

# AQUACULTURE HEALTH

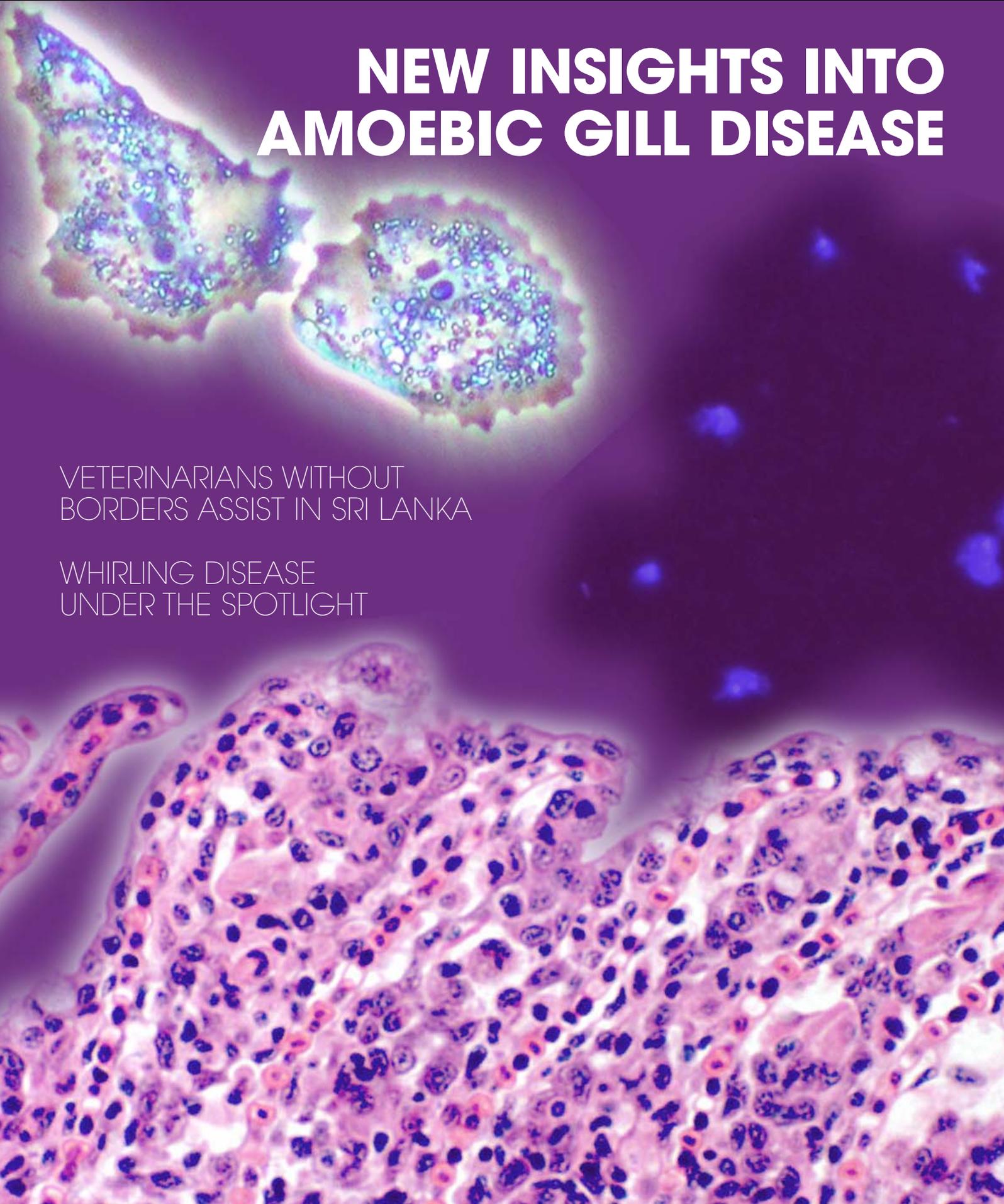
I N T E R N A T I O N A L

ISSUE 5 MAY 2006 NZ\$10.00

## NEW INSIGHTS INTO AMOEBIC GILL DISEASE

VETERINARIANS WITHOUT  
BORDERS ASSIST IN SRI LANKA

WHIRLING DISEASE  
UNDER THE SPOTLIGHT



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INTERNATIONAL

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# AQUACULTURE HEALTH INTERNATIONAL - ONE YEAR ON

DR SCOTT PEDDIE, EDITORIAL DIRECTOR

It is hard to believe that a year has passed since *Aquaculture Health International* was launched. Since “going live” in Bali in May 2005, we have published around 30 articles covering a wide range of disease issues in both fish and shellfish species. With contributions from leading experts, we have been able to provide an eclectic mix of cutting edge articles from across the globe.

Our reader response has been very favourable to date. *Aquaculture Health International* has a growing number of subscribers in Asia, South America, North America, Australia and New Zealand. Perhaps not surprisingly, our main concentrations of readers are found in Norway, Chile, the UK and the USA. All sectors of industry and academia are represented and include biologists, veterinarians, policy makers and fish and shellfish farmers.

Although we have the capacity to produce a printed version of the magazine alongside the pdf version, we have found the costs of producing the former and distributing it to an international audience prohibitive. As a consequence, you will have noticed that we have concentrated on producing the online version - this has several advantages. The first is that we have not been restricted in the amount of material we can include in each issue, so we have been able to increase the content issue by issue. Secondly, we can reach a wider audience more effectively.

Looking to the year ahead, we want to increase the scope of the magazine to include more articles and news items on the ornamentals industry. We also want to provide more practically orientated articles to engage more fully with those at the “coal face” of the aquaculture industry. So in essence, we want to make the magazine more responsive and relevant to our increasingly diverse readership. In order to do this we need as much feedback from you, the reader, as possible. So I would encourage you to contact me with any suggestions you might have. Are there any areas of fish or shellfish health you want us to focus on? Do you want to highlight your area of research or special interest by writing an article for us? If so, I'd be delighted to hear from you!

## EFFECTIVE COMMUNICATION

Those of us living in the UK would have found it hard not to be aware that a dead swan infected with H5N1 was found on our shores at the

end of March. The dreaded H5N1 strain of bird flu had arrived. Thankfully it appears to be an isolated case in a non-native whooper swan, and in mid-April the authorities relaxed some of the emergency biosecurity restrictions put in place.

Why are we discussing bird-flu in a magazine devoted to fish and shellfish health, I hear you ask? Well, it seems to me that the UK occurrence highlights some important points for those of us involved in aquatic animal health. Firstly, it demonstrates the importance of effective communication between those on the ground and the regulatory authorities. That a member of the public was sufficiently aware of the bird-flu threat prompted them to report the dead swan to the relevant agency. In the aquaculture industry, the first line of defence is almost always the fish farmer; an appreciation of what constitutes normal mortality rates, feeding response and overall behaviour in their stock is essential. Together with both passive and active surveillance methods, simple routine on-farm monitoring by knowledgeable staff on the ground helps to ensure that potential disease threats are identified quickly and can be dealt with effectively. Perhaps we take this for granted in the developed regions of the world. The situation in other areas of the globe can be more challenging - there can be particular difficulties in disseminating basic health information to small-scale farmers operating in isolated areas devoid of an effective communications infrastructure.

The second point also relates to communication, but this time between experts and the public. Although scientists are sometimes accused of being notoriously poor at communicating complex science to a general audience, it seems to me that this time the message has got through. The buying public has taken on board the message that poultry and poultry products are safe to eat, therefore there has been no substantial shift in consumer behaviour to date, at least in the UK.

So the message is clear. Good communication between key stakeholders in the food production industry with respect to potential or actual disease issues is of increasing importance. Animal health scientists play a key role in the chain of communication, therefore their skills in this area are progressively coming to the fore. So perhaps we should all ask ourselves this question: am I ready for the communications revolution? ■



**AQUACULTURE HEALTH**  
INTERNATIONAL

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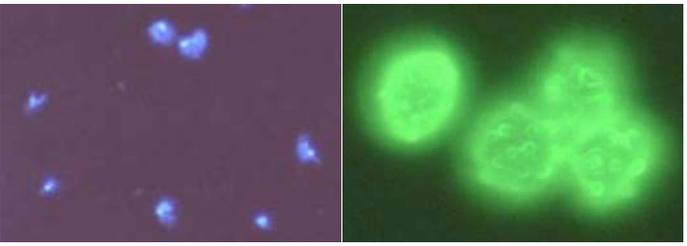
# INSIGHTS INTO AMOEBIC GILL DISEASE PATHOGENESIS

BY DR RICHARD MORRISON, ASSOC PROF BARBARA NOWAK, DR PHILIP CROSBIE, DR MARK ADAMS, DR ANDREW BRIDLE (UNIVERSITY OF TASMANIA) AND DR MATTHEW RISE (UNIVERSITY OF WISCONSIN-MILWAUKEE)

Atlantic salmon aquaculture in Tasmania, Australia has expanded since its inception and approximately 17,000 tonnes of fish are now harvested annually.

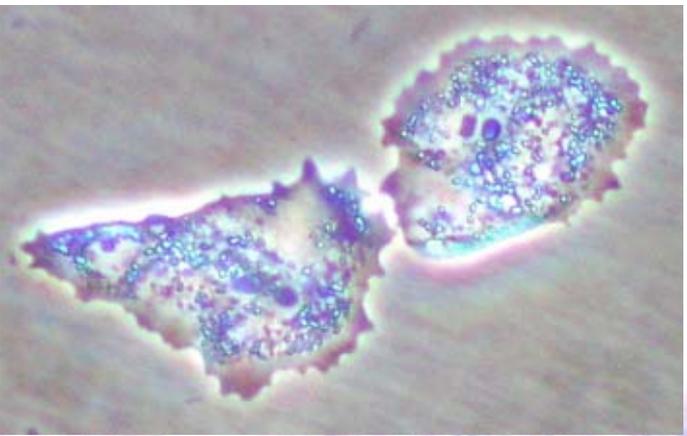
This has provided a stable economic benefit to Australia's island state and revitalised many regional municipalities. However, production in the seawater phase is restrained by a recurrent parasitic condition, amoebic gill disease, or AGD.

AGD can be easily mitigated, yet the cost is something farmers could do without. This article describes aspects of AGD, together with the outcomes of recent studies within our research group at the University of Tasmania, or UTAS.



NEOPARAMOEBEA PEMAQUIDENSIS LABELLED WITH DNA-SPECIFIC FLUORESCENT DYE

NEOPARAMOEBEA PEMAQUIDENSIS BOUND BY FLUORESCENTLY LABELLED ANTIBODY



A LIGHT MICROSCOPE IMAGE OF NEOPARAMOEBEA PEMAQUIDENSIS



GROSS AGD PATHOLOGY. NOTE THE WHITE LESIONS

## AMOEBIC GILL DISEASE (AGD) – THE TASMANIAN PARADIGM

As the name suggests, amoebic gill disease develops after amoebae *Neoparamoeba* spp. colonise and replicate on the gills of susceptible fish. In turn, host epithelial cells adjacent to the amoebae proliferate unabated until death.

Salmonids grown in Tasmanian waters are susceptible to AGD, but the condition has also been reported in Atlantic salmon, rainbow trout, Chinook salmon, coho salmon, turbot and seabass in North America and/or Europe.

In Tasmania, Atlantic salmon smolts were first transferred to seawater during the mid-1980s. Subsequently, AGD outbreaks occurred and were responsible for demoralising losses. Fortunately, freshwater bathing is an effective treatment, and the development of commercial-scale freshwater bathing allowed the industry to prosper.

Bathing involves moving all fish from a net-pen into fresh water contained within a canvas liner for three or four hours, then releasing them back into a net-pen. This is an expensive process and reportedly accounts for up to 20 percent of production costs. It is also limits production, as fish must be farmed within reach of a suitable source of fresh water.

AGD is observed in association with 35 parts per thousand salinity and may be detected year-round in these conditions. However, outbreaks are most prevalent during the warmest months of the year.

During this time, all fish are repeatedly bathed in fresh water, as often as monthly. Surveys of marine sites around Tasmania indicate that *Neoparamoeba* spp. is ubiquitous, and therefore in the long run the industry would prefer to adopt preventative measures such as vaccination.

To this end, current collaborative research supported by the Australian Government Cooperative Research Centre programme is aimed at producing an anti-AGD vaccine. In addition, our research group at UTAS is complementing the vaccine development initiative by investigating AGD pathogenesis.

## COMBINING FISH AND CHIPS TO LEARN MORE ABOUT AGD

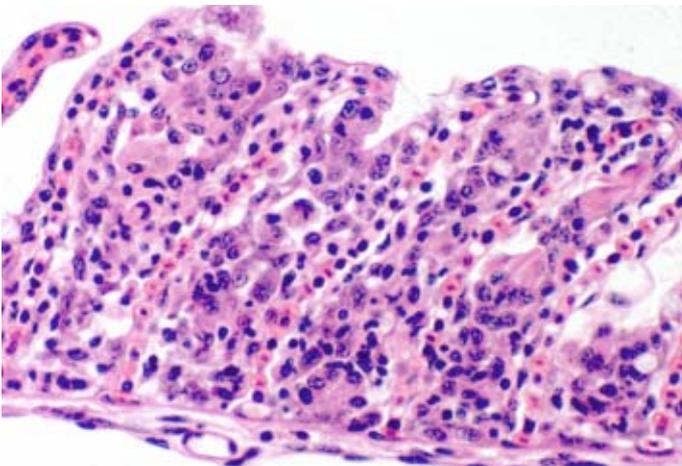
While AGD histopathology is well described, molecular pathways affected by AGD are yet to be identified. Our collaborators at the University of Victoria in Canada have developed a salmonid DNA micro-array chip. This is a glass slide that has been coated with 16,006 microscopic DNA spots, representing thousands of individual genes.

It is used to detect genes that are switched on or off in response to various parameters such as disease. Lists of affected genes can be compiled to identify related genes within molecular pathways that are affected.

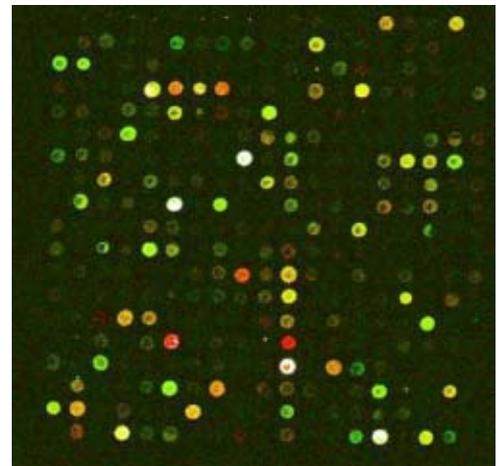
Recently, we used this cDNA micro-array chip to identify nearly 200 genes that were switched on or off during AGD. A gene called as AG-2 is consistently switched on in AGD-affected gill tissue, and interestingly, the equivalent gene is known to be strongly expressed in human cells that are rapidly multiplying.

This observation was confirmed using quantitative PCR, and it was also shown that as AG-2 up-regulation was restricted to AGD

**FRESHWATER BATHING OF ATLANTIC SALMON AFFECTED BY AGD**



**LEFT:  
AGD  
HISTOPATHOLOGY.  
NOTE THE AMOEBA  
ASSOCIATED WITH  
HYPER-PROLIFERATIVE  
TISSUE**



**RIGHT:  
A MICRO-ARRAY SLIDE  
AFTER BINDING OF  
FLUORESCENTLY  
LABELLED DNA PHOTO  
COURTESY OF GLENN  
COOPER, UVIC**

lesions relative to unaffected tissue.

Human AG-2 has been shown to block the activity of the p53 tumour suppressor protein that can control the growth of human cells. Therefore p53 and p53-regulated gene expression was also tested by quantitative PCR. This data not only showed that the p53 tumour suppressor gene is switched off in affected tissue, permitting uninhibited cell division, but also p53-regulated genes were affected.

AGD pathogenesis is certainly a complex process, given the number of affected genes, but p53 and as AG-2 may be fundamental to the development of this condition. Knowing how AGD works may well help in the design of a vaccine.

Research on AGD is continuing with the support of the Tasmanian Salmonid Growers Association, and is a collaborative effort between the University of Tasmania, the University of Technology, Sydney and CSIRO Marine and Atmospheric Research. Micro-array chips are available for purchase.

See [www.uvic.ca/cbr/grasp](http://www.uvic.ca/cbr/grasp)

**ACKNOWLEDGEMENTS**

A UTAS/UVic collaborative team conducted the micro-array research. Thanks go to Glenn Cooper and Prof Ben Koop at UVic for ongoing support. This research formed part of a project of the Aquafin Cooperative Research Centre, and received funds from the Australian government's CRCs Programme, the Fisheries R&D Corporation and other CRC participants.

**FURTHER READING**

Morrison RN, Cooper GA, Koop BF, Rise ML, Bridle AR, Adams



**DR RICHARD MORRISON  
WITH PHD STUDENT  
BENITA VINCENT**

MB, Nowak BF (2006). Transcriptome profiling the gills of amoebic gill disease (AGD)-affected Atlantic salmon (*Salmo salar* L). A role for the tumour suppressor protein p53 in AGD-pathogenesis? *Physiological Genomics*, Feb 21 [Epub ahead of print]

von Schalburg KR, Rise ML, Cooper GA, Brown GD, Gibbs RA, Nelson CC, Davidson WS and Koop BF (2005). Fish and chips: Various methodologies demonstrate the utility of a 16,006-gene salmonid micro-array. *BMC Genomics* 6. pp126-144

# DIAGNOSTIC LABORATORY SERIES

SERIES EDITORS: DR DAVE GROMAN AND DR FRANCK BERTHE  
(ATLANTIC VETERINARY COLLEGE AT THE UNIVERSITY OF PRINCE EDWARD ISLAND, CANADA)

A variety of service, research and teaching laboratories exist worldwide which support the aquaculture industry. These laboratories often offer disease screening and diagnostic services, with various levels of testing and quality assurance. In addition, some laboratories may not engage in pro-active international marketing. As a result, many aquaculture companies and their fish health service providers are not always aware of the range of laboratory resources available in the global marketplace.

This series of laboratory articles will provide *Aquaculture Health International* readers with a guide to diagnostic laboratories which offer regional, national or global "routine - fee for service" veterinary diagnostic services to finfish, mollusc and crustacean producers and their veterinary service providers.

In addition, the articles will focus on affiliations that these diagnostic laboratories may have with universities, government agencies and institutes linked to aquatic health training or research. The articles will seek input from each laboratory as to their strategic goals and operational philosophy.

This objective services review, in combination with subjective input on management philosophy, will provide readers with a

balanced description of the laboratory, and will ultimately help aquaculture veterinary professionals to make informed decisions on selecting appropriate diagnostic service laboratories, aquatic health training and research programmes.

To accomplish this, we will provide a formative review of the services provided by each laboratory, with the approval and assistance of the company, programme or laboratory management. To this end, we have developed an aquatic health diagnostic services evaluation checklist which will detail information on the type and scope of services offered:

- quality assurance programmes
- referral options
- reporting methods
- client base, and
- the cost of testing.

We will strive to capture a thorough description of the diagnostic component of the laboratory, with a capsulated summary of services provided. If a laboratory prefers not to participate in the series, we will only provide a description based on published information, public advertising or government documentation.

## FEATURED DIAGNOSTIC LABORATORY: AquaTechnics Inc (Carlsborg, Washington, USA)

BY DR RALPH ELSTON

AquaTechnics, a private aquatic animal diagnostic and environmental evaluation service, has operated from Sequim, Washington since 1995. Founded by Dr Ralph Elston, AquaTechnic's mission is to assist aquaculture clients in managing aquatic animal health, reducing waste and minimising environmental impacts from farmed aquatics production, and maximising clients' operating profits.

The company also assists a variety of clients in environmental restoration projects and environmental damage assessments, many of which involve aquatic animal health issues. Clients range from small shellfish farms to Fortune 500 companies, and services are provided for companies in North and South America, Asia, Europe and Australia.

AquaTechnics also serves regional and national governmental agencies and non-governmental organisations. Its philosophy emphasises diligence, quality, value and follow-through, resulting in a high level of repeat customer business. The company's prime objective is to find permanent solutions to client problems.

Company staff members have assisted high-intensity shellfish production facilities by identifying bacterial pathogens and formulating management and remediation procedures involving water treatment, sanitation protocols and feeding and bacterial management. In addition, staff members have identified several previously unrecognised infectious disease agents that may affect both cultured and wild stocks, including bacterial pathogen designated NP-1, and were the first to identify the OIE-reportable molluscan disease agent *Mikrocytos mackini* in the United States.



### FACILITIES AND STAFF

AquaTechnics started as a home-based business in 1995, but quickly grew to a dedicated business location with office, laboratory and wet laboratory space in Sequim, Washington, located on the Olympic Peninsula. An expansion is currently underway in a newly purchased property adjacent to the existing facility.

Ralph and Heidi Elston are the president and vice-president of the company, which employs four to six additional staff members and has a web of contractor-associates who provide ancillary services to clients upon demand.

Technical staff members include:

- Dr Ralph Elston, a specialist in molluscan and fish health and

environmental assessment

- Dr Arthur Gee, a specialist in molecular genetics and bacteriology
- Karen Humphrey, a registered pharmacist and laboratory operations supervisor
- Hans Daubenberger, PCR specialist, and
- Dr James Humphrey MD

The company also employs laboratory technical specialists.

Associated collaborators and consultants include Dr Paul Frelief, DVM (DACVP), PhD, Dr Barbara Watrous, DVM (DACVR), Dr Jerry Heidel, DVM (DACVP), PhD and Dr John Pitts, DVM.

Facilities and hardware include a necropsy and histology preparation laboratory, a microbiology laboratory, bacterial fermentation and concentration facilities, a PCR laboratory, bright field, fluorescent and stereo microscopy and a variety of routine laboratory equipment. The facilities include a wet laboratory building with recirculating marine life support systems permitted by the state authority for use with non-indigenous pathogens.

## SERVICES PROVIDED

Diagnostic services are provided primarily to shellfish (mollusc) farms, but a smaller portion of clientele produce freshwater and marine finfish and shrimp. AquaTechnics has developed a reputation for identifying and solving persistent and difficult problems in aquatic animal production, particularly related to animal health.

Services include on-site evaluations and laboratory-based evaluation utilising technologies as indicated in the aquatic health diagnostic services evaluation checklist. A combination of classical and molecular methods is used to optimise diagnostic efficiency.



By emphasising a differential diagnostic approach and applying appropriate technology, the company has been successful in elevating health standards on clients' marine farms and in reducing the effects of infectious diseases and inferior environmental quality.

Of particular importance to hatchery and nursery clients has been the identification of pathogenic bacteria and protozoa which can persistently and opportunistically infect early life stages of molluscs. In addition, AquaTechnics maintains reference samples for both histological and molecular diagnosis of many OIE-reportable diseases and other diseases of regional significance, including an archive of bacterial pathogens. ▶

### Aquatic health diagnostic services evaluation checklist

This table summarises the level and diversity of aquatic diagnostic testing provided, as well as information on methods of pathogen or agent confirmation, laboratory quality assurance, referral services, reporting options, client base and cost of testing. (See key below.)

CATEGORY	CLINICAL VISITS	WATER QUALITY	PHYCOLOGY	NECROPSY	CLINICAL CHEMISTRY	HAEMATOTOLOGY	CYTOLOGY	HISTOPATHOLOGY	ELECTRON MICROSCOPY	BACTERIOLOGY	MYCOLOGY	VIROLOGY	PARASITOLOGY	TOXICOLOGY	SEROLOGY	ENDOCRINOLOGY
Fin-Fish	F	S	S	F	S	S	S	F	S	F	S	S	F	S	S	S
Mollusc	F	S	S	F	S	S	S	F	S	F	S	S	F	S	NA	NA
Crustacean	F	S	S	F	S	S	S	F	S	F	S	S	F	S	NA	NA
Agent ID	S		M				M	M	M	M,C,I,G	M,C,I,G	M,C,I,G	M,C,G	AC	I	AC,I
Q A	IN		IN	IN	IN,EX	IN	IN,EX	IN,EX	IN	IN,EX	IN,EX	IN,EX	IN	IN,EX	IN	IN,EX
Referral	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Reporting	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE	PFE
Client Base	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I	R,N,I
Cost (\$)	\$	\$	\$	\$,P\$	\$	\$	\$	\$	\$	\$	\$	\$	\$	\$	\$	\$

#### KEY

Scope of testing

Full testing available = F

Selective testing = S

Pathogen/agent ID

By morphology = M

By culture = C

By immunology/serology = I

By genomic / molecular = G

By analytical chemistry = AC

Quality assurance scheme

Internal quality control = IN

External quality assurance/ring testing = EX

Certification = ISO

Referral testing

Available = A

Not available = NA

Reporting options

By post = P

By fax = F

By email = E

By website = W

Client base

Regional = R

National = N

International = I

Cost of services

Full cost recovery = \$

Partial subsidy = P\$

Full subsidy = F



AN AQUATECHNICS TECHNICIAN AND SCIENTIST AT WORK

THE AQUATECHNICS LABORATORY IN SEQUIM, WASHINGTON

**Related services.** As a result of implementing successful health management programmes, clients have requested the company assist them in farm site selection, establishing environmental compliance programmes, assessing environmental damage and meeting regulatory authority requirements. These services are often provided for the company's aquaculture clients and also for other clients where analytical and scientific depth is required for marine environmental health and damage assessment.

**Shellfish High Health Guideline.** Under the auspices of a Pacific Shellfish Institute (Olympia, Washington, USA) study funded by the National Marine Fisheries Service Saltonstall-Kennedy Programme, Dr Elston developed a Shellfish High Health Programme which has been implemented through AquaTechnics' assistance to shellfish producers.

The programme is a set of health-oriented guidelines for shellfish farmers endorsed by the Pacific Coast Shellfish Growers Association. It has been incorporated into the requirements of the Animal and Plant Health Inspection Service, or APHIS, of the US Department of Agriculture for the certification of shellfish farms as qualified export farms in regard to meeting standards of shellfish health.

APHIS currently recognises only seven farm sites on the western coast of the United States that utilise the Shellfish High Health Programme as export-eligible mollusc farms for live molluscan product destined for receiving waters.

Through implementation of the Shellfish High Health Program, incorporation of other APHIS health surveillance requirements, and farm examination by a network of AquaTechnics cooperating veterinarians, qualifying farms may maintain their status for APHIS certification of shellfish health and health certificates required for admission of live animal products by importing national governments.

**APHIS Certification and Farm Surveillance.** To help farm clients establish and maintain their ability to export healthy shellfish stock, AquaTechnics is an APHIS-approved laboratory for the

interstate transfer support and farm management health maintenance purposes.

**Regional Surveillance.** AquaTechnics also assists various state and local governments by conducting testing to support infectious disease surveillance of wild stock fish and shellfish. Through such regional surveillance, AquaTechnics' staff first reported the presence of *Mikrocytos mackini*, an OIE-reportable disease, in the United States.

The company has worked with state and federal agencies and private farms to not only conduct additional surveillance but also to assess and manage risk from this disease through farm risk evaluation procedures and formal risk assessment and risk management procedures. In collaboration with the Pacific Shellfish Institute, AquaTechnics' staff conducted a formal risk assessment and risk management process and workshop on managing *Mikrocytos mackini*, the causative agent of Denman Island disease of Pacific oysters.

## COLLABORATIONS AND LINKS

AquaTechnics is linked to the University of Washington by support of graduate students and participation in research programmes headed by the university and the Pacific Shellfish Institute. AquaTechnics is also linked to Pacific Lutheran University in Tacoma, Washington, Washington State University and Oregon State University by providing educational opportunities and collaborative research and diagnostics.

AquaTechnics conducts Small Business Innovation Research grant projects funded by the US Department of Agriculture and other federal agencies, where such projects are directly tied to assisting the company's client base.

For further information contact the company president, Dr Ralph Elston. Telephone +1 360-681-3122, fax +1 360-681-3123 Email [ralph@aquatechnics.com](mailto:ralph@aquatechnics.com) Mailing address, AquaTechnics Inc, PO Box 687, Carlsborg, WA 98324 USA. Office and laboratory location and courier address is at 455 West Bell Street, Sequim, WA 98382 USA See [www.aquatechnics.com](http://www.aquatechnics.com)

# SOME PERSPECTIVES ON SPRING VIRAEMIA OF CARP

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

Imagine you're standing in your back yard looking at your fish pond when two men in a sinister-looking car drive up your driveway.

"Are you the owner of this pond?"

"Why, yes I am," you reply.

One of them holds out a piece of folded paper and says, "This is to serve as notice that these premises are under quarantine for spring viraemia of carp, and will shortly be depopulated. You may from this point forward only keep fish here under the purview of our organisation, and you will not keep fish here until we give you clearance to do so."

Could this happen to you? Yes, it could.

Firstly, I would like to expand my thoughts on the above scenario, which is surely uncommon, but nevertheless possible. The virus that causes such a ruckus is called spring viraemia of carp, or *Rhabdovirus carpio*. It can kill tens of carp in a group of a hundred.

Yes, I said SVC might be responsible for the loss of whole hand nets full of fish affected by this virus. My professional experience with SVC has been that it can kill nearly five percent of affected fish. Wow! Imagine losing five out of every hundred fish to this virus. Worse, because the virus may be endemic in a fish population, you could lose over five percent of your livestock every spring and probably not even notice.

Those are the worst kinds of losses, because they remain unmeasured and they don't calculate into your losses or expenses. Fortunately, there is a "treatment" that eliminates the virus affecting the fish, and testing for SVC is now relatively reliable. In the United States, fish with the virus are (as required by law) reported to Federal agencies, and incinerating or burying the infected fish solves the problem. The owner is spared the anxiety of taking any further risk of selling infected fish to the public because their business will be terminated, and re-opened at the convenience of the regulators.

## SVC SYMPTOMS

SVC looks like a bacterial infection because the virus by itself in vivo is not usually effective at killing pond fish without a bacterial co-morbidity. So the signs are indistinguishable from bacterial sepsis, dropsy or furunculosis.

Other signs that suggest the virus may be present can include dead or dying fish in small numbers over the course of years, making it indistinguishable from the normal operation of a fish business.

The purpose of my discussion of SVC is to try and convey my opinion and experience with this rarely encountered viral condition. This experience comes directly from a fish farmer whose fish were



A FISH WITH SVC

diagnosed with SVC. He told me that they were losing a few fish in a particular pond and, "just to be on the safe side," they sent fish out for diagnostics.

By the time the results came back as SVC - they'd lost a few fish and sold the rest - it was 12 weeks after the fish were showing some symptoms that the government came into their lots and shut them down.

The upshot to the story is that the government has since passed into law an indemnification policy, and payments are made to farmers who lose everything to the test and elimination programmes instituted on their farms.

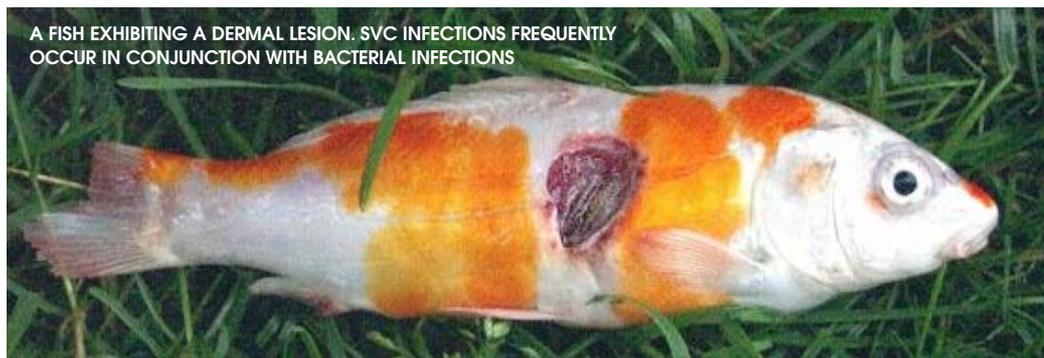
Whether the lost fish are accurately assessed for financial impact or whether you can survive being closed down until the government indemnifies you is a risk you can avoid.

## IN CONCLUSION

Spring viraemia of carp is a reportable viral disease of cyprinids, including goldfish and carp. The impact of the virus on livestock is not catastrophic, having unknown morbidity and usually mild mortality. However, the regulatory effect of the discovery of the virus on a farm lot is catastrophic, with the assignment of 100 percent morbidity and therefore 100 percent mortality.

I acknowledge that I am trivialising the virus, but I am not trivialising the regulation of it.

See [www.koivet.com](http://www.koivet.com)



A FISH EXHIBITING A DERMAL LESION. SVC INFECTIONS FREQUENTLY OCCUR IN CONJUNCTION WITH BACTERIAL INFECTIONS

# A US PERSPECTIVE ON SELECTED BIOTECHNOLOGICAL ADVANCEMENTS IN FISH HEALTH

## PART 1: VACCINES

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This article is the first part of a two-part series looking at selected biotechnological advances in aquaculture. The second part will look at genetic stock improvement, biosecurity tools and alternative protein sources in fish diets.

Remarkable biotechnological advancements have been made in the aquaculture industry in the past five years. These advancements in areas such as fish vaccines, improved genetic stock, biosecurity tools and alternative protein sources in fish diets are necessary to meet the rapid growth of the aquaculture industry worldwide, and the ever-increasing demand for fish and other seafood.

These contributions to the future of the industry are fully interactive, and focus on supplying large amounts of fish and seafood to the worldwide consumer with minimal impact on the environment. The benefits to the aquaculture industry are shown in Figure 1. The aim of this two-part article is to review some of these important biotechnological contributions.

During the past decade, aquaculture production has significantly increased in many parts of the world. From 1992 to 2001, total seafood supply increased by 29.8 percent, while the supply of wild captured fish increased by only 8.3 percent (FAO 2002). Seafood provides 16 percent of animal protein consumed by humans.

The United Nations' Food and Agriculture Organisation has reported that global aquaculture is increasing by 11 percent per year and is the world's fastest-growing food producing sector. The FAO also forecasts the demand for seafood to increase by 3.3

billion pounds or 35 to 50 percent by the year 2010 (FAO, 2004). More than 38 million people are employed in aquaculture and associated industries worldwide, and 131 aquatic species are currently being commercially cultured.

In the United States, channel catfish production represents a rapidly growing sector. Six hundred and sixty million pounds were produced in 2003, yielding \$675-690 million in sales. (See [www.usda.mannlib.cornell.edu](http://www.usda.mannlib.cornell.edu))

### US PRODUCTION

The leading state producers of channel catfish in the United States are Mississippi, Alabama, Arkansas and Louisiana. The majority of catfish is processed into frozen whole fillets, nuggets, fingers and value-added products. The commercial catfish industry is responsible for over 48 percent of the value of aquaculture production in the United States (See [www.usda.mannlib.cornell.edu](http://www.usda.mannlib.cornell.edu)).

The consumption of tilapia is becoming more popular in the US and Europe. Tilapia production in the US has increased more than 20 percent between 1991 to 1998. The US production of tilapia is about 9100 tonnes annually. (See [www.ag.arizona.edu/azaqua/ata.html](http://www.ag.arizona.edu/azaqua/ata.html))

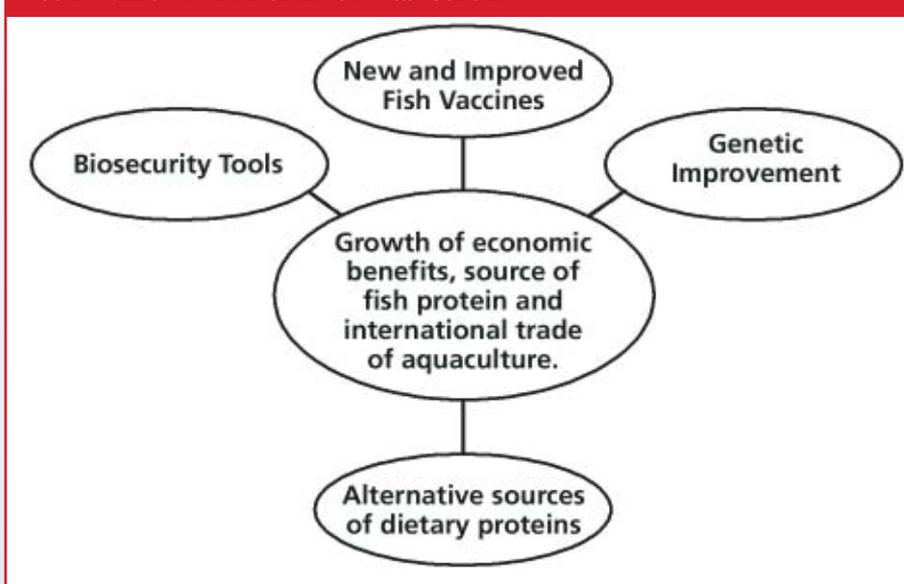
The value of imported frozen tilapia-fillets was \$176 million in 2005. The cultivation of fish will continue to be a growing industry that supplies an important source of food, employment and domestic and international trade, worldwide.

### DISEASES AND PRODUCTION SYSTEMS

The incidence and emergence of new infectious diseases has almost paralleled the growth of the aquaculture industry. The greater impact of infectious diseases on production is likely the result of production husbandry practices, intensive culture at high fish densities, lack of health management practices and 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 economic impact of fish disease is

FIGURE 1. SELECTIVE ADVANCEMENTS IN AQUACULTURE



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 results in the destruction of healthy fish in the affected area to control the epizootic from spreading to other regions or countries. The risk of disease in channel catfish and tilapia is very high due to the highly intensive production systems they are reared in.

Aquaculture production systems are very diverse, and vary from net cages to ponds, from fresh to marine water, from small to large production systems and from open water to re-circulating water (anonymous, 2004). Catfish are raised in earthen levee ponds that vary in size from 4.5-7ha (11 to 17 acres), and the water is from groundwater or well sources, with ponds at a depth of 1-2m. The ponds are aerated using paddle wheels or other mechanical devices.

The temperature tolerance of catfish is 4-35° Celsius and optimal growth temperature is 25-27° Celsius. The production cycle is 16-18 months. Catfish fry are stocked in fingerling ponds or grow-out ponds at 10 to 14 days post-hatch. The stock density in grow-out ponds is 6000 to 12,000 per acre, and the fish are harvested by selective seining at about 1 pound for processing (anonymous, 2004; Tucker and Hargreaves 2004).

Tilapia is produced in hot water re-circulating enclosed tanks or raceways in the colder regions of the US, and outdoors in ponds, tanks and raceways without heated water in warmer regions. The temperature tolerance of tilapia (16-32° Celsius) determines what type of production will be needed (anonymous, 2004).

Optimal growing temperatures and conditions for channel catfish and tilapia are also optimal bacterial growth conditions. This makes the need for vaccine protection against bacterial diseases of channel catfish and tilapia of utmost importance.

## VACCINE DEVELOPMENT

Antibiotics have long been used to control many bacterial diseases. However, they have been found to have many problems and are generally not a satisfactory solution. The major problems are antibiotic-resistant bacteria, as well as antibiotic contamination of the food chain and environment. Sick or infected fish are less likely to eat, which makes the antibiotic treatment less efficacious, as delivery of antibiotic treatment to fish usually occurs in the feed.

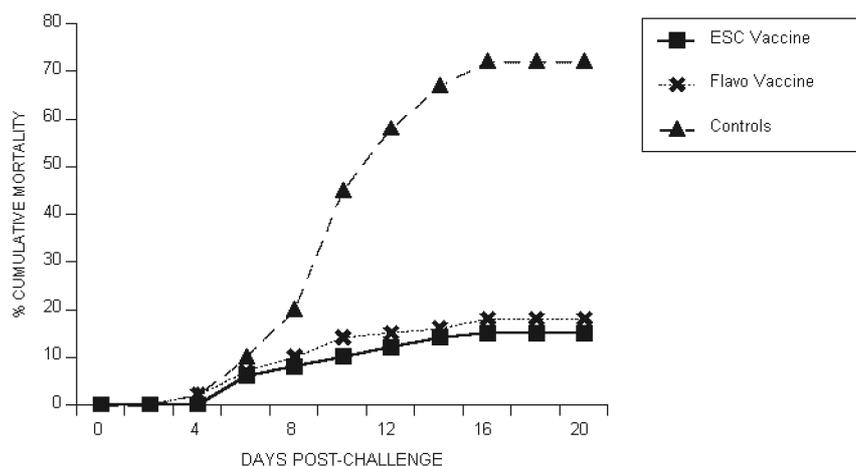
Finally, many countries, including the US, have adopted stringent policies prohibiting the use of toxic chemicals and antibiotics to treat morbid or apparently healthy fish.

The use of vaccines in humans, food animals and pets to prevent disease is a common practice (Klesius and Shoemaker 1999, Klesius et al 2001, Klesius et al 2004). Similar biotechnological advancements in the development of vaccines for disease prevention in cultured fish are on the rise.

From 1976 to the present, the numbers of commercially available, safe and efficacious fish vaccines has increased from one to more than 14 (Somerset et al 2005). The majority of these vaccines are for the prevention of bacterial diseases. However, several vaccines are also available to prevent viral diseases.

The majority of the available bacterial vaccines are of the killed type (ie, the infectious agent(s) are inactivated or killed). Killed vaccines are primarily administered by injection and the duration of their protection is limited, unless an adjuvant is added. This means of immunisation is costly because of the need to handle and inject each fish.

FIGURE 2: EFFECT OF IMMUNISATION WITH ESC AND FLAVOBACTERIUM ATTENUATED VACCINES ON SURVIVAL OF CHANNEL CATFISH INFECTED WITH LIVE *E. ICTALURI* AND *F. COLUMNARE*



### Attenuated bacterial vaccines

A biotechnological breakthrough was the development of attenuated bacterial vaccines. These 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 (Klesius and Shoemaker 1999; Wise et al 2000) and fry as young as seven to 10 days post-hatch (Shoemaker et al 1999). This immunisation will last the life of their production cycle as opposed to a shorter duration of only six months for a killed vaccine. Finally, *in ovo* immunisation of channel catfish eggs (US Patent 5,153,202) with attenuated enteric septicaemia of catfish ESC vaccine resulted in protection against ESC in fingerlings (Shoemaker et al 2002).

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.

Examples of the first US licensed attenuated bacterial vaccines are those against enteric septicemia of catfish (ESC) and columnaris disease of catfish. These 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 B1).

*Edwardsiella ictaluri* is the bacterium responsible for ESC which costs the catfish industry about \$50-60 million annually (Wagner et al 2002). Columnaris disease is caused by the bacterium *Flavobacterium columnare*, and costs the catfish industry about \$40 million annually. (Wagner et al 2002). Both diseases are generally found together, compounding these industry losses.

The Agricultural Research Service, USDA licensed both vaccines to Intervet, Inc of Millsboro, Delaware, which commercialised the vaccines. The ESC and columnaris vaccines are commercially labelled AQUAVAC-ESC® and AQUAVAC-COL®, respectively.

The economic impact of the ESC vaccine is an increase of producer profit by \$1706 per acre, and a significant reduction in loss due to disease (Intervet). The effectiveness of these vaccines is shown in an experimental vaccine trial (Figure 2).

The cumulative mortality in the channel catfish immersion trial with fish vaccinated at 10 days post-hatch was significantly

## SELECTED BIOTECHNOLOGICAL ADVANCEMENTS – VACCINES

lower than for the non-vaccinated controls. The fish were challenged with *E ictaluri* and *F columnare* at 30 days post-vaccination. The results show that both vaccines significantly increased the survival of the immunised channel catfish (Shoemaker and Klesius 1997, Klesius and Shoemaker 1999).

### Oral immunisation

Oral immunisation is also a relatively recent biotechnological advancement. Vaccination of larger fish is compromised by their aquatic environment. Vaccines must be delivered on a mass scale to be effective, thus oral vaccination is appealing. The key to oral vaccination is to protect the vaccine components from destruction by the fish's digestive tract so that the antigens are able to penetrate the intestinal lining and stimulate an immune response.

Encapsulation of the vaccine components or development of a fish vaccine "pill" that contains and protects the vaccine components appear to be plausible solutions for the successful development of an oral immunisation method. 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 increases the gastric pH of the fish intestine (Vandenberg 2004).

Currently, the ARS-patented (US Patent 6,379,677) *Streptococcus iniae* vaccine (Klesius et al 2000, Klesius et al 2002) was incorporated into Oralject™ and fed to tilapia twice for one day to satiation. The *S iniae* Oralject™ vaccine was efficacious following challenge with live *S iniae* in the orally immunised tilapia.

*Streptococcus agalactiae* is another major pathogen of tilapia that causes serious economic losses in many species of fresh water, marine and estuarine fish worldwide. A highly efficacious *S agalactiae* vaccine (US patent pending) was developed using formalin-killed cells and extra-cellular products (Evans et al 2004a, 2004b).

Increased global trade is dependent on the continued advancement of biotechnological contributions

The vaccine was shown to confer protection for up to 180 days post-vaccination and the protection was correlated with specific antibody response to *S agalactiae* (Pasnik et al 2005a). Furthermore, antibody specific for the *S agalactiae* extra-cellular product was found to be correlated with the protective immune response (Pasnik et al 2005b).

A specific antibody response appears to confer protection for both the *S agalactiae* and *S iniae* vaccines (Shelby et al 2002). 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 killed vaccines.

### DNA vaccines

Deoxyribonucleic acid (DNA) vaccination is another example of biotechnological advancement (Lorenzen and LaPatra 2005) 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's immune system to provide protection against the pathogen.

Intramuscular injection of DNA vaccines against the important

viral diseases of salmon such as infectious haematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) (Lorenzen and LaPatra 2005) has resulted in protection in laboratory trials.

Very limited success has been reported for bacterial pathogens with DNA vaccines. Issues with safety and mass methods of delivery of DNA vaccines have not been fully resolved.

### SUMMARY

The aquaculture industry is growing rapidly thanks in part to the biotechnological advancements in many areas of the industry. This paper has focused on the ability of the aquaculture sector to provide a continuous and increasing supply of high-quality healthy fish protein to the consumer. The recent biotechnological advancements that include vaccine development will continue to improve production efficiency.

Increased global trade of aquaculture products is dependent on the continued advancement of these and other such biotechnological contributions. Thus, the common goal of these achievements is to ensure increased economic benefits through providing safe and healthy sources of fish protein for the consumer worldwide.

### ACKNOWLEDGEMENTS

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## CANADA: REVISED APPROACH TO MALACHITE GREEN

Health Canada recently conducted a scientific assessment to determine the risks to public health associated with the presence of trace amounts of malachite green and its metabolite, leucomalachite green, in fish products.

Health Canada is advising the public that the probability of serious adverse health consequences associated with the daily consumption of fish containing trace amounts of malachite green and leucomalachite green is remote.

Based on Health Canada's assessment, and the possibility that malachite green may be an unavoidable contaminant, Health Canada is advising that on an interim basis, fish containing malachite green and leucomalachite green residues of one part per billion (ppb) or less may be sold in Canada, provided that an investigation rules out intentional use of malachite green in the fish production lifecycle.

Fish products containing malachite green or leucomalachite green at levels above 1ppb will not be allowed to be sold in Canada. The interim guideline, which remains until adequate scientific data can be generated, applies equally to imported and domestic products.

Since it is unclear to what extent malachite green may be an unavoidable contaminant, further investigation will be conducted into possible sources.

Health Canada has a policy of zero-tolerance of deliberate use of malachite green in food-fish, and says it acknowledges the remoteness of health risks associated with the consumption of trace amounts of malachite green and leucomalachite green. It also ensures that Canadians have a safe and nutritious supply of fish.

Malachite green is only approved in Canada for use in aquarium fish. It is not approved for use in fish intended for human consumption.

See [www.inspection.gc.ca](http://www.inspection.gc.ca)

## INTERNATIONAL: THE RESPONSIBLE USE OF ANTIBIOTICS

The Food and Agricultural Organisation has published a report entitled "The responsible use of antibiotics in aquaculture". The document was commissioned by the Fish Utilisation and Marketing Service of the FAO's Fisheries Division, and aims to raise awareness of the antibiotic resistance issue in fish farming and related sectors. The promotion of the prudent use of medicines is an important facet of the FAO Code of Conduct for Responsible Fisheries.

The document contains a review of the contribution of antibiotics used in aquaculture to anti-microbial resistance using a risk analysis framework. It also contains some recommendations for responsible conduct in this area with a view to minimising risk.

See <ftp://ftp.fao.org/docrep/fao/009/a0282e/a0282e00.pdf>

## ROMANIA: VHS OUTBREAK REPORTED TO THE OIE

The Romanian National Sanitary Veterinary and Food Safety Authority has filed a report with the OIE, the world organisation for animal health, detailing an outbreak of viral haemorrhagic septicaemia (VHS) in the Arges region of the country.

The condition has affected rainbow trout (*Oncorhynchus mykiss*) and grass carp (*Ctenopharyngodon idella*) farmed intensively in a 6000sq m pond system. The reported morbidity rate is 80 percent and the mortality rate is between 35 and 40 percent.

The Romanian authorities claim the trout originated from embryonated spawn imported from Denmark and introduced into

the pond in March 2005 without the approval of the Sanitary Veterinary and Food Safety Directorate in Arges. The origin of the grass carp is unknown.

See [www.oie.int/aac/eng/en\\_fdc.htm](http://www.oie.int/aac/eng/en_fdc.htm)

## INTERNATIONAL: INNOVATIVE COMPENDIUM LAUNCHED

Source: [www.fishupdate.com](http://www.fishupdate.com)

CABI has launched the Aquaculture Compendium, an encyclopaedic, multimedia information tool covering all aspects of aquaculture production and health. Until now, there has been no single source of information for the millions of people involved worldwide in the industry.

The unique compendium took information specialists and scientists three years to develop. It is available on the Internet and will be released on CD-ROM.

CABI information specialist Martin Parr, who led the project, said, "We're the first to bring together in one place comprehensive information, images, maps and authoritative articles from around a hundred different organisations involved in the aquaculture industry."

The Aquaculture Compendium contains information on 300 commonly cultured aquatic species, including finfish, crustaceans, molluscs, algae and live feeds. The data sheets cover all aspects of their biology and production, including their taxonomy, life cycle, reproduction, nutrition, feeding and environmental requirements, genetics, behaviour, distribution, and post-harvest handling and marketing.

Aquaculture production statistics and other economic data from the Food and Agricultural Organisation and other authoritative sources are integrated and linked to a graphic interface, allowing the user to specify and generate visual presentations. The database also includes hundreds of linked data sheets on the main diseases and disorders of these species - from the disease's host range and geographic distribution to its economic impacts, and from its diagnosis, prevention and treatment, through to food safety issues.

For those involved in treatment, the Compendium incorporates a quick reference database of international veterinary drugs, vaccines and pesticides. "The compendium also brings together issues and themes of critical importance to aquaculture today," says Parr. "This makes it an absolute must-have for anyone involved in determining policy, investing in the industry or solving aquaculture industry-related problems."

Major industry concerns such as the likely impact of aquatic invasive species, the problem of aquatic pests and diseases in trade, and the sustainable management of aquatic resources are covered through a unique electronic library of around 1000 articles, book chapters and data sets from internationally respected sources. Some are previously published, but many have been specially commissioned for the compendium. This gives users the latest take on hot topics in the industry, including valuable insights into global trends.

The compendium is an initiative of a global development consortium of technical institutions, development-assistance agencies and private-sector companies.

CABI is an international not-for-profit organisation that generates and disseminates information in the applied life sciences via printed publications and electronic information products. It also provides research, consultancy and training services.

See [www.cabicompendium.org/ac](http://www.cabicompendium.org/ac)

## UK: WORKSHOP EXPLORED SEA LICE IN FARMED SALMON

Forty aquaculture industry professionals gathered in Inverness earlier this year to discuss the treatment of sea lice. The meeting focused on treatment through the use of medicated feed, with emphasis on optimising treatment practices and novel developments.



Organised by the Schering-Plough Animal Health Corporation's Aquaculture Business Unit, the event attracted veterinarians, health managers and biologists from salmon farms throughout Scotland. Representatives from major feed companies, including Skretting, Ewos and Biomar, as well as academics and independent consulting veterinarians from Scotland, Ireland and Norway provided additional perspectives on managing the disease.

Tony Wall, the director of Fish Vet Group, Scotland, Dr Solveig Gaasø, a veterinarian with Marine Harvest in Norway, and Hamish Rodger, practice principal of Vet Aqua International in Ireland, presented their field experiences with sea lice, and the challenges of effective control programmes, including appetite issues in relation to optimum dosing.

Dr Gordon Ritchie, the fish health technical manager of the Marine Harvest's Technical Centre in Stavanger, Norway, discussed the variability in medicated feed intake and the company's global experience in optimising medicated feeding practices.

Crawford Revie, a quantitative epidemiologist at the University of Strathclyde, Glasgow, introduced the concept of mathematical modelling as a pragmatic tool in treatment strategies.

Dr Jon Erik Juell, Head of Research Group, Fish Welfare in Aquatic Production, Institute of Marine Research in Bergen, Norway, presented some of the concepts developed on fish behaviour, and how these may impact in-feed treatments such as response to stimuli.

The speakers and participants then split into teams to develop and present practical approaches to optimising the success of in-feed treatments and further enhancing the welfare and performance of farmed fish affected by sea lice.

"It was a very timely and useful meeting," Tony Wall said. "The range of competencies from various countries and areas of expertise ensured all avenues of in-feed medication were explored.

From a personal point of view, the extensive discussions should be very beneficial in further ensuring safe and efficacious use of in-feed medicines with regards to the fish, the consumer and the environment."

### ASIA/PACIFIC: AQUATIC ANIMAL DISEASE REPORT RELEASED

The fourth quarter (October-December 2005) Aquatic Animal Disease Report produced by NACA was published in April. As well as the usual country-specific disease surveillance returns, the report contains details of the new reporting procedure, together with details of how to fill in the revised returns form.

The foreword also contains an overview of two new AusAid-supported aquatic animal health projects within the region. The first is entitled "Strengthening aquatic animal health capacity and biosecurity in ASEAN". The second, "Operational guidelines on responsible movement of live food finfish in ASEAN" is also profiled.

See [www.enaca.org/Health](http://www.enaca.org/Health)

### CANADA: SYNDEL LABORATORIES SOLD

Syndel Laboratories Ltd has been sold to Aquatic Life Sciences Ltd.

"After 28 years of successful service to the fisheries and aquaculture sector, it is time to expand the horizons of Syndel as provider of approved fish health products both here and abroad," said Monty Little, the president and founder of Syndel. "By combining the efforts of Syndel and Aquatic Life Sciences we will be better able to respond to the needs of a growing industry."

Little will remain with Syndel for a period of transition and as a stakeholder in the subsidiary company, Syndel Asia. Syndel becomes a sister company to Western Chemical Inc. of Ferndale, Washington.

"It just makes sense," says the chief executive officer and president of Aquatic Life Sciences, Steven Becker. "By combining the resources of the two companies, both of which have been serving the fisheries and aquaculture industry for almost 30 years, we are collectively able to focus our efforts on meeting the growing needs of our domestic and international clients."

Prior to the sale, Syndel was a distributor of Western Chemical products. "The philosophies and practices of the two companies are a perfect match; they both focus on supplying quality approved products and superior service," says Becker.

Both Syndel and Western Chemical have been developing aquaculture chemicals and pharmaceutical products for a worldwide market for decades. "The product lines are developed with the goal of being consistent and valuable to the industry. We are market driven," says Becker. Their products are available in more than 40 countries to supply the expanding fisheries and aquaculture industries.

For more information, contact Dr Jim Powell, manager of business development, Syndel Laboratories Ltd at + 1 (800) 830-4886, email [powell@syndel.com](mailto:powell@syndel.com), or Steven Becker at +1 (800) 283-5292, email [stevenb@syndel.com](mailto:stevenb@syndel.com)

### NORWAY: PROJECT TO IMPROVE CARP WELFARE

The research institute AKVAFORSK is to participate in a new EU project called EuroCarp to make carp less susceptible to disease and stress. Gene technology will be used in selective breeding so that the carps' beneficial genes are passed on more effectively. This has never been tried before in carp.

The project is directed by Zsigmond Jeney of HAKI, a Hungarian research institute, and has partners in Great Britain and Russia, as well as Norway. In addition to the EU, the Research

Council of Norway is funding the portion of the project being conducted by AKVAFORSK.

Carp is one of the most important fish species in the world, both in volume and value, and in Europe it is the second most widespread aquaculture species after rainbow trout. Little has been done so far in the field of genetics to improve the health of carp, despite several threatening diseases in Eastern Europe.

After the carp genes are mapped out, it will be possible to recognise the gene profiles of fish with various characteristics. Researchers can then identify the fish that demonstrate high resistance to disease without testing disease resistance in practice. This will be a valuable addition to the selective breeding programme for carp, and the knowledge will be put to practical use by implementing a selective breeding programme.

Combining biotechnology with traditional breeding methods for carp is a completely new approach. Kari Kolstad, the head of the breeding and genetics division of AKVAFORSK, is responsible for AKVAFORSK's contribution to the programme. "We will design this in such a way so that the methods in biotechnology are used as effectively as possible. We will do this by developing new experimental and statistical methods for combining gene technology and traditional breeding."

The researchers hope to gain information about the biological processes that make one fish more disease-resistant than another. This information can lead to the development of alternative tests or vaccines.

AKVAFORSK has already begun to establish a selective breeding programme for carp in Serbia, and knowledge gained from the EU project will be used in this programme.

## FDA APPROVES AQUAFLO<sup>®</sup>

The recent approval by the United States' Food and Drug Administration of Aquaflor<sup>®</sup> (florfenicol) type A medicated article for the catfish marked a significant milestone in the industry's combined efforts to make more products available for public and commercial aquaculture, said Schering-Plough.

"Schering-Plough invested more than five years bringing this product to market, but we couldn't have done it without the teamwork and commitment from our development partners," said Dr Richard Endris, the research programme manager with the Schering-Plough Animal Health Corporation, the company that developed Aquaflor.

Dr Patricia Gaunt, associate professor, aquatic animal health at Mississippi State University College of Veterinary Medicine, who played a significant role in the development of Aquaflor for US channel catfish, approached Schering-Plough Animal Health in 1997 about a pilot project using florfenicol in catfish. It was funded and completed the following year.

Shortly thereafter, Rosalie, "Roz" Schnick, the national coordinator for aquaculture new animal drug applications, proposed gaining approval of Aquaflor<sup>®</sup> in a variety of fish species, including channel catfish, through the Federal-State Aquaculture Drug Approval Partnership Project under the auspices of the International Association of Fish and Wildlife Agencies.

A self-described "matchmaker", Schnick worked with state and federal agencies as well as other organisations to support Aquaflor, which last fall became the first in-feed antibiotic licensed for US aquaculture in more than 20 years.

"Not only was Aquaflor<sup>®</sup> approved in the United States, but it also became one of the first drugs to meet new FDA requirements for antimicrobial resistance and microbial food safety," says Schnick.

The result is that the US catfish industry has a new tool for

managing disease. Dr Gaunt helped Schering-Plough Animal Health conduct the necessary studies on efficacy, residue depletion and catfish safety. Dr Craig Tucker, head of the National Warmwater Aquaculture Centre in Stoneville, provided information to assess the environmental safety of Aquaflor in pond systems.

Another research group, the Upper Midwest Environmental Sciences Centre, US Geological Survey, in La Crosse, Wisconsin, carried out the pivotal target animal safety study as part of the cooperative effort.

Dr William H. Gingerich of UMESC says his group partnered with Schering-Plough Animal Health and other members of the aquaculture team to develop the safety data for catfish required by the FDA's Centre for Veterinary Medicine.

"The cooperation among all the groups involved was unprecedented," he said. "We were extremely pleased to be considered by Schering-Plough Animal Health to be a member of their team."

Dr Gaunt agreed. "There was good communication and a strong desire by everyone involved to make more products available for US aquaculture. I think the level of cooperation with Aquaflor<sup>®</sup> will serve as a valuable model for other new products and registrations," she said.

The FDA's Centre for Veterinary Medicine also loaned its expertise. "CVM has provided direct guidance to assure a more efficient approval process for aquaculture drugs," says Schnick. "The agency has also helped to educate us on how to meet requirements, and really formed a partnership with us through the drug approval process.

"Cooperation between this large number of groups is certainly unique, as is the major role played by Schering-Plough and their steadfast efforts and investment to get a new aquaculture drug approved," said Dr Tom Bell of the US Fish and Wildlife Service and its Aquatic Animal Drug Approval Partnership Programme.

In the end, however, it was the dedication and commitment of specific individuals who were leading the various teams. For example, Gaunt credits Endris with coordinating the efforts of all the scientists involved.

"He watched over the approval process every step of the way, making sure that the necessary studies were completed and reports submitted to the FDA in a timely fashion."

She also credits Dwayne Holifield of Delta Western, a major feed company based in Indianola, Mississippi, for his role in manufacturing medicated feeds for the clinical studies as well as the analytical studies.

"There was no method in place for manufacturing FFC catfish medicated feed when we began. Dwayne literally began from scratch and helped develop a reproducible method for preparation of feed that was accepted by the FDA."

With the approval of Aquaflor, aquaculture not only has the first new drug in more than two decades, it has exclusive Veterinary Feed Directive status from the FDA to regulate its use and ensure its long-term effectiveness. "This approval model for new drugs allows for mutually beneficial partnerships to form," says Schnick, "and that can only benefit aquaculture."

Mississippi State University and AADAP continue working jointly with Schering-Plough Animal Health to develop studies on *Flavobacterium columnare*, the second leading cause of catfish disease, which is also an important pathogen of salmonids. UMESC and others are also working on other Aquaflor label claims that will benefit all US aquaculture.

*For more information on Aquaflor, catfish farmers should contact their veterinarian, diagnostician or feed mill, or Schering-Plough Animal Health at 1-800-521-5767. See [www.Aquaflor-USA.com](http://www.Aquaflor-USA.com)*  
Aquaflor<sup>®</sup> is a registered trademark of the Schering-Plough Animal Health Corporation.

## EU: ORAL VACCINE RECEIVES MORE RECOGNITION

Fourteen more EU member states of the European Union had given their approval for the Schering-Plough Animal Health Corporation to begin marketing AquaVac® ERM Oral, the company said on April 21. The booster vaccine improves the control of enteric redmouth disease, or ERM, in rainbow trout.

Used successfully in the United Kingdom since 2001, AquaVac® ERM Oral vaccine prolongs immunity against ERM throughout the production cycle, eliminating the need for antibiotic treatment.

“Extensive field trials have shown excellent results,” says Chris Gould, PhD, the manager of global technical services (salmonids) for Schering-Plough Animal Health Aquaculture, based in the UK.

UK farms using the vaccine as part of a comprehensive control strategy have avoided any significant losses due to ERM throughout the production cycle, Gould says.

“The vaccine has produced no adverse side effects, nor has it hindered growth or feed efficiency. Since the oral vaccine is added to feed, you don’t have to stress fish by handling them.

“In addition, farmers have reported high palatability and have found the oral vaccine easy to administer.”

Gould says farmers using the vaccine also have more marketing flexibility because there is no withdrawal time required with the vaccine as there is with antibiotics. It also gives farmers a drug-free option for ERM control at a time when consumers are urging fish and livestock producers to minimise or eliminate the use of antibiotics, he says.

AquaVac® ERM Oral vaccine, the world’s only licensed oral ERM

vaccine, can be mixed into any type of feed, either at the feed mill or on the farm. It is intended for use about four to six months after initial vaccination with AquaVac® ERM, an immersion vaccine administered at the hatchery when fish weigh between 2g and 5g, Gould says.

“Since the early 1980s, when it was licensed for use in Europe, AquaVac® ERM immersion vaccine has reduced ERM deaths in farmed rainbow trout by an estimated 75 percent.

“However, the immersion product’s duration of immunity is unlikely to extend through the entire production cycle, and antibiotic treatment for ERM is often still needed late in the cycle.”

He says boosting the fish with AquaVac® ERM Oral vaccine enables fish to maintain immunity against ERM for the entire production cycle. (*See sidebar*)

### ANTIGEN PROTECTION

The efficacy of AquaVac® ERM Oral vaccine is due to a new, patented technology known as the Antigen Protection Vehicle (APV), which ensures that antigens in the vaccine get to the hind gut, where they can initiate an immune response against *Yersinia ruckeri*, the cause of ERM.

In the past, antigens in oral vaccines were degraded in the acidic environment of the stomach, rendering them ineffective, but the APV solves this problem, says Gould. AquaVac® ERM Oral vaccine can be used with AquaVac® Ergosan<sup>®</sup>, a natural feed ingredient based on seaweed that helps ensure good nutrition, enabling the fish to mount an optimal immune response.

AquaVac® ERM immersion vaccine and AquaVac® ERM Oral vaccine comprise a comprehensive ERM control plan known as the Total Protection Strategy, developed by scientists at Schering-Plough Animal Health.

“For rainbow trout, the strategy provides the most efficient and cost-effective way to protect stock from ERM, but vaccination timing is crucial to success,” Gould says.

Individual farming methods and seasonal and disease cycles must be considered. Toward that end, Schering-Plough Animal Health Aquaculture provides individualised technical support to fish farmers using AquaVac® vaccines to assure they get the best results, he says.

European member states that recently granted a marketing authorisation to AquaVac® ERM vaccine are Austria, Czech Republic, Germany, Slovakia, Slovenia, Poland, Denmark, Norway, Greece, Spain, France, Ireland, Italy and Portugal. These states also agreed to recognise the UK marketing authorisation for AquaVac® ERM Oral through the mutual recognition procedure and marketing authorisations that Portugal and Denmark have already granted.

“As the vaccine becomes more widely available throughout Europe, we expect to see vastly improved ERM control in the rainbow trout farming industry,” Gould says.

For more information, contact Chris Haacke, marketing manager, Global Aquaculture, Schering-Plough Animal Health, at +44 (0) 1799 528 167 or by email at [chris.haacke@spcorp.com](mailto:chris.haacke@spcorp.com).

See [www.spaquaculture.com](http://www.spaquaculture.com)

\*AquaVac® and Ergosan are worldwide trademarks of Schering-Plough Ltd or any affiliated company.

### USA/INTERNATIONAL: DATABASES AVAILABLE ONLINE

The AVMA has spearheaded the development of a unique resource for veterinarians, potential clients and others to locate diagnostic veterinarians and diagnostic laboratories. It has been released after more than a year of development and testing. Individuals seeking the services of aquatic veterinary medicine can now access more than 2000 veterinarians and 100 diagnostic laboratories for no charge at [www.AquaVets.com](http://www.AquaVets.com).

### FIELD TRIALS SHOW VACCINE'S EFFICACY

The results from extensive field trials show how AquaVac® ERM Oral vaccine boosts the protective effects of AquaVac ERM immersion vaccine, providing optimal protection against enteric redmouth disease (ERM) in farmed rainbow trout, says Chris Gould, PhD, the manager of global technical services (salmonids) for Schering-Plough Animal Health.

For instance, at one intensive trout farm in the UK, 16 of 17 rainbow trout batches vaccinated only with the immersion vaccine at the hatchery still required antibiotic treatment five to nine months later for control of ERM.

None of the batches of fish that received the full Total Protection Programme, consisting of AquaVac ERM immersion vaccine followed three to six months later by AquaVac® ERM Oral booster vaccine required treatment.

One batch that was booster-vaccinated outside the correct time frame required a single antibiotic treatment to maintain full disease control. This clearly demonstrates the need for carefully designed Total Protection Programmes if the industry is going to move toward antibiotic-free trout farming, Gould says.

The effectiveness of AquaVac® ERM Oral vaccine is due to the antigen protection vehicle, or APV, a patented technology, says Gould. The APV ensures that antigens in the vaccine are protected from stomach acids until they can get to the hind gut, where they initiate an immune response that prevents a clinical ERM outbreak.

Information about the veterinarians and laboratories involved with aquatic species was previously known only through word-of-mouth, said Dr David Scarfe, assistant director of the AVMA Scientific Activities Division. "Two recent US national animal disease emergency declarations, one affecting food-producing salmon, the other for ornamental koi and other carp, have clearly heightened the awareness of these veterinary needs," Dr Scarfe said.

The databases were initiated with funds from the Department of Agriculture as a resource for reducing disease risks to commercial aquaculture. The databases incorporate all disciplines of veterinary medicine that involve any aquatic species, from crustacean and molluscan invertebrates to finfish, reptiles, amphibians and marine mammals.

"The AVMA recognised that aquaculture is the fastest growing segment of agriculture," said the president of AVMA, Henry Childers. "More than 47 percent of all animal protein consumed is seafood, a third of which is farmed, yet no single source of information on how to locate aquatic veterinarians or diagnostic laboratories existed. This has left aquaculture producers, animal owners, government agencies and other veterinarians at a disadvantage."

Users can search the online databases using several criteria such as person or laboratory name, location, species type served, or disease and diagnostic test type. With password-protected access, veterinarians and laboratories can update their profile at any time, and new veterinarians and laboratories can easily register.

As part of registration, veterinarians and laboratories can choose whether they want their information made public, and also if they want to subscribe to AquaVetMed, an e-mail news service moderated by the AVMA.

During the test phase of the AquaVets.com project, Dr Scarfe said, the developers received a large number of requests from companion animal practitioners to participate. "We always assumed that there were a large number of companion animal veterinarians servicing aquatic animal clients, but the response was quite surprising."

Consequently, the developers will examine the possibility of adding modules to AquaVets.com for online continuing education, disease surveillance, diagnostic laboratory submissions and certificates of veterinary inspection, he said.

The AVMA, Aquaculture Underwriting and Management Services, and GlobalVetLink LLC developed the databases as part of the National Risk Management Feasibility Programme for Aquaculture. The Department of Agriculture Risk Management Agency's Federal Crop Insurance Corporation through the Mississippi State University provided partial support.

A brochure on the resources available at [www.AquaVets.com](http://www.AquaVets.com) may be obtained by calling the AVMA at + 1 (800) 248-2862, ext 6636.

## AquaVets.com

### NORWAY: MAPPING PARTS OF THE ATLANTIC COD IMMUNE SYSTEM

Source: Marit Seppola, Fiskeriforskning, Norway

Scientists at Fiskeriforskning are mapping some of the important parts of the immune system in Atlantic cod. The results could be important for the aquaculture industry.

The objective of this mapping is to characterise genes involved in the innate immune system of Atlantic cod. By increasing the knowledge of how the immune system works, our chances of



ATLANTIC COD  
(PHOTO BY FRANK GREGERSEN, FISKERIFORSKNING)

preventing diseases in the fish increases.

Scientist Marit Seppola at Fiskeriforskning, who is studying the innate viral defence in Atlantic cod, says that they are injecting synthetic dsRNA in the fish. "Afterwards, we will attempt to study which genes could be involved in the early defence against viruses.

"This is only a simulated viral infection, but it is used because it generally causes a stronger immune response than an ordinary viral infection," says Seppola. "Nevertheless, the results can give us indications about which genes are important for fighting viral infections.

"To find the genes that are important in the immune system, we have created a subtractive suppression hybridization library that consists of a collection of genes. At present the collection contains 330 different genes and we are about to study some of these genes more closely," she says.

One of the genes Seppola has worked with is called interferon stimulated gene 15 (ISG15). It appears that the gene expression is up-regulated as much as 800 times during a simulated viral infection. Recent results also indicate that bacterial infection causes an increased gene expression of Atlantic cod ISG15.

"We can measure this increase already the first day after injecting dsRNA, which can implicate this gene as an important part of first defence against viral infections. This can mean that we are about to reveal some of the tightly regulated key mechanism of the innate immune system in Atlantic cod. But there is still much further work, because the immune system is complex," says Seppola.

"This is time-consuming research, because there has not previously been performed much research involving genes in the immune system of Atlantic cod. Our work has also shown that there is little gene homology between Atlantic cod and the more studied fish species like Atlantic salmon and catfish. With this project, we hope to generate knowledge to better understand the immune system in Atlantic cod," she says.

The project is collaboration between Fiskeriforskning and the Norwegian College of Fishery Science in Tromsø. It is financed by the Research Council of Norway and Fiskeriforskning and will run over five years until 2008.

Contact Marit Seppola (+47) 77 62 90 00.

### ECUADOR: AQUA BOUNTY LAUNCHES SHRIMP FEED ADDITIVE ONTO THE MARKET

Aqua Bounty Technologies, a biotechnology company focused on health and productivity applications in the aquaculture sector, has received regulatory approval for Shrimp IMS, its lead product, in Ecuador. Ecuador is the leading producer of shrimp in the Western hemisphere and the second country to approve the product, following Mexico.

An initial commercial shipment of Shrimp IMS has been made to Aqua Bounty's licensee in Ecuador, Karakoram, a South American pharmaceutical supplier working to distribute Shrimp

IMS in cooperation with Exportachura de Alimentos S.A. ("Expalsa"), the leading aqua feed manufacturer in Ecuador. Aqua Bounty plans to introduce IMS to the six Latin American shrimp producing countries during 2006 with additional product launches in Asian markets planned for 2007.

The global shrimp industry generates \$13 billion in annual revenues and is growing at a compound annual rate of 5%. The expansion into Ecuador marks a major milestone in the Company's growth strategy to exploit this market potential.

Shrimp IMS is a feed additive administered through the full shrimp life cycle that reduces mortality by enhancing the animal's natural immune system; the shrimp's disease-fighting haemocyte production is increased by up to 250 percent. In field trials conducted over several years in Mexico and Ecuador, the Company has confirmed that Shrimp IMS is effective in the presence of a number of common shrimp diseases, including the Taura Syndrome and White Spot viruses, both implicated in the catastrophic \$280-million collapse of the Ecuadorian shrimp industry in 1999. White Spot virus, in particular, has caused over \$1 billion of production losses in the Americas since the 1990s. It is the most lethal, widespread viral disease of shrimp. The trials demonstrated survival improvements of 70- to 80 percent over untreated populations. In Mexico, during the first year of commercial use, benefits to farmers were calculated at \$2.50 return on investment per dollar spent on treatment.

"Ecuador has done a remarkable job rebuilding its position in the shrimp market since the loss of production in the late 1990s," said Elliot Entis, CEO of Aqua Bounty Technologies. "It is again among the top farmed shrimp exporters in the western hemisphere and an important supplier to the U.S. market. With an effective immune stimulants like Shrimp IMS now available through established market channels, biosecurity is likely to rise in priority in both the regulatory and farming communities and help reduce the risk of catastrophic epizootic events in the future."

Aqua Bounty Technologies is a Boston-based biotechnology company selling diagnostic tools, feed additives and therapeutants to the global aquaculture industry. Pipeline products include viral blockers, antifungals, fish vaccines and transgenic salmon, trout and tilapia that grow more quickly than standard farmed fish.

## USA: WATER MOULD LINKED TO ESTUARINE FISH

Source: Suzi Fraser (See [www.aquafeed.com](http://www.aquafeed.com))

A new study led by NOAA scientists provides strong evidence that the water mould known as *Aphanomyces invadans* is the pathogen responsible for seasonal outbreaks of skin ulcers and lesions observed in menhaden and other estuarine fish along the eastern coast of the United States. The study was reported in the peer-reviewed journal *Applied and Environmental Microbiology*.

Large fish lesion events in the 1990s, initially linked to the dinoflagellate *Pfiesteria piscicida*, caused widespread concern over the safety of seafood and recreational waters. These concerns caused many people to avoid the coast and to avoid eating seafood. An independent study published in 2003 in the journal *Ocean and Coastal Management* estimated that lost revenues for the tourism, restaurant and seafood industries exceeded \$100 million.

Scientists from NOAA's Centre for Coastal Fisheries and Habitat Research in Beaufort, North Carolina, led the research, along with experts from the Florida Fish and Wildlife Conservation Commission, the North Carolina Division of Water Quality, the Virginia Institute of Marine Science at The College of William and Mary, and North Carolina State University's College of Veterinary Medicine.

The scientists developed two very specific tests or assays to

detect the *A invadans* water mould - one using sensitive polymerase chain reaction (PCR) procedures, the other using fluorescent in situ hybridisation (FISH). They first validated these assays in the laboratory before applying them to Atlantic menhaden taken from the Pamlico and Neuse River estuaries in North Carolina.

The results of both assays were the same - all lesioned menhaden tested positive for *A invadans*. "The FISH assay is the first molecular assay to provide unambiguous visual confirmation that water mould from the ulcerated lesions were exclusively *A invadans*," said Mark Vandersea of NOAA, the corresponding author of the study. Until this study, scientists had been unable to positively identify *A invadans* as the only species of water mould responsible for causing the lesions in wild caught fish.

The new study supports a growing body of research evidence, including recently collected data by researchers in southern Asia and Australia, indicating that *A invadans*, rather than *Pfiesteria*, is the major cause of ulcers and lesions in fish. *A invadans*, however, should not be considered the sole cause for ulcerative lesions. Stress, poor nutrition, and certain parasite, bacterial and viral infections are all capable of producing similar lesions. Further studies will be needed to determine how natural *A invadans* infections are caused.

"The PCR and FISH assays developed in this study now make it possible to screen the large numbers of environmental samples needed to identify alternative hosts and sources of *A invadans* infections," said NOAA scientist Wayne Litaker.

"Identifying the sources and the conditions promoting the growth and transmission of the pathogen will help resource managers better predict when lesion events are likely to occur and perhaps develop effective mitigation strategies."

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# CONFERENCES AND MEETINGS

## NORTH AMERICA

### Advanced Concepts in Epidemiology, Surveillance and Biosecurity.

AVMA Convention 2006, Hawaii, July 19-21

The American Veterinary Medical Association will host a three-day workshop in Hawaii from July 19 to 21 in conjunction with the 2006 AVMA Convention (July 15-19). The instructors include Dr Angus Cameron (AusVet Animal Health Services, Australia), Dr Nora Wineland (USDA-APHIS-VS, USA), Dr Don Lightner (University of Arizona, USA) and Dr Allen Riggs (Hawaii Department of Agriculture, USA). Dr A David Scarfe from the AVMA will moderate the proceedings.

#### THE WORKSHOP

This workshop will present concepts and methodologies for aquatic animal surveillance and biosecurity, and discuss the implications of recent changes to the OIE Manual. This is an advanced course targeted at those responsible for national, regional and on-farm biosecurity, and the design, establishment or management of aquatic animal disease surveillance systems, and the analysis of surveillance data. The workshops will emphasise finfish and crustacean aquaculture.

#### THE APPROACH

The approach used at the workshop will be based on a combination of:

- lecture-style presentations to introduce key concepts
- small group and full group discussions
- exercises working with problems given by the presenter
- exercises based on participants' own background and workplaces
- computer-based exercises to practice data analysis (if resources and time permit)
- utilise the principles learned in table-top exercises to developing practical and applied surveillance and biosecurity programmes applicable for aquaculture operations
- utilise the applied programmes to design a specific on-farm surveillance and biosecurity programme for an operating aquaculture farm, and
- utilise the information from validated diagnostic tests, clinical observation of animals and disease surveillance data to determine the status of disease at any point in time, and to issue Certificates of Veterinary Inspection (Health Certificates) for the movement of animals.

#### WORKSHOP OUTLINE

*Day 1 – Principles for on-farm biosecurity programme design and application*

Didactic interaction outlining the practical application of

disease diagnostics, epidemiology/surveillance necessary for assessing the disease status of animal production systems (emphasising all agriculture and aquaculture animal species) and developing practical on-farm biosecurity programmes. It will specifically cover:

- introduction to surveillance
- objectives of surveillance
- context of surveillance – trade, WTO, OIE
- implications of changes to the OIE code
- sources of surveillance data
- monitoring endemic disease
- demonstration of freedom from disease

*Day 2 – Designing on-farm aquaculture biosecurity programmes*

Utilising the epidemiology principles covered in Workshop I, design disease surveillance and biosecurity programmes that are applicable for assessing, determining and certifying the status or freedom of important diseases on finfish and crustacean aquaculture operations. It will specifically cover:

- approaches to collecting surveillance data
- components of an effective disease surveillance system
- data collection and management options
- information systems, feedback and sustainability
- aquatic animal surveys
- survey design
- sampling issues
- analysis of surveillance data
- approaches to the analysis of non-random data sources
- analysis of monitoring data
- group discussions, and the
- development of surveillance systems by participants

*Day 3 – Implementing on-farm finfish and crustacean aquaculture biosecurity programmes*

Participants will be transported to a working aquaculture operation. Acting as group consultants, participants will develop an on-farm disease surveillance and biosecurity programme using the principles and designs covered in Workshops I and II.

The farm owner will implement this biosecurity programme with the assistance of his/her local attending veterinarian. If feasible, the instructor or attending veterinarian may provide participants with periodic disease status and biosecurity reports over the next 12 months, to gauge the efficacy of the biosecurity programme.

While the programme primarily serves aquatic veterinarians, state and federal officials, and non-veterinarian professionals and producers involved in aquatic animal health programmes are encouraged to participate. As enrolment is restricted to 40 participants, online pre-registration is very strongly encouraged. See [avmaconvention.org/](http://avmaconvention.org/)

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shadowne, barramundi, cockles, crabs, flatfish, goldfish, kingfish, lobsters, mussels, oysters, prawns, snapper, snappers.

VIPAC04

Registrants will also be able to participate in other convention aquatic veterinary continuing education and wetlab programmes from July 15 to 19.

*For further information, contact Dr David Scarfe, email [dscarfe@avma.org](mailto:dscarfe@avma.org)*

### 5th International Symposium on Aquatic Animal Health

San Francisco, USA. September 2-6

The 5th ISA AH programme will emphasise the multi-disciplinary nature of aquatic animal health and includes 12 plenary lectures, more than 200 oral presentations and up to 150 posters. Plenary lectures by outstanding speakers of international stature will address topics of broad interest.

See [www.fisheries.org/fhs/isaah\\_2006.htm](http://www.fisheries.org/fhs/isaah_2006.htm)

### EUROPE

#### 4th International Veterinary Vaccines and Diagnostics Conference

Oslo, Norway. June 25-29

The IVVDC has become an important meeting place for regulatory authorities, pharmaceutical companies and the scientific community. It provides an excellent opportunity to be updated on recent progress and future perspectives in the fields of vaccinology and diagnostics. The conference includes a specific session on fish vaccines.

See [www.ivvdc.org](http://www.ivvdc.org)

### OCEANIA

#### International Symposium on Veterinary Epidemiology and Economics

Cairns, Australia. August 6-11

Includes sessions on aquatic animal health, together with a pre-symposium workshop entitled Aquatic Animal Epidemiology.

See [www.isveexi.org](http://www.isveexi.org)

### ASIA

#### First International Symposium on Viral Nervous Necrosis of Fish

Hiroshima, Japan. November 28-December 1

This symposium will be the first opportunity to review, collate and discuss the current information and knowledge on various aspects of viral nervous necrosis (VNN) and its causative agents, Nodaviruses.

It is expected to attract scientists active in this particular field from many parts of the world, including Europe, North America, Oceania and Asia.

See [www://home.hiroshima-u.ac.jp/fishpath/vnn2006](http://www://home.hiroshima-u.ac.jp/fishpath/vnn2006) ■

# THE ADVANTAGES OF VACCINE ACTIVATED WHITE SHRIMP

CHRIS HAACKE & ROBIN WARDLE (SCHERING-PLOUGH ANIMAL HEALTH, AQUACULTURE BUSINESS UNIT, UK)

Commercial trials have identified significant production and feed efficiency gains when white shrimp hatcheries gave a vibriosis vaccine simultaneously with an alginate-based complementary feedstuff. The results indicate key advantages at every stage of production from larval through harvest when compared with a control sample. The financial impact demonstrated advantages of over US\$40,000 in harvest values alone.

The trial conducted in Sinaloa, Mexico, by Schering-Plough Animal Health's aquaculture business unit, utilised the company's AquaVac<sup>®</sup> Vibromax<sup>®</sup> vaccine and AquaVac Ergosan<sup>®</sup> complementary feedstuff. The company supplies both products as an integral part of its 'Total Protection Strategy' approach to aquaculture health.

The trial included in excess of 51 million shrimp and measured performance across three separate production phases. Improved performance values, including mortality, feed intake, feed conversion and yield in the trial, gave an increase of over 15% in crop value and a recorded improvement of US\$8,657 per million PLs stocked.

Dr. Victoria Lopez-Doriga PhD, Clinical Trials Manager with Schering-Plough Animal Health explains the background. "We had previously trialled AquaVac Vibromax vaccine and AquaVac Ergosan together at commercial sites in Thailand. The results had already shown us significant performance uplift right through the growth stages. The shrimp were stronger and more active, they had more appetite and feed intake levels were higher and feed conversion improved. We decided to undertake a larger trial to see if the initial results would be replicated in the different farming environment encountered in Mexico."

In Sinaloa the established production system is divided into three stages, hatchery rearing, post larval rearing and grow-out. Due to the temperature profile of the region there is a single grow-out season lasting from May to October. This region is in

## Vibromax<sup>®</sup> feeding regime

TABLE 1

Production Stage	AquaVac Ergosan Grams/tank/day	Vibromax Ml/Tank/day	Artemia fed
PL 9	25	375 ml	50 Nauplii per PL
PL 10	25	375 ml	50 Nauplii per PL
PL 11	30	375 ml	60 Nauplii per PL
PL 12	30	375 ml	60 Nauplii per PL
PL 13	35	375 ml	72 Nauplii per PL
PL 14	35	375 ml	72 Nauplii per PL
PL 15	35	375 ml	86 Nauplii per PL
PL 16	35	375 ml	86 Nauplii per PL
PL 17	35	375 ml	103 Nauplii per PL
PL 18	35	375 ml	103 Nauplii per PL

common with many production areas and is at risk to Whitespot virus disease out breaks.

The trial population was 51.054 million nauplii, purchased from a single independent hatchery. For larval rearing they were stocked into 7 m<sup>3</sup> tanks with 250 nauplii per litre. The results in Sinaloa confirmed the success of previous trials.

## PHASE 1: SURVIVAL RATES 10.3% SUPERIOR TO NON-ACTIVATED SAMPLE

At production phase 1 (Zoea 1 - Post Larvae 8,) AquaVac Ergosan<sup>™</sup> was added directly to the tanks as a feed supplement at a level from 5 grams per tank per day at Z1 to 20 grams per tank per day at PL8. Survival rates in the AquaVac Ergosan<sup>™</sup> sample averaged 71.3% in comparison with 61.6% control sample.

Dr. Lopez-Doriga confirms "We know that Vibrio is associated with high mortality in hatcheries and grow-outs. It is for this reason that AquaVac Vibromax was developed. We have now developed a program for administering the vaccine to shrimp ►

## Summary of PL performance

TABLE 2

	Survival at PL 22	Size grams	FCR
Vaccinated Average	82.42%	0.01719	1.62
Control Average	83.36%	0.01452	1.69



Schering-Plough Animal Health  
Aquaculture

# Giving Nature a Hand



ORAL

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**AquaVac Ergosan\***

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**AquaVac Vibromax "Activated" Shrimp PLs  
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[www.spaquaculture.com](http://www.spaquaculture.com)

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## THE ADVANTAGES OF VACCINE ACTIVATED WHITE SHRIMP

post larvae which we call 'Activated PLs'. The cost benefit for producers is highly measurable, particularly when optimum production and margins are threatened by viral challenge.

### PHASE 2: INCREASED APPETITE, 18.4% LARGER PLS AND IMPROVED FCR

At phase 2 (Post Larvae 9 - Post Larvae 18) raceways were stocked with 3.75 million PLs each. At this stage the vaccination process with AquaVac Vibromax commenced. Vibromax was introduced via feeding by bio-encapsulation in artemia (feeding rates are shown in Table 1). It is critical that only instar 2 Artemia nauplii are used for this process. Increased appetite levels were evident at this stage. Dr. Lopez- Doriga makes an important point, "Where shrimp at this stage are looking for more feed it is essential to make additional feed available. Maintaining feed to appetite will reduce the risk of cannibalism and maximises growth. "The trial showed that AquaVac Vibromax improved the potential of the shrimp, allowing them to convert feed more efficiently and grow larger" she adds.

It is worth noting that whilst the survival rates at the end of phase 1 were generally the same, the PLs coming forward into Phase 2 were a significant 18.4% larger.

During phase 2 both activated and un-activated shrimp PLs were exposed to a Vibrio challenge and subsequently treated with an antibacterial disinfection. It is recognised that Vibrio infection will affect growth and performance of shrimp. The trial suggests that the Vibromax Activated PLs displayed a response to the challenge and in addition it was observed that they returned to feed sooner than the non-activated control populations. The growth rate, size and FCR of the Activated PLs were superior. The cumulative benefits at this stage of the trial included improved survival, 18.4% larger PLs and a superior FCR in the activated groups (Table 2).

### PHASE 3: 16% INCREASED HARVEST VALUE, HIGHER AVERAGE WEIGHT, IMPROVED FCR AND ENHANCED REALISABLE VALUE DUE TO MORE SHRIMP IN HIGHER WEIGHT BANDS

The trial at phase 3 sought to evaluate performance of the Activated PLs in the grow-out phase. Ponds were stocked at two different densities. During this stage the farm was subjected to a White spot viral disease outbreak. Typically White spot viral disease presents a major challenge to economic production in the grow-out phase. The consequential financial losses can be large. In this case over 62% mortality was recorded in the low density

ponds and 68% in the higher density ponds. The only management option is to harvest the shrimp early. This results in shrimp size and weight well below their optimum, effectively raising the average cost of production and limiting achievable prices, margin and overall profitability. The Activated PLs were harvested last as they showed a higher resistance to the disease. However at the end of the period the total mortality in both Activated and standard control groups was the same.

When the results were collated, the differences in performance between the activated and non-activated samples continued. The average mean weight of the activated PLs was 1.78 grams higher at 18.70 grams. Most significantly in activated PL populations five out of nine (5/9) ponds were harvested in the 31/35 (20 gram plus) size category but none of the control ponds reached this size, while only 2/9 were in the smaller 41/50 size category compared to 5/9 nine of the standard control ponds. All ponds were harvested early due to the White spot disease problem although the Activated PLs were considered resistant enough to be kept a few days longer. The daily FCR was 2.153 for the activated and 2.34 for the non-activated giving a further economic advantage calculated as being worth \$1000 per million PLs stocked.

### HARVEST RETURNS

The AquaVac Vibromax activated population was harvested with an increased biomass of 6.61% and the total crop value which was 17.1% higher when compared directly with the standard control population (Table 3).

For the standard control group the average end weight was 17.07 grams and harvest values were US\$268,627 at an average price of US\$6.82 per kilo (Table 3).

The harvest values for the AquaVac Vibromax group were an average end weight of 19.04 grams and total harvest value of US\$314,625 at an average price of US\$7.57 per kilo (Table 3).

The cumulative effect of these improvements in the activated PLs compared to the standard control PLs was worth US\$45,998 for the activated population (Table 3).

The trial indicates that the implementation of an Activated PL programme utilising AquaVac Ergosan and AquaVac Vibromax produced more, bigger and stronger shrimp throughout the growth cycles. Shrimp grew quicker, converted more efficiently and as a result of the increased numbers of shrimp in larger size bands they achieved superior returns at harvest. ■

\* AquaVac, Ergosan and Vibromax are worldwide trademarks of Schering Plough Ltd, or any affiliated company.

TABLE 3

Total Harvest	Biomass (Kgs)	Average weight	Size Category	Price per Kilogram	Harvest Value US \$	Harvest Value US \$ per Million PLs seeded
Activated PLs	41845.64	19.04	0.00	7.57	314,625	47,796
Controls	39250.83	17.07	0.00	6.82	268,627	40,539
Benefit of AquaVac* Vibromax* Activated PLs	2594.81	1.97	0.00	0.75	45,998	7,257

# VETERINARIANS WITHOUT BORDERS ASSIST IN SRI LANKA

BY DR MARK SHEPPARD AND DR CRAIG STEPHEN (VETERINARIANS WITHOUT BORDERS, CANADA)



DR MARK SHEPPARD TOURS  
AQUACULTURE FACILITIES

**V**eterinarians Without Borders/Vétérinaires sans Frontières – Canada (VWB) is a new organisation that aims to work with disadvantaged communities around the world to foster the well-being of livestock, domestic animals and wildlife, and to promote public and ecosystem health.

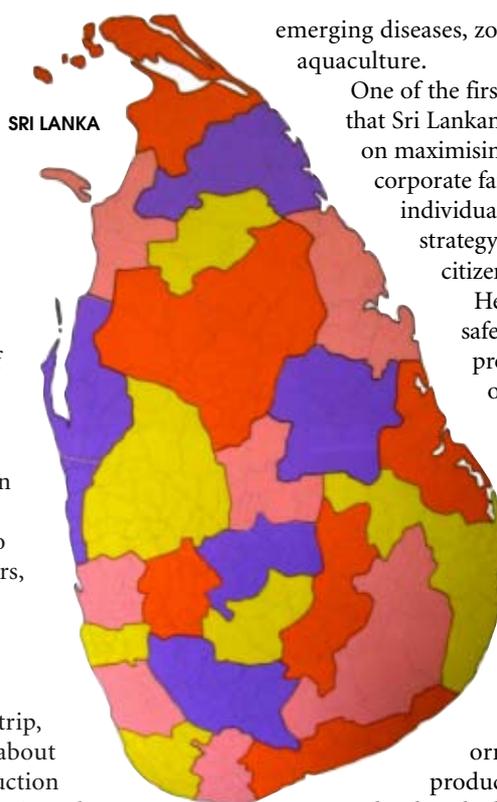
VWB – Canada's inaugural project recently became a successful reality in Sri Lanka thanks to generous donations. VWB was asked by the Sri Lankan Department of Animal Production and Health (DAPH) to explore how VWB could contribute to Sri Lanka's veterinary public health and fish health capacity. This trip took place between November 20 and 30, 2005. Drs Craig Stephen and Mark Sheppard were invited to tour parts of the country and meet educators, regulators and farmers to conduct a needs assessment of all stakeholder groups, and discover if partnerships between Canadian veterinarians and Sri Lankans could be developed. Over the course of their 10-day trip, the two veterinarians focussed on learning about the Sri Lankan veterinary and animal production systems, and the challenges they faced in exotic and

emerging diseases, zoonotic disease control, food safety and aquaculture.

One of the first and most important lessons learned was that Sri Lankan agriculture and aquaculture is not focused on maximising profits and production of integrated and corporate farms. Rather, it is focused on supporting individual farmers as a critical part of the national strategy for improving the health of individual citizens and lifting people from poverty.

Helping smallholder farmers produce enough safe and secure food for their families, as well as producing a small surplus of fish to sell to others to generate a modest income, is the prevailing agricultural model of Sri Lanka. However, there is also an eye on developing export markets into which the local farmers, as well as the few larger-scale producers, could sell their products.

There were three main aquaculture interests within this trip. The freshwater inland food fishery (comprising tilapia and carp production in seasonal earthen ponds), the freshwater ornamental fish market, and shrimp production for export. Even though the goal is to develop the latter two industries for exports to



## VETERINARIANS WITHOUT BORDERS ASSIST IN SRI LANKA



Europe and North America, the overall strategy still includes having family farms and local cooperatives produce fish for sale into these markets, rather than focusing on developing larger corporate farms.

Our VWB/VSF team toured a variety of aquaculture sites starting with a visit to a national fisheries research centre of National Aquatic Research Agency (NARA). The aquaculture, fisheries and oceanography programs at NARA were halted December 2004 when a 4-foot wall of water and mud flooded labs, destroyed critical analytical equipment as well as their research vessel. Rebuilding has been slow but steady. NARA's latest projects involve developing a basic and affordable farmer-made fish feed that can be used for ornamental fish culture and the subsequent sale of fish into the pet trade. NARA also

supports the export of shrimp and ornamental fish by conducting water quality testing for pathogens-of-concern in transport water of animals during export.

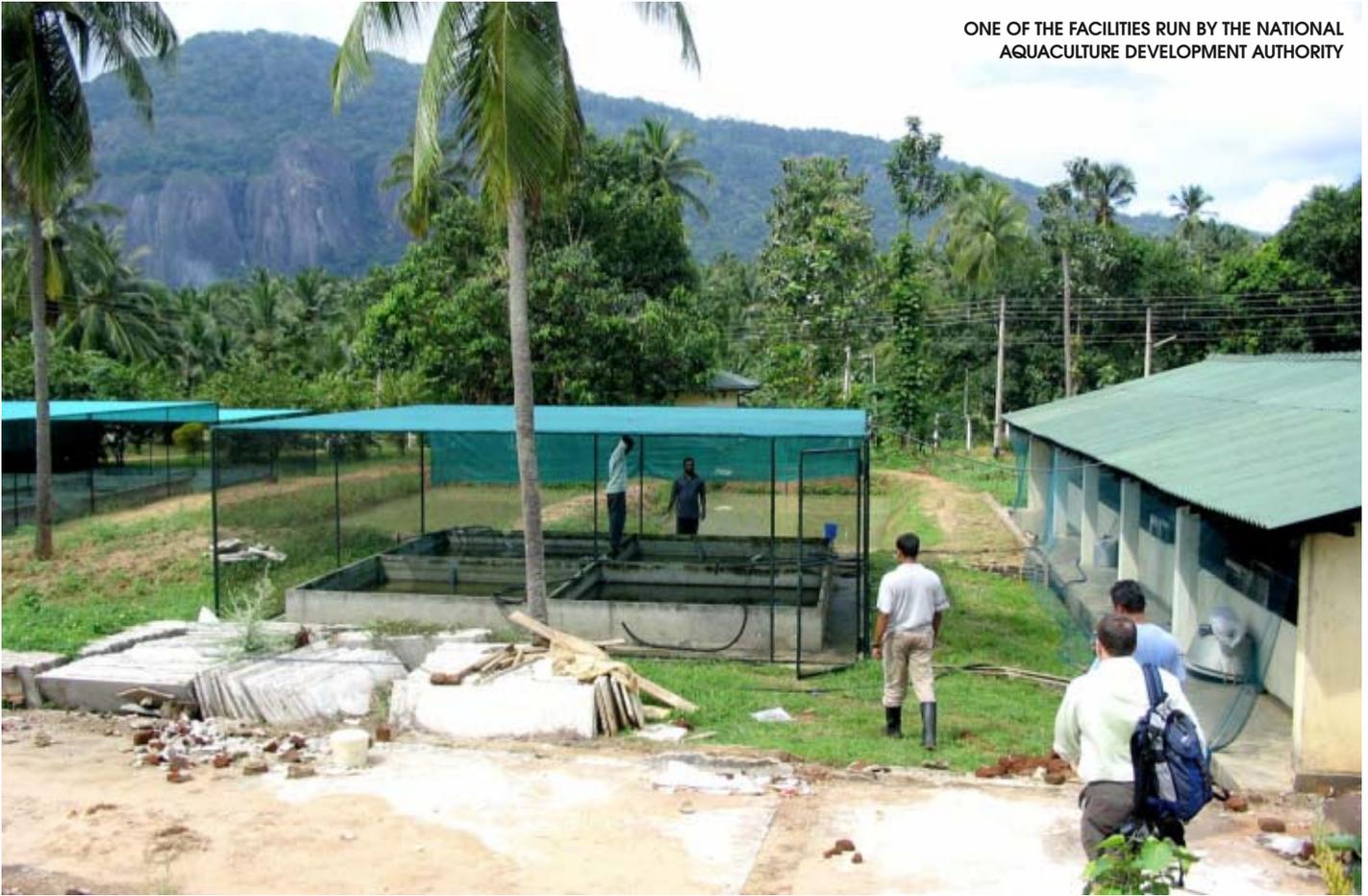
## TWO LOCATIONS

Inland, Sheppard and Stephen visited two locations run by the National Aquaculture Development Authority (NAQDA). The first site was an experimental farm developing broodstock for ornamental fish culture. The ornamental fish trade not only reduces the use of wild-caught fish for the pet industry but also is a significant contributor to the agricultural economy. Our VWB team toured this new facility and consulted with the staff about fish health management, diagnostic needs and various treatment strategies.

The second NAQDA site was a carp and tilapia brood farm and hatchery that provides numerous types of fry to cooperative farming groups and local farmers. They in turn grow the fish for community consumption. The grow-out phase of this industry takes place in "seasonal reservoirs" (natural and man-made large, earthen ponds) that become filled with fresh water during the rainy seasons and eventually dry up after seven to eight months.

As with the ornamental industry, support for diagnostics and fish health management is currently limiting the size and output capacity of this food-producing industry. Increasing the role of fish health specialists in fish management plans and operations will be a key to increasing fish survival and productivity.

The final facet of the aquaculture tour involved marine shrimp farming. Our VWB team visited one large commercial operation as well as many small farms that consisted only of one or two ponds. Shrimp farming is a redeveloping and growing sector that the recently elected government has recognised as a target industry.



In years past the shrimp industry of Sri Lanka was largely unregulated, and many farmers succumbed to widespread outbreaks of white spot disease. In fact, many ponds remain inactive and largely abandoned today, awaiting rejuvenation under recent recommendations and revised regulations set forth by the current government.

The goal of the government is to facilitate the rebuilding of 150 shrimp farms to bring annual production back to 2500 to 3000 metric tonnes. The shrimp farms are located on the western coast of Sri Lanka adjacent to natural lagoons and the historic “Dutch Canal” built in the 1400s.

As part of the response to the historical outbreak of white spot disease, a large project is under way to dredge several key areas of the Dutch Canal. This canal is the main water source for many shrimp farms, but its flow had been interrupted by the accumulation of silt and solids from shrimp farm waste and sediment piled on retaining dikes.

In addition to the dredging project, government extension officers are working with farmers to change waste and water management practices to reduce the sharing of pathogens between sites. Water management will be addressed by coordinating shrimp production cycles based on pre-determined zones and based on six-month grow-out seasons throughout the sector.

Much of our exploration of veterinary public health in Sri Lanka took place in Kandy city, a World Heritage city located in the mountainous interior. Many key facilities and people are headquartered here, including the veterinary school at the University of Peradeniya, the federal Veterinary Research Institute (which serves as the primary diagnostic laboratory for the country) and the head offices of the Department of Animal Production and Health (DAPH).

Here plans were developed for partnerships in food-borne disease, training in field epidemiology and new initiatives in



public health and aquaculture.

The VWB/VSF-Canada trip to Sri Lanka culminated in an unexpected invitation to meet with the newly re-elected Minister of Agriculture, who emphasised his support for our work and the importance of agriculture production for the health and prosperity of the average Sri Lankan.

This meeting highlighted for us the strong interest in partnering with Canadian veterinarians to help improve animal production and public health as a major way to combat poverty and improve the quality of life in Sri Lanka. ■

*Kind support for the ongoing project in Sri Lanka was provided by Aeroplan, Schering-Plough Animal Health and Dr Chas Povey, all of Canada, and Marine Harvest, Japan.*

# AN ONLINE CERTIFICATE FROM WISCONSIN

BY DR MYRON J KEBUS (WISCONSIN DEPARTMENT OF AGRICULTURE TRADE & CONSUMER PROTECTION, DIVISION OF ANIMAL HEALTH, USA); DR MICHAEL T COLLINS (UNIVERSITY OF WISCONSIN-MADISON, SCHOOL OF VETERINARY MEDICINE, USA)  
DR JEANNETTE MCDONALD (UNIVERSITY OF WISCONSIN-MADISON, WISTREC, USA)



ABOVE LEFT: THE EXPERTS INVOLVED IN DELIVERING THE ONLINE PROGRAMME  
ABOVE: A STUDENT EXAMINES A SPECIMEN TO GAIN VALUABLE PRACTICAL EXPERIENCE

It originated with foresight. The state of Wisconsin has a large fish farming and sport fishing industry, and in 1999 the state hired its first aquaculture veterinarian to oversee this growing market.

Rather than try to cover the whole state singlehandedly, the new aquaculture veterinarian decided to deputise veterinarians around the state, freeing the central office to focus on progressive efforts rather than limiting itself to an endless array of sample collection and submission.

In partnership with Wisconsin's School of Veterinary Medicine, the state first established a continuing education short course, and more recently an online *Fish Health Medicine Certificate Programme*. The long-term plan was to train individuals to recognise fish diseases and be able to provide health services to fish farms.

The new, online programme was adapted from the intensive, two-day short course that provided practical training in field techniques for sample collection and field diagnostics.

The initial programme, called Aquaculture Veterinary Medicine for Practitioners, was developed by the Wisconsin Department of Agriculture, Trade and Consumer Protection (commonly referred to as DATCP), in collaboration with the University of Wisconsin-Madison School of Veterinary Medicine (UW SVM). All veterinarians in Wisconsin who wish to be certified to issue fish health certificates or a Report of Veterinary Health Assessment of Fish were required to complete this course.

The course has been adapted for online delivery through the continuing education portal of UW SVM. (See [www.vetmedce.org](http://www.vetmedce.org)). It is constructed as a series of six modules.

The first five modules can be taken at any time and anywhere over the Internet using narrated Powerpoint presentations and

supplemental reading materials delivered using new educational technology software. Each module is followed by a test that is automatically generated and computer-scored. The successful completion of each module results in the award of CE credits that can be used to satisfy state veterinary licensing requirements.

The final module is a one-day, hands-on practice of the principles and techniques learned in the five online modules, with the course instructor judging final proficiency. Passing the final module will result in the awarding of the Fish Health Medicine Certificate.

The content for these modules is a collaborative effort between Dr Myron Kebus, State Fish Health Veterinarian at DATCP and Dr Michael Collins, professor at the UW SVM, with special contributions by:

- Dr Grace Karremann, president of the Association of Aquaculture Veterinarians of British Columbia, Canada
- Dr Julie Bebak-Williams, director of Aquatic Animal Health at the Freshwater Institute in Shepherdstown, West Virginia, USA, and
- Dr Jill Rolland, aquaculture specialist with USDA-APHIS Veterinary Services, Maryland, USA.

Dr. Jeannette McDonald and her staff at WisTREC (Wisconsin's Technology Resource for Educating Care-Providers) at the University of Wisconsin produced the modules.

## INTENSIVE DETAIL

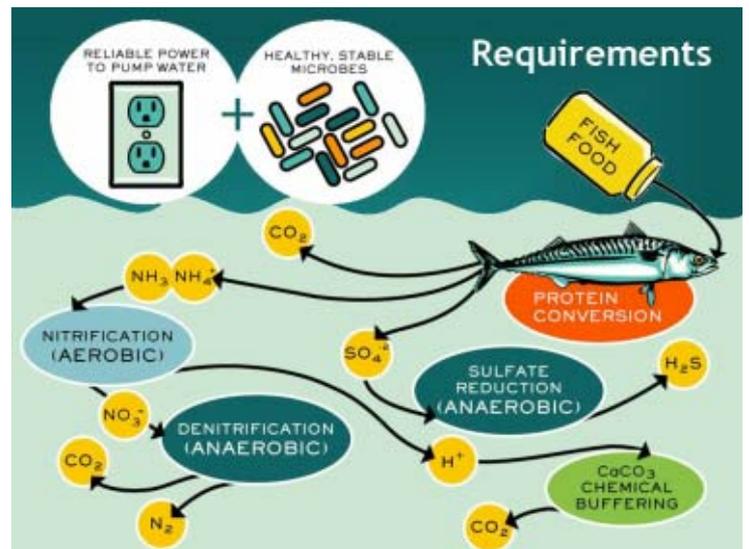
Module one of the programme introduces the course. It provides basic information on the US aquaculture industry and discusses a national partnership designed to reduce risks related to fish farming. Module two provides more detail on risk management



ABOVE: STUDENTS DISCUSS WATER QUALITY ISSUES DURING A PRACTICAL CLASS

LEFT: DR MYRON KEBUS UNDER THE SPOTLIGHT

FAR LEFT: WATER QUALITY MANAGEMENT IS AN INTEGRAL COMPONENT OF THE COURSE. STUDENTS LEARN ABOUT WATER CHEMISTRY IN INTENSIVE RECIRCULATION SYSTEMS



and biosecurity on fish farms, and describes best management practices for raising fish.

The third module focuses on water quality, the aspect of fish farming that is most novel to veterinarians and the most critical to fish health. The fourth explains how to conduct a fish health inspection in order to test for a select set of pathogens controlled by government regulations. Module five describes a practical “how to” method of performing a veterinary health assessment, or VHA, on fish.

The VHA is a relatively novel new tool for the surveillance and management of fish health problems. Wisconsin’s VHA originated as a fish health/condition assessment procedure developed in 1976 by Ron Goede from the Utah Division of Wildlife Resources. It was designed to assess the health of fish reared in state and federal fish hatcheries for stocking.

In 1994, Dr Kebus modified the procedure and developed the Wisconsin Veterinary Health Assessment for evaluating fish on commercial fish farms.

In 1999, as the director of the newly created Fish Health Programme at DATCP, he began to develop the VHA as an objective measure of fish health. This gives fish buyers and regulators of fish movement a tool for making sound decisions.

The VHA can be used for all fish species, but specifically fills a void of health inspections of non-salmonids. While voluntary in most situations, the VHA can also be used to satisfy some state regulations.

In Wisconsin, they use the VHA to meet the health requirements for fish that are imported to the state or will be stocked in public waters. In 2004, DATCP reviewed 243 VHAs submitted by 23 veterinarians and four non-veterinary inspectors. A growing



LEARNING HOW TO MEASURE DISSOLVED OXYGEN LEVELS IN A HOLDING TANK

## AN ONLINE CERTIFICATE FROM WISCONSIN



number of other states are finding it a useful tool to reduce the risk of moving diseased fish.

## GOALS AND REGISTRATION

The development of the certificate programme was funded by the USDA Risk Management Programme, administered through Mississippi State University. Training accessibility and self-sustainability are two critical elements. The primary goals are to increase the number of well-trained veterinarians available to fish farmers to help them prevent economic losses due to disease and improve fish farm biosecurity, ie limit the chances of the introduction of diseased fish to farms or public water.

While the training is targeted at veterinarians, anyone is welcome to take the online training. Within the US, we hope that the certificate programme will grow and one day be used by all states.

The programme could easily be adapted to the needs of other countries, and international collaboration to expand training of veterinarians in fish diseases is welcome.

For more information, contact Dr Myron Kebus at [myron.kebus@datcp.state.wi.us](mailto:myron.kebus@datcp.state.wi.us)

## AQUACULTURE AND VETERINARIANS IN WISCONSIN

Though fish producers and veterinarians in most states recognise that there are important diseases (eg spring viremia of carp, largemouth bass (*Micropterus salmoides*) virus, and heterosporis of non-salmonid fish, there are very few examples in the US of health standards and health forms for non-salmonid fish, and most states are still struggling to develop standards and forms.

Wisconsin chose to proceed based on its state law, which specifies that veterinarians are “certified” to perform specific, specialised veterinary activities, in contrast with “accredited” (limited to veterinarians who work for federal animal health programmes, such as the US Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS). Examples of veterinary activities that require certification include Johne’s Disease testing in cattle, chronic wasting disease testing in deer, and fish disease testing.

By training and certifying private practice veterinarians, DATCP privatised many of the on-farm fish health services that in other states are performed by government employees. This has allowed DATCP staff to work on progressive efforts, as opposed to working solely on sample collection and submission for disease testing, and provides more accessible and more knowledgeable local services for fish farmers.

As a result of its decision, Wisconsin has developed a fish health certificate for all fish introduced or imported to waters of the state. Since 1999, all fish stocked into waters of the state, and from 2001, all fish imported to Wisconsin, have been required to meet health standards. A valid Report of Veterinary Health Assessment of Fish must be provided prior to stocking or importing non-salmonid fish. This report is viewed as a first step in developing health certificates for non-salmonids. Producers have viewed the assessments favorably, largely because they highlight positive as well as negative findings, and they are practical, quick and affordable.

DATCP has assisted the Wisconsin aquaculture industry since 1988, initially through the Division of Agricultural Development. The fish health programme includes registration of fish farms, certification of veterinarians, rules for health standards for fish introduced into public waters, and issuance of fish import permits. A key component of the programme’s success has been the close working relationship among DATCP, fish farmers and veterinarians.

## A GROWING INDUSTRY

As Wisconsin’s markets for farm-raised fish have grown, the need to keep fish healthy on the farm has increased. There are more than 350 commercial fish farms and 1739 hobby fish farms in the state. There are also 14 state, two federal and four tribal hatcheries. The farmers see the investment in fish health as an investment in the future of the \$17.5 million aquaculture industry in Wisconsin.

According to the USDA 2002 Census of Agriculture (USDA NASS 2002), Wisconsin ranks third in baitfish production and seventh in trout production in the US, and has led the US in efforts to research the commercial rearing of yellow perch (*Perca flavescens*).

A large number of fish are transported into Wisconsin from 26 states and Canada. In 2003, Wisconsin imported 726 metric tonnes of fathead minnows (*Pimephales promelas*), 202 tonnes of golden shiners (*Notemigonus crysoleucas*), 272 tonnes of white sucker (*Catostomus commersoni*), 374,000 walleye (*Stizostedion vitreum*), and six million yellow perch (*Perca flavescens*).

A person importing live fish or fish eggs into Wisconsin must first obtain an annual import permit from the DATCP. The DATCP only issues the permit to the Wisconsin facility that will receive the imported fish when it has approved a valid veterinary health assessment report.

A copy of the permit is sent to those who will deliver the fish from out of state, and the hauler is also required to carry a copy during transport.

Since 1997 DATCP has issued over 750 import permits for fish originating from 26 states and Canada. In 2003, 105 fish import permits were issued, similar to the number issued in 2001 and 2002.

The DATCP Wisconsin maintains a list of aquaculture veterinarians who are accredited and certified to perform regulatory services on Wisconsin fish farms. Wisconsin has trained and identified over 75 private veterinary practitioners who are certified to issue a Report of Veterinary Health Assessment of Fish to private aquaculture, state-run aquaculture, and tribal fish hatcheries in nine states.

The online Fish Health Certificate Programme is another example of Wisconsin’s leadership role in fish health protection in the United States.

WHIRLING DISEASE RESEARCH  
FOCUSES OFTEN ON THE  
VARIABILITY IN SUSCEPTIBILITY  
AMONG TUBIFEX TUBIFEX, THE  
ALTERNATE HOST OF THE PARASITE  
(PHOTO COURTESY OF LEAH  
STEINBACH ELWELL)



# DEVELOPMENTS IN WHIRLING DISEASE RESEARCH THROUGH THE NATIONAL SYMPOSIUM

BY KAJSA STROMBERG, (WHIRLING DISEASE INITIATIVE, MONTANA WATER CENTER, USA)

When the first national Whirling Disease Symposium was held in 1995, there was a great sense of urgency among coldwater fisheries managers, aquaculturalists and a concerned public.

Whirling disease, caused by the myxozoan parasite *Myxobolus cerebralis*, had recently emerged as a major threat to wild trout in the United States. Since that time, intensive research has increased knowledge about the parasite, its two hosts and the potential for managing the disease.

Although investigations about the complicated disease seem to raise more questions for every one that is answered, research results presented at the 12th annual symposium have yielded new information that can be put into practice on the ground.

The first detection of *M. cerebralis* in the United States occurred in Pennsylvania in 1956 (Hoffman 1962). The parasite was also detected at nearly the same time in California. Researchers suspect the parasite was introduced from Europe, where it is native, in a shipment of infected frozen trout (Bartholomew and Reno 2002). The parasite soon became widely distributed as a result of inadvertent stocking of infected fish and the use of infected fish for food in hatcheries.

Low levels of infection in wild fish and a lack of visible signs of disease allowed the parasite to spread largely unnoticed. By 1970, the parasite had been detected in 10 states. By 1990, it had spread to an additional seven states, from the eastern to the western coast of the country. Currently, the parasite has been reported in 23 states.

However, despite this widespread distribution, prior to 1994 observations of clinical whirling disease were largely limited to aquaculture facilities, and population-level impacts were not documented in the wild.

## IMPACT OF WHIRLING DISEASE

In the years following its introduction, impacts of whirling disease on public and private aquaculture enterprises were severe, in part as a result of fish health regulations imposed to control the further spread of the parasite.

During that time, the detection of the parasite could signal the destruction of infected trout stocks, expensive renovations and even the closure of facilities. Millions of fish were destroyed and the economic costs were high. Regulations attempted to keep pace with the spread of the parasite, restricting the transfer of fish and fish parts.

However, the parasite continued to spread through inadvertent introductions and *M. cerebralis* was detected in an increasing number of waters. The consequences of *M. cerebralis* for aquaculture in the United States continue to be severe in many cases.

For example, in the state of Utah, the total value of trout sales in Utah was reduced nearly 29 percent during 2005. This is largely attributed to whirling disease, since six private hatcheries went out of business in 2005 due to detection of the parasite, according to the Utah Department of Agriculture and Food.

The first observations of the effect of whirling disease on wild fish populations occurred in the early 1990s in Colorado and Montana. Biologists observed declines in wild rainbow trout, and by 1994 whirling disease was identified as the primary cause. Reductions in recruitment of 90 to 100 percent were observed in the worst cases, and biologists, managers, and anglers became very concerned.

The parasite was already widespread, and its distribution continued to increase. In addition, it was well known that

## DEVELOPMENTS IN WHIRLING DISEASE RESEARCH THROUGH THE NATIONAL SYMPOSIUM

management and eradication in aquaculture facilities was highly difficult and expensive. Concerns were raised that *M cerebralis* might decimate wild trout and salmon populations throughout North America and run producers out of business.

### THE NEED FOR FURTHER RESEARCH

In this atmosphere of urgency and concern, the first national Whirling Disease Symposium was held in Denver, Colorado in 1995. This annual event, organised by the Whirling Disease Foundation, is the main gathering for whirling disease-related scientific research.

Scientists from a wide spectrum of scientific fields, including pathology, parasitology, fish ecology and genetics, attend the meeting each year. It provides a forum to share the latest research developments and management lessons, and facilitates cooperation and strategic planning.

This year marked the 12th annual Whirling Disease Symposium, and it is evident that research has become increasingly sophisticated and that the cooperative research programmes have matured. There is no single solution to the problems of whirling disease, and it's clear that a variety of approaches will be required.

As the scientific community struggles to provide the answers needed by managers and policy-makers, research focus areas continue to shift. The primary drivers of research related to whirling disease are the Whirling Disease Foundation and the Whirling Disease Initiative, each with their own priorities and strategies, but complementary and cooperative.

In the beginning, during the 1990s, research was mainly focused on basic questions of biology, ecology, diagnostics and treatment. Research revealed more information about the parasite's complicated life cycle, and deepened our understanding of the factors that influence whether the parasite becomes established in a locale, and causes infection and mortality. Research has also provided tools for detecting the parasite at very low levels in fish and in the wild.

This achievement has resulted in a regulatory programme that is much more efficient at preventing further parasite spread as a result of inadvertent movements of infected fish, and also in providing better information on where the parasite life cycle had become established. As this information was obtained, attention shifted to the study of management applications of these results and large-scale, watershed level investigations that would provide more insight on how the parasite spreads and under what conditions it results in disease.

### THE 2006 WHIRLING DISEASE SYMPOSIUM

Research presented during the 2006 symposium focused on where *M cerebralis* infection occurs and why, particularly emphasising aspects of resistance and susceptibility of the parasite's two hosts: salmonid fishes and *Tubifex tubifex* worms.

One series of investigations examined the relationships between land use, habitat characteristics, *T tubifex* genotype and the prevalence of *M cerebralis*. There are multiple, distinct genetic lineages of *T tubifex*

(Beauchamp et al 2002).

Lineages are determined by mitochondrial DNA (mt 16S) sequences that indicate genetic relationships, but are not necessarily indicators of specific resistance or susceptibility to the parasite. However, there is some indication that genetic differences may reflect variations among *T tubifex* worm populations with regard to their ability to support the parasite and produce the triactinomyxon life stage.

This variability between lineages may therefore be an important factor in determining infection rates among fish (Baxa et al 2006). This recent discovery has generated considerable interest in the implications for management of *M cerebralis*. Future studies are planned to investigate the mechanisms for resistance in *T tubifex* and how this information can be applied.

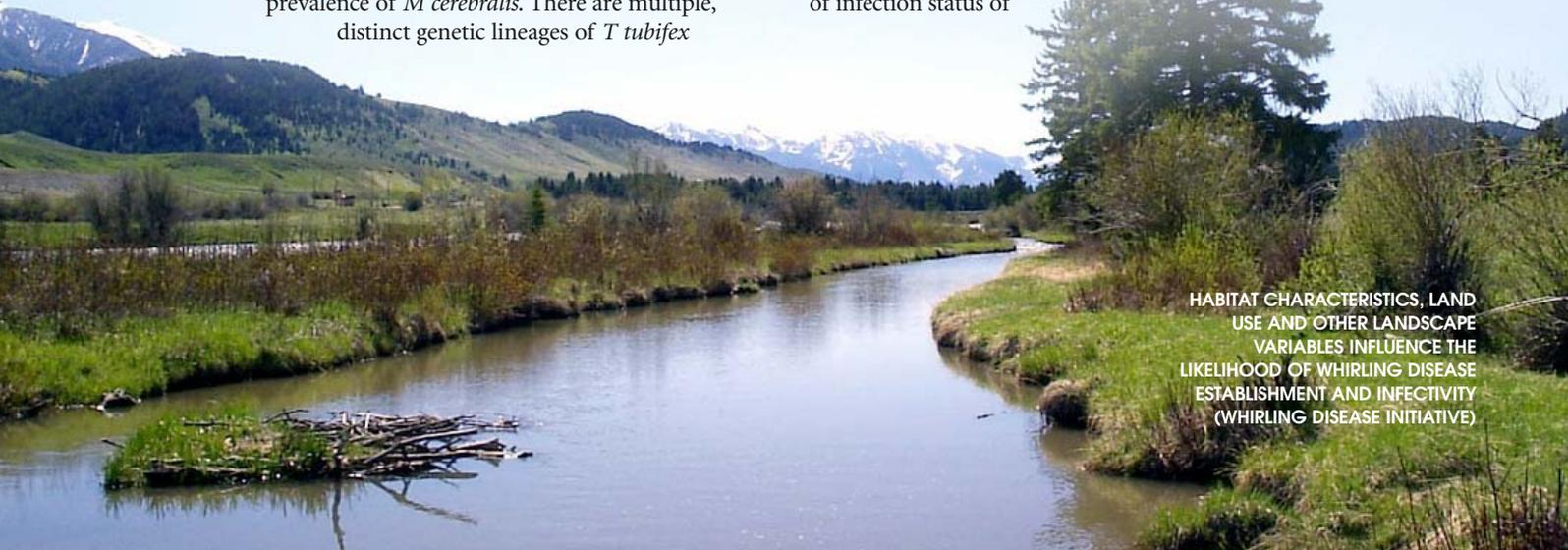
Studies regarding the salmonid host also focused on resistance to *M cerebralis* infection. Naturally occurring resistance is being investigated on the Madison River, Montana, where researchers hypothesise that selective processes are yielding a surviving population of fish that is more resistant to *M cerebralis* infection (Vincent 2006). Despite a high parasite concentration, the severity of infection in young rainbow trout in the Madison River in 2004 and 2005 is much lower than during the 1990s. However, many of these fish are not surviving to reproductive age due to factors still unclear. Research is continuing to evaluate the possibility of a developing resistance and what implications it may have for management.

Another line of research on resistant trout focuses on a domestic strain of rainbow trout known as the Hofer strain developed in Germany. These fish have been identified as having a high degree of resistance to whirling disease (Hedrick et al 2003). Crosses between this domestic strain and naturalised strains, like the Colorado River rainbow trout, are being evaluated for potential stocking into parasite-positive waters (Schisler et al 2006).

Pilot introductions have taken place in the Gunnison River, Colorado and survival will be carefully tracked and evaluated. These fish may provide managers with a sportfish that will survive in waters where infection levels are high. Additional studies investigated the mechanisms of resistance, evaluating the physical processes involved and genetic analyses.

Advances in *M cerebralis* diagnostics have been helpful for researchers, and have provided managers with highly sensitive tools for detecting low parasite levels in fish. However, the increasingly diverse questions being asked have presented challenges that require development of a new tool.

One area of interest has been in the development of non-lethal sampling methods, and one of the methods presented at this year's symposium could provide an indicator of infection status of



HABITAT CHARACTERISTICS, LAND USE AND OTHER LANDSCAPE VARIABLES INFLUENCE THE LIKELIHOOD OF WHIRLING DISEASE ESTABLISHMENT AND INFECTIVITY (WHIRLING DISEASE INITIATIVE)

a fish population by measuring antibody levels (Adkinson et al 2006).

Other researchers are investigating methods to identify the parasite in soil and fecal material through molecular techniques that have already proved successful for detection in fish and water (Gates and Guy 2006, Steinbach Elwell et al 2006).

Risk assessments, habitat studies and manipulations continued to be vital research areas in 2006. Hatchery operations were in the spotlight, with a case study from Utah's Springville fish hatchery, where composting provided a valuable disposal option for infected fish (Cavender and Wilson 2006).

## LOOKING TO THE FUTURE

Whirling disease is nothing if not complicated. With multiple hosts and parasitic forms, *Myxobolus cerebralis* has introduced new complexities to the management of salmonids in the wild and in hatcheries. Since the parasite first appeared in the United States in 1956, research has advanced significantly, yet the parasite and the disease it causes continues to spread. Each year we add a few more pieces to the puzzle and solve a few more questions.

As time goes by, the perspectives shift. What once was commonly viewed with panic is now more often viewed with resignation. What once was considered solely a fish health issue is increasingly considered in a context with other aquatic nuisance species, gaining attention broadly on a national scale collectively as a major emerging threat.

The full impact of whirling disease in the US may not yet have been observed, nor have all the questions been answered. Meanwhile, through an unprecedented collaboration of scientists, fishery managers, biologists and aquaculturalists, we continue to increase our understanding of the disease biology and develop measures for control and management.

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NEW DIAGNOSTIC TECHNIQUES ARE IMPROVING THE DETECTION OF *M. CEREBRALIS* IN AQUACULTURE AND WILD SETTINGS (WHIRLING DISEASE INITIATIVE)

myxospores from sediment utilising a soil texture centrifuge technique and sodium hexametaphosphate: at what level does sediment quantity in a sample interfere with extraction efficiency?

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# SALMON – WHERE IS THE CORRECT INJECTION SITE?

BY ARILD KOLLEVÅG (INTERVET NORBIO AS, NORWAY)

In recent years, between 160 and 170 million salmon and rainbow trout have been vaccinated each year in Norway. The vast majority of these were vaccinated by injection (intra-peritoneal) with oil-based vaccines. Recent experience in the field has shown that the quality of the vaccination procedure *per se* may have a big influence on how the fish progresses through the whole production cycle, in terms of both the efficacy of the vaccine to protect against the targeted diseases and any side-effects that may arise from the vaccination.

The most serious problems often arise when  $S_0$  smolts are being vaccinated, and when a large number of fish are being vaccinated during a short period. Under these circumstances, the quality of the vaccination procedure *per se* may be reduced, and a higher proportion of the fish are of a small size, which in turn increases the risk of incorrect vaccination. Indeed, even apparently insignificant deviations from the recommended injection site can reduce the vaccine efficacy and also result in a higher incidence of side-effects (ie local reactions in the internal viscera).

Over the years, Intervet Norbio AS has performed several vaccination audits in Norway. Vaccination techniques have certainly become better, but there is still room for improvement. The greatest challenge is to perform the vaccination correctly on every fish on every day of the vaccination period.

It is also worth mentioning that new legislation (Norwegian Regulation of Aquaculture Management, No. 55) entered into force in Norway in December 2004. The new legislation requires closer inspection of the vaccination process when automatic vaccination equipment is used. It is also possible that similar legislation will be enacted in other fish vaccination countries in the future.

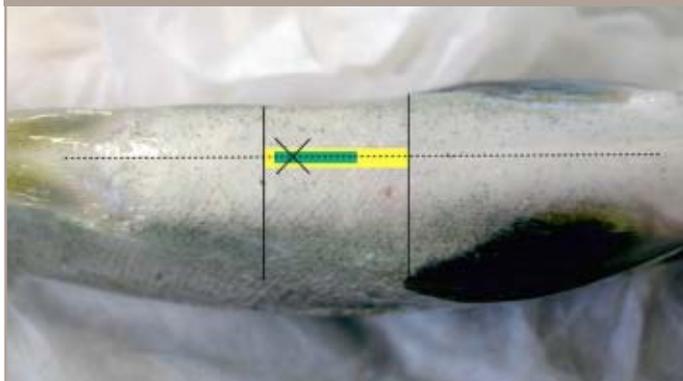
## RECOMMENDED PLACEMENT

The recommended position of the injection site is in the midline of the abdomen, one pelvic fin length in front of the base of the pelvic fins.

Intervet Norbio now has extensive research data showing that water temperature and fish size are key factors that may influence the development of local reactions in vaccinated fish.

Optimally, the temperature should be below 15° Celsius from

**FIGURE 1.** Optimal injection site. The fish shall be injected in the abdominal midline, slightly in front of the pelvic bone (that lies beneath the flesh) and one fin length in front of the base of the pelvic fins (marked with an X). The green area depicts the ideal area for injection and the yellow area depicts the outer limits for acceptable deviations to this



the time of vaccination until several weeks after sea transfer, and the individual fish size at vaccination should be  $\geq 35$ g. A high and/or sudden increase in the water temperature during this window will possibly contribute to a greater incidence and severity of local reactions in the fish.

Although it is likely that some of the link between (small) fish size and greater side-effects is due to fish size *per se*, undoubtedly some is also due to operators injecting the vaccine in the wrong position in fish that are below the recommended size. See Figure 1.

## SITE DEVIATION

Section 55 of the Norwegian Regulation of Aquaculture Management states that the percentage of deviation from the correct site of injection shall not exceed 0.1 percent. In practice, it will be hard to attain such a low percentage deviation, but the rule of thumb should be to keep the deviation as close as possible to 0.1 percent.

A deviation in the injection site occurs when the vaccine is deposited in a way where it does not float freely in the abdominal cavity. When this occurs, it usually means that the injection site was outside the recommended injection area. Other reasons could be that the depth of the needle was not correct (Figure 2, adjustment of the length of the needle) or that the angle of the needle was not optimal. These types of injections can lead to improper vaccination and/or unwanted damage.

### Examples of deviations in the injection site are:

- Incorrect position of the injection site (Figure 3), where the injection is given outside the yellow area depicted in Figure 1.
- Too shallow an injection (Figure 4). The vaccine is injected directly into the muscle or between the muscle (abdominal wall) and the peritoneum.
- Too deep an injection (Figure 5). The vaccine is injected into the internal organs, such as the appendix (pyloric caecae), spleen, liver or intestines, and may even reach the kidney.
- Tears and bleeding at the injection site (Figure 6). The needle causes wounds in the skin or muscle on the outer surface or on the inside of the abdominal wall.

**FIGURE 2.** Adjustment of the length of the needle. The needle should penetrate 1mm to 2mm into the abdominal cavity



**FIGURE 3.** The injection site is placed too far forward, resulting in injecting inside the pyloric caecae. This may also occur if a vaccinator “forces” the needle



**FIGURE 5.** Depositing the vaccine into the intestine raises the danger of a reduced vaccine efficacy



- Wrong timing of injection. The vaccine is injected when the needle is on its way into or out of the abdominal cavity.

**The usual causes of deviation of the injection site include:**

- Uneven size of fish. If the difference between the small and big fish in the group to be vaccinated is too great, this could lead to either shallow or deep injections.
- Lack of validation of the vaccination technique. The deviation in the injection site during the vaccination period will not be recognised and corrected.
- The speed of vaccination is too high. This could lead to reduced precision of vaccination.
- Lack of routine adjustment of the equipment for each individual vaccinators, together with long working days. This creates a higher risk of human error with vaccination.
- Not withholding feed for a period before vaccination increases the risk of injecting the vaccine into the internal organs.
- Vaccination with improperly honed needles or needles that are blunt. This leads to tears and bleeding at the injection site.

**CONSEQUENCES OF A DEVIATION**

- Mortality after vaccination. Injection of the vaccine into internal organs may lead to acute mortality.
- Reduced efficacy. Injection of the vaccine into the muscle or internal organs, or incomplete injection of the correct vaccine volume, can result in insufficient protection against diseases after vaccination.
- Side-effects (local reactions). Injecting the vaccine into the pyloric caecal region or into the stomach or intestines can lead to a traumatic local inflammatory reaction as a result of leakage of contents from the intestines into the abdominal cavity.

**FIGURE 4.** Vaccination with a needle that is too short or placement of the injection site is too far back. The vaccine is deposited between the peritoneum and the abdominal wall



**FIGURE 6.** Tears and bleeding at the injection site due to using damaged or blunt needles, or by too rough handling. It raises the danger of vaccine leakage or wounds that can lead to infection



- Downgrading of fish quality at harvest as a result of pigmentation/melaninisation in the muscle. Injecting vaccine directly into the external muscle or between the membrane (peritoneum) covering the inner side of the abdominal cavity and the abdominal wall (Figure 3).
- “Unvaccinated” fish. This occurs when the vaccine has been deposited inside the front or hind intestine, or inside the stomach. The vaccine would then be flushed out with the faeces a short time after vaccination. When a high percentage of fish are vaccinated in this way, there is the additional risk that the group protection (or “herd immunity”) will not take place.

**SUMMARY**

Correct placement of the injection site is best attained when the fish to be vaccinated are equal in size and when trained personnel using good work practices perform the vaccination.

The vaccination should be performed in cooperation with the fish health service and the vaccine manufacturer. Routine auditing of the vaccination procedure is crucial for attaining a consistently good quality vaccination process.

Correct vaccination of fish  $\geq 35g$  in size and at water temperatures below 15° Celsius with a quality vaccine will normally result in fish with good protection against disease and a low incidence and severity of side-effects. This will ensure the farmer gets good production performance and correspondingly good margins.

Importantly, it also means that in Norway, the farmer can ensure good animal welfare standards in accordance with the Norwegian Regulation of Aquaculture Management as founded on the Norwegian Law of Animal Welfare. ■



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