health managers concerned with *Perkinsus* species and haplosporidian parasites, including *Bonamia* species, worldwide.

Four PhD-level scientists are affiliated with the SPL:

- Eugene Burreson, head of the SPL and professor of marine science
- Kimberly Reece, associate professor of marine science (molecular diagnostics of *Perkinsus* sp, molecular phylogenetics)
- Ryan Carnegie, assistant research scientist (molecular diagnostics of *Bonamia* sp., molecular phylogenetics), and
- Corinne Audemard, assistant research scientist (real-time PCR of *Perkinsus* sp).

In addition, the SPL includes Nancy Stokes (molecular diagnostics of haplosporidians), Rita Crockett (histopathological diagnoses) and two technical support staff for histopathology and molecular biology.

As an OIE reference laboratory, VIMS SPL provides diagnostic support services and identification confirmation to researchers and governments worldwide for haplosporidians and Perkinsus sp using PCR and in situ hybridisation. The SPL also provides reference material in the form of histological slides and positive control DNA.

SURVEILLANCE

The SPL provides shellfish pathogen surveillance for the state of Virginia. The programme includes quarterly oyster sampling along a salinity gradient in the James River, an annual fall survey of all oyster beds in Virginia, and an annual importation of susceptible oysters to monitor for *H nelsoni* disease pressure.

Periodic surveillance for the QPX pathogen in *M mercenaria* also occurs at the request of the hard clam culture industry.

DIAGNOSTICIAN RITA CROCKETT SHUCKS OYSTERS FOR DISEASE ANALYSES

SPL STAFF PROCESS OYSTERS FOR PERKINSUS MARINUS DIAGNOSIS BY THE THIOGLYCOLLATE CULTURE TECHNIQUE

THE EXCELLENCE OF VIRGINIA'S SHELLFISH PATHOLOGY CENTRE

THE LOCATION OF THE SHELLFISH PATHOLOGY LABORATORY WITHIN THE VIRGINIA INSTITUTE OF MARINE SCIENCE

THE LOCATION OF VIMS

EDUCATIONAL LINKS

VIMS SPL personnel teach formal courses in the School of Marine Science on diseases of marine organisms, systematics and phylogenetics, and genomics, as well as other relevant courses in conjunction with other VIMS faculties.

Graduate students are an integral part of the research the SPL conducts on shellfish diseases. In addition, as part of our OIE Reference Laboratory mandate, VIMS SPL personnel train visiting researchers and students from around the world in histopathological and molecular diagnostics of shellfish pathogens.

RESEARCH LINKS

Much of the research conducted by SPL staff is funded by the US government and is directed toward developing improved diagnostics for known or emerging pathogens, and toward increasing our understanding of the biology and pathogenicity of disease agents.

Examples include:

- documenting through molecular techniques that *H nelsoni* is an introduced pathogen, and working toward resolution of its life cycle
- developing specific PCR and in situ hybridisation assays for two new species of *Bonamia* recently discovered in North Carolina
- field studies to determine the infection window and seasonal cycle of *Bonamia* sp in North Carolina
- developing specific DNA probes for *Perkinsus marinus*, *P olseni* and *P chesapeaki*
- the synonymisation of *Perkinsus chesapeaki* and *P andrewsi*, and
- developing real-time PCR for detection of *P marinus* in water samples.

The SPL has also collaborated in developing *P marinus* and *H nelsoni*-tolerant oyster stocks for *C virginica* aquaculture that have been crucial for the growth of this industry, and studies the impact of *P marinus* and *H nelsoni* on restored populations of *C virginica* in Virginian waters.

The laboratory also works with researchers around the world in the identification and molecular characterisation of new and emerging parasites of molluscs and crustaceans.

RELEVANT WEBSITES

VIMS Virginia Institute of Marine Science - www.vims.edu
EAAH VIMS Department of Environmental and Aquatic Animal Health - www.vims.edu/env
SPL VIMS Shellfish Pathology Laboratory - www.vims.edu/env/research/shellfish/index.html

Contact details:
Dr Eugene M Burreson, Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, PO Box 1346, Gloucester Point, VA 23062 USA
Phone +1 804 684 7015. fax +1 804 684 7796, email gene@vims.edu

NEW DISEASE IDENTIFIED IN NORWAY'S FARMED COD

BY DR ANNE-BERIT OLSEN, JARLE MIKALSEN AND DR DUNCAN COLQUHOUN, (NATIONAL VETERINARY INSTITUTE, NORWAY)

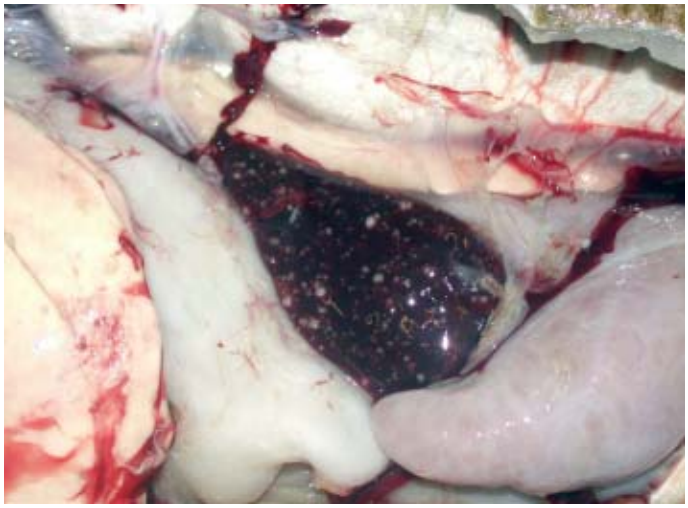


FIGURE 1: SPLEEN LESIONS IN AN INFECTED FISH

FIGURE 3: HEART LESIONS ARE ANOTHER SYMPTOM OF THE CONDITION

A previously undescribed granulomatous disease in Atlantic cod *(Gadus morhua L)* related to the presence of a Gram-negative, facultative intracellular bacterium belonging to the genus *Francisella* has recently been identified in Norway.

The initial outbreak was identified in a population of mature cod weighing 2-4kg held in an enclosed natural seawater "basin" on the western coast of Norway. Increased levels of mortality were registered in July 2005 at a water temperature of around 14.5°C and peaked in August. Accumulated mortality reached approximately 40 percent in the five months from July to November. Other pathogenic agents were not thought to have contributed significantly to this total.

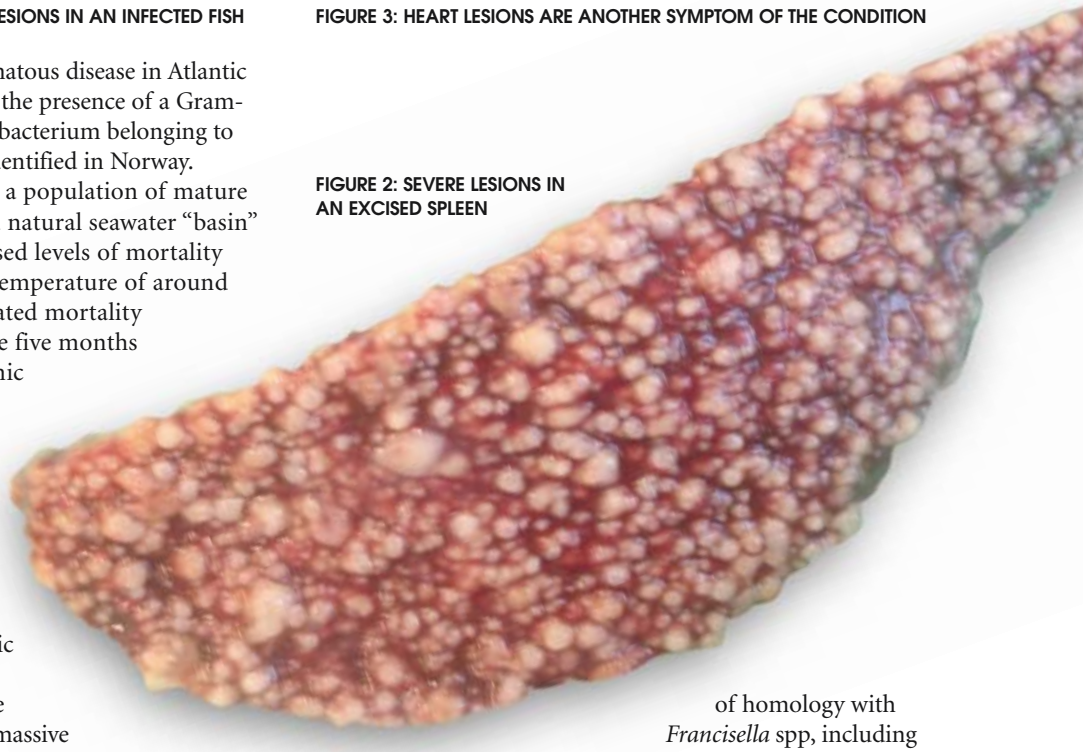


FIGURE 2: SEVERE LESIONS IN AN EXCISED SPLEEN

CLINICAL AND PATHOLOGICAL FINDINGS

Externally, the fish appeared to have a generally emaciated condition. Some individuals displayed raised haemorrhagic nodules in the skin. All moribund fish caught for examination showed extensive internal gross lesions, with moderate to massive occurrence of white, partly protruding nodules of various sizes in the spleen (see Figures 1 and 2), the heart (Figure 3), kidney and liver.

The spleen was enlarged and sero-haemorrhagic ascites and thickened intestinal mucosa were observed. Extensive chronic granulomatous inflammation with multiple granuloma in all organs was the main histopathological finding. Few to numerous small Gram-negative bacteria were found intracellularly in the granulomas.

IDENTIFICATION OF ISOLATED BACTERIA

The bacterium associated with the infection does not grow on standard microbiological media, but grows well in cell culture and on media with a high cysteine content. A nearly complete 16S ribosomal RNA sequence was obtained showing a high degree of homology with *Francisella* spp, including isolates previously identified in fish in Taiwan (*tilapia*) and Japan (three-lined grunt).

Although the present isolate has yet to be fully described, the phenotypical evidence so far available comprises compelling evidence for its inclusion within the genus *Francisella*. Of the limited temperatures tested, the best growth was identified at 22°C, with very weak growth registered at 30°C and no growth at 37°C, suggesting that the bacterium is probably incapable of surviving within a mammalian host.

OCCURRENCE

So far three outbreaks have been confirmed by bacterial isolation. Histopathological findings consistent with those described above have been identified in several other cases currently under microbiological investigation. ■

FISH IMMUNOLOGY UNDER THE SPOTLIGHT IN SCOTLAND

BY DR ALLISON CARRINGTON (SCOTTISH FISH IMMUNOLOGY RESEARCH CENTRE, UNIVERSITY OF ABERDEEN, UK)

An ever-increasing consumer demand for fish and shellfish products, together with diminishing yields from many traditional marine and inland capture fisheries, has encouraged a rapid expansion of the aquaculture industry.

In the past 10 years over 25 percent of the total world supply of finfish and shellfish was derived from aquaculture, and with the growing pressure to reduce fishing of many marine stocks this trend looks set to continue.

Infectious diseases are a major impediment to the development, productivity and profitability of successful commercial fish farms that depend heavily on a commitment to animal health. Intensively farmed stocks can be significantly affected by infectious diseases that occur as intermittent, random events in wild populations.

The establishment of a comprehensive, cost-effective programme of vaccination or prophylactic treatment, negating the long-term use of antibiotics and the possibility of micro-organisms developing resistance to them, is essential in any farming environment.

However, fish are a low-value species and, despite the fact that in recent years vaccines have become a major factor in combating fish diseases and have made a major contribution to improving fish health in aquaculture, cost-effective vaccines for many piscine diseases have not yet been produced or approved for use.

The Scottish Fish Immunology Research Centre (SFIRC) was opened in June 2003 with support from the Scottish Higher Education Funding Council. The centre combines expertise from the University of Aberdeen, the University of Stirling and the FRS Marine Laboratory in Aberdeen with the aim of strengthening collaborative research in fish health in Scotland and addressing fundamental and applied issues relating to fish health. One objective of this research is the development of novel effective vaccines, immunostimulants/adjuvants, and antiviral/antimicrobial reagents for use in farmed fish. We are also developing multiplex tests to monitor immune responses in both vaccinated and diseased fish.

IMMUNE SYSTEM

The fish immune system has evolved a vast array of defence mechanisms against invasion by pathogens. However, although genetic differences between bony fish and higher vertebrates are relatively small, with many of the cellular systems being similar, the structure of the fish immune system is different to that of mammals.

Add to this is the fact that there are approximately 24,000 different species of bony fish (and even more fish species if cartilaginous fish are to be included), with considerable morphological variation between them, and it is easy to understand why the immune processes in fish are poorly understood, with relatively few immune cell populations from fish having been isolated and characterised.

To date, immunological studies on fish have concentrated principally on species of economic importance such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), catfish *(Ictalurus punctatus*), sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) cod (*Gadus morhua*), common carp (*Cyprinu carpio*) and grass carp (*Ctenopharyngodon idellus*), although this list is growing rapidly.

STATE OF THE ART EQUIPMENT AT THE SCOTTISH FISH IMMUNOLOGY RESEARCH CENTRE'S FACS MACHINE

One of the aims of the SFIRC is to study the role or roles of immune cell subpopulations and to identify and monitor the factors that regulate their activation and function.

AREAS OF EXPERTISE

Flow cytometry is an excellent method for making rapid measurements on cells as they flow, one by one, in a fluid stream through a detection point.

The first commercial flow cytometers were available in the early 1970s and have since been developed to evaluate a wide range of cellular parameters (ie size and granularity) and functions. They are mainly used in hospitals or research laboratories to measure levels of cell surface and/or internal proteins by labelling these proteins with fluorescent dyes.

Measuring the patterns of expression of these proteins is termed phenotyping, and as well as being an essential component of the diagnosis of a variety of diseases in humans, analysis of these proteins can also provide valuable information about the general status of the immune system in both mammals and fish. Additionally, many flow cytometers allow single-cell types to be identified and separated from the population being studied, enabling further specific analysis to be carried out.

Although we work with a number of fish species, the majority of the work carried out at SFIRC is on salmonids. As part of our ongoing research into the fish immune system, we are currently working to identify fish cell surface proteins that can be used as markers for single-cell populations such as cluster of differentiation (CD) antigens.

CD antigens can be present only on a single cell population such as CD3, which is present only on T-cells, on more than one cell type; CD45, which is present on all leucocytes, or only on cells in a particular state of activation; and CD212, which is present on activated T-cells and activated natural killer (NK) cells.

To date more than 262 CD antigens have been identified in mammals, and this number is still growing. In contrast, only a small number have yet been characterised in fish, and it is not yet

CONFERENCES AND MEETINGS

NORTH AMERICA

Aquaculture America 2006
Riviera Hotel and Casino, Las Vegas, USA. February 13-16
See www.was.org/meetings/ConferenceInfo.asp?MeetingCode=AA2006

37th Annual International Association for Aquatic Animal Medicine (IAAM) Conference
Nassau, Bahamas. May 6-10
See www.iaaam.org/meeting/meeting.html

10th International Congress of the International Society of Developmental and Comparative Biology
Charleston, South Carolina, USA. July 1-6
See www.isdci.org/meetings/php

5th International Symposium on Aquatic Animal Health
San Francisco, California, USA. September 2-6
See www.fisheries.org/fhs/isaah_2006.htm

American Veterinary Medical Association 2007 Convention Aquatic Medicine Programme
Washington DC, USA. July 14-18, 2007
See www.fisheries.org/fhs/avma_2007.html

SOUTH AMERICA

Aqua Sur 2006
Puerto Montt, Chile. March 22-25
See www.aqua-sur.cl

EUROPE

Aquaculture Today 2006
Sheraton Grand Hotel & Spa, Edinburgh, Scotland. March 28-30
See www.aquaculturetoday.co.uk

Fish Immunology/Vaccination Workshop
Wageningen, The Netherlands. April 18-22
See www.cbi.wur.nl/UK/fish_workshop/

OCEANIA

International Symposium on Veterinary Epidemiology and Economics
Cairns Convention centre, Cairns, Australia. August 6-11
See www.isveexi.org

Australasian Aquaculture Conference 2006
Adelaide Convention Centre, Adelaide, South Australia. August 27-30
See www.australian-aquacultureportal.com/austaqua/aa06.html

ASIA

The Second International Symposium on Cage Aquaculture in Asia
The International Conference Centre, Zhejiang University, Hanzhou China. July 3-8
(See item in News this issue) See www.caa2.org

---

known if all the CD antigens present in mammals are found in fish, and vice versa, or whether those that are present in fish function in the same way as their mammalian counterparts.

Fluorescently labelled antibodies generated against CD antigens can be used in flow cytometry to mark cells of interest, providing evidence of which cell types are present in a mixed population and their state of activation.

We are also working to identify and produce antibodies against cytokines - small, secreted proteins that act to mediate and regulate the activity of cells. They are produced by cells in response to immune stimuli such as disease or vaccination, and typically act at very low concentrations over short distances and short time spans.

Cytokines act by binding to specific cell membrane receptors to alter the behaviour (gene expression) of the target cell in a number of ways, including increasing or decreasing the expression of membrane proteins (including CD antigens), cell proliferation and secretion of other effector molecules.

Despite the fact that cytokines are not expressed on the cell surface, they can be detected while still inside the cell, again by using fluorescently labelled antibodies and flow cytometry, allowing their production to be monitored. Up and down regulation of specific cytokines following vaccination or disease will also be able to be monitored by use of multiplex assays.

The expression and subsequent actions of a number of cytokines is of great interest to immunologists. The production of antibodies against fish cytokines, specific cell-surface markers and other immune factors will enable us to expand our knowledge of the cellular expression of these proteins, helping us to elucidate how the fish immune system behaves in both naïve and vaccinated or infected fish. Ultimately, this knowledge can be used to develop vaccines or other prophylactic treatments for use in aquaculture.

UNITED STATES: ANIMAL HEALTH COMPANY LAUNCHES US WEBSITE

The Schering-Plough Animal Health Corporation has launched a new website, www.aquaflor-usa.com to keep the United States aquaculture industry abreast of the latest developments with Aquaflor® (florfenicol). The company says it is the first antibiotic approved for use on US fish farms in more than 20 years.

The site, produced specifically for the US market, includes a newsroom section with educational articles, announcements, company news releases and a new series of questions and answers.

Producers can use the web site to access HTML and download PDF versions of product literature, as well as a handbook on using Veterinary Feed Directive drugs, an exclusive status the Food and Drug Administration assigned to Aquaflor® and all new in-feed drugs to ensure correct usage and long-term effectiveness. VFD forms required by the FDA are also available for downloading.

The scientific articles section features a downloadable PDF file of the Aquaflor® Monograph, which summarises all major US trials conducted to date to support the product's use in catfish, as well as links to several technical articles presented by scientists at industry meetings in recent years.

An industry links page allows quick access to additional information from industry support services and the news media serving US aquaculture.

Aquaflor® Type A Medicated Article is said to be a fast-acting, broad-spectrum, highly palatable antibiotic premix developed specifically for aquaculture. It has been used worldwide to treat highly infectious bacterial diseases in salmon, trout and other farm-raised aquatic species.

It was recently approved in the United States for controlling mortality in catfish due to enteric septicaemia associated with *Edwardsiella ictaluri*. Schering-Plough Animal Health says it is pursuing additional claims for use in other farm-raised aquatic species produced in the United States.

See www.schering-plough.com

CANADA: PUBLICATION SHOWCASES AQUACULTURE RESEARCH

FRONT COVER OF THE CANADIAN AQUACULTURE R&D REVIEW

Capamara Communications Inc, the publishers of the popular trade journals Northern Aquaculture and Hatchery International, has recently released the Canadian Aquaculture R&D Review 2005. Available as a free download on the Northern Aquaculture website (see www.naqua.com), the review contains over 150 summaries of recent research projects on salmon, trout, charr, oysters, mussels and marine species, plus full-length features on completed projects across Canada.

Several project summaries are of interest to fish health professionals, including:

- Nodavirus research in Atlantic cod and haddock
- the effects of water temperature on cod and haddock health
- tracing the origin of sea lice infecting wild juvenile salmon
- disinfecting processing blood water
- using tidal modelling to predict ISA (Infectious Salmon Anaemia) dispersal
- sea lice vaccine research
- genomics of aquatic animal diseases
- the development of new rapid typing methods for fish pathogens
- finding better diagnostic tests for IHN (Infectious Haematopoietic Necrosis), and
- testing the relationship between stress, vaccination and Kudoa in Atlantic salmon.

SEA LICE RESEARCH IS A PROMINENT FEATURE OF THE CANADIAN R&D SCENE

EU: DIPNET WEBSITE UPDATES

Since the last issue of *Aquaculture Health International* went to press, DIPnet (Disease Interactions and Pathogen exchange between farmed and wild aquatic animal populations - a European network) has updated its website with three new newsletters:

- Reports on Vertical Transmission of Fish Diseases now Available on Web (Newsletter 32).
- Open Workshop: Review of Evidence for Pathogen Transmission and Disease Interactions between Wild and Farmed Shellfish in Europe (Newsletter 31).
- Global Epidemiology: Worldwide Distribution of IPNV is Not Only a Consequence of Aquaculture and Commercial Movement of Cultured Fish (Newsletter 30).
- Effects of pathogenic *Vibrio tapetis* on defence factors of susceptible and non-susceptible bivalve species - two recently published results (Newsletter 29).
- A Review of the Norwegian National Action Plan Against Salmon Lice on Salmonids: the effect on wild salmonids (Newsletter 28).
- DIPnet Epidemiology Seminar: surveillance of aquatic animal diseases (Newsletter 27).
- Salmon Lice Spread by Currents - but where do they go? (Newsletter 26).

See www.dipnet.info/index.htm

AUSTRALIA: INTERVET ENTERS AQUATIC ANIMAL HEALTH MARKET

Intervet (Australia) Pty Ltd is based in Bendigo, a large town in central Victoria, about 200km northwest of Melbourne. The site consists of a vaccine manufacturing plant, plus warehousing, technical, sales, marketing and administration departments.

All types of vaccines are manufactured, ranging from live freeze-dried vaccines for dogs, cats and poultry to inactivated

INTERVET AUSTRALIA

THE BARRAMUNDI VACCINATION TEAM AT WORK AT THE DARWIN AQUACULTURE CENTRE

cattle, pig and sheep vaccines. The company continues to invest heavily in vaccine development, with no fewer than 12 new vaccines for five animal species in various stages of development and registration.

Servicing almost exclusively the Australian animal health industry, Intervet Australia offers a broad range of vaccines and pharmaceuticals, with approximately 40 percent of the business being generated from vaccines manufactured in Bendigo. A new pilot laboratory was completed in July 2005 to help reduce time to market.

One area of animal health that Intervet Australia had not yet explored was that of aquatic animal health (AAH) relating to the aquaculture industry. While this sector was obviously growing in Australia, its size was previously considered too small. However, with government-supported aquaculture production increasing rapidly in Australia, and with a need for vaccine solutions to finfish diseases, Intervet Australia looked at the possibility of making full use of its GMP standards and marrying it to Intervet's existing AAH expertise. Indeed, the Australian government recently brought in legislation to ensure that all fish vaccines are manufactured according to GMP standards.

In early 2005, Marine Harvest, which has a large barramundi operation in the Northern Territory, sourcing their fingerlings from the Darwin Aquaculture Centre, approached Intervet Australia to manufacture an autogenous *Streptococcus iniae* vaccine. The vaccine had to be autogenous because Australia's strict quarantine laws do not allow any importation of vaccines or vaccine strains without lengthy, rigorous and often expensive evaluations. With the help of Intervet Norbio Singapore, the first batch was manufactured, and used to successfully vaccinate 220,000 fish in July 2005. Since then, vaccination has been employed as part of Marine Harvest's health management programme.

Intervet Australia says it has since had several more requests for fish vaccines, typically for barramundi and salmon, against a variety of bacterial pathogens.

Although the Australian aquaculture market remains small in global terms, it continues to grow with increasing financial support from both state and federal governments. The company says it plans to become a key player in the industry as it evolves.

CHILE: CHILEAN MANAGERS STUDY NEW TRENDS

As part of their 'AquaTrends 2005' trip in August, a group of fifteen Chilean managers in the salmon industry travelled through four European countries to learn about the most advanced research and production facilities of several European-based suppliers of vaccines (Intervet International, The Netherlands), pigments (DSM, The Netherlands and Switzerland) and fish feed (EWOS, Norway).

They also visited Aqua Nor 2005 in Trondheim, Norway, where they saw the latest technologies for the sector and met international colleagues. AquaTrends 2005 was the initiative of Intervet Veterinaria Chile Ltda (Andres Engelbreit, General Manager and Oscar Parra, Product Manager, Aquatic Animal Health) but was organised jointly by the three supplier companies.

The first leg of the trip involved a visit to the Intervet International facilities in Boxmeer. The group listened to a corporate presentation about Akzo Nobel and heard details about the role Intervet played in the veterinary field for different animal species, including aquatic animals.

The group was informed about the latest advances to fight important diseases affecting salmon and trout production in Chile, including IPN, vibriosis, furunculosis and SRS. They also toured the Boxmeer site, including seeing "behind the doors" of some vaccine production facilities, and became acquainted with the procedures Intervet applied relating to sterility, biosecurity, automation, good practices and quality control in its GLP laboratories and GMP production buildings.

They also met various Intervet staff, including the Boxmeer site manager, Jan van Raaij, the director of corporate communications and affairs, Dr Sabine Schueller, and scientists in the virology, bacteriology, pathology and other laboratories.

The group then travelled to Switzerland and France to see DSM production plants, and to Norway to call into EWOS and AquaNor.

THE AQUATRENDS GROUP DURING THEIR VISIT TO INTERVET INTERNATIONAL BV IN BOXMEER

CHINA: INTERVET SUPPORTS MAJOR CONFERENCE

Cage aquaculture has a long history in Asia, but its potential has been far from reached, especially for off-shore cage culture in open sea.

The first cage culture symposium was successfully held more than five years ago, and the aquaculture community will meet again this year in Hangzhou, China to discuss the recent advances, potential, challenges and problems of cage aquaculture in Asia. CAA2 will be held from July 3 to 8, and will discuss:

- recent advances and innovations in cage culture technology
- cage design, structure and materials
- site and species selection
- nutrition, feed, feeding technology and management
- disease prevention and health management
- economics and marketing
- sustainable management and development
- policy and regulation
- constraints to cage culture development, and
- conflicts between cage culture and other stakeholders.

Intervet is an active participant for the symposium. Besides being one of the supporting organisations, Intervet staff will share their research findings and experience in the field of fish health, including a presentation on Health management practices for cage aquaculture in Asia - a key component for sustainability, given by Dr Zilong Tan as one of the keynote speakers.

Alistair Brown will speak on the success of salmon farming with the help of vaccination technology, and also serve on the expert panel for an open forum that will involve the business sector, farmers, government officers, scientists, NGOs and other stakeholders to tackle practical issues related to cage culture.

Intervet will also participate in the international trade exhibition held in conjunction with the symposium.

For further details, including submission of papers (deadline March 31), see www.caa2.org

IRELAND: SEMINAR DISCUSSES PERFORMANCE AND PROFITABILITY IN AQUACULTURE

Alltech hosted a one-day seminar for experts and representatives of the major companies and research institutes in the aquaculture industry at its European headquarters in Ireland on November 29. Over 50 experts from 17 countries attended the meeting.

The third annual European Aquaculture meeting discussed the latest developments of this fast-growing industry. Areas of discussion included organic mineral nutrition, gut health status and morphology, and new molecular tools to investigate gene expression and nutrition.

The speakers included Professor Giovanni Bernadini, of the University of Uninsurbia, Italy, who presented a paper on the use of molecular tools to investigate welfare, growth and performance in sea bass and other species of commercial interest. He described the identification of over 1400 genes in sea bass and, through gene expression, related these to stress and environmental conditions.

Dr Simon Davies of the University of Plymouth in the United Kingdom spoke on gastrointestinal morphology in cultured fish and the effect of Bio-Mos® on gut integrity: new perspectives. Dr Davies explained the role of gut function, prebiotics and mannan oligosacharides, which beneficially affect the host by selectively stimulating improved gut morphology and function and altering microbiota.

Dr Turid Morkore of the Nutrition Group, Akvaforsk, Norway, discussed the dietary impact on fish quality of farmed Atlantic salmon. Dr Morkore emphasised the financial importance of the flesh colour, fat content and gaping losses. She reported that early trial results with Alltech's Salmon Pak, which includes Bio-Mos® and Bioplex®, reduced significantly the gaping losses that are responsible for 38 percent of salmon fillet rejection at processing.

"Alltech has a clear vision on how it can contribute to this industry with its products in order to ensure a better performance and thus higher profits for the aquaculture industry players," said the European technical manager, John Sweetman.

Timm Neelsen, the European aquaculture coordinator, added that "Products such as Bio-Mos®, Bioplex® and Sel-Plex® have shown great results in the diets of aquaculture." He also explained the importance of numerous independent trials.

NORWAY: PUMPKINSEED *(LEPOMIS GIBBOSUS)* INFECTED WITH NON-NATIVE MONOGENEANS

(Source: Erik Sterud, researcher, National Veterinary Institute, Norway)

Pumpkinseed *(Lepomis gibbosus)* heavily infected with gill monogeneans were recently found in a pond outside Oslo. The monogeneans belonging to the family Ancyrocephalidae are non-native parasites not previously found on fish in Norway. The effects of the introduced fish and parasites are unknown.


PUMPKINSEED *(LEPOMIS GIBBOSUS)*


ERIK STERUD

Pumpkinseed, a fish native to North America, were introduced to European waters more than 100 years ago. The origin of the fish found in a pond outside Oslo is unknown. The gills of the four fish examined were heavily infected (50-100 parasites) with two ancyrocephalid monogeneans.

The parasites have tentatively been identified as *Haplocleidus* sp and *Onchocleidus* sp, both common parasites of pumpkinseed. The host specificity and potential effect upon native fish species is not known. There is apparently no risk of natural dissemination of fish from the pond, but the risk for anthropogenic disseminations must be regarded as high.

The Directorate for Nature Management in Norway will support the National Veterinary Institute in a project dealing with parasites on introduced fish, where the parasites of pumpkinseed will be studied more closely.

USA: SCHERING-PLOUGH RECEIVES APPROVAL FOR AQUAFLOR® (FLORFENICOL)

The Animal Health Division of the Schering-Plough Corporation has received approval from the US Food and Drug Administration to begin marketing Aquaflor® in the United States to control mortality in catfish due to enteric septicemia (ESC) associated with *Edwardsiella ictaluri.*

Schering-Plough claims that Aquaflor® is a highly palatable, fast-acting antibiotic proven worldwide to be effective against a wide range of bacteria in several aquatic species. It is the first in-feed antibiotic to be approved for US aquaculture in more than 20 years.

Aquaflor®'s sister product, Nuflor® (florfenicol), has been used successfully in the United States since 1996 to treat respiratory disease in beef and non-lactating dairy cattle.

After reviewing data on Aquaflor®, the FDA's Centre for Veterinary Medicine concluded that the meat derived from catfish that were fed florfenicol was safe for human consumption when the fish were fed according to the approved label (CVM Update, www.fda.gov/cvm/catfishapp.htm)

The centre said Aquaflor® was reviewed under its Guidance for Industry 152, evaluating the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern.

The agency also determined that Aquaflor® can be used in food-producing animals without creating a public health risk from antimicrobial resistance. The product is limited to use by veterinarians, a stipulation that will reduce the likelihood of resistance developing.

Unlike sulfa drugs and tetracyclines, Aquaflor® was developed specifically for use in food animal species. Schering-Plough claims that US studies show that Aquaflor® can be used with no setbacks in feed consumption or growth, and that its short, 12-day

withdrawal period leaves producers with ample marketing flexibility.

"Aquaflor® has proved to be safe and highly effective, with good palatability, so we have high hopes for it in the US aquaculture industry," said Dr Patricia Gaunt, associate professor, aquatic animal health with the Mississippi State University College of Veterinary Medicine.

In tank trials conducted at the university, catfish fingerlings challenged with ESC had a cumulative death rate of only 0.8 percent, compared with 60 percent for challenged, untreated fish. In the same study, treated fingerlings showed an infection rate of only 1.7 percent, compared with 72.3 percent for untreated controls.

Palatability trials show that fish consume feed medicated with Aquaflor® at the same rate as unmedicated feed - even when Aquaflor® was used at 10 times the recommended dose rate.

Aquaflor® has been used to treat fish species around the world since the early 1990s. Its first introduction was in Japan for use in yellowtail and other local species, and it was subsequently introduced in Europe, Canada and Chile for treating furunculosis in salmon.

Additional approvals have been granted or are anticipated over the next several months in Latin American and Far Eastern countries for shrimp and species of finfish such as tilapia. Additional claims and indications are also being sought in Europe.

See www.spaquaculture.com and www.aquaflor-usa.com.

(Aquaflor and NUFLOR are registered trademarks of the Schering-Plough Veterinary Corporation)

MOROCCO: *BONAMIA OSTREAE* OUTBREAK UPDATE

Details of the final report, submitted to the OIE by the Animal Production Department, Ministry of Agriculture and Rural Development, Rabat, on the outbreak of *Bonamia ostreae* in farmed flat oysters (*Ostrea edulis*) in Laayoune Province, can be accessed via the Aquatic Animals Commission website.

See www.oie.int/aac/eng/en_fdc.htm

PACIFIC ISLANDS: MODEL IMPORT RISK ANALYSIS (IRA) FRAMEWORK DEVELOPED

(Source: Jean-Paul Gaudechoux, Fisheries Information Adviser, Secretariat of the Pacific Community, New Caledonia, e-mail jeanpaulg@spc.int)

The Aquaculture Section has engaged a team of international consultants to undertake two import risk analyses (IRAs) involving the proposed introduction of aquatic species. The team, led by J Richard Arthur, included Melba Bondad-Reantaso, Edward Lovell, David Hurwood and Peter Mather.

The risk analyses were developed to serve as models for consideration by other Pacific Island countries for future translocations. The IRA process can significantly reduce the risks associated with translocation that might occur from a poorly planned and executed introduction or some unanticipated result. This is a valuable tool for implementing proper bio-security measures, and constitutes a best practice approach for quarantine and translocation.

The IRA process formulated for the Pacific has two approaches. Unlike the traditional approach, which focuses on pathogenic diseases, the model framework for the Pacific includes both a pathogen and an ecological risk analysis. The ecological component recognises the high value attributed to biodiversity in the Pacific.

LARVAL *MACROBRACHRIUM ROSENBERGII* CULTURE AT THE MINISTRY OF FISHERIES AND FORESTRY AQUACULTURE CENTRE, NADURULOULOU, FIJI

The pathogen risk analysis examines the potential risks due to pathogen introduction, along with the movement of the commodity, identifies hazards (pathogens) requiring further consideration, and recommends ways to reduce the risk of introduction to an acceptable level. The analysis was conducted using a qualitative approach with six risk categories (high, moderate, low, very low, extremely low, negligible).

The ecological risk analysis focuses on the invasiveness and "pest potential" of the species to be translocated, and considers the likelihood of its escape and/or release into the natural environment, and the nature and extent of any potential ecological impacts that could stem from escape or release. To assist in assessing the ecological risks, a questionnaire and decision-making process was used.

The first risk analysis concerned the introduction of blue shrimp *(Litopenaeus stylirostris)* from Brunei Darussalam to Fiji.

See the SPC's website

www.spc.int/aquaculture/ site/publications/ documents/ Stylirostris_BruneiFiji.pdf

A second, separate report analysed the risk associated with the proposed introduction of giant river prawn *(Macrobrachium rosenbergii)* from Fiji to the Cook Islands.

See www.spc.int/aquaculture/ site/publications/documents/ Macrobrachium Rosenbergii1.pdf

PRAWN FARM LOCATED IN NAVUA, OUTSIDE OF SUVA, FIJI

THE POWER OF PURIFIED NUCLEOTIDES

BY JOHN WHITEHEAD (WYRESIDE, UK),
DR SIMON WADSWORTH (EWOS INNOVATION, NORWAY) AND IAN CARR (EWOS LTD, UK)

The use of a proprietary formula of concentrated and purified nucleotides in EWOS boost® for farmed fish results in better gut physiology, better fish performance and a better bottom line for the fish farmer. This article explores the mechanism behind this revolution in bioscience.

Through a focused research programme, EWOS has recognised the unique "power" of nucleotides to form an integral part of their dietary strategies for farmed salmon and other aquaculture species.

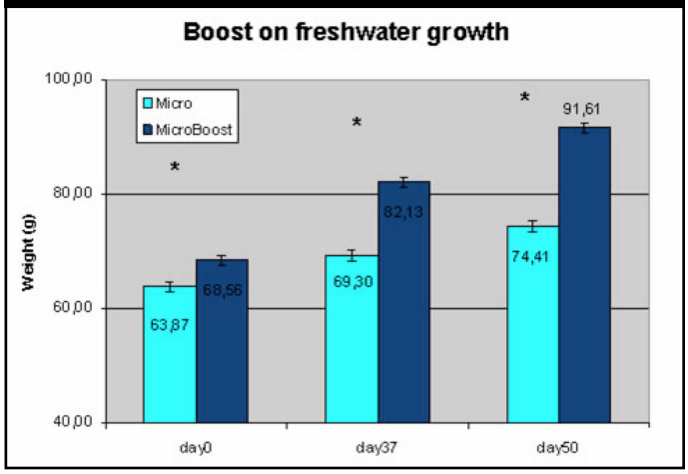
THE POWER OF NUCLEOTIDES

Initially the aquaculture industry considered nucleotides to be non-essential for use in aquaculture feed programmes. It was assumed that they would be supplied through natural dietary intake and produced in sufficient amounts to meet their physiological needs via *de novo* synthesis.

Research in Switzerland and by EWOS Innovation in Norway has confirmed that this is not totally correct, with many circumstances under which there are distinct advantages to dietary supplementation of nucleotides in order to transcend physiological requirements. Improved gut physiology provides for improved nutrient uptake and consequently better growth rates (see Figure 1).

Fish absorbing extra nutrients more efficiently have more resources for a variety of additional requirements, such as during vaccination, reproduction, disease challenges, limited food supply and environmental and metabolic stress. During such times, dietary nucleotides become conditionally essential nutrients, as they spare the fish the metabolically taxing and energy-depriving cost of *de novo* synthesis, which substantially accelerates their availability.

FIGURE 1: EFFICACY. EWOS BOOST® HAS BEEN REPEATEDLY SHOWN TO IMPROVE GROWTH RATES THROUGH IMPROVING GUT PHYSIOLOGY IN FARMED FISH. (TRIPLICATE TANKS, 100 ATLANTIC SALMON *SALMO SALAR* SAMPLED PER TANK, P<0.01 ANOVA)



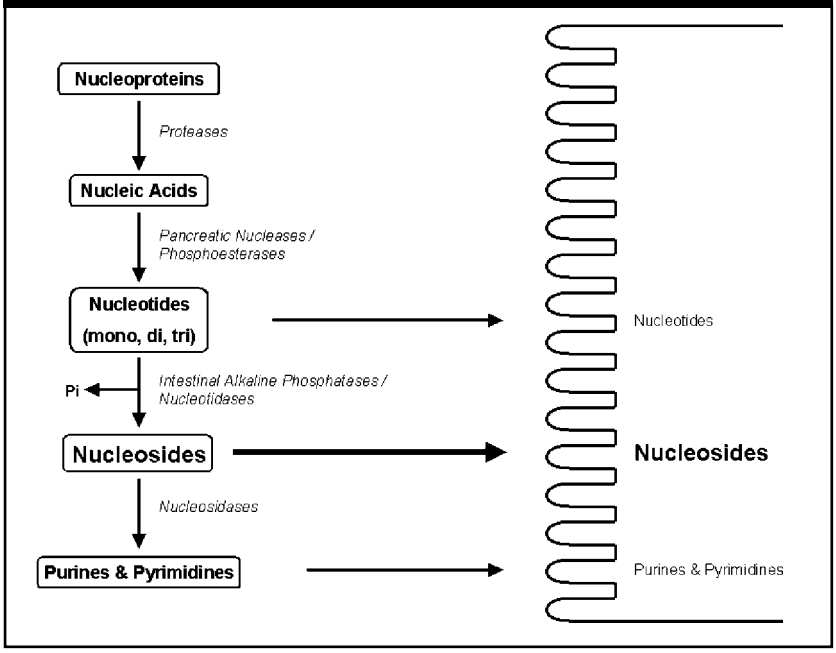
Although repeated scientific research did confirm some remarkable and measurable benefits from nucleotide supplementation (see further reading section), recognising those advantages and obtaining concentrated and universally efficacious nucleotide formulas for commercial applications was something quite different. It became apparent that the primary benefits of nucleotide supplementation occurred during times of high metabolic demand caused by stress, immune challenges, reproduction, transfer, and rapid physiological development and growth.

The quest to produce an optimal formula of nucleotides with their derivatives that would provide an efficient dietary pathway to varied beneficial effects in animal culture became a daunting task. Nucleotides are the essential building blocks of DNA and RNA, and are needed in very large quantities in order to facilitate the endless process of creating new cells for the body.

As they are primarily provided through natural nutrition, they need first to be absorbed through the intestinal wall before they are made available for this process. Some nucleotide components are more readily absorbed than others, and in addition, natural nucleotides found in many substances are divergent and protected from absorption by specific binding proteins. This permits only about 30 percent of them to be available for the animal (Figure 2).

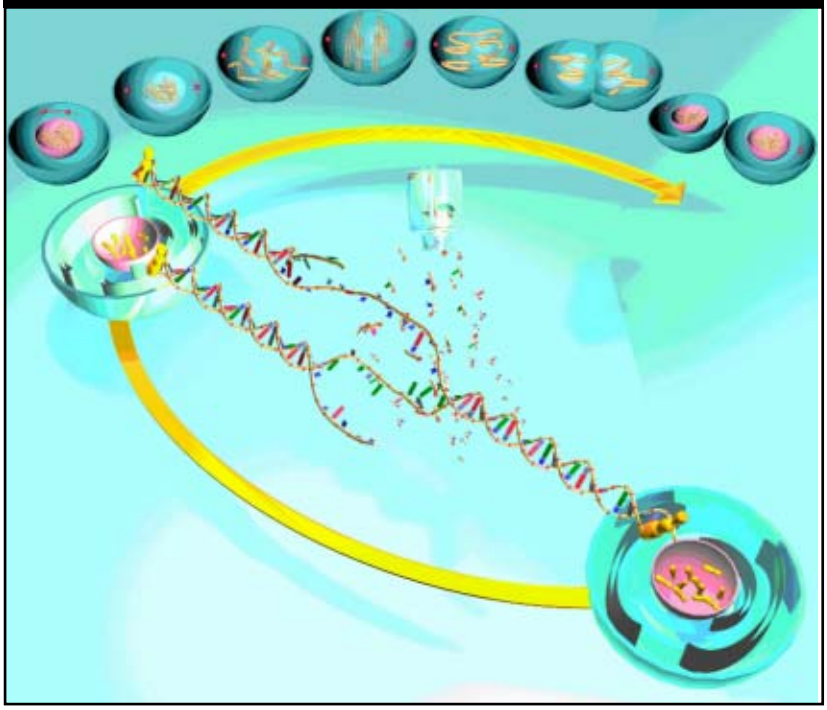
Tenacious scientific inquiry by Swiss biochemists resulted in a discovery which increased the availability of nucleotides to over 95 percent by purifying them and eliminating the protective proteins. They also identified the most limiting nucleotides and those which are ▶

FIGURE 2: AVAILABLE NUCLEOTIDES. THE ABSORPTION IN THE INTESTINAL TRACT OF THE VARIOUS NUCLEOTIDES AND THEIR DERIVATIVES FROM FEED MATERIALS. THE RATE AND EFFICIENCY OF THE ABSORPTION VARIES, WITH NUCLEOSIDES HAVING THE HIGHEST ABSORPTION RATE, FOLLOWED BY NUCLEOTIDES AND PURINES AND PYRIMIDINES. NUCLEIC ACIDS AND NUCLEOTIDES ENCASED BY PROTECTIVE PROTEINS HAVE LITTLE OR NO ABSORPTION



THE POWER OF PURIFIED NUCLEOTIDES

FIGURE 3. SUPPLEMENTATION. ORAL SUPPLEMENTATION OF THE RIGHT BALANCE OF ALL KEY NUCLEOTIDES IN A PURIFIED FORM REDUCES THE TIME AND ENERGY REQUIRED FOR BUILDING DNA AND CELL PROLIFERATION IN THE BODY, PARTICULARLY DURING PERIODS OF ELEVATED DEMAND.



most readily absorbed through the intestinal wall.

Furthermore, the Swiss found that an abundant and readily available natural nutrient was the perfect resource to obtain and process the entire spectrum of purified nucleotides and nucleosides. Additional research focused on varied physiological differentiations among species which require discrete nucleotides leading to unique nucleotide formulas for fish, and EWOS boost® became a reality.

These discoveries, along with the increasing scientific research into the use of nucleotides in all animal species and humans, led to the international debut of the EWOS boost® feed programme for salmon. This programme utilised the benefits of a specific aquaculture nucleotide blend to form the core of the EWOS programme targeting "health through nutrition".

This has subsequently led to the further development and launch of other EWOS' special dietary formulations for a variety of life-stages and different aquaculture species. Across the spectrum of fish culture, nucleotide biotechnology has allowed EWOS to help fish farmers

- control disease more effectively
- lessen the negative effects of stress
- improve production and reproduction
- increase growth and survivability
- reduce parasite infestation
- improve feed conversion
- accelerate immune response, and
- reduce mortality rates

giving "healthier fish and a healthier bottom line".

AQUACULTURE-SPECIFIC NUCLEOTIDES

Fundamental research into the role of the different nucleotides and their specific effect on overcoming stress and challenges in aquatic animals resulted in the design of "aquaculture-specific" combinations of nucleotides forming the basis of EWOS boost®.

Repeated trials in many aquaculture species have clearly demonstrated that dietary nucleotide products are proven only to be efficacious if they contain exact, highly concentrated and differentiated formula of nucleotides. If not, substantial efficacy is not always obtained or obtainable (see Figure 3). Therefore, the assumption that any good diet or aqua supplement claiming merely "to contain nucleotides" is similarly efficacious, or even beneficial, is certainly incorrect.

THE MECHANISM FOR NUCLEOTIDES

Cell proliferation describes the reproduction or multiplication of all living cells. This process, by which cells divide, is imperative to life of all organisms and is fundamental to their biological functions. For just a single cell to divide, it is necessary for its DNA to be duplicated. Amazingly, every strand of DNA contains approximately three billion nucleotides!

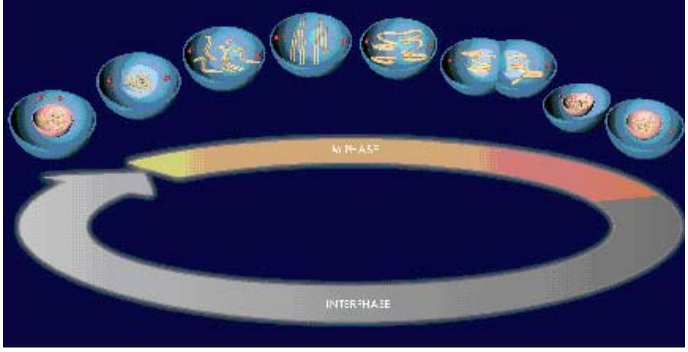
When considering the fact that most fish must produce millions of new cells every second simply to maintain the status quo, it is quite easy to understand that during times of extraordinary stress, such as growth, reproduction, environmental change or challenge, combating disease and recovery from injury, trillions of additional nucleotides must be readily available for cell proliferation. However, since the organism must first produce these nucleotides, this continual process is slow and metabolically taxing (see Figure 4).

Most cells are capable of producing sufficient nucleotides to maintain a satisfactory supply to the organism for normal metabolic activities and life. For a healthy fish, this constant re-supply of nucleotides is very well balanced and is appropriately adjusted in response to occasional stress. However, an increased production of nucleotides takes time and energy, and taxes the fish's supply of basic raw materials to produce more nucleotides. The fish's own production of nucleotides is based on average requirements, with allowances only for occasional short-term increase in response to growth, health challenge etc.

For a fish to maintain good health, much depends on how quickly it can adapt to changing conditions. It does this by increasing the defence cells specific to an invading organism or disease. However, only very few defence cells recognise these viral invaders or errant conditions and move to destroy them.

But the speed by which the body is able to produce new defence cells depends essentially on the availability of the right nucleotides. If the body has to first produce the specific nucleotides, valuable time is lost during which the disease condition progresses or the invader can multiply unhindered. However, if enough differentiated nucleotides are available, the production of the defence cells begins immediately, allowing the

FIGURE 4: CELL DIVISION. THE MULTIPLICATION OF CELLS REQUIRES THE DOUBLING OF INFORMATION STORED IN THE FORM OF DNA. OVER THREE BILLION NUCLEOTIDES (ALL FORMS) ARE REQUIRED FOR EACH CELL. TIME AND ENERGY ARE REQUIRED TO SYNTHESIS EACH NUCLEOTIDE, INVOLVING UP TO 14 BIOCHEMICAL STEPS.



Success with EWOS boost® became a catalyst to explore scientifically the potential benefits of nucleotide supplements in aquatic diets for other species

body to begin the fight against the infection or errant condition during its initial stages.

Dietary nucleic acids, from which the body's source of dietary nucleotides is obtained, are found in ingredients of both plant and animal origin. However, these nucleotides are at quite a low concentration, especially in plant-based proteins. Also, their inherent protective proteins further limit their availability to the organism. Therefore, the ingestion of additional selected pure nucleotides will ensure a much greater and readily available supply of these cell-building blocks needed by the body during times of elevated demand.

EWOS' research scientists used this knowledge in the formulation and development of EWOS boost® and supporting programmes targeting better health through nutrition. This knowledge also continues to be the foundation of EWOS' continuing research and development into providing optimum nutritional value in their current and future aquaculture feed programmes.

NATURAL IMMUNITY

The underlying reason for the success of purified nucleotide-supplemented aquaculture diets, and for the wide range of desirable, observable and measurable effects they have, lies in the fact that the added nucleotides facilitate an accelerated immune response.

An immune response is simply the ability of an organism to mount an effective defence against malignancies and invading micro-organisms (antigens) by producing immunoglobulins (antibodies). In general, the immune response is activated through the production of millions of specialised white blood cells (B-lymphocytes or B cells, plasma cells, and T-lymphocytes or T cells). Since this cell proliferation depends on the ready availability of nucleotides, it becomes clear why the immune response is accelerated when more nucleotides are made available.

A strong immune system is equally important for fish in their ability to respond to other stress factors, such as injury, sudden environmental changes, physical exertion and growth, to name a few. Such pressures tax the immune system and, consequently, the ability to survive and adequately react to traumatic changes during its lifetime.

EWOS BOOST® - DRIVEN BY RESULTS

In other common aquaculture species such as shrimp, nucleotide programmes have demonstrated a highly significant effect on health and performance parameters of interest to commercial producers. Its perceptive scientists measured the value of nucleotide supplements by incorporating an exclusive nucleotide formula into high quality and cost-effective diets for salmon for various cycles during their development.

The results of these trials were dramatic. When comparing various groups of fish fed standard versus nucleotide-supplemented feed, the nucleotide diets, administered anywhere from three to 10 weeks, showed a number of significant zootechnical advantages, including:

- "a remarkable 21 percent" increase in length of intestinal villi, as well as increased surface area, as shown in micrographs
- an 11.8 percent to 12.1 percent increase in fry growth rate after three weeks of nucleotide feed, and 25.53 percent after six weeks
- 9.2 percent increase in mean weight of fish fed for eight weeks (14.7 percent higher with added vitamin C)
- 38 percent reduction in sea lice infestation
- 18 percent decrease in mortality in fish infected with *Vibrio anguillarum*, and a
- 95 percent survival rate (versus 79-84 percent) after vaccination and challenge with *Aeromonas salmonicida*.

As a result, EWOS developed and marketed its unique EWOS boost® diet and now has a leading world market share among competing formulas, with sales of EWOS boost® fast approaching 10 percent of the company's worldwide yearly feed sales.

In other aquaculture species such as shrimp, nucleotide programmes have also demonstrated a highly significant effect on health and performance parameters of concern and interest to commercial producers. Trials in Asia, India and South America have shown a significant reduction in the effects of stress, reduced mortality, higher growth and feed efficiency.

EWOS BOOST® - HARNESSING THE POWER OF NUCLEOTIDES

Success with EWOS boost® became a catalyst to explore scientifically the potential benefits of nucleotide supplements in aquatic diets for other species. As a result of other research and development programmes, EWOS has expanded the use of its product into other phases of salmon development, and unique dietary programmes for hatcheries and diets for cod, halibut, turbot, sea bass, sea bream, yellow tail, tuna and amberjack.

EWOS boost® has also gained approval for certain organic accreditation, allowing it to be used in the process of rearing fish in organic systems, where access to medication is strictly limited.

Over the last two years, significant investment has been made in developing a routine analytical process that accurately determines the level and quality of the individual nucleotides present in EWOS boost®. This process ensures consistency in the quality of the nucleotide formulas used.

The use of dietary nucleotides to positively effect growth, survivability, disease challenges, stress resistance and other factors in fish and animal farming is continually expanding. EWOS has played a most important and instrumental role in making this happen through its significant commitment to an ongoing scientific research programme at EWOS Innovation in Norway, and its continuous quest to develop and manufacture the very best dietary programmes for aquaculture.

See www.ewos.com or contact per.sveidqvist@ewos.com

FURTHER READING

The Journal of Nutrition, January 1994 Supplement; Nucleotides and Nutrition, Volume 124, Number 1S

PERSPECTIVES ON KOI HERPES VIRUS IN US ORNAMENTALS

BY DR ERIK JOHNSON (JOHNSON VET SERVICES, GEORGIA, USA)

TAKING A BLOOD SAMPLE FROM AN ANAESTHETISED KOI

I am a veterinarian in Georgia with a specialty in fish health. Over the years, my caseload has refined itself to one principally composed of pond fish of economic or sentimental importance.

I do not treat food fish, nor do I know much about them. I have become very aware of the problems facing the ornamental hobbyist and their suppliers, but I am grateful that those of us treating pet fish are not hamstrung by some of the limitations facing the food fish industry.

I'm sure that the food fish veterinarian is sometimes jealous of the liberty we have in terms of medications for our ornamental patients. But still there must surely be jealousy the other direction, in that for the food fish practitioner, it's "okay" to lose a few fish, because few specimens of trout or salmon are formally named, and sacrifices for a diagnosis can be easily made.

In the private practice of ornamental fish health, sometimes the fish you want to sacrifice for a diagnosis is "the" fish they specifically want you to save. So there are advantages and disadvantages of practicing either food fish medicine or ornamental fish medicine. There are trade offs.

Since I do not practice food fish medicine, I wanted to write a document that informed and entertained on specific issues developing in my area of experience. I will therefore mention some pertinent issues to let you know what's going on in that "other world" of fish health.

I want to discuss koi herpes virus (hereafter referred to as KHV) in the first of this series of articles. I hope you find my interpretations and thoughts enlightening, informative and helpful.

HISTORY OF KHV

KHV was first described as early as 1996 in Japan. Researchers identified it as a Corona virus and it was a serious but narrowly experienced event, killing a lot of a few fish groups, and then seemingly disappearing. It showed up again in a group of Japanese fish being held in England.

The fish were being moved en masse to Israel, because it was unknown whether some as yet undetected intoxication or water quality issue was causing their rather rapid demise.

The fish went to Israel, where they contaminated (and killed) a formidable amount of fish on a koi farming kibbutz. Israeli researchers were forthright and published mightily on the virus, and the preponderance of information we have on the koi herpes virus comes from the original work done in Israel - and so the virus is erroneously referred to as an Israeli phenomenon.

A KOI INFECTED WITH KHV

WHAT IS IT

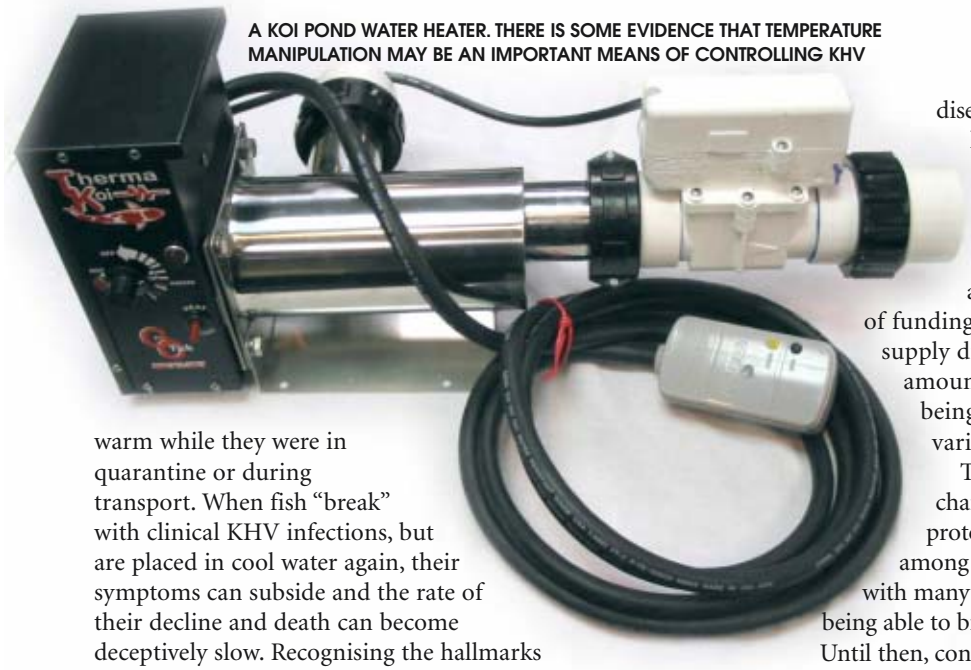
KHV is a herpes virus that features very high morbidity and nearly 100 percent mortality. The virus is spread to vulnerable fish under stress, usually cohabited in confined facilities. Crowding and co-morbidity with Costa or other pathogens seems to increase its virulence. The KHV virus has certain temperature-related "windows" of opportunity or action that have been instrumental in its occurrence and control.

I have been professionally involved in countless cases in North America, and most of them feature the same sorts of hallmarks:

- introducing and mixing of new fish without quarantine
- fish have come from cold water, usually under 70°F, and
- fish have been transported and have Costia co-morbidity.

Most fish are of Asian origin. However, I have seen few cases from Japan. From personal experience it seems that the incidence in Taiwan, Malaysia and China is ten-fold higher.

Many cases were confounded as far as their recognition, because the fish came from spring-fed, cool ponds and were only



A KOI POND WATER HEATER. THERE IS SOME EVIDENCE THAT TEMPERATURE MANIPULATION MAY BE AN IMPORTANT MEANS OF CONTROLLING KHV

warm while they were in quarantine or during transport. When fish "break" with clinical KHV infections, but are placed in cool water again, their symptoms can subside and the rate of their decline and death can become deceptively slow. Recognising the hallmarks of KHV infection can help identify the disease, regardless of the rate of their demise.

CLINICAL SIGNS

The main clinical signs of KHV are:

- fish skin begins to peel, especially at the nape of the head
- fish become reddened and appear abraded overall
- gills are damaged by the removal of the epithelial cells, secondary infections rapidly set in and gills summarily necrose
- lack of oxygen causes the fish to become fearless and unaware
- internal organs may liquefy, and an unusually copious amount of fibrin can be found in these peritoneal cavities, and
- the kidney is damaged, but fish often die before that effect is grossly observable.

DIAGNOSTIC TESTS

The proper diagnosis of KHV is via any of three diagnostic tests. The PCR (polymerase chain reaction) test uses an enzyme-linked immunosorbent assay and detects crucial segments of specifically identifiable DNA from the virus.

Another test can actually detect circulating antibodies via agglutination and is wonderful for finding out whether a fish has recently been infected or not.

The third test is called in situ hybridisation, and it uses enzyme markers to find and illuminate viral DNA or particles in fixed tissues. This last test doesn't just prove the presence of the virus as the PCR test does. The in situ hybridisation test proves actual infection because it demonstrates the virus inside the cell.

The importance of the tests:

PCR testing reliably shows the presence or absence of the virus on tested samples. It does not prove infection, and the absence of virus particles doesn't conclusively prove a lack of infection. The presence of the virus at detectable levels varies as the infection progresses.

Agglutination testing holds promise on very expensive koi as a pre-purchase examination; to wit, before the purchase of a valuable fish, the buyer should require an agglutination (antibody) test be run to ensure they are buying non-exposed/non-infected specimens.

If no antibodies are found in a mature fish in good clinical condition, it is assumed (with all that that implies) that the fish is not infected and has not recently been infected, as antibodies can circulate for up to a year after infection.

Finally, in situ hybridisation is a way of looking at various tissues to see where and if the virus "hides" in neural, meningeal, brain or other tissues latently. It also proves for research purposes that a fish that you're bleeding for agglutination testing and antibody studies was in fact infected when that has been experimentally attempted. It's the only and best "proof" that the fish actually contracted the disease and can therefore contribute meaningfully to latency and sustainable antibody studies.

Vaccine research is underway at several universities, heavily funded by scant hobbyist donations and industry participation. Very little support for KHV research has come from the actual breeders and vendors of koi. The majority of funding has come from hobbyists, and companies that supply dry goods such as food to the industry. The amounts raised are disappointing, and the funds are being distributed to many different organisations for various studies.

The result will be that the research needed to characterise the virus and develop immunisation protocols, technologies and resources will be splintered among many different research facilities, which will end up with many entities having all the pieces, but no one entity being able to bring them to bear on the virus and its eradication. Until then, control of the virus and its effects to save fish lives and restore collections of fish have been developed.

WATER TEMPERATURE MANIPULATION

Because of the above industry-funding allocation snafus, I wanted to contribute some thoughts and experiences on the "control" of KHV. The virus is easily quelled by heating infected fish to 83-86°F. I have found that heating infected fish at a rate of one degree Fahrenheit per hour is fast enough and slow enough to permit control of the viral symptoms without killing the fish via too-rapid warming.

As I often say, aeration while heating is supposed to be absurdly aggressive, short of creating a Jacuzzi effect that tosses the fish out. Within a short time of achieving temperatures in the low eighties Fahrenheit, fish begin to exhibit more normal body comportation, and success rates have been nothing short of amazing. Survivors by heat treatment have not been infective for years after their recovery. However, careful examination of tissue samples and various agglutination tests are only underway, and not yet available.

I am presently waiting for the University of Georgia to start and conclude studies on my behalf, and funded by me to grow KHV in carp fin cell lines and then demonstrate the virus particles in the media. Then, in a highly oxygenated environment, I want the cultures to be heated gradually (one degree per hour) to 83°F, and a second tray to 86°F. Then I want these cultures to be examined via electron microscopy again to show the condition of the virus in the cells.

This study would conclude two things:

1. In the absence of the koi immune system in the cell lines, it could no longer be argued that the koi immune system accounts for the beneficial effects of heating the virally infected fish to 86°F.
2. We may be able to show that the virus (being heat labile) is denatured/coagulated at those temperatures. The results would create a high confidence interval that heating koi is an effective way of ridding the fish of the virus, and so heating could become the accepted and traditional "cure" for KHV without the half-million dollar investment in vaccine research.

We could use the money that is saved for a trip to Cabo San Lucas Mexico for all concerned parties and margaritas for a month. Or, seriously, we could put the money saved into better drugs to control other important diseases such as Costae. Perhaps a tame toluidine stain or a perfect dosing regimen for Acriflavine?

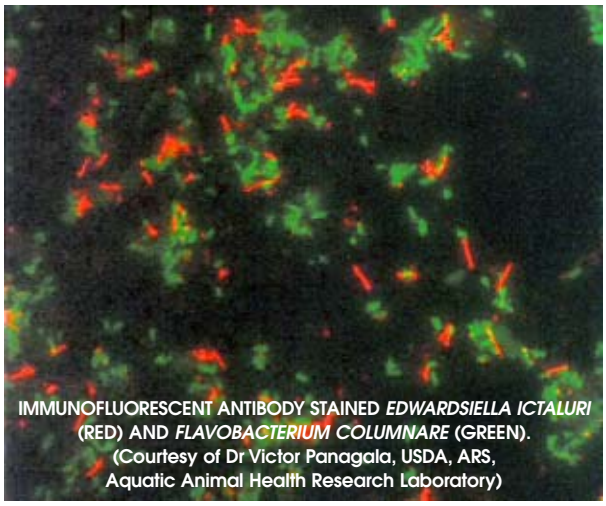
I thank you for your attention. I look forward to contributing to this journal more often. To do that most propitiously, it would be great if you wrote to the publisher and editor with your thoughts on what I could bring you from the microcosmic world of ornamental fish medicine.

See www.koivet.com

A US PERSPECTIVE ON ADVANCEMENTS IN FISH VACCINE DEVELOPMENT

BY DR PHILLIP KLESIUS, DR JOYCE EVANS AND DR CRAIG SHOEMAKER
(UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURE RESEARCH SERVICE, AQUATIC ANIMAL HEALTH RESEARCH LABORATORY, AL AND CHESTERTOWN, MD)

During the past decade, aquaculture production has significantly increased in many parts of the world. Seafood provides 16 percent of the animal protein consumed by humans. From 1992 to 2001, the United Nations' Food and Agriculture Organisation (FAO) reported that total seafood supply increased by 29.8 percent, whereas the supply of wild-captured fish increased by only 8.3 percent. The FAO also reported that global aquaculture is increasing by 11 percent per year and is the world's fastest growing food-producing sector. More recent FAO figures have also confirmed this trend.



IMMUNOFLUORESCENT ANTIBODY STAINED *EDWARDSIELLA ICTALURI* (RED) AND *FLAVOBACTERIUM COLUMNARE* (GREEN). (Courtesy of Dr Victor Panagala, USDA, ARS, Aquatic Animal Health Research Laboratory)

The incidence and emergence of new infectious diseases has almost paralleled the growth of the aquaculture industry. The increasing impact of infectious diseases on production is likely to be the result of sub-optimal production husbandry practices, intensive culture at high fish densities, the lack of health management practices and the introduction of sick fish to healthy populations.

The movement of fish, eggs and genetic material from country to country has resulted in the introduction of new diseases for which the fish have little or no resistance. The overall economic impact of fish diseases is difficult to determine, but may be as high as 10 to 15 percent of the total value of fish production worldwide.

Certain diseases may destroy the entire production chain, and often result in the destruction of healthy fish in the affected area in an effort to control the epizootic from spreading to other regions or countries.

The aquaculture industry has made remarkable biotechnological advancements in the past five years. These advancements in areas such as fish vaccines are necessary to meet the rapid growth of the aquaculture industry worldwide. The use of vaccines for humans, food animals and pets to prevent disease is a common practice. Similar biotechnological advancements in the development of efficacious fish vaccines for disease prevention in cultured fish are on the rise.

From 1976 to the present, the number of commercially available, safe and efficacious fish vaccines has increased from one to more than 14. The majority of these vaccines are to prevent bacterial diseases. However, several vaccines are also available to prevent viral diseases.

The majority of the available bacterial vaccines are of the killed type (i.e. the infectious agent(s) are inactivated or killed). Killed vaccines are primarily administered by injection and are therefore costly because of the need to handle and inject each fish.

KILLED STREPTOCOCCAL VACCINES

Streptococcus iniae and *Streptococcus agalactiae* are major pathogens that cause serious economic losses in tilapia and numerous species of freshwater, marine and estuarine fish worldwide.

Efficacious *S iniae* (US Patent 6,379,677 B1) and *S agalactiae* (patent pending) vaccines were developed and patented by the Agricultural Research Service, Aquatic Animal Health Research Laboratory at Auburn, AL and Chestertown, MD using formalin-killed cells and concentrated extra-cellular products.

A specific antibody response appears to confer protection for both the *S iniae* and *S agalactiae* vaccines. The finding that extra-cellular products of these Gram-positive streptococci are important immunogens that confer protective immunity following immunisation is a notable advancement in the development of efficacious killed vaccines. Indeed, commercial vaccines are now on the market.

ATTENUATED VACCINES

The development of attenuated bacterial vaccines was a biotechnological breakthrough. Attenuated vaccines are made by changing virulent pathogens so they retain the ability to infect and cause the host to mount an effective immune response without causing mortality, adverse reactions or reverting to the virulent form.

Attenuated vaccines can be successfully administered by bath immersion, a cost-effective method of mass immunisation of large numbers of fish. Equally important, attenuated vaccines can be successfully used to immunise fingerlings and fry as young as seven to 10 days after hatching. This immunisation will last the life of their production cycle, as opposed to a shorter duration of about six months for a killed vaccine.

Examples of the first US-licensed attenuated bacterial vaccines are those against enteric septicemia of catfish (ESC) and columnaris disease of catfish. These attenuated vaccines were developed and patented by the Agricultural Research Service, USDA, Aquatic Animal Health Research Laboratory at Auburn, AL (US patents 6,019,981 and 6,881,412). *Edwardsiella ictaluri*, the causative agent of ESC, costs the catfish industry about US$50-60 million annually.

Columnaris disease caused by the bacterium *Flavobacterium columnare,* costs the catfish industry about $40 million annually. Both diseases are generally found together, compounding these industry losses. The Agricultural Research Service, USDA, licensed both vaccines to Intervet, Inc, Millsboro, DE which commercialised the vaccines.

The ESC and columnaris vaccines are commercially labeled Aquavac-ESC® and Aquavac-COL®, respectively. The economic

ESC IMMUNISED CHANNEL CATFISH
(ARS photo news story, Agricultural Research, May 2005)

impact of the ESC vaccine is an increase of producer profit by $1706 per acre and a significant reduction in loss due to disease. The results show that both vaccines significantly increased the survival of the immunised channel catfish.

DNA VACCINES

Deoxyribonucleic acid (DNA) vaccination is another example of a biotechnological advancement to protect fish from pathogens. The basis of a DNA vaccine is the delivery of a gene encoding for a protective vaccine antigen. The vaccine gene is expressed by the host muscle cells to produce the vaccine antigen, which in turn stimulates the host immune system to provide protection against the pathogen.

Intramuscular injection of DNA vaccines against the major viral diseases of salmon, such as infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) has resulted in protection in laboratory trials. Moreover, a commercial variant of the former was approved by the CFIA in Canada last year.

IN OVO AND ORAL VACCINATION

Different methods of administration for mass vaccination can be employed to maximise the protection conferred by different vaccine types. *In ovo* immunisation of channel catfish eggs (US patent 6,153,202) with attenuated ESC vaccine resulted in protection against ESC in fingerlings.

This is the earliest life stage at which fish have been successfully immunised with an attenuated vaccine. The commercial use of *in ovo* immunisation would allow for a very cost-effective method of mass vaccinating fish.

Oral immunisation is also a recent biotechnological advancement. Vaccines must be delivered on a mass scale to be effective, thus oral vaccination, like *in ovo* vaccination, is appealing.

The basis of oral vaccination is to protect the vaccine components from destruction by the fish digestive tract so that the antigens are able to penetrate the intestinal lining and stimulate an immune response.

PerOs Technologies, Inc, of St Nicolas, Canada, has developed its patented Oralject™ technology that prevents the degradation of the vaccine's components by digestive enzymatic function and decreases the gastric pH of the fish intestine.

Currently, the ARS patented *Streptococcus iniae* vaccine (US patent 6,379,677 B1) was incorporated into Oralject™ and fed to tilapia. The *S iniae* Oralject™ vaccine was efficacious following challenge with live *S iniae* in the oral-immunised tilapia.

VACCINATION AS PART OF THE AQUATIC ANIMAL HEALTH MANAGEMENT PLAN

The practice of culturing finfish is dependent on the employment of health management and biosecurity measures in which vaccination is an integral tool for the producer. Thus, vaccines are a management tool in aquatic animal health management and biosecurity plans to prevent disease outbreaks and the introduction of economically devastating pathogens into the producer facilities.

Increased global trade of aquaculture products depends on the continued advancement of these and other such biotechnical contributions.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Laura McGinnis and Lisa Biggar for their helpful editorial assistance. The use of a trade or manufacturer's name does not imply endorsement by the US Department of Agriculture.

FURTHER READING

Hill B 2005. The need for effective disease control in international aquaculture. In: *Midtlyng P* (ed) Progress in Fish Vaccinology. 3rd International Symposium on Fish Vaccinology. CAB Press, Basel, Switzerland. pp 3-12

Horne M 1997. Technical aspects of the administration of vaccines. In: Gudding R, Lillebaug A, Midtlyng P, Brown F (eds) Fish Vaccinology. Developments in Biological Standardisation. Vol 90. Karger, Basel, Switzerland. pp 79-89

Klesius P, Evans J and Shoemaker C 2004. Warm water fish vaccinology in catfish production. Animal Health Research Reviews 5. pp 305-311

Klesius P, Evans J, Shoemaker C and Pasnik D (in press). Streptococcal vaccinology in tilapia aquaculture. In: Lim C, Webster C (eds) Tilapia: Biology, Culture and Nutrition. Haworth Press, Binghamton, NY.

Lorenzen N and LaPatra S 2005. DNA vaccines for aquacultured fish. Scientific and Technical Review International Office of Epizootics 24. pp 201-213

Vandenberg G 2004. Oral vaccination for finfish: academic theory or commercial reality? Animal Health Research Reviews 5. pp 301-304

WHIRLING DISEASE RESEARCH AT YELLOWSTONE NATIONAL PARK

BY AMY ROSE (FORMERLY OF THE WHIRLING DISEASE INITIATIVE, USA),
SILVIA MURCIA AND JULIE ALEXANDER (MONTANA STATE UNIVERSITY, USA)

CAGES WERE PLACED IN PELICAN CREEK TO TEST SENTINEL FISH FOR EXPOSURE TO *M CEREBRALIS*

STREAM FLOW WAS AMONG THE HABITAT CHARACTERISTICS MEASURED IN YELLOWSTONE LAKE TRIBUTARIES

The Whirling Disease Initiative was established in the USA by an Act of Congress in 1997. Its purpose is to conduct research that develops practical management solutions to maintain viable, self-sustaining wild trout fisheries in the presence of the whirling disease parasite.

The initiative's ultimate clients are state, tribal and federal fisheries management agencies and the constituencies they serve.

Yellowstone National Park, located in the western United States, is the stronghold for the native Yellowstone cutthroat trout *(Oncorhynchus clarki bouvieri)*, a species that is increasingly rare outside of the park. The adfluvial population of Yellowstone cutthroat trout associated with Yellowstone Lake is the largest inland population in the world (Koel et al, 2005). Yet even within the park, this population of trout is challenged by several factors.

Whirling disease, caused by the myxozoan parasite *Myxobolus cerebralis,* is one of three major factors contributing to recent trout population declines in Yellowstone Lake and its spawning tributaries. The other factors, predation by non-native lake trout *(Salvelinus naymaycush)* and years of drought, play a significant role and are addressed elsewhere. In this article, we address the ongoing problem of whirling disease, and the research strategies designed to assist in managing Yellowstone cutthroat trout within Yellowstone National Park.

PART 1: FROM DISCOVERY TO LARGE-SCALE INVESTIGATIONS

Myxobolus cerebralis, the causative agent of whirling disease, was first detected in Yellowstone National Park in Yellowstone cutthroat trout from Yellowstone Lake in 1998. Since then, efforts have been directed at determining the severity and distribution of *M cerebralis* in the lake and its tributaries.

From 1999 to 2001, a large-scale investigation was conducted focusing on histological analysis of by-catch adult Yellowstone cutthroat trout and sentinel fry exposures in spawning streams over a wide range of water temperatures and flow regimes. Bycatch adults were obtained during gillnet operations targeting invasive lake trout in Yellowstone Lake. An examination of more than 1500 fish revealed the prevalence of the parasite to be approximately 20 percent in the northern section of Yellowstone Lake to 10 percent in the southern arms.

Dr Todd Koel, supervisory fisheries biologist for the park and a whirling disease researcher, oversees much of this investigation. Koel monitors waters throughout the park, including the tributaries of Yellowstone Lake. He also conducts research projects and coordinates the Yellowstone Whirling Disease Research Programme.

This programme consists of a team of researchers from the park, Montana State University, the University of Wyoming, the US Geological Survey Western Fisheries Research Centre, the US Fish and Wildlife Service-Bozeman Fish Health Laboratory and the states of Idaho, Montana and Wyoming.

The programme has been funded primarily by the Whirling Disease Initiative, the Whirling Disease Foundation and the National Park Service.

Pelican Creek is a major spawning tributary for the trout in Yellowstone Lake. It became a priority for whirling disease research when monitoring data from the investigation revealed that the disease had nearly wiped out the cutthroat population.

The loss of cutthroat trout in Pelican Creek created a gap in the natural food chain, with trophic level implications. Birds and bears have been required to change their feeding habits (Koel et al, 2005). On a social level, anglers have lost a favourite trout fishery which was often touted as the best cutthroat fishery in the world. ▶

WHIRLING DISEASE RESEARCH AT YELLOWSTONE NATIONAL PARK

Pelican Creek is now closed to angling to allow the cutthroat to rebound, and to prevent the potential movement of the parasite into other drainages in the park.

SOLUTIONS THROUGH RESEARCH

In an effort to understand the ecology of the disease and improve management options for Yellowstone cutthroat trout, researchers are investigating whirling disease risk in three of the lake's tributaries. This research is being conducted by Silvia Murcia, a researcher for the Yellowstone Whirling Disease Research Programme. She is a graduate student pursuing a doctorate in fish and wildlife management at Montana State University-Bozeman.

Under the advice of premiere whirling disease researcher Dr Billie Kerans, Murcia's research focuses on spatio-temporal variation in whirling disease risk to the Yellowstone cutthroat trout in three spawning tributaries of Yellowstone Lake: Pelican Creek, Clear Creek and the Yellowstone River.

One puzzling aspect of this issue is why the problem is so severe in Pelican Creek. Murcia postulates that the disease severity results from a combination of environmental factors such as naturally occurring high water temperatures, low flow regimes, movement of bison and elk adding organic material to the water, and angler movements.

These are some of the factors contributing to a warm, silty habitat ideal for tubificid and whirling disease proliferation. Murcia's research, which began in 2001, analyses whirling disease presence in sentinel and wild-reared Yellowstone cutthroat fry by testing for parasite DNA using polymerase chain reaction (PCR) analyses and evaluating infection severity by histology. For potential correlations to disease prevalence and severity, she also looks at the physical and chemical features of the study streams.

To establish the infection risk among wild native cutthroat, Murcia is examining current and historical adult trout spawning data to assess the start, peak and end dates of spawning, and approximate adult cutthroat mortality after spawning. She hopes her research will answer questions regarding fish health and diagnostic, management and control methods for whirling disease in native cutthroat trout and other vulnerable salmonids.

The results from her research and the larger-scale investigation should assist the park and other regional fisheries managers to understand and control whirling disease in a variety of stream types. Quantification of environmental characteristics preferred by *M cerebralis* in the Yellowstone Lake basin will assist in predicting probable high-risk sites for infection. Of particular concern are tributary basins with landscape-level characteristics similar to Pelican Creek, such as the Beaverdam, Trail and Chipmunk Creeks in the remote southern and southeastern arms of Yellowstone Lake.

Murcia and Dr Kerans will use this data to develop a whirling disease risk assessment model that could be applied to a range of watersheds across the Intermountain West. The goal is to provide fish biologists and managers with risk assessment tools to identify management actions to reduce disease risk, increase public awareness and lessen the chances of this non-native pathogen invading other systems.

PART II: FILLING IN THE GAPS

Another student of Dr Kerans, Julie Alexander, is currently completing research on Pelican Creek and other tributaries with variable whirling disease risk (2004 to 2007).

Alexander's work complements Murcia's by investigating the ecology of the oligochaete host, *Tubifex tubifex*. She is examining the potential for using high-resolution thermal imagery and habitat characteristics to detect high tubificid abundance and possible "hot spots" of *M cerebralis* infection.

Alexander is pursuing her PhD in biology with a focus on parasite-mediated *T tubifex* ecology. The objectives of her study are to quantify *M cerebralis* infection risk in Pelican Creek using *T tubifex* and sentinel fish exposures to measure variation among tubificids and habitat, and to assess the potential for developing a tool for detecting *T tubifex* and *M cerebralis* in Pelican Valley, using a combination of remotely sensed and habitat data.

THE PARASITE *M CEREBRALIS* DIGESTS CARTILAGE AND CAN CAUSE SEVERE SKELETAL DEFORMITIES

Alexander determined the distribution of *M cerebralis* using infection prevalence in tubificids collected along the length of Pelican Creek and adjacent tributaries, and infection prevalence and severity in sentinel fish. Habitat characteristics known to influence *M cerebralis* infections were measured and compared to tubificid data. Relationships among infection prevalence, tubificids and habitat were compared to remotely sensed thermal data from the NASA Atlas sensor.

Alexander found that although *M cerebralis* and tubificids had non-uniform distributions, tubificids in more than 90 percent of sampling locations were infected with the parasite. These intense infection levels in Pelican Creek made it nearly impossible to draw conclusions regarding the parasite's ability to establish and proliferate; therefore, Alexander's study area has expanded to cover 11 nearby cutthroat spawning tributaries in addition to the highly infected Pelican Creek.

Another component of Alexander's investigation involves documenting actinosporean varieties and numbers that may be present. Actinosporeans, the waterborne spore phase of the parasite expelled by the worm host, have presented yet another mystery to researchers in the park. In 2001, collaboration with the US Geological Survey Western Fisheries Research Centre showed that 20 of 3037 tubificids collected in Yellowstone National Park released actinospores.

Molecular analyses indicated that none were *M cerebralis*. The team is now interested in determining what other myxozoans are present in tributaries within the park. If there are new and different myxozoans in the system, the potential for new and different problems, either affecting fish or other living parts of the system, creates an additional management concern.

Alexander's field work is expected to fill gaps identified by the principal investigators, Drs Kerans and Koel. The results are hoped to further explain the effects of variation in host ecology, assemblage community and habitat on whirling disease risk in Yellowstone cutthroat trout.

The National Park Service and many other organisations in the United States are working to restore native salmonids. Knowledge of the relationships among the environment, tubificid susceptibility and whirling disease risk could improve the prioritisation process of stream restoration and increase the probability of costly restoration success. Murcia's and Alexander's work will contribute to these larger-scale investigations. In addition, this quantitative data should improve the ability of managers to carry out risk assessments, particularly for native cutthroat trout, in other backcountry areas.

As whirling disease spreads, techniques for management in these areas will be needed, and it is hoped that Yellowstone National Park will provide a successful model for such areas.

For more information, see the Whirling Disease Initiative website www.whirlingdisease.montana.edu

FURTHER READING

Koel TM, Bigelow PE, Doepke PD, Ertel BD and Mahony DL 2005. Non-native lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30. pp10-19

NOCARDIA SERIOLAE - A CHRONIC PROBLEM

BY DR MARK SHEPPARD (SAKANA VETERINARY SERVICES LTD, CANADA)

This article was first published by Intervet in their Aquatic Animal Health Newsletter 10 (May 2005)

Marine nocardiosis is a long-term and problematic bacterial infection of warm-water fish, and eventually presents itself as an underlying debilitating factor in many types of fish. Affected fish often have other concurrent or secondary infections.

Nocardiosis begins as a "silent infection", developing undetected for months in fry or juvenile fish. The duration of the infection is a long-term (chronic) phenomenon. *Nocardia* bacteria multiply slowly within fish tissues before any visual symptoms arise, and certainly before lethargy and death rates increase.

The typical outcomes within affected fish populations are poorly performing yearling and pre-harvest fish, elevated feed conversion rates, emaciation and rising mortality rates near the end of the summer.

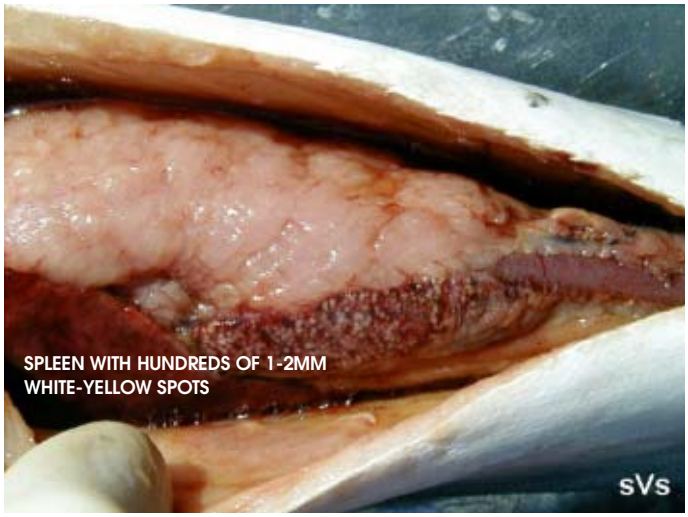
CAUSATIVE AGENT

Many *Nocardia* species are found in the terrestrial and marine environments, but *Nocardia seriolae* (previously *N kampachi*) is considered the most likely pathogen of *Seriola* fish. The bacteria do not stimulate a septicaemic reaction or an acute immune response. Rather, *Nocardia* is thought to progressively invade (and perhaps dwell and multiply inside) various types of fish host cells, including white blood cells.

Relatively limited information about the microbiology, chemistry and patho-physiology related to *Nocardia* is published. This may be due to the inherent problems of researching slow-growing microorganisms.

TRANSMISSION AND EPIDEMIOLOGY

The initial exposure of fry to *Nocardia* is the likely result of the fry consuming uncooked fish tissues (live, raw or frozen) or by the horizontal transmission of *Nocardia* from sick fish. Amberjack and yellowtail juveniles fed raw fish or moist pellets are likely the first to be infected, so the use of raw, low quality trash fish should be avoided when rearing fish of any type. The infection develops silently as the bacteria multiply slowly over months within major organs such as the spleen, kidney and liver.



SPLEEN WITH HUNDREDS OF 1-2MM WHITE-YELLOW SPOTS

ABOVE: A CLASSIC NOCARDIAL LESION SHOWING WHITISH-YELLOW IRREGULARLY-SHAPED MASSES AT THE BASE OF THE GILL FILAMENTS

BELOW: A COALESCING CLUSTER OF NOCARDIAL SKIN ABSCESSES. THESE DRY ABSCESSES PROTRUDE INDIVIDUALLY OR IN GROUPS, EACH CONTAINING MASSIVE NUMBERS OF *NOCARDIA* BACTERIA. MANY BURST, LEAVING A "DRY" YELLOW SKIN ULCER

Cohabitation with infected or diseased fish is also a contributing factor of this disease. Research indicates that yellowtail sharing tank space with sick juveniles (previously injected with live *Nocardia*) eventually exhibit internal pathology (white spots in their spleens) after three months of cohabitation, yet no external visual symptoms are evident.

On the other hand, the injected cohort fish began dying within two weeks of their intraperitoneal injections. Various shellfish populations have also been shown (by RT-PCR) to contain genetic material indicative of *Nocardia* and *Mycobacterium*. However, the question remains whether the shellfish should be considered an environmental and contributing source of these pathogens, or simply accumulators of bacteria from affected finfish populations.

In marine finfish culture, nocardial infections appear to ▶

progress more quickly during the summer months, when water temperatures reach 24°C or more, but the mortality due to *Nocardia* is more commonly experienced in autumn and early winter, perhaps as the fish becomes overwhelmed and its immune system wanes.

CLINICAL SIGNS AND GROSS PATHOLOGY

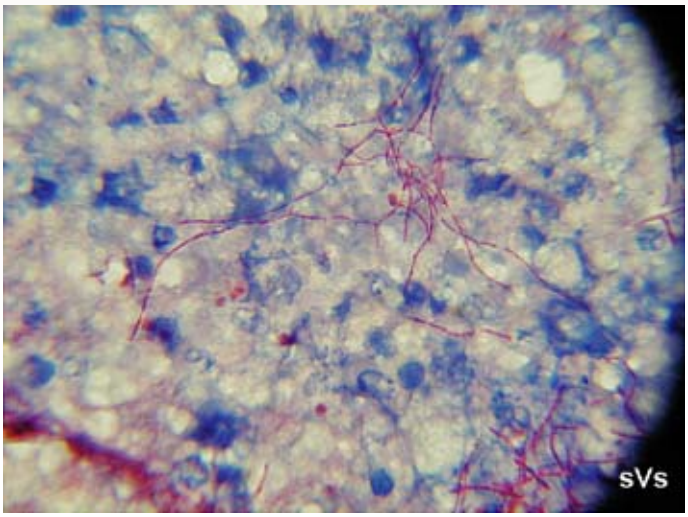
The visual symptoms of this disease vary somewhat. The typical external lesions are thin fish, skin nodules (focal, multifocal or coalescing), skin ulceration, opercular erosion and irregularly shaped fleshy white masses at the base of the gill filaments. The internal pathology of nocardiosis is easily confused with other "white spot-forming" diseases such as mycobacteriosis ("fish tuberculosis") and photobacteriosis (formerly *Pasteurella* or "pseudo-tuberculosis"), especially if mixed infections exist.

The white-yellow granulomata are usually 1-2mm in size. The spots are most obvious in the spleen, kidney and liver, but can be found in any tissue. Fish that mount a significant immune reaction to the disease eventually "heal" somewhat and exhibit hard black spots (melano-macrophage accumulations) in place of the white spots in the liver and adipose tissues. Brown-black crusty plaques often develop on the dorsal inner surface of the swim bladder.

MICROBIOLOGY

The bacterium is thread-like, beaded and branching. It is variable staining when using Gram's stain and the bacteria are acid-fast positive (pink). The culture and isolation of *Nocardia* is relatively easy, yet somewhat tedious. Several types of agar and broth media will support nocardial growth, but these media also support the growth of other, faster-growing species of bacteria.

The use of selective antibiotic agar-tube media, such as



A SPLEEN IMPRINT OR "STAMP" (X1000, ACID-FAST STAIN) SHOWING BRIGHT PINK, THREAD-LIKE BRANCHING AND BEADED *NOCARDIA SERIOLAE*

Lowenstein-Jensen, is most efficient to isolate *Nocardia* directly. The incubation time may range from four to 10 days depending on incubation temperatures of 25°C to 35°C. The colonies appear dry and "stacked". The results of *in vitro* antibiotic sensitivity testing tend to be ambiguous and misleading. In general, *Nocardia* appears to be inherently resistant to most commercially available antibiotics when challenged *in vitro* and *in vivo*.

DIAGNOSIS AND PRIMARY ON-SITE TESTS

A thorough visual examination of the fish is always the best way to begin an assessment. Feel the skin and body wall for lumps and ulcers. Upon cutting through the firm skin nodules, one will find a dry, grey-yellow, inspissated abscess. Lift the operculum to look for pale gills and irregular whitish lumps at the base of the filaments.

Gill, kidney or spleen imprints or "stamps" are easily collected

COLONIES OF *NOCARDIA SERIOLAE* ISOLATED ON LOWENSTEIN-JENSEN SELECTIVE (ANTIBIOTIC) AGAR

(in duplicate), dried and stained using Gram's or an acid-fast stain. Five-millimetre sections of the same tissues are helpful for a histological diagnosis when preserved in 10 percent buffered formalin.

MANAGEMENT AND CONTROL

The best prevention and control of this disease would be through vaccination. However, Nocardia vaccine development remains experimental. To date, I am unaware of the development of a commercially available efficacious antigen-adjuvant combination that will prevent nocardial infections in fish. Therefore the early detection of silent infections among juvenile live fish is the goal. However, the efficacy and practicality of detecting sub-clinical nocardiosis remains questionable in that the surveillance for infection may involve expensive experimental tests such as mucous testing by polymerase chain reaction (PCR) and antibody serology.

The use of drugs to control Nocardia is controversial. Environmentally, consumers are not in favour of drug treatments. From a fish production viewpoint, it is very difficult to ensure that fish consume sufficient volumes of medicated feed to achieve a therapeutic daily dose.

In making the attempt, the fish may reduce their daily food intake and slow their weight gain, thus creating another cost to the farmer. Overall, the cost-effectiveness of antibiotic therapies to control nocardiosis in finfish is debatable.

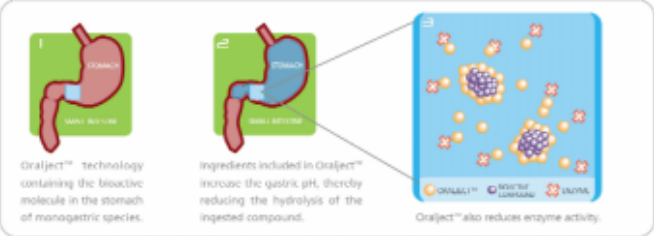
Antibiotic doses need to be high, and the duration of treatment must be extended to the point that the use of antibiotics is largely impractical. That said, it is speculated that the application of two prophylactic treatments applied to asymptomatic juvenile fish may be useful. Using specific antibiotics that can penetrate fish cells (when in high serum concentration) for an extended period of time (10 to 14 days) may interfere with in vivo nocardial development.

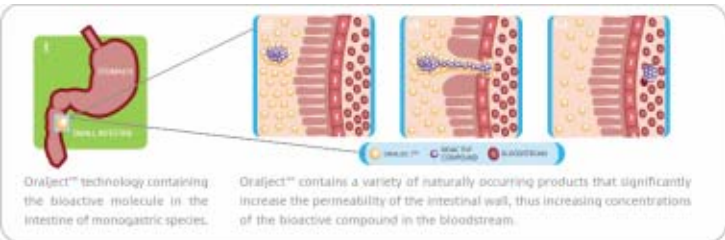
CONTINUED ON PAGE 28 ▶

PEROS
WE DELIVER THE FUTURE

INNOVATIVE HEALTH SOLUTIONS TO GLOBAL AQUACULTURE

BY EMILIANO RABINOVICH (PEROS SYSTEMS TECHNOLOGIES, MONTREAL, CANADA)





HOW ORALJECTᵀᴹ WORKS

The pharmaceutical world has long aspired to be able to orally deliver bioactive compounds. The solution, however, has been hindered by seemingly insurmountable biological and technical limitations.

According to the chief scientific officer of PerOs, Dr Grant Vandenberg, Oraljectᵀᴹ is an answer to the ongoing quest for natural methods of delivering safe and non-invasive treatments for multiple diseases and other therapeutic applications.

Oraljectᵀᴹ permits the oral delivery of therapeutic molecules, including antigens (vaccines), antibiotics, peptides and nutraceuticals, without costly and complicated encapsulation systems. Oraljectᵀᴹ is said to be the first means of delivering large molecules in a cost-effective, accurate, safe and efficient manner.

The intensification of livestock production and the increase in stocking densities has created new opportunities for disease organisms to flourish and threaten the health of both animal and human populations. The increasing exploitation of and dependency on animal resources has meant that disease risks that did not exist or were unthinkable 40 to 50 years ago are now commonplace. The housing and living conditions of intensively farmed animals such as pigs, poultry and fish provide significant exposure to bacteria and viruses greatly enhance the risk of disease.

Oral administration of vaccines and other bioactive substances for livestock industries is by far the most sought after method of delivery. Oral vaccine delivery requires no change in normal animal husbandry or handling, and thus eliminates the stress associated with other methods of administration.

Oral vaccination is the only method suitable for the rapid and simultaneous mass immunisation of bioactive compounds to a large population, which is essential for preventing and controlling outbreaks of disease.

PerOs has decided to develop and adapt the Oraljectᵀᴹ technology to provide solutions in the aquaculture market. In 2004 we founded the first Chilean biotechnological company specialising in developing aquaculture products for the local market.

PerOs Aquatic was established in the Puerto Montt region of Chile, the core of Chilean aquaculture. PerOs Aquatic will manufacture, commercialize and provide support for PerOs' different products and become the springboard for commercializing the Oraljectᵀᴹ technology throughout the world.

PerOs Aquatic will build a manufacturing plant and a field service office in the Puerto Montt region to support its initial commercial activities.

There is potential to combine Oraljectᵀᴹ with a large variety of applications, and PerOs is constantly developing new applications for different aquatic species such as tilapia, sea bass and shrimp, and for other monogastric species such as swine, poultry and companion animals.

PerOs is working all over the world to develop new applications and actively searching for new opportunities. We have conducted tests in Canada, USA, Chile and Norway, and plan to start new tests in Ecuador, Mexico, Venezuela, Australia and the Southeast Asian region.

ORALJECTᵀᴹ TECHNOLOGY

Oraljectᵀᴹ is an innovative oral delivery system for bioactive compounds such as vaccines, peptides and antibodies to monogastric species (fish, swine, poultry, companion animals and potentially humans).

It is a combination of naturally derived anti-nutritional factors that reduce overall digestive capacity, along with those that increase intestinal absorption, providing a novel method to transport bioactive compounds intact to the sites of intestinal absorption and increase intestinal uptake.

Compounds given orally tend to be broken down by acid and enzymes in the digestive tract, thus neutralising their bioactivities and consequent benefits. In order for these compounds to be delivered efficiently, it is necessary to avoid this intrinsic digestive function.

Furthermore, a compound's ability to penetrate the intestinal wall varies from one molecule to another, thereby affecting the amount and the nature of the bioactive compound that can be delivered into the bloodstream.

Oraljectᵀᴹ can efficiently bypass the enzymatic process by feeding a bioactive compound of interest, along with a cocktail of factors that temporarily suppress the digestive enzyme function and increase gastric pH. This permits intestinal uptake of the compound, allowing it to achieve the desired biological effect.

ORALJECTᵀᴹ ADVANTAGES

Oraljectᵀᴹ is said to be very easy to administer, and does not require any specialists or specific skills. A single meal is simply replaced with Oraljectᵀᴹ. This simplicity opens a new door to mass inoculation. However, the key advantage of this technology is that it is cost-effective. Regular injection not only requires ▶

INNOVATIVE HEALTH SOLUTIONS TO GLOBAL AQUACULTURE


AQUACULTURE IN CHILE


FISH MANAGEMENT IN ACTION

expensive specialists and equipment, but also generate significant indirect costs for producers (estimated to be as high as the direct costs) as a result of livestock stress, mortalities, adhesion, necrosis and eating disorders. With no needles and a simple administration procedure, Oraljectᵀᴹ technology provides a straightforward, cost-effective solution that requires only minimal human labour, the company says.

This technology is very flexible and adaptable. The Oraljectᵀᴹ delivery platform can be applied to a wide range of bioactive compounds in monogastric species, including vaccines, peptides, proteins and antibodies. This versatility makes Oraljectᵀᴹ the ideal solution for a large variety of applications around the world.

REGULATORY ASPECTS

All the compounds of Oraljectᵀᴹ are classified as Generally Regarded as Safe, a key advantage that simplifies regulatory approval for new applications.

"During the next few years we will be developing a great variety of applications for the aquatic health market in the Americas, Europe and Southeast Asia, and we'll try to consolidate our presence in key markets such as Chile and the United States," says the chief executive officer of Per Os, Jean-Simon Venne.

"Another big step forward will be developing applications for the shrimp market due to the large economic losses in this market caused by diseases. We are really satisfied with the results obtained up to date. In the short run, PerOs will become a global leader in drug delivery systems."

See www.perosbio.com

PerOs Systems Technologies Inc, known as PerOs, is a Canadian biotechnology company that develops oral drug delivery systems under the name Oraljectᵀᴹ.

CONTINUED FROM PAGE 26

NOCARDIA SERIOLAE – A CHRONIC PROBLEM

Control and prevention through husbandry and good management practices is the best approach for nocardial infections. Avoid uncooked fish feeds (live, raw or frozen) when rearing fish of any age or type. Feed only dry, cooked feed. Reduce shellfish fouling (ie barnacles) on floats and ropes near finfish cages whenever possible. Disinfect hands and marine equipment, practice strict diving hygiene between pens, farm sites and rearing areas, and minimise fish stress as much as possible.

KEY REFERENCES

Fukuda Y 2001. Prefectural disease report - PCR and *in vitro* comparisons of nocardiosis, mycobacteriosis, odan. 11, pp 131-139 (in Japanese)

Itano T and Kawakami H 2002. Drug susceptibility of recent isolates of Nocardia seriolae from cultured fish. *Fish Pathology* 37, pp 152-153 (in Japanese with English abstract)

Kusuda R and Nakagawa A 1978. Nocardial infection of cultured yellowtail. *Fish Pathology* 13. pp 25-31 (in Japanese with English abstract)

Miyoshi Y and Suzuki S 2003. A PCR method to detect Nocardia seriolae in fish samples. *Fish Pathology* 38. pp 93-97

Sako H 1988. Survival of fish pathogenic bacteria in seawater. *Bulletin of the National Research Institute of Aquaculture* 13. pp 45-53 (in Japanese with English abstract)

Sheppard ME 2004, 2005. A photographic guide to diseases of yellowtail (*Seriola*) fish. 64 pages. ISBN 0-920225-14-4, 0-920225-15-2

AQUATIC ANIMAL HEALTH AT THE UNIVERSITY OF TASMANIA

BY DR BARBARA NOWAK (SCHOOL OF AQUACULTURE, UNIVERSITY OF TASMANIA, AUSTRALIA)

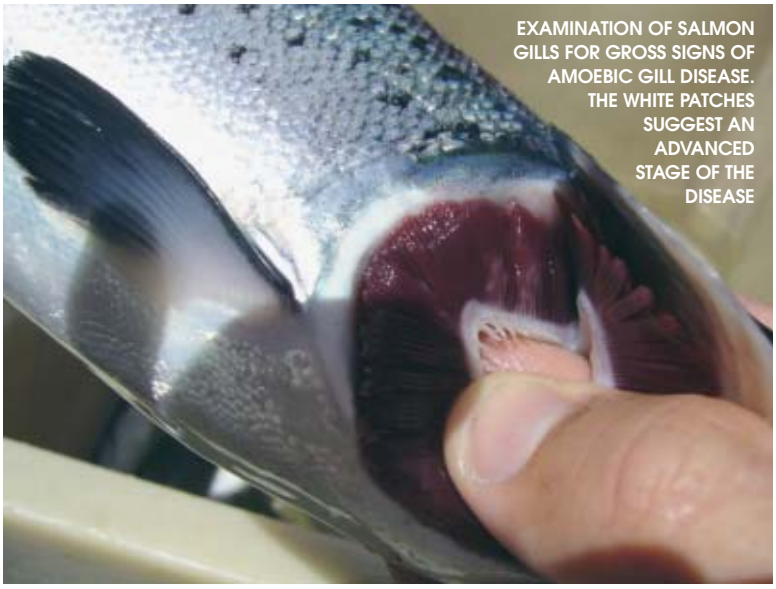
Rapid development of aquaculture has to be supported by an increased capability in aquaculture teaching and research. Australia recognised this as early as the 1980s, when courses were first developed in Launceston, Tasmania. These courses became an important part of the new University of Tasmania in 1991 and undergo a continuous cycle of review and revision to ensure their relevance. The School of Aquaculture remains the only university school in Australia dedicated to teaching all aspects of aquaculture, and is widely recognised as a centre for teaching and research excellence. In the 1990s it was the National Key Centre for Teaching and Research in Aquaculture, and was funded for the maximum nine years.

Today, its status is further strengthened by being part of TAFI, the Tasmanian Aquaculture and Fisheries Institute and one of the biggest partners in the Cooperative Research Centre for Sustainable Aquaculture of Finfish (Aquafin CRC).

Over the years many students, industry partners, researchers and staff have made huge contributions to the shape and direction of the school, and graduates are found throughout the Australian industry and around the world.

It is the only place in Australia to offer degrees entirely in aquaculture. Students have access to the best on-campus aquaculture facilities in Australia, with pilot scale fish and shellfish production. The school houses temperate and tropical fish and shellfish species, including salmon, trout, barramundi, seahorses, prawns, an algal culture and planktonic production unit, marine fish and mollusc hatcheries and sophisticated bio-filter systems.

All academics specialise in the areas they teach and are active researchers. The School of Aquaculture maintains strong and direct links with the aquaculture industry and is believed to be the largest base of aquaculture research in the southern hemisphere.



EXAMINATION OF SALMON GILLS FOR GROSS SIGNS OF AMOEBIC GILL DISEASE. THE WHITE PATCHES SUGGEST AN ADVANCED STAGE OF THE DISEASE

Aquatic animal health is an integral component of undergraduate teaching. The 13-week unit is compulsory for all degree students. Disease diagnosis and treatment are discussed, with the main emphasis placed on health management. Host, environment and pathogen relationships and fish immunology are examined. Problem-solving exercises provide case histories for various species. Associate degree students complete a more applied Fish Health Management unit. This unit provides students with an understanding of aquatic animal health issues, including basic principles and on-farm disease diagnosis, control and treatment. Both units have a significant practical component, ranging from measuring immune variables to investigating diseases.

THE HANDS-ON COMPONENT IS ESSENTIAL IN AQUATIC ANIMAL HEALTH EDUCATION AT ALL LEVELS. UNDERGRADUATE STUDENTS DURING THEIR PRACTICAL IN THE AQUACULTURE CENTRE

In addition to the undergraduate courses, short courses are run for researchers and fish health professionals. The School of Aquaculture runs fish histopathology courses twice a year. The workshop is designed to suit postgraduate students and postdoctoral fellows who are interested in learning histopathology, but also as an introduction to aquatic animals for veterinarian pathologists or as a revision for experienced pathologists.

Classes are taught in small groups of about four persons to accommodate different levels of experience. Streaming of participants according to experience increases the opportunity to maximise benefits for each group.

Conference microscopes, slide projectors and computer software illustrate fish histopathology. The workshop programme includes examples from a ▶

THE TUNA HEALTH WORKSHOP FOCUSES ON INVESTIGATING MORTALITIES AND SAMPLE SUBMISSION. INDUSTRY PARTICIPANTS EXAMINE THE EXTERNAL SURFACES OF FISH BEFORE DISSECTING AND COLLECTING SAMPLES

variety of fish species, both marine and freshwater.

The use of histopathology in disease diagnosis and its use in research are also discussed, and participants are encouraged to bring their own slides for discussion. Additionally, fish immunology, fish parasitology and aquaculture epidemiology courses are organised on demand.

While these short workshops are open to industry participants, the School of Aquaculture also organises specialised workshops for the aquaculture industry, in particular the salmon and tuna sectors. They are sometimes run off-campus, closer to the fish farms, to allow industry participants to have easier access.

These workshops are designed to be practical, and focus on issues of interest to the industry, such as the investigation of mortalities and submission of samples, stress and health in fish, parasites of tuna, use of aquaculture epidemiology in farm management and research.

The school offers a range of undergraduate and postgraduate degrees. The one-year Graduate Diploma in Aquaculture would suit recently qualified degree graduates or people seeking to retrain to follow a new career.

While most graduate diploma students have a background in biological sciences, candidates with other degrees (including chemistry, computer science and arts) have successfully completed the graduate diploma and are now working in the aquaculture industry or are involved in research.

The graduate diploma can be credited as the course work towards a master's degree by course work and dissertation (total two years). A large proportion of masters and honours projects address aquatic animal health issues.

Many PhD students specialise in aquatic animal health, working on areas including fish immunology and fish pathophysiology, as well as multidisciplinary projects linking health and nutrition, for example. While amoebic gill disease is one of the commonly used disease models, bacterial diseases and other parasitic conditions are also studied. The projects range from field work to laboratory-based basic science projects.

LIGHT MICROSCOPY IS A CONVENTIONAL TOOL FOR EXAMINING FISH SAMPLES. BLOOD SMEARS, MUCOUS SMEARS, TISSUE IMPRINTS AND HISTOLOGY SLIDES ARE USED IN AQUATIC ANIMAL HEALTH PRACTICALS

The School of Aquaculture accepts applications for both a traditional start in February and a mid-year start in July. Research degrees, either MSc or PhD, can be started at any time of the year. The next fish histopathology course is scheduled for February 6 to 8, with another planned for later this year.

See www.utas.edu.au/aqua