AQUACULTURE HEALTH INTERNATIONAL

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COULD THE KHV BE A **WHITE KNIGHT?**

RED MARK SYNDROME IN BRITAIN

BIOTECHNOLOGICAL ADVANCEMENT IN FISH HEALTH

SHRIMP INDUSTRY IN INDIA

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ON THE COVER

Cell culture at Benchmark Biolabs

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TWO YEARS ON AND GOING STRONG

DR SCOTT PEDDIE, EDITORIAL DIRECTOR

It's hard to believe that two years have passed since the release of the first issue of *Aquaculture Health International* in May 2005. Over those two years the magazine has expanded from an initial sixteen pages of content to over forty for most subsequent issues. We have published a plethora of articles on a wide-range of topics from expert contributors across the globe – around ninety articles in total from contributors as far afield as Venezuela, Serbia, Australia, India, Thailand, Ireland, Norway, the USA and Chile, to name but a few.

Species coverage has been diverse, although we have had a particular focus on the most commonly farmed species, including salmon, tilapia and shrimp. More unusual focus articles have dealt with sterlet in Serbia, homarid lobster in the UK and the work of aquaculture and veterinary charities across the globe. International and national aquatic animal health policy and the ever-present and increasingly important issues related to biosecurity have had extensive coverage. We have also taken the decision to highlight commercial trial results and new product development of interest to the sector world-wide. Major diseases have been covered in-depth by noted experts and include extensive articles on Streptococcus, Epitheliocytis and Amoebic Gill Disease to name but three.

Also, under the highly professional guidance and editorship of Dave Groman and Franck Berthe from the Atlantic Veterinary College, University of Prince Edward Island, we have published a series of in-depth diagnostic laboratory features on independent fish and shellfish diagnostic service providers in the USA, Canada, Chile, Thailand and the UK. We have also focused on the excellent work carried out by the research and development departments of major companies such as Intervet, PerOs and Lallemand.

At *Aquaculture Health International* our vision is to continually expand the range of articles, news items and book reviews we offer to our diverse range of subscribers working at the cutting-edge of aquaculture health in both academia and commerce. We hope that contributors, old and new, will continue to provide an interesting insight into the multi-faceted world of fish and shellfish health, and as always, we look forward to hearing from you, wherever you are and whatever your field.

All that remains for me to say is to thank the advertisers and numerous contributors that have supported the magazine and its development over the last two years. With your help we can continue to reach a wide and ever-expanding audience and ensure that the global aquaculture community is aware of the excellent and innovative work that is happening in this exciting field.

THE CSIRO-AUSTRALIAN ANIMAL HEALTH LABORATORY, GEELONG IS A HIGH-SECURITY DISEASE LABORATORY BUILT FOR RESEARCHING AND DIAGNOSING EXOTIC DISEASES OF AUSTRALIAN ANIMALS

KOI HERPES VIRUS: DREADED PATHOGEN OR WHITE KNIGHT?

DRS KENNETH A MCCOLL, AGUS SUNARTO, LYNETTE M WILLIAMS AND MARK ST J CRANE
(CSIRO-AAHL FISH DISEASES LABORATORY, GEELONG, AUSTRALIA)

Disease associated with koi herpes virus (KHV) was first described in common carp (*Cyprinus carpio carpio*) in Israel in 1998, although retrospective studies have since shown that the virus was responsible for mortality in common and koi carp (*Cyprinus carpio koi*) from about 1996.

Since then, the virus has spread throughout much of Europe and Asia, and to South Africa and the United States, and represents a serious threat to important carp industries. Consequently, in many areas of the world, significant resources are currently being directed toward developing control strategies for the disease.

By contrast, common carp in Australia is an introduced pest, and KHV represents one of a number of potential weapons that might be used in a multi-pronged attempt at controlling them.

THE AUSTRALIAN SCENE

There are approximately 300 species of freshwater fish in Australia, a small number by comparison with other similar-sized landmasses. Of these, at least 43 alien fish species are recognised in Australian inland waters, with 31 of these species now thought to be present as self-sustaining populations.

While there are no native salmonid, percid or cyprinid fish in Australia, the alien species include representatives of these families (for example, rainbow trout, brown trout, redfin perch and carp). In addition, highly undesirable species, such as *Tilapia* sp and *Gambusia* spp, are also present.

Cyprinidae is the largest family of freshwater fishes in the world, with over 2000 species in 210 genera. Native species occur in all the tropical and temperate regions except Australia, New Zealand, Madagascar and South America.

However, they have been introduced to each of these previously cyprinid-free locations. Apart from carp, a number of other cyprinids have also been introduced to Australia, including goldfish (*Carassius auratus*), rosy barb (*Puntius conchonius*), roach (*Rutilus rutilus*) and tench (*Tinca tinca*).

Carp were first introduced to Australia in the 1850s as an ornamental fish, but had little impact until the 1960s, when the Boolara strain gained access to the Murray River in southeastern Australia. This particular strain adapted very well to Australian conditions (aided by floods in 1974 and 1975), and the carp population expanded dramatically.

Although there have been no systematic surveys of the distribution of carp in Australia, they are found throughout the Murray-Darling system, and all of southeastern Australia (Figure 1).

They are said to comprise 80 to 90 percent of the fish biomass of the Murray-Darling basin, and have favoured rivers that are highly regulated for irrigation. Carp have also been found in Tasmania and Western Australia, the former state having staged a decade-long eradication programme in the Ramsar-listed wetland (Interlaken) at Lakes Crescent and Sorell.

While four strains of carp have been described in Australia – koi, Prospect, Yanco, and Boolara – it is the latter which has been responsible for the massive extension of the range of carp. As an example, numbers of the Yanco strain in the Murrumbidgee Irrigation Area (MIA) changed very little from the time of its introduction. However, following the invasion of the Boolara strain, carp numbers in the MIA increased dramatically.

ENVIRONMENTAL ISSUES

So, why all the fuss about carp in Australian river systems, and why is there now such an effort to control them? Davidson (2002) has

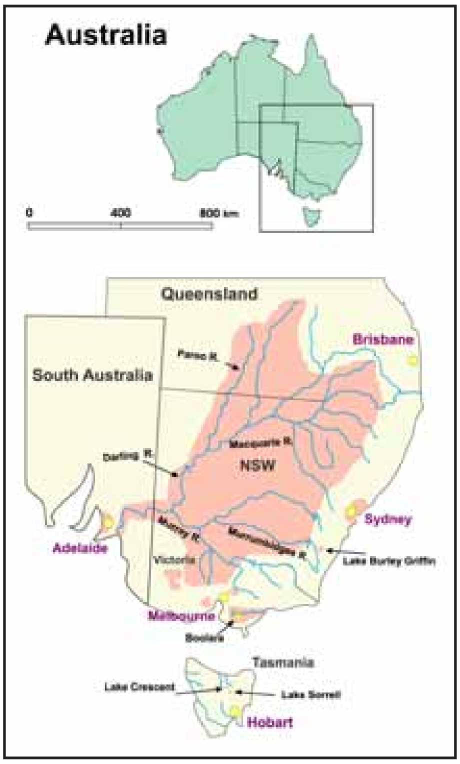

FIGURE 1: DISTRIBUTION OF CARP IN SOUTHEASTERN AUSTRALIA. THE GEOGRAPHICAL RANGE IS REPRESENTED BY THE SHADED AREA AND INCLUDES TWO LAKES IN TASMANIA

dubbed the carp the "rabbit of the river", an epithet that is perhaps an indicator of:

- **fecundity.** A female carp may produce a million eggs per year and can spawn a number of times in a year on a variety of substrates and environmental conditions.
- **adaptability.** While they prefer warm, still waters, they may be found in virtually all aquatic environments, including saline waters, except free-flowing rivers and mountain streams.
- **carrying capacity.** Carp have been known to dominate fish assemblages, in some cases up to 96 percent of the fish biomass in parts of the Murray-Darling Basin. In addition, carp are omnivorous and there are no native detritivorous fish to compete for food resources.

Because of the ubiquitous nature of carp in many areas of Australia, they are considered to be a national problem. Nevertheless, Davidson also says carp may be "the first scapegoat to have fins and scales", that is, perhaps carp are not responsible for all of the problems that have been attributed to them.

There has been very limited research on the environmental impact of carp in Australia, and very little of the information that is available has come from controlled experiments. In the years since their introduction, many environmental problems have been attributed to carp, but the only confirmed cause-and-effects have been:

- increased water turbidity. The feeding mechanism of carp, (sucking mud from the substrate, and then sieving it to detect food) no doubt increases turbidity
- a reduction in submerged vegetation, due to direct and indirect effects (uprooting of plants, and a reduction in light, respectively), and
- effects on the frequency of algal blooms, due to increased nutrients in the water.

Despite claims to the contrary, there is apparently little evidence for a role for carp in erosion of riverbanks, spread of disease, and declines in the number of native fish. In fact, declines in the numbers of various native fish have been documented prior to the expansion of carp, and it is likely that these declines are the result of human intervention (eg regulation of river flows, eutrophication of waters and increased salinity).

Carp are probably favoured by the declining state of many of our river systems, and therefore it is now considered that carp are not only one of the causes of the environmental degradation of our inland waterways, but also that their proliferation is an effect of the degradation.

Nevertheless, while hard evidence for the damaging effect of carp may presently be lacking, there is little doubt that the very high absolute, and relative, numbers of carp has a seriously deleterious effect on Australian freshwater ecosystems. For this reason, a variety of approaches have been examined in an attempt to control carp.

CONTROL STRATEGIES

Potential future strategies for controlling carp include methods that have been used in the past, together with other new and innovative approaches that are currently being evaluated through the Invasive Animals Cooperative Research Centre. Realistically, it is likely that controls will probably rely on a combination of approaches, and it should be known that there will be no "silver bullet" for controlling common carp.

Commercial harvesting and poisoning. This approach probably has little effect on established populations of carp, due to their fecundity. It appears that the increased resources that are available to those carp that escape a harvest, or a poisoning campaign, simply allow very rapid repopulation by the survivors. In other words, even a decimated population will bounce back very quickly.

"Daughterless carp". The underlying principle of this approach is to control carp by genetically interfering with sex ratios and biasing towards male-only offspring. If the approach proves successful, over many decades there would be a gradual reduction in the number of female carp in wild populations, with a concomitant reduction in the overall number of carp.

Immunocontraception. Any approaches to reducing carp fertility are at a very early stage of development, and would require much more work before being considered for implementation.

Environmental restoration. There is increasing interest in the idea of restoring the health of rivers, and in the process making them less attractive to carp and more appropriate for native fish. The reintroduction of carnivorous native fish, in particular, may contribute to a reduction in carp numbers by predation on young carp.

Physical controls. Carp in Australia have predictable season spawning movements, and display innate jumping behaviours that lend them to trapping and screening technologies. These are currently being investigated and trialed in the IA CRC.

Chemical controls. The large amount of ecological work done on carp in the wild shows that carp release and respond to chemical cues, including pheromones and environmentally borne cues. The IA CRC is investigating the isolation and evaluation of these potential cues.

Biological control with infectious agents. While spring viraemia of carp virus has been considered a potential candidate in the past, there are documented limitations with it. For that reason, attention in Australia has more recently focussed on a possible role for KHV.

POTENTIAL BIOLOGICAL CONTROL

Although there is some continuing debate over the taxonomy of KHV, it is currently recommended that it be classified as a herpes virus. Certainly, at a genetic level, it appears to be most closely related to two other herpes viruses of cyprinids – one causing carp pox, and the other goldfish haematopoietic necrosis – so it is now officially known as cyprinid herpes virus 3 (CyHV-3). Further research may yet provide some taxonomic surprises.

Regardless of the nature of the virus, however, observations recorded during natural outbreaks of KHV-associated disease, especially when combined with research results on the virus, ▶

LESIONS ASSOCIATED WITH FIELD CASES OF KHV INFECTION IN CARP.

have revealed a number of features that make KHV attractive as a potential biological control agent for carp in Australia.

1. It is known to cause high mortality in carp and koi carp. Not only are farmed carp susceptible to disease, but wild populations are also susceptible – an indication of this virus's virulence and an essential property if KHV is to be effective as a biological control agent for wild carp populations.

2. The susceptibility of a number of other species (including both cyprinids and non-cyprinids) has been tested in the laboratory, and not only are they insusceptible to KHV, but there is also no evidence that they are even capable of developing a subclinical infection during which they might transmit the virus to susceptible carp.

The susceptibility of a variety of species, both native and introduced, that are important in Australian aquatic ecosystems still needs to be tested, but at present, KHV appears to be highly specific for a particular species, ie carp and koi carp.

An characteristic of KHV is that laboratory work has indicated that carp are susceptible to very low levels of virus

3. Both field and laboratory evidence suggests that KHV will affect carp of any age. For example, it has been estimated that in natural outbreaks of disease, mortality may vary from 70 to 100 percent, the implication being that many, if not all, age groups are affected, (although it is difficult to understand how such figures can be obtained with any accuracy in a natural environment).

Meanwhile, laboratory studies have suggested that although larvae may be insusceptible, very high mortality will occur in fish weighing between 2.5g and 6g. The mortality then apparently declines as fish become bigger and older.

Obtaining a more precise understanding of this age-related mortality would be very important for any future attempts to develop mathematical models of the potential effect of KHV on carp in Australia.

4. A very attractive characteristic of KHV when considering its potential as a biological control agent is that laboratory work has indicated that carp are susceptible to very low levels of virus. Given that in an aquatic environment large concentrations of virus might be diluted very rapidly, even in a slow-moving stream, this would seem to be an essential requirement for a possible biological control agent. There appears to be little, if any, available information on the potential for vertical transmission.

The outcome of KHV infection of carp is very dependent on environmental temperatures, with maximum losses tending to occur when water temperatures range from 17°C to 28°C.

Outside this range, mortality drops off considerably, eg, laboratory studies have suggested that the virus is relatively innocuous at 13°C or 30°C. Once again, these observations have important implications for the use of KHV as a control agent in Australia, where carp are said to survive in waters ranging in temperature from 2°C to 30°C.

While the innocuity of the virus at low temperatures is probably due to the concomitant low body temperatures of the host (and therefore slow virus replication in host cells), the lack of virulence at higher temperatures more likely reflects the level of stability of KHV at those temperatures.

There is only limited information available on the persistence of infectious virus in water at different temperatures, and this deficiency will need to be addressed in order to fully understand the potential of KHV as a biological control agent.

SUMMARY

It is the specificity of KHV for carp, the sensitivity of carp to infection with the virus, the subsequent high mortality in the host species (even in wild populations) and the capacity to affect carp of many ages that make KHV such an attractive option for the biological control of carp in Australia.

Because KHV is still not present in Australia (and is therefore a so-called exotic virus), the IA CRC has funded the Fish Diseases Laboratory at the high-security CSIRO-Australian Animal Health Laboratory to examine the potential of KHV as a biological control agent for carp in Australia.

These preliminary studies will be strictly confined to the laboratory, and even if the results are encouraging, it is likely to be many years, and after much public consultation, before the virus would be considered for use in a multi-pronged attempt to control carp in Australia.

FURTHER READING

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Koehn JD and MacKenzie RF 2004. Priority management actions for alien freshwater fish species in Australia. NZ J Mar Freshwater Res 38 (3). pp457-472

Roberts J and Tilzey R eds 1997. Controlling carp – exploring the options for Australia. CSIRO Land and Water, Griffith

BOOK REVIEW:

Basic Atlas of Normal and Abnormal Blood Cells in Farmed Tilapias

Atlas Básico de las Células Sanguíneas Normales y Anormales en Tilapias Cultivadas

CD-ROM edited and published by Patterson Peddie Consulting Ltd, Carrickfergus, United Kingdom (2007). ISBN: 978-0-9553926-2-7
Contact info@pattersonpeddie.com for further information.

The authors of this CD-ROM are our esteemed colleagues Drs David and Gina Conroy. The CD-ROM is a bilingual document in Spanish and English and contains 32 pages of text and 61 full-colour figures. It describes and illustrates in a very easy manner the different types of red and white blood cells that may be detected in blood films stained by Giemsa's method, as much in healthy as in diseased tilapias.

The figures are of an excellent quality, and facilitate recognition of the blood cells in smears stained by Giemsa's method, obtained from tilapias. This extremely practical document will be of great value and utility for tilapia farmers and their technical personnel, since it shows certain cell types present in tilapias affected by nutritional and infectious problems.

The product has 17 key references to the literature on aspects of tilapia haematology. This CD-ROM is highly recommended to all of the tilapia farming community for its regular use on the farm or in other centres of production.

– TSU Eugenio García-Franco, President,
Venezuelan Aquaculture Society, Valencia, Venezuela

This is excellent material for those with an interest in tilapia haematology; it is very didactic, and easy to consult. This CD-ROM can be used as an effective guide to the identification of the different types of blood cells in tilapias. It makes the science of tilapia haematology/blood cells accessible, and serves as a quick tool for diagnosing different pathological conditions in tilapias.

– Dr Gustavo A Alvis,
National Aquaculture Director, SOLLA SA, Colombia

WEBSITE REVIEW:

OESA (Spanish Aquaculture Observatory)

The Ministry of Education and Science of the Spanish government, in close collaboration with universities and other aquaculture concerns and services in Spain, has published an interesting and very useful series of six texts, in Spanish, dealing with different aspects of aquaculture for use as training material for technical and professional personnel.

The texts concerned include:

1. Reproducción en Acuicultura (Reproduction in Aquaculture)
2. Alimentación en Acuicultura (Feeding in Aquaculture)
3. Nutrición en Acuicultura I (Nutrition in Aquaculture I)
4. Nutrición en Acuicultura II (Nutrition in Aquaculture II)
5. Patología en Acuicultura (Pathology in Aquaculture)
6. Genética en Acuicultura (Genetics in Aquaculture)

Each book is a collaboration by a number of well-known Spanish and international authors, and is well illustrated with photographs and figures. A bibliography is included in each text. The publications are intended to be used in the training of aquaculture personnel, and the contents of each book comply with this stated aim.

The publications are available on-line in pdf format. Further information can be obtained by contacting observatorio-acuicultura@csic.es. They can be highly recommended for consultation and use by Spanish-speaking aquaculture professionals.

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THE SHRIMP INDUSTRY IN INDIA

BY SATYAJIT S BELSARE, SANTOSH N KUNJIR, SOMNATH R YADAV
(COLLEGE OF FISHERY SCIENCE, SEMINARY HILLS, NAGPUR, MAHARASHTRA STATE, INDIA)

By virtue of its vast geographical stretch and varied terrain, India supports a wide variety of inland and coastal wetland habitats. Its vast coastline of 8118km, distributed in nine coastal states and four union territories, has a wide variety of natural coastal ecosystems. The eastern coast is low-lying and dominated by lagoons, marshes and deltas, while the western coast is dominated by rocky shore.

India has an exclusive economic zone (EEZ) of 2.02 million sq km and a continental shelf area of 0.506 million sq km. The potential yield of the marine fishery resources of India is 3.9 million tonnes. The fish production of India during the year 2003-2004 was 6.4 million tonnes, of which the marine sector contributed 2.94 million and inland 3.46 million tonnes.

The fisheries sector has played an important role in the national economy through food supply, employment generation and income. During 2002-03, the fisheries sector contributed Rs 35,482 crores to the total gross domestic product, forming 1.43 percent of the total.

There is currently a danger of declining capture fishery production due to overexploitation of fishery resources and habitat degradation. Aquaculture, which entails managed farming or culture of organisms in fresh, saline or brackish water, is seen as a viable means of fisheries diversification, increased fish production and rural employment.

Attracted by the short development periods, high return on investment and good international demand for shrimp in developed nations, shrimp farming has expanded rapidly. Thus shrimp farming has changed from traditional, small-scale business into a global industry.

In India, commercial shrimp farming, which is restricted to monoculture of the black tiger shrimp, *Penaeus monodon*, started gaining a foothold during the mid-1980s. It was a relatively late start in India; by this time, shrimp farming had reached a peak in most of the neighbouring Asian countries, especially the Philippines, Indonesia, Taiwan, China and Thailand.

TABLE 1: POTENTIAL AREA AND AREA UNDER SHRIMP FARMING IN EACH STATE

STATE	POTENTIAL AREA	AREA DEVELOPED	% OF POTENTIAL AREA
WEST BENGAL	405,000	50,405	12.44
ORISSA	31,600	12,877	40.75
ANDHRA PRADESH	150,000	76,687	51.12
TAMIL NADU	56,000	5,286	9.44
PONDICHERRY	800	130	16.25
KERALA	65,000	14,106	21.70
KARNATAKA	8,000	1,910	23.87
GOA	18,500	310	1.68
MAHARASHTRA	80,000	1,281	1.60
GUJARAT	376,000	2,271	0.60
TOTAL	1,190,900	165,263	13.88

The boom period of commercial shrimp culture in India started in 1990, with shrimp farmers from the states of Andhra Pradesh and Tamil nadu on the eastern coast, and Gujarat on the western coast, taking up this activity on a huge scale. The area under shrimp farming increased from 65,000ha in 1990 to 165,263ha in 2004 (See Table 1).

Most of the coastal states in India were new to commercial shrimp farming, and there was unplanned and unregulated mushrooming of farms around available water sources. This led to negative impacts such as serious ecological degradation, socio-economic problems, social tension, mangrove destruction, isolation of villages and salination of agricultural land and freshwater wells.

Moreover, in 1995-96, the outbreak of white spot disease (WSD) played havoc with the industry, and its repeated occurrence demoralised shrimp farmers.

A TYPICAL SHRIMP FARM IN INDIA

BROADCASTING OF FEED

A HEALTHY SHRIMP

As a result, the Aquaculture Authority of India was constituted under Section 3 (3) of the Environment (Protection) Act, 1986 as per the judgment delivered by the Supreme Court of India on December 11, 1996. The primary objective of this authority, functioning under the administrative control of the Ministry of Agriculture in the Indian government, is regulating shrimp farming in the coastal areas and promoting sustainable shrimp farming.

PRESENT STATUS

It is now generally agreed that good management practices can make shrimp farming highly sustainable, and the procedures and methodologies for sustainable shrimp farming are now being practised in India with demonstrably effective results. These include effective and holistic farm management practices, production of hatchery and disease-resistant shrimp seed, domestication of broodstock and diversification, including alternative candidate species.

Major research initiatives in the past decade have led to the introduction of modern diagnostic tools such as polymerase chain reaction (PCR) techniques to check the presence of white spot virus in the shrimp post-larvae prior to stocking, and helped reduce the risk of disease outbreak. The Central Institute of Brackish Water Aquaculture at Chennai has developed a rapid-diagnostic PCR test kit that has substantially reduced the cost of testing. Presently, PCR testing laboratories have been established under government and private sectors (Table 2).

TABLE 2: STATE DISTRIBUTION OF PCR LABORATORIES

STATE	NO. OF PCR LABS
GUJARAT	1
MAHARASHTRA	2
KARNATAKA	4
KERALA	11
TAMIL NADU	21
ANDHRA PRADESH	41
ORISSA	5
WEST BENGAL	2
TOTAL	87

The introduction of protocols relating to good management practices and bio-security are additional developments that have considerably reduced the chances of the spread of WSD. Of the many good management practices that are currently in vogue and adopted by the farmers in the country, low stocking densities have proved to be successful in attaining sustainability.

It is now reported that a high percentage of farmers are adopting low stocking densities in the country and enjoying a high success rate as a result. In terms of economics, too, the low stocking densities are preferred. Adoption of low stocking densities will be one of the key elements of sustainability in the years to come, and needs to be promoted among the shrimp farmers.

Feed accounts for around 60 percent of the operating costs of shrimp culture, and the development and use of compound feeds has been a major advancement in the successful expansion of shrimp farming. Bio-remediation through various microorganisms, enzymes and probiotics added to the feed has proved to be useful.

Availability of good quality feed has not been a serious problem. Along with feed quality, feed management techniques are also important for shrimp farming profitability. The best shrimp feed can be an expensive fertiliser if not managed properly.

Realising the potential demand that exists for shrimp feed, several Indian and foreign joint-venture companies have established feed mills. India has about 28 shrimp feed mills, both big and small, ▶

TABLE 3: STATE DISTRIBUTION OF SHRIMP FEED MILLS

STATE	NUMBER
KERALA	1
TAMIL NADU	1
ANDHRA PRADESH	25
WEST BENGAL	1
TOTAL	28

SOURCE WATER AS A DUMPING GROUND FOR WASTE DISCHARGE

with an installed capacity of about 0.38 million tonnes and an annual production and sale of about 150,000 tonnes (Table. 3). The price of feed ranges from US$1 to $1.22.

Of all the ingredients, fish meal is the most critical ingredient, as it is required in large quantities. The shrimp farming sector has received severe criticism in recent years for excessive use of fish meal when marine fish catches are declining. It therefore becomes important to develop fish meal substitutes over time. Several researchers have tried to replace fish meal, either partially or completely, with different animal and plant protein byproducts, but have met with limited success.

In terms of seed availability, until the mid-1980s only a few government hatcheries were operating. Thus, seed availability was a major bottleneck. During the late 1980s, the Marine Product Export Development Agency demonstrated large-scale hatchery technology at the shrimp research and production centres TASPARC and OSPARC. Since then, growth in the shrimp hatchery sector has been phenomenal. With the establishment of 351 state-of-the-art hatcheries with a total capacity of 14.3 billion post-larva per annum, this problem seems to be temporarily solved. However, the availability of quality seed still persists.

The fast pace of development in the shrimp farming sector has brought to focus the use of a wide variety of drugs, chemicals, antibiotics and hormones by fin and shellfish farmers. The use of such drugs and chemicals is indiscriminate, and is mostly due to the lack of awareness among the farmers and a strong marketing strategy by the drug and chemical manufacturing companies.

The easy availability of a large quantity of inferior quality chemicals has also led to an indiscriminate use of antibacterials and other chemicals. These products are sold to farmers to enhance the productivity of the water bodies, increase resistance in fin/shellfish, promote growth and protect against diseases such as WSD. Bacterial drug resistance (Karunasagar *et al* 1994) and tissue residues are some of the serious problems that need to be addressed.

Aquaculture waste management has become a very serious issue. The majority of the farms use source water as a dumping ground for waste discharge (See above). Raw effluent rich in organic matter and waste feed is released directly into water sources without any treatment or settlement. This has led to problems with disease transmission and water quality.

Crop losses due to natural calamities like flooding and cyclones have become common. The absence of buffer zones and the destruction of mangroves further aggravated these problems. The tsunami that struck the southeastern coast of India on Boxing Day 2004 is an example of this.

Resource mobilisation and financing for developing shrimp farming has been relatively well organised, because shrimp farming is no longer seen as a traditional occupation but as a capital-intensive industry. Public and private banks are extending loans to the industry on a priority basis. Several private companies have gone public and have raised sufficient funds through public issue of equity shares.

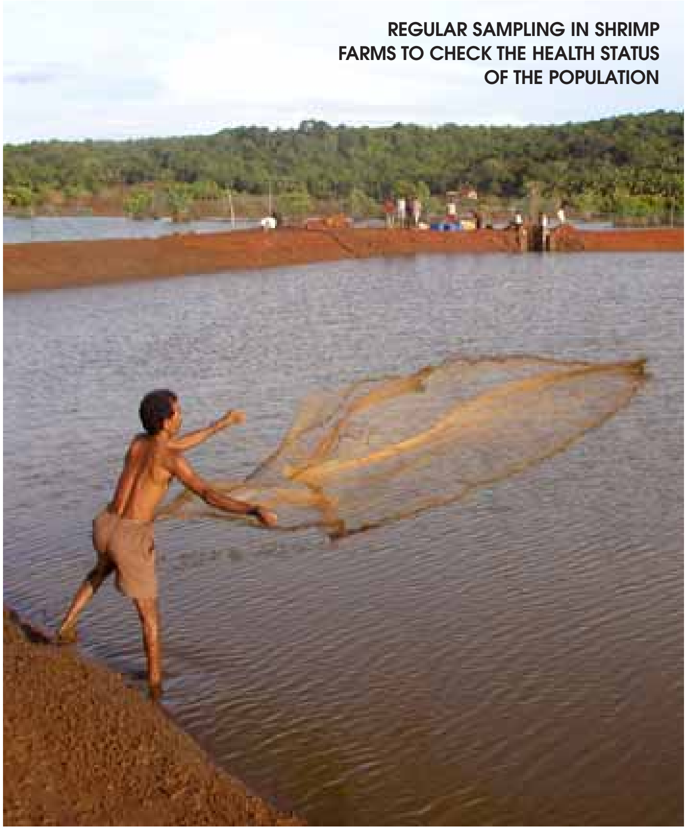
Insurance companies are also involved, with risks associated with natural calamities and diseases being insured and crop losses protected. Several national and international corporate houses and private companies have realised the enormous potential for investment opportunities, and have taken advantage of the government's liberalisation policy by setting up shrimp hatcheries and farms.

The concept of satellite and franchise farming as promoted by big industrial houses is well established and is working reasonably well. In these systems, the industrial house acts as the nucleus for several hundred small and medium-scale extensive farmers. Technical input in the form of consultancy, and other inputs in the form of seed, feed and disease and water quality management are provided.

The company buys back the end product at the prevailing market price. In addition to satellite farming, many small companies have long-term buy-back arrangements with big industrial houses and multinational companies.

EXTENSION SUPPORT

Small farmers who own less than 2ha of water area comprise more than 90 percent of the shrimp farming sector in India. Most of them



REGULAR SAMPLING IN SHRIMP FARMS TO CHECK THE HEALTH STATUS OF THE POPULATION

HARVESTING WITH THE HELP OF A BAG NET

are poor and illiterate. Further, about 100,000 shrimp farmers are distributed in the coastal area of the country.

Under this scenario, the extension requirement for building capacity and creating awareness among the farmers is enormous. Very few institutions like the MPEDA, the Brackishwater Fish Farmer's Development Agency, central fisheries research institutes and fisheries colleges provide technical support in this sector.

Case studies conducted by various research institutes have also indicated that a major source of information for farmers is the consultants or technicians of the input dealers. The formation of "aqua clubs" in some shrimp farming areas in Andhra Pradesh Gujarat and Tamil Nadu is a significant step, and the state governments should promote such movements to encompass all of India's shrimp farmers.

HEALTH MANAGEMENT CONSTRAINTS

Indian shrimp farming is still in its infancy. The research effort that has gone into shrimp disease studies has been very limited, and hence very limited progress has been made towards health management. The majority of the health management techniques and chemo-therapeutic practices that are being followed are largely adopted from South East Asian countries. Some of the important health issues that require immediate attention are:

- Dependence on a common water source is the root cause of many problems. The majority of the farms are owned by small farmers (<2ha), and it is difficult for them to make provisions for reservoir and sedimentation tanks. Lack of reservoir and sedimentation tanks makes it impossible to undertake prophylactic measures.
- Insufficient number of disease diagnostic centres and trained personnel for disease diagnosis, especially on the western coast of India.
- Lack of awareness among farmers on the need for proper disposal of dead shrimp and waste water during and after a disease outbreak, and failure to implement routine health monitoring programmes.
- Disease certification programmes are not widely practised throughout India. No effective regulations exist to check the misuse of antibacterials and other therapeutic chemicals.

RECOMMENDATIONS

Research and developmental work in the following areas would help evolve standard scientific health management tools:

- explore the use of specific pathogen-free broodstock and healthy post-larvae
- explore the development and use of specific pathogen-resistant broodstock
- determine the carrying capacity of brackish water sources before developing farms and earmarking areas for shrimp farming
- establish more disease diagnostic centres in shrimp farming belts, especially on the western coast of India
- create a national centre of excellence in shrimp disease research, and
- initiate work on kinetics of antibacterials in shrimp tissue, tissue residues, bacterial drug resistance, etc.

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STUDIES ON RED MARK SYNDROME IN THE UNITED KINGDOM

BY DRS DAVID VERNER-JEFFREYS, MYRIAM ALGOET, STEVE FEIST, KELLY BATEMAN, EDMUND PEELER (CEFAS WEYMOUTH LABORATORY, WEYMOUTH, UK), AND EDWARD BRANSON (RED HOUSE FARM, MONMOUTH, UK)

FIGURE 1: RAINBOW TROUT AFFECTED BY RMS

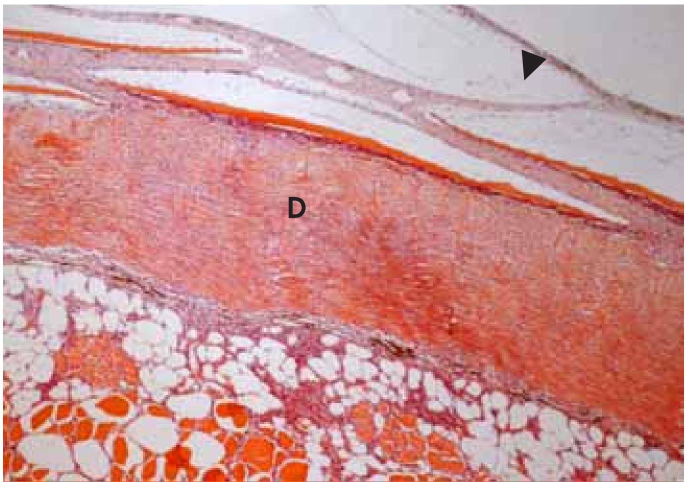
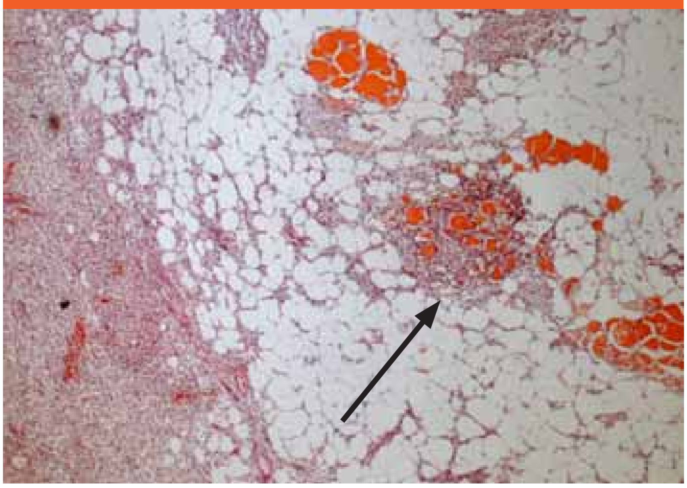


FIGURE 2: SECTIONS OF SKIN AND MUSCLE TAKEN FROM AN RMS LESION. NOTE EROSION OF THE EPIDERMIS (ARROW HEAD), INFLAMMATORY CELLS IN THE DERMIS (D) AND INTO THE UNDERLYING ADIPOSE TISSUE AND MUSCULATURE, AND ASSOCIATED MYOFIBRILLAR DEGENERATION (ARROW)



Red mark syndrome, or RMS, is a transmissible disease of rainbow trout characterised by the appearance of multiple ulcerated skin swellings of varying intensity on the flanks of affected fish (Figure 1).

It shares some similarities with strawberry disease (SD), not least that the infectious agent responsible has yet to be definitively identified[1]. However, there are enough differences from SD, in terms of both its epidemiology and pathological effects, to regard RMS as a separate condition.

RMS causes losses to farmers in that affected fish are downgraded at harvest. There are also some reports of increased losses of RMS-affected stock during grading and other stressful procedures.

HISTORY

The condition was first noted in Scotland in 2004. In early 2005, RMS was diagnosed for the first time in fish farmed in England. Farmers in both Scotland and England report that the disease is prevalent at low temperatures (less than 12°C), and early signs of the condition can include severe scale loss, prior to the emergence of the characteristic external lesions.

DISEASE INVESTIGATIONS

As part of our remit to investigate new and emerging diseases of farmed and wild fish, outbreak investigations of the two English farms suffering from RMS were instigated. Epidemiological analysis indicated that the condition on both farms originated in stocks of fish sourced from the same Scottish hatchery. This hatchery had, itself, suffered from RMS outbreaks in the past.

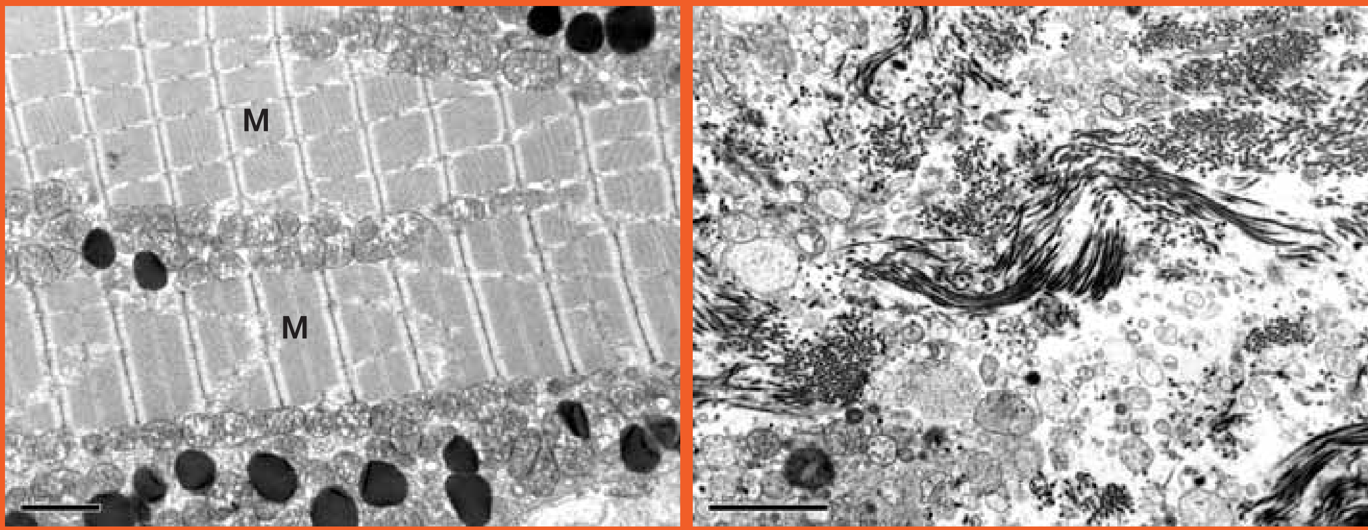
There were also indications that the condition had spread from the imported, infected stock to other batches of fish on both farms. Samples of RMS-affected fish from both farms were returned to the Cefas Laboratory for further tests, in an effort to learn more about the disease.

The affected fish presented with a range of lesions of differing severity. However, despite extensive analysis, including histopathological investigation of preserved material (by light and electron microscopy), as well as mycology, bacteriology and virology, no single potential disease agent could be consistently isolated from affected fish.

Histopathologically, the condition was characterised as a non-suppurative dermatitis with extensive lymphocytic infiltration. Interestingly, when lesions were examined, it was noted that even in the very early stages, areas of necrosis and associated inflammatory foci were seen well below the external epithelium in the dermal layer. What this means, in essence, is that the disease works "inside out" (see Figures 2 and 3).

The first observable stage of the disease is necrosis in the area where the scales are attached to the dermis. Only after this has taken place will scales then be raised or removed and the integument breached, with formation of the characteristic external lesions.

FIGURE 3: TRANSMISSION ELECTRON MICROSCOPY OF AN RMS LESION



A: NORMAL STRUCTURE OF THE MYOFIBRILS (M) IN LONGITUDINAL SECTION WITH PROMINENT MITOCHONDRIA AND DARK STAINING LIPID DROPLETS.

B: TEM OF INFLAMMATORY FOCUS, REVEALING CELLULAR NECROSIS AND DISASSOCIATED CONNECTIVE TISSUE FIBRES. NO PATHOGENS WERE DETECTED

Farmers note that lesions are often more obvious after grading or other stressful handling procedures, which fits with these findings. Another observation was heart pathology in up to 20 percent of the fish examined.

We have run a transmission trial at the Weymouth Laboratory in an effort to see if the disease can be passed to unaffected fish in the laboratory. When we cohabited 20 RMS-affected fish (average size 410g) with 40 naïve rainbow trout (average size 75g) in the same tank, only nine of the naïve fish survived to the end of the 96-day trial.

Mortalities in the naïve group were not attributable to a particular disease agent and finished by day 42. Of the remaining nine survivors, four showed external lesions characteristic of SD and RMS. Although this data is interesting, it also serves to illustrate the difficulties of working with a disease where a causative agent has not been identified.

Currently there is no evidence that the mortalities in the trial were associated with the syndrome. It is entirely possible that the RMS-affected fish were also carrying other diseases that killed the naïve fish. Although it appears likely that the condition was transmitted from the RMS-affected fish to the surviving naïve fish, this cannot be stated with certainty since a confirmatory diagnosis, apart from the presence of external lesions, is lacking.

The trial also provided other useful information. Out of the 80 RMS-affected fish transported to our laboratory, only one died during the trial, and surviving fish showed signs that the initial lesions had healed.

We also noted no difference between a group of RMS-affected fish that were maintained on the diet they had previously been fed at the farm, and a group fed a control laboratory diet. Both groups appeared to recover from the condition, suggesting that a dietary link was unlikely.

RECOMMENDED TREATMENTS

Treatment is complicated by the lack of an identified disease agent. Farmers report that RMS responds to application of in-feed antibiotics, particularly oxytetracycline (OTC). Nevertheless, this should be considered a temporary measure because continued application of OTC is to be discouraged, as it may lead to the emergence of antibiotic resistance.

Another problem with treating with OTC is that the withdrawal period is 700 day-degrees. It is also reported that affected fish will often spontaneously heal themselves if left untreated, as found in our tank experiments.

There is no evidence that supplementing the diet with vitamin C will affect the progression of RMS, unlike SD, where encouraging results have been reported.

LIKELY CAUSES

Although no pathogen has been consistently associated with the condition, observation that the condition responds to antibiotic treatment suggests a bacterial cause or influence, as with SD.

FUTURE WORK

Agreement on "case definitions" for RMS, SD and other possibly linked conditions is being sought to enable accurate description, differential diagnosis and tracking of the spread of the diseases. There is a proposal by Hugh Fergusson (Institute of Aquaculture, Stirling) to refer to both these conditions as strawberry disease. The condition we refer to here as RMS could then be termed coldwater strawberry disease, and what is more commonly understood to be SD in the UK could be termed warm water strawberry disease.

It is possible that the similar clinical signs for both syndromes are caused by a generalised hyperinflammatory host reaction in the skin layers to the presence of different initial causes, such as different bacterial antigens.

If farmers note the appearance of symptoms similar to those described in their stock, they should contact the Cefas Fish Health Inspectorate for further advice.

ACKNOWLEDGEMENT

Funding for these studies was provided by Defra under contracts FC1151 and FC1166.

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EDITOR'S NOTE: Since this article was first published, Edward Branson has sadly died.

MYCOBACTERIOSIS AS A POTENTIALLY IMPORTANT TILAPIA DISEASE

BY PROFESSOR DAVID CONROY (PHARMA-FISH SRL, MARACAY, ESTADO ARAGUA, VENEZUELA)

Mycobacteriosis (also known as piscine tuberculosis) is a disease produced by non-motile Gram-positive rods belonging to the genus *Mycobacterium* that have the property of resisting the destaining action of mineral acids and alcohol. For this reason they are referred to as acid-alcohol-fast bacteria (AAFB).

Various species and nominal species of mycobacteria have been reported as causing spontaneous cases of mycobacteriosis in a wide variety of wild, cultured and ornamental fish of fresh water and marine origin. A now-classic list of susceptible species included many cichlids (Nigrelli and Vogel, 1963).

In this respect, it is important to mention a report by Chávez de Martínez (1990: cited by Martínez-Palacios and Ross, 1994, and Chávez de Martínez and Richards, 1991) referring to the detection of cases of mycobacteriosis in “mojarras” (*Cichlasoma urophthalmus*) being cultured in Mexico, and where a clear relationship was established between the infection and a deficiency of vitamin C in the feed.

This is just one concrete example of the importance which mycobacterial infections might acquire in aquaculture activities involving native and introduced species of cichlids - including tilapias - in the Americas.

Roberts and Mathieson (1979: cited by Roberts and Sommerville, 1982) reported cases of spontaneous mycobacteriosis in wild specimens of *Sarotherodon andersonii* and *Tilapia sparmanii* from the Okavangu swamp in Botswana.

Roberts and Haller (unpublished data cited by Roberts and Sommerville, op cit) detected cases of spontaneous mycobacteriosis, caused by *Mycobacterium fortuitum*, in Nile tilapias (*Oreochromis niloticus*) under conditions of intensive culture in Kenya. Although the mortality levels in this latter case were not high, Roberts and Sommerville (*op cit*) expressed very clearly their belief that mycobacteriosis could be the cause of very significant mortalities in tilapias, particularly in cases where these fish were fed on raw fish and/or fish offal.

Noga et al (1990) described cases of mycobacteriosis in Mozambique tilapias (*Oreochromis mossambicus*). The affected tilapias showed small focal granulomas in the kidney, liver and spleen. The present authors are aware of spontaneous cases of mycobacteriosis which occurred in juvenile red tilapia hybrids raised in a production centre in a Central American country, and from which *Mycobacterium fortuitum* was isolated.

In Venezuela, Cabrera and Jiménez (2004) obtained 11 isolates of mycobacteria from samples of the skin and superficial muscle tissue of fish from 60 feral specimens of *O mossambicus* that were collected from two sites in Lake Valencia. The sampled tilapia ranged between 304g and 555g in weight, and between 19cm and 34cm in length. Reported salient clinical signs included patchy areas of necrosis on the fins, and pale livers with localised haemorrhagic foci. The Venezuelan isolates were initially identified as *M chelonae*, *M fortuitum* and *M marinum*.

Mycobacteriosis is thought to be acquired through the ingestion of AAFB present in the environment, and which usually have their origin in detritus derived from dermal lesions, faeces etc shed by fish that are already infected. The disease can also be transmitted when non-infected fish nibble at the corpses of fish that have died from the infection.

The possibility of mycobacteria being transmitted by the feeding of infected raw fish and offal must also be considered (Roberts and Sommerville, 1982). The characteristic clinical signs include loss of normal skin pigmentation, fraying of fins, anorexia, lethargy, emaciation and stunted growth.

The eyes may be swollen (exophthalmos) or have a sunken appearance, and skeletal abnormalities (eg lordosis, kyphosis, scoliosis) may also be present. The body surface frequently displays areas of localised ulceration.

Internally, there is evidence of a generalised granulomatosis, which permits the presence of numerous greyish-white or yellowish-white nodules to be detected in the kidney, liver, spleen and other organs. This disease has a chronic course, and clinical signs may not always become apparent until the condition is quite advanced in its development.

The infection produces a typical chronic inflammatory response in the fish, where the bacteria themselves are surrounded by macrophages. The individual granulomas have a necrotised centre containing bacteria and cellular detritus, often accompanied by numerous melano-macrophages.

In cases where bacteriological studies have been undertaken on tilapias affected by mycobacteriosis, the species *Mycobacterium chelonae*, *M fortuitum* and *M marinum* have been isolated and identified. These three species of mycobacteria are considered to be potentially pathogenic to man (Frerichs, 1993).

As a basic precautionary measure, therefore, it is essential that anyone who may have to handle tilapias (including personnel in the processing plants who eviscerate and fillet the fish) wear rubber gloves and other protective clothing in order to avoid any accidental infection. It must be pointed out that mycobacteria in fish material (eg muscle tissue and viscera) are capable of surviving freezing.

The diagnosis of mycobacteriosis in tilapias must include a careful and systematic examination of the kidney, liver, spleen and other organs to detect the presence of any type of granuloma or nodule. Where granulomas are observed, duplicate smears should be taken from the affected organ or tissue, carefully heat-fixed, and stained by the Gram technique and by the modified technique for acid-alcohol-fast bacteria described by Bullock (1971), respectively.

The stained smears are examined microscopically, and the detection of Gram-positive and acid-alcohol-fast rods, which appear bright red against a blue background, is sufficient to establish a presumptive diagnosis of mycobacteriosis in the fish. Bacteria that are not acid-alcohol-fast stain blue with the staining technique recommended by Bullock.

Additional presumptive evidence for the presence of this disease is obtained by fixating representative material and preparing it for examination as histological sections. The recommended staining

TABLE 1: BASIC DIFFERENTIAL CHARACTERISTICS OF MYCOBACTERIUM SPP ISOLATED FROM TILAPIAS

TEST	REACTION		
	M chelonae	M fortuitum	M marinum
Morphology	rod	rod	rod
Gram stain	+	+	+
Acid-alcohol-fast stain	+	+	+
Motility	-	-	-
Catalase	+	+	+
Pigment production	-	-	+ (Ph)
Type of growth	R	R	S
Nitrate reduction	-	+	-
Tween 80 hydrolysis	-	-	+
Tellurite reduction	+	+	-
Arylsulphatase (3 days)	+++	+++	
Arylsulphatase (21 days)			+
Growth on MacConkey agar	+	+	-
Growth at 20°C	+	+	+
Growth at 37°C	-	+/-	+
Growth at 45°C	-	+/-	-
Runyon Group	IV	IV	I

R = rapid growth. S = slow growth. Ph = photochromogen

procedure for these sections is the Fite-Faraco technique or the Kinyoun technique. In both cases, the acid-alcohol-fast bacteria stain bright red, and certain of the histological changes that have occurred in the tissues can also be observed in the sections.

Fish mycobacteria are susceptible to isolation in special culture media such as Löwenstein-Jensen's medium, Petragnani's medium and Middlebrook's LH10 agar, but attempts to isolate them *in vitro* are not always crowned with success.

The tissues intended for isolation purposes must previously be treated by the Petroff technique to concentrate the mycobacteria, following which the centrifuged material is carefully neutralised and inoculated onto the corresponding culture medium, which must be incubated for up to three months at 25 to 30°C.

Identification of piscine mycobacteria to the species level is a complicated and time-consuming procedure which - for public health reasons - must be entrusted to a laboratory duly approved by the competent authorities of the country concerned to undertake such procedures.

For quick reference purposes, and as a simple guide, certain of the basic differential characteristics of *Mycobacterium chelonae*, *M fortuitum* and *M marinum* have been summarised. (See Table 1.)

The prevention of mycobacteriosis in tilapias (as well as in other types of farmed fish) must include adopting and implementing very strict hygienic measures on the farm or in the production centre. The environmental and rearing conditions must be maintained at their optimum levels.

Equipment such as nets and rubber boots must be thoroughly surface-disinfected before use and immediately afterwards by immersion for 20 minutes in a fresh aqueous solution of a commercial iodophor compound with a pH of seven and a concentration of active iodine (as I2) of 150ppm.

At the conclusion of the disinfection process, any residual iodine can be neutralised by adding crystals of sodium thiosulphate. The chronic nature of mycobacteriosis in fish means that it is often too late for any remedial action to be taken once the first cases have been observed and diagnosed.

The application of antimicrobial compounds in an attempt to treat established cases of this infection in tilapia farming facilities is unadvisable, as the results are not always fully successful, and the application of such compounds could contribute to the appearance of antimicrobial-resistant strains of the bacterium.

It is important to understand that mycobacteriosis is only one of several infections which produce manifestations of granulomatosis in tilapias. For that reason, great care must be exercised to differentiate this disease from others such as staphylococcosis, streptococcosis, "piscirickettsiosis"/francisellosis and ichthyophonosis, which also produce a somewhat similar clinical picture.

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INTERNATIONAL: SPANISH VERSION OF DIPNET SUMMARY

A Spanish language version of the DIPNET non-technical summary is now available for downloading at the project website. The translation has been accomplished by Dr Nacho de Blas of the Veterinary Faculty at the University of Zaragoza.

See www.dipnet.info and look under Document, subsection Reports and Project Deliverables.

SCHERING-PLOUGH ACQUIRES INTERVET

Schering-Plough is to acquire Organon BioSciences NV, the human and animal health care businesses of Akzo Nobel NV, for approximately US$11 billion in cash ($14.4 billion based on the closing exchange rate on March 9).

The acquisition was part of the strategy to transform Schering-Plough into a global high-performance company, said the chairman and chief executive officer, Fred Hassan.

"It is the right deal at the right time," Hassan said. "Schering-Plough will become a leading animal health care company, with premier biologics capabilities."

Intervet is one of the top three animal health care companies globally, with sales of its products to treat a broad array of animals and disease states reaching approximately $1.5 billion in 2006.

The businesses are strong and complementary. Intervet's products include:

- Nobivac, a range of canine vaccines
- Panacur, a de-wormer
- Bovilis, a bovine biological for disease control and eradication, and
- Nobilis, a poultry vaccine to keep flocks free from infectious disease.

Schering-Plough Animal Health has a strong business in treatments for cattle and companion animals, including Nuflor, an antibiotic for cattle, swine and fish; Banamine, an anti-inflammatory for cattle, horses and swine, and Otomax, a canine otic ointment.

Schering-Plough Animal Health and Intervet will continue to operate at their existing locations.

Boxmeer in the Netherlands will become the headquarters of the global animal health business, which will maintain a strong pharmaceutical leadership team in the United States.

"We expect a smooth and efficient transition that will allow us to unlock more value from the Organon BioSciences products and pipeline than would have been possible on a stand-alone basis," said Hassan. "We have great respect for the talented people of Organon BioSciences, and look forward to working together as we continue to build a new kind of health care company."

The sale of Organon BioSciences was a major milestone in the history of Akzo Nobel, said the chief executive officer of Akzo Nobel, Hans Wijers, "It is a fundamental step towards our goal of creating a focused international industrial player. At the same time, we are convinced that we have found a good home for Organon BioSciences."

The partnership with Schering-Plough would give more scope to develop Organon BioSciences' "unique capabilities," said Wijers.

The transaction, announced on March 12, is expected to close by the end of the year.

Schering-Plough said it expected to achieve annual synergies of $500 million within three years. It will finance the acquisition through a mix of cash, debt and equity. The transaction is subject to regulatory approvals. Shareholder approval was not required.

UK: CONSUMERS APPROVE SALMON WELFARE

UK consumers perceive salmon as having one of the best animal welfare standards, according to Freedom Food.

Its report, entitled Consumer Attitudes to Animal Welfare, says salmon have the second-top living conditions, with their environment described as "similar to nature". Highlighting the best and worst animal living conditions, salmon is positioned behind the first-placed dairy cattle and ahead of pigs, beef cattle, sheep, turkeys, ducks, chickens and laying hens.

The report by Freedom Food, the farm assurance and food-labelling scheme of the Royal Society for the Prevention of Cruelty to Animals, included a survey of 1000 British shoppers from different age groups and regions in the UK.

The report suggests that more than half of the population is buying at least one or two higher welfare products a week, and that one out of four UK consumers would be willing to pay an extra 10 percent for higher welfare standards.

"Salmon farmers are acutely aware of the public perception of food production, and it is great news that salmon is perceived to have one of the best welfare standards for farmed animals," said the chief executive of the Scottish Salmon Producers' Organisation, Sid Patten.

The reality is that salmon are farmed to the highest standards of welfare, with best practice principles applied at every stage of production

"The industry has repeatedly challenged a sustained campaign from ill-informed critics for many years which has been designed to damage its progress.

"The reality is that salmon are farmed to the highest standards of welfare, with best practice principles applied at every stage of production according to the Code of Good Practice for Scottish Finfish Aquaculture." These principles were the result of many years of research and development, and the application of the highest standards in health management.

Patten said the report confirmed the responsible use of veterinary medicines, and the negligible impact of medicines on the marine environment.

"It is therefore extremely encouraging to see that the negative messages perpetuated by some commentators have been dismissed by the large majority of consumers."

The managing director of Loch Duart Ltd, Nick Joy, whose fish are marketed under the brand name Sustainable Salmon Company, said the report confirmed his company's own research that consumers were willing to pay more for higher welfare and environmentally friendly products.

"It is encouraging to see that the consumer is aware of the excellent welfare practiced on salmon farms." ■

RESEARCH

A US PERSPECTIVE ON SELECTED BIOTECHNOLOGICAL ADVANCEMENTS IN FISH HEALTH

Part II: Genetic stock improvement, biosecurity tools and alternative protein sources in fish diets

DRS PHILLIP H KLESIUS, JOYCE J EVANS, CRAIG A SHOEMAKER AND CHHORN LIM
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This article is the second of a two-part series reviewing selected biotechnological advances in aquaculture. Part one was published in Issue 5, and explored advances in vaccine development and production.

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 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 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 1.5 million metric tonnes, or 35 to 50 percent, by 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 2002, Asia led the world in aquaculture production, with about 12.4 million tonnes, while Europe, North, Central and South America together produced 1.2 million tonnes (See www.usda.mannlib.cornell.edu). In recent years, other countries are joining the surge in the aquaculture industry. For example, salmon aquaculture grew 159-fold between 1980 and 2004 from 4783 tonnes to 761,752 tonnes. The largest salmon-producing countries are Norway, Chile, the United Kingdom and Canada.

Another example of rapid growth within the aquaculture industry is channel catfish in the United States. Six hundred and sixty million pounds were produced in 2003 yielding $675-690 million in sales (See www.usda.mannlib.cornell.edu). The states with the leading production of channel catfish are Mississippi, Alabama, Arkansas and Louisiana. The greatest 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).

FIGURE 1: HARVEST OF NWAC CHANNEL CATFISH

The consumption of the tropical fish tilapia is becoming more popular in the US and Europe. Tilapia production in the US increased more than 20 percent between 1991 and 1998. The US production of tilapia is about 9100 tonnes annually (See www.ag.arizona.edu/azaqua/ata.html). The value of imported frozen tilapia-fillets was $176 million in 2005. The cultivation of tilapia will continue to be a growing industry that supplies an important source of food, employment and domestic and international trade worldwide.

INFECTIOUS DISEASES

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 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 result 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 their rearing in highly intensive production systems.

GENETIC IMPROVEMENTS IN CATFISH

Improved genetic stocks have played a key role in the growth of aquaculture, especially in channel catfish. The Agricultural Research Service, USDA, Catfish Genetics Laboratory at Stoneville, Mississippi, conducted research to enhance the genetic potential of channel catfish (Weaver-Missick 2004). The result of this research was the development of the USDA 103 channel catfish line, which exhibited excellent growth compared to other lines of channel catfish.

The USDA 103 line was released to producers through a joint effort of the USDA, ARS and the Mississippi Agricultural and Forestry Experiment Station under the name (National Warmwater Aquaculture Centre) (NWAC) 103 in 2001. (See www.msucares.com/aquaculture/catfish/nwac103.html).

CRAIG SHOEMAKER, JOYCE EVANS AND PHIL KLESIUS

Figure 1 shows the harvest of NWAC channel catfish (ARS news service photograph). The NWAC 103 line is a faster growing channel catfish with a shorter production cycle. This channel catfish line is estimated to consume 10 percent more feed and grow 10 percent faster than other catfish lines.

BIOSECURITY TOOLS

Biosecurity may be defined as the protection of animals, plants, humans and their associated activities and the environment from the unwanted impacts of biological agents, including disease and pests. How to properly biosecure aquaculture is a major issue in many countries where the aquaculture industry is very susceptible to unwanted infectious agents and pests. The unwanted introduction of infectious agents or pests could disrupt current and future aquaculture industries. At present, aquaculture now provides 16 percent of the world's animal protein. Among biotechnological advancements important to biosecurity are the development and use of rapid and early infectious agent detection tests and use of vaccines (Klesius *et al* 2006).

The need for international surveillance of fish pathogens has led to the development of rapid and dependable methods to identify infectious agents. Rapid detection and identification of an infectious agent in fish means that the infectious agent can be detected and identified in sub-clinically infected fish (ie, fish harbouring the pathogen but not showing overt disease signs).

The effective treatment or the elimination of these sub-clinically infected fish means that the spread of the disease to healthy fish could be controlled. The movement of live fish, genetic material and fish products aids the possibility for the movement of infectious agents within the live animal or processed products. International trade provides significant economic benefits but carries the risk of the introduction of disease.

The strategy is to detect the infectious agents in the live fish, genetic material or fish products prior to their movement. Thus, rapid detection is needed to manage the risks of disease transmission in international trade. These rapid detection tests need to be able to detect as few as 10 cells of an infectious agent in live fish, genetic material and/or fresh or frozen fish products.

Traditional methods for diagnosis/detection of fish pathogens have relied on culture and biochemical identification tests (days to weeks) or antibody detection, using a variety of assays that include neutralisation, enzyme-linked immunosorbent assays (ELISA), agar gel diffusion and complement fixation (hours to days).

More recently, new molecular assays such as monoclonal antibodies (Panangala *et al* 2006) and polymerase chain reaction (PCR) (Bilodeau *et al* 2003, Welker et al 2005, Panangala *et al* 2007) or variations of DNA amplification techniques (Yeh *et al* 2005) are being frequently used to detect fish pathogens.

In the early 1970s, the method to develop monoclonal antibodies was discovered (Köhler and Milstein 1975). Monoclonal antibodies are identical copies of an antibody that can specifically react with antigenic substance(s) of infectious agents. An infectious agent present in live fish, genetic material or fish products is identified by its reaction with a monoclonal antibody specific for that pathogen. Many pathogen-specific monoclonal antibodies are readily available commercially or in diagnostic test kits.

Monoclonal antibodies are used to improve or develop ELISA methods or immunofluorescent antibody tests (IFAT) that can be used to rapidly screen for infected fish. A wide variety of diagnostic ELISA assays were developed to identify and diagnose specific pathogens. The signal molecule incorporated onto the monoclonal antibody can be an enzyme, gold particles, bioluminescent compounds or fluorescent dye.

Depending on the kind of monoclonal antibody signal molecule, the antigen-antibody complex can be detected by

- photometrical apparatus through a substrate chemical reaction
- an electron microscope
- a bioluminescent apparatus through light emissions, or
- a light microscope through visible fluorescent reaction.

The advent of PCR in the mid-1980s revolutionised molecular biology methods for detecting pathogens. The basic application of PCR is the amplification of small amounts of template DNA or RNA into large quantities in a few hours. More recently, a new method of PCR was developed called real-time PCR.

Real-time PCR allows for actual visualisation of the increase in the amount of the DNA as it is being amplified. Polymerase chain reaction methods must be able to discriminate between microbes based on a signal from specific nucleic acid sequences found in their DNA or RNA. Detection tests utilising PCR are being used in biosecurity applications, such as detecting potentia lly harmful viruses in the shrimp aquaculture industry, to monitor for potential problems and to assist in selecting specific pathogen-free stocks (Lightner 2005).

Several of these identification techniques are rapid (30 minutes or less), sensitive and accurate (ie, they reduce the number of false- ▶

positive reactions) (Bilodeau *et al* 2003, Welker *et al* 2005, Yeh 2005, Panangala *et al* 2007).

Future biotechnological advancements are expected and needed for biosecurity applications both on and off the farm in order for international trade of aquaculture products to continue to grow. Examples of future advancements are the further development and application of biosensors and nano-technologies for continuous modes of identification of pathogens that may be introduced into an aquatic environment. A recent review provides additional information on advances in diagnostic tools (Schmitt and Henderson 2005).

ALTERNATIVE PROTEIN FEED SOURCES

The cost of feeding fish can represent 40 to 60 percent of the total production cost, depending on the species, production system intensity and feeding practices employed. Currently, aquaculture feeds are very dependent on fish meal to meet their critical protein requirements. The global supply of fish meal is not increasing, but static, and it is likely that the amount of available fish meal may decline in future years.

This trend, coupled with the growth of the aquaculture industry, requires that an alternative source of protein other than fish meal be discovered. Cottonseed, soybean meal and canola protein concentrate have been shown to be among the lower-cost alternatives to fish meal. Fish diets may contain about 38 percent replacement of fish meal with non-nutritive additives (flavour enhancers and enzymes) and nutritive additives (the amino acids lysine and methionine).

The development and production of nutritionally improved alternative protein sources through molecular techniques and new processing methods is necessary. Current biotechnological advancements are protein concentrates from grains and oilseeds, single cell proteins produced by methanotrophic bacteria and products of fermentation (Hardy 2004).

Increased global trade of aquaculture products is dependent on the continued advancement of these and other such biotechnological contributions

Webster and Lim (2002) provide complete information on nutritional requirements and feeding practices of commercially important aquaculture organisms. The importance of nutrition (nutrients, feed and feed additives) on the ability of fish to resist infectious organisms has generated a better understanding of the interactions between nutrition and fish health (Lim and Webster 2001). These advancements may help meet the current projection that 50 percent of food consumed in the US will be from organic sources (ie, free of chemicals, antibiotics and other additives) by 2020 (Miller 2004).

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. Recent biotechnological advancements, including genetic improvement of fish stocks, development and implementation of biosecurity tools and the development and use of alternative fish feed protein sources have and 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

The authors would like to thank Crystal Braden and Lisa Biggar for their help in preparing this manuscript.

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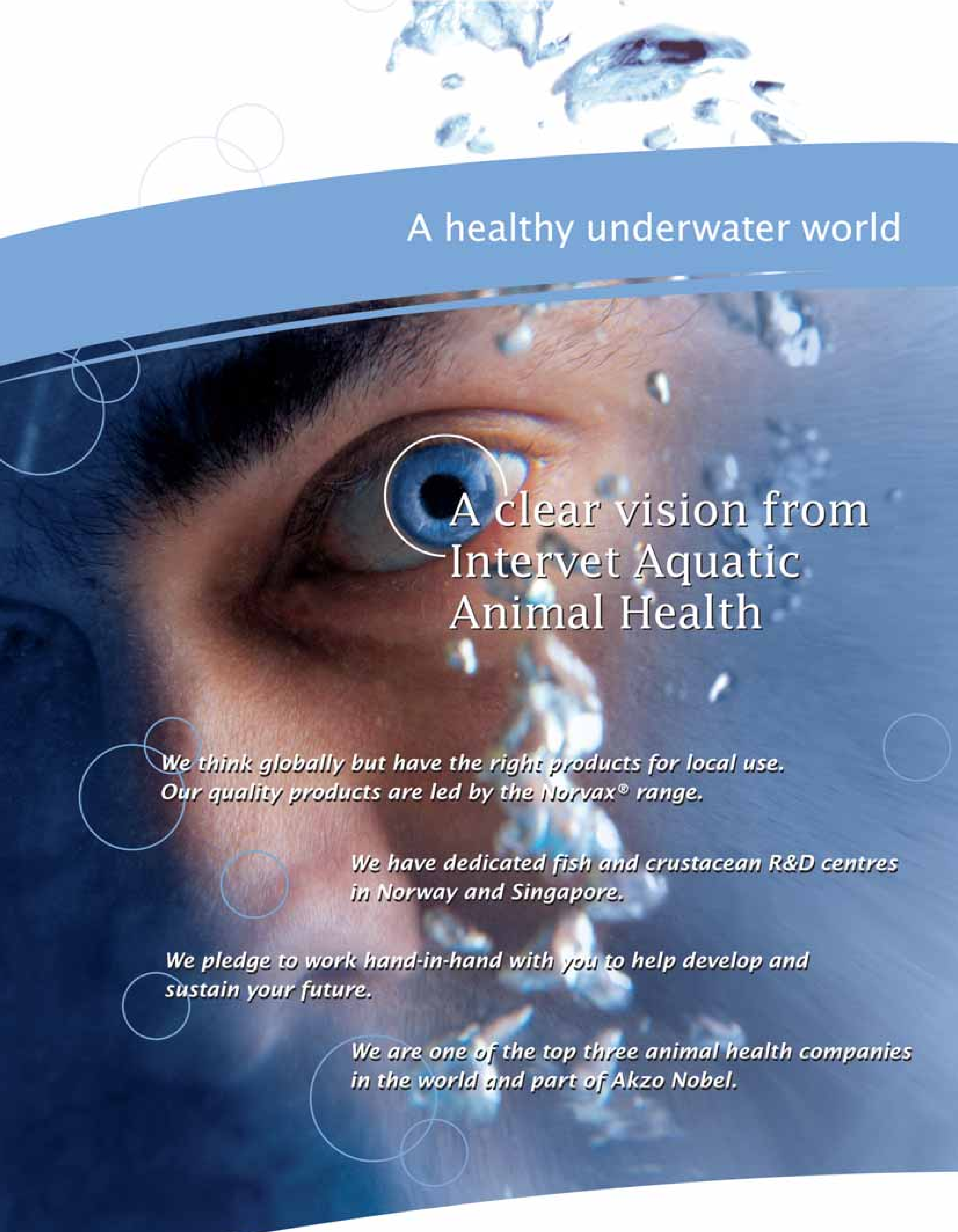

A healthy underwater world
A clear vision from Intervet Aquatic Animal Health
We think globally but have the right products for local use. Our quality products are led by the Norvax® range.
We have dedicated fish and crustacean R&D centres in Norway and Singapore.
We pledge to work hand-in-hand with you to help develop and sustain your future.
We are one of the top three animal health companies in the world and part of Akzo Nobel.

SUB-LETHAL CHANGES IN DANUBE STERLET

BY DR MIRJANA LENHARDT, DR VESNA POLEKSIC, GORCIN CVIJANOVIC AND IVAN JARIC (INSTITUTE FOR BIOLOGICAL RESEARCH, BELGRADE, SERBIA)

Six species from the family Acipenseridae are native in the Danube River and are listed in the Convention on International Trade of the Endangered Species (CITES). Among them is sterlet (*Acipenser ruthenus* Linnaeus, 1758).

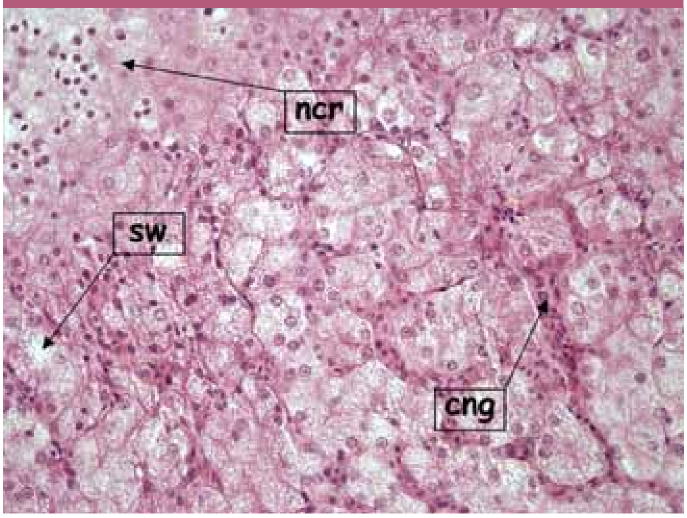
Modification of the river bottom, dam construction, overfishing and pollution has lead to a decrease in sterlet population size in the Danube River. Since sterlet is an economically important species, and like other sturgeon species has predictable migration patterns, it has always been a target of commercial fisheries on the Danube River.

With river regulation and construction of the Djerdap I and Djerdap II dams in 1970 and 1984 respectively, sterlet began to decline constantly. Unfortunately, it is not just these problems that the sterlet has to contend with.

Pollution, as another extremely negative factor, is especially present in sections of the Danube River near big cities where there is no proper treatment of sewage and industrial wastewaters. In order to determine the impact of these polluted sites on sterlet, a survey was conducted below the sewage outlets of Belgrade, Serbia.

Histopathological effects were assessed on gills, skin and liver. All changes were sub-lethal, and the most pronounced effects of toxic and organic pollution were on liver (congestion of sinusoids, necroses - see Figure 1) followed by changes in the gills (hypertrophy and lifting of secondary epithelium, as well as circulatory changes – Figure 4) and skin (increase of mucous production – Figures 2 and 3). Pectoral fin sections, which have been used for age

FIGURE 1;
LIVER WITH CHANGES: CNG – CONGESTION OF SINUSOIDS, SW – CLOUDY SWELLING OF HEPATOCYTES AND NCR – NECROSIS



FIGURES 2 AND 3: SKIN WITH CHANGES: HPP – HYPERPLASIA OF MUCOUS CELLS IN THE EPITHELIUM; EPR – EXCESSIVE MUCOUS PRODUCTION, AND DMG – DAMAGE OF SURFACE EPITHELIAL CELLS

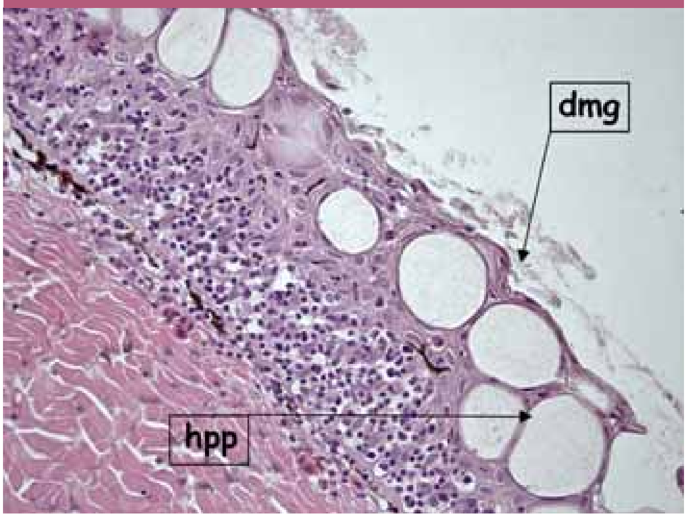
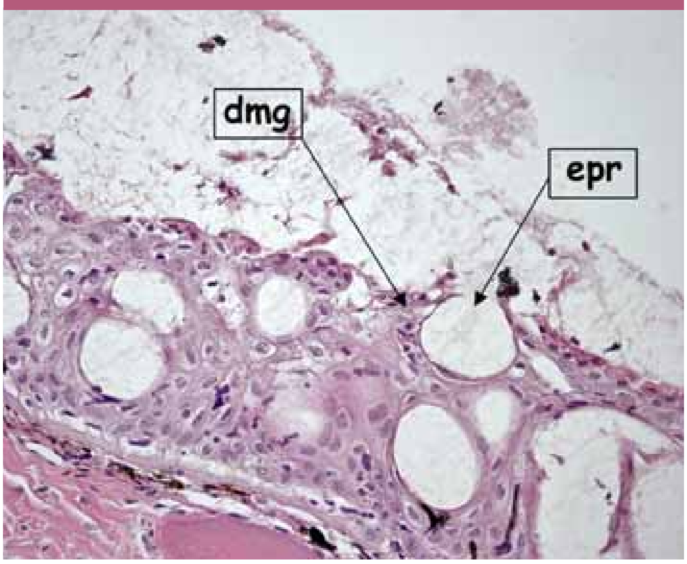


FIGURE 4: GILLS WITH CHANGES: HTR – HYPERTROPHY AND LIFTING OF SECONDARY EPITHELIUM, AND CCH – CIRCULATORY CHANGES

FIGURE 3



determination, showed visible malformation (Figure 5). These deformities were quantified as the angle of lateral torsion (Figure 6) and were related to several factors, such as nutrient imbalance (in captivity often caused by differences in water quality), fin spine abrasion (against tank walls) and high temperature.

Analysis of age structure showed that natural population consists mostly of younger specimens (0^+, 1^+, 2^+), which can be attributed to over-fishing. This age structure raises another important question: where should we turn to for a solution to the problem of declining natural populations?

Aquaculture and its development arise as, perhaps, the best possibility. Reared sterlet could help bolster natural populations, not only with restocking, but also by providing the market with a much soughtafter resource, and therefore reduce pressure on wild sterlets.

Nevertheless, whether the reared specimens are suitable for survival in the wild is a point for discussion, and should always be considered before embarking on such a programme of action. In an attempt to improve knowledge on this matter, we compared morphological features of reared and wild sterlet.

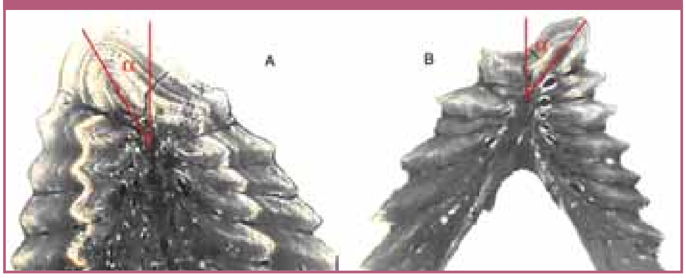
Both samples originate from the Danube, with wild specimens caught near Belgrade and reared obtained from a culture facility in Homokmegy, Hungary. Measured differences showed a consistent pattern - all length-related proportions were larger in wild specimens, while width-related proportions were larger in cultured specimens. In other words, fish reared in aquaculture have a "stockier" body shape than those reared in the wild.

Also, the pectoral fin was significantly shorter in cultured specimens, which is in concordance with findings of other authors. Sterlet use the posterior portion of the pectoral fins to initiate ascent or descent. Further research is needed to reveal whether the shorter pectoral fin in cultured sterlet could have a negative effect on survival success when they are used for stocking of open waters, especially in large rivers.

For more information on sturgeons in Serbia, see www.sturgeons.info

FIGURE 5: VISIBLE DEFORMITY ON THE RIGHT PECTORAL FIN – THE SO-CALLED "FIN CURL"

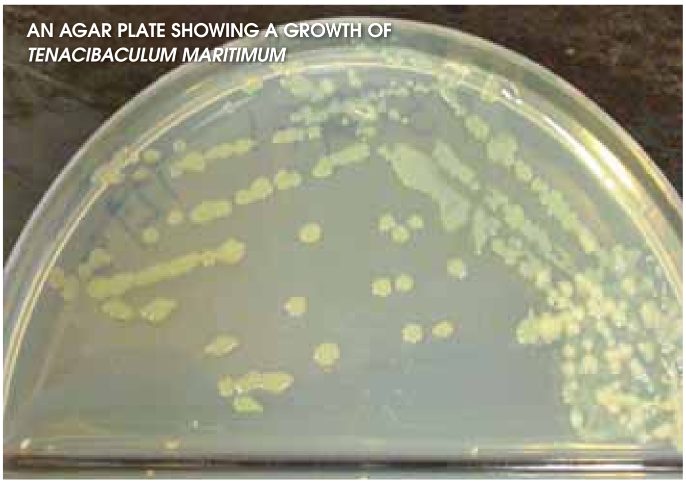
FIGURE 6: PECTORAL FIN SPINE SECTIONS OF TWO STERLET SPECIMENS WITH VISIBLE DEFORMITIES. A - DEFORMITY ANGLE A) AGE CLASS 1+, A=23.95; B) AGE CLASS 0+, A= 36.23



VACCINATION OF ATLANTIC SALMON (*SALMO SALAR* L) AGAINST *TENACIBACULUM MARITIMUM*

BY DRS REBECCA VAN GELDEREN, JEREMY CARSON AND BARBARA F NOWAK
(SCHOOL OF AQUACULTURE, TASMANIAN AQUACULTURE AND FISHERIES INSTITUTE, UNIVERSITY OF TASMANIA, AUSTRALIA)

REBECCA VAN GELDEREN ON A FISHING EXPEDITION



AN AGAR PLATE SHOWING A GROWTH OF *TENACIBACULUM MARITIMUM*

A GRAM STAIN PLATE SHOWING *TENACIBACULUM MARITIMUM*

Tenacibaculum maritimum (formerly *Flexibacter maritimus*) is a well-known pathogen in a number of cultured fish species worldwide. It is a marine bacterium that causes necrotic lesions on the body, head, fins and gills, with erosive lesions on the external surface as the prominent clinical sign.

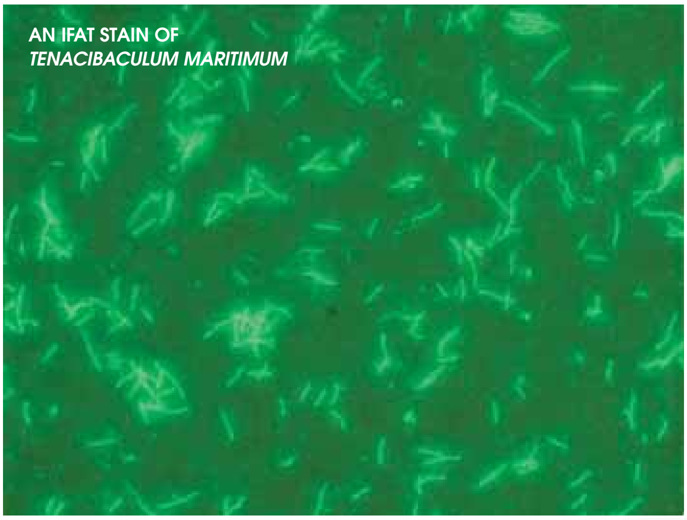
A large outbreak of the disease, causing significant mortalities, occurred on salmon farms in Tasmania in 1988 and 1989. Management strategies have reduced the incidence and severity of the disease. However, smaller outbreaks continue to occur. There is limited information on the biological factors that could initiate further outbreaks of the disease, and patterns of infection are variable. However, it is known that outbreaks occur more often in years with higher summer temperatures and more sunny days.

Antibiotics are undesirable for prolonged use, with problems of bacterial resistance and unknown tissue residence times. There is also inadequate evidence of their efficacy under outbreak conditions. Therefore, development of a safe and effective vaccine against *T maritimum* was the focus of this research.

The first step was to characterise 20 isolates of *T maritimum*. Selection of isolates for later pathogenicity and vaccine trials also required the determination of similarities and differences below the species level, particularly properties that enable production of the disease in the host (antigenic differences) and those that can affect vaccine efficacy.

The following tests were carried out for each isolate: whole cell protein profiles, lipopolysaccharide profiles, extracellular product (ECP) profiles, hydrophobicity and the indirect fluorescent antibody test, or IFAT.

The next step was to determine the pathogenicity of the organism and confirm Koch's postulates. Also, a model of infection was developed so that the efficacy of the experimental vaccines could be established. Three pathogenicity trials were undertaken to observe differences in culture methods, differences


AN IFAT STAIN OF *TENACIBACULUM MARITIMUM*


A CLOSE-UP OF A SALMON INFECTED WITH MARINE FLEXIBACTERIOSIS


A FISH INFECTED WITH MARINE FLEXIBACTERIOSIS. NOTE THE LESIONS AND THE POOR CONDITION FACTOR OF THE INFECTED FISH


A MARINE FLEXIBACTERIOSIS LESION ON AN ATLANTIC SALMON

in the virulence of isolates and a comparison of doses to set up a model of infection and determine the LD60 for the vaccine trial.

The possible role of extracellular products in the pathogenicity of *T maritimum* and its toxicity was also investigated *in vivo* by injecting different doses of ECP into the peritoneal cavity of Atlantic salmon. The ECP was found to be toxic and caused internal necrosis and haemorrhaging.

Finally, a vaccination trial was conducted using an injectable formalin de-activated vaccine, and the same vaccine and an adjuvant (Freund's incomplete). Some protection was provided with the vaccine alone (RPS = 22-27.7 percent), but significant protection was achieved with the vaccine and adjuvant (RPS = 78-79.6 percent).

More research is necessary on alternative adjuvants, further characterisation of isolates, role of toxins such as extracellular products (ECPs) and lipopolysaccharide (LPS), and the duration of protection for the vaccine. But this PhD project provides a basis for developing a vaccine against marine flexibacteriosis for the Tasmanian salmonid industry.

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THE FISH HEALTH UNIT OF THE MARINE INSTITUTE, IRELAND

DR NEIL RUANE (FISH HEALTH UNIT, MARINE INSTITUTE, IRELAND)

The Marine Institute was set up under the 1991 Marine Institute Act with the following role: To undertake, to coordinate, to promote and to assist in marine research and development and to provide such services related to research and development that, in the opinion of the institute, will promote economic development and create employment and protect the marine environment.

The institute is now the national agency responsible for marine research, technology development and innovation in Ireland. We seek to assess and realise the economic potential of Ireland's 890,308sq km marine resource, promote the sustainable development of the marine industry through strategic funding programmes and essential scientific services, and safeguard our marine environment through research and environmental monitoring. The vision of the Marine Institute is "a thriving maritime economy in harmony with the ecosystem and supported by the delivery of excellence in our services".

The institute provides a broad range of statutory services related to marine research development and other Irish and European Union legislation. The statutory services include technical monitoring and advice to the Department of Communications, Marine and Natural Resources (DCMNR), the Food Safety Authority of Ireland (FSAI), the Department of Agriculture and Food (DAF) and the European Commission (EC).

In order to provide this broad range of services, the institute is organised into a number of service areas, including fishery science, aquaculture and catchment management, ocean sciences, and the marine environment and food safety services, among others.

SERVICES PROVIDED

The Marine Environment and Food Safety Division of the Marine Institute provides scientific services and advice on fish and shellfish health, seafood safety and the status of the marine environment for clients such as the aquaculture and fishing industries, the DCMNR, FSAI, EC, ICES and the Oslo-Paris Commission (OSPAR). It carries out monitoring programmes to meet Ireland's EU commitments in fish/shellfish health, shellfish safety, residues and contaminants in seafood.

The Fish Health Unit supports the aquaculture industry and the inland fisheries sector in maintaining Ireland's superior fish health status. It provides both statutory services (in line with EU directives), diagnostic support and is involved in research collaborations with both national and international research and third-level institutes.

STATUTORY SERVICES AND DIAGNOSTICS

The FHU carries out monitoring on behalf of the DCMNR under EU legislation. Directive 91/67/EEC deals with the placing on the market of live fish and gametes, and governs the majority of fish health monitoring. The objective of this directive is to allow free trade within the European Community, while ensuring that countries can maintain their health status. Under this directive, routine monitoring is carried out for viruses causing infectious haematopoetic necrosis (IHN) and viral haemorrhagic septicaemia (VHS) in fish, and the parasites *Bonamia ostreae* and *Marteilia refringens* in shellfish.

Directive 2004/453/EC granted Ireland additional guarantees in relation to freedom from the diseases bacterial kidney disease (BKD), *Gyrodactylus salaris* and spring viraemia of carp (SVC) and the FHU is obliged to test regularly for these diseases in order to maintain this freedom. A new directive 2006/88/EC has now been published and will come into effect on the August 1, 2008, replacing directives 91/67, 93/53 and 95/70. Under directive 96/23/EEC, the FHU screens for the presence of antimicrobial residues in fish muscle tissues.

The FHU, which is the National Reference Laboratory in Ireland for fish and shellfish diseases, offers a diagnostic service to the aquaculture industry, the wild fish sector and private veterinarians.

FINFISH HEALTH MONITORING

On behalf of the DCMNR, the FHU monitors the health of all stocks of farmed fish that are moved within the country. All marine and freshwater sites in the country are inspected at least once per year. This generally involves sampling 30 fish and an examination of fish health, movement and medicine usage records. Fish undergo a full post-mortem examination with tissues taken for histological, microbiological and virological examination. A report is then issued to the producer after all the tests have been carried out.

FIGURE 1: AN ATLANTIC SALMON SMOLT SUFFERING FROM PANCREAS DISEASE. NOTE THE FAECAL CASTS IN THE INTESTINE AND A LACK OF FATTY TISSUE AROUND THE PYLORIC CAECAE

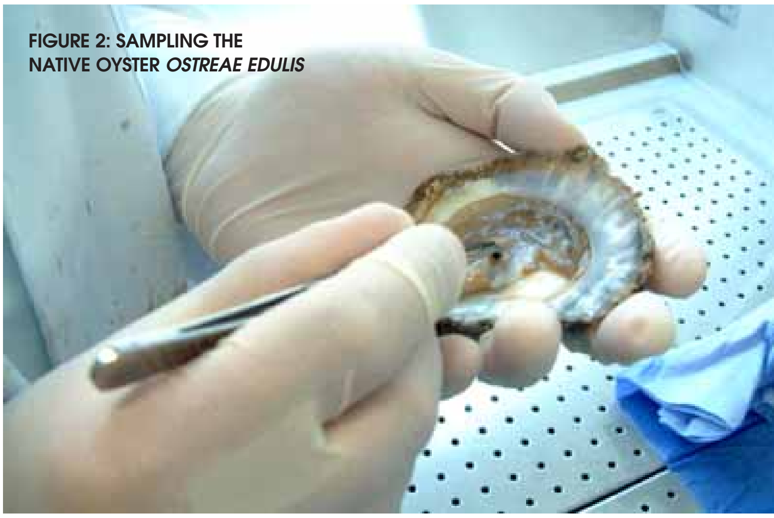


FIGURE 2: SAMPLING THE NATIVE OYSTER *OSTREAE EDULIS*

FIGURE 3: WILD LOBSTERS INFESTED WITH A GILL COPEPOD PARASITE

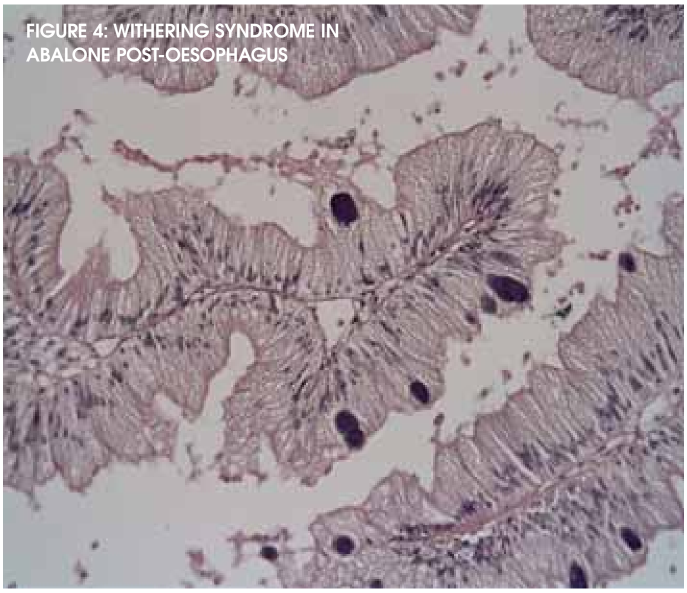


FIGURE 4: WITHERING SYNDROME IN ABALONE POST-OESOPHAGUS

Apart from the diseases listed under EU legislation, the FHU also routinely tests for other diseases that are of economic importance in Ireland, namely pancreas disease (PD) and infectious pancreatic necrosis (IPN), furunculosis, enteric redmouth and vibriosis, etc as well as investigating unexplained mortalities of farmed and wild fish and shellfish.

SHELLFISH HEALTH MONITORING

All movements of shellfish within the country are strictly controlled by the DCMNR. Shellfish may move only under permit, and movements of susceptible species from *Bonamia*-positive areas to *Bonamia*-negative areas are prohibited. At least 30 native oysters, *Ostreae edulis*, are sampled from each growing area in the country twice per year.

Under the terms of the EU legislation and of each aquaculture license awarded in the country, all "abnormal mortalities" must be reported immediately to the FHU. An investigation is carried out immediately to determine the cause of the problem and a written report is furnished to the grower in question. Apart from *B ostreae* and *M refringens* testing, the FHU tests for other important shellfish diseases such as brown ring disease and withering syndrome.

DIAGNOSTIC SERVICES

The Fish Health Unit has a modern, well-equipped laboratory which facilitates its national and international commitments in relation to diagnosing and controlling fish and shellfish diseases. The five main sections within the unit are parasitology, histology, bacteriology, virology and molecular diagnostics. Each require different areas of technical expertise and equipment.

Parasitology
Gross pathology and necropsy
Wet-mount preparations

Histology/histopathology
Sampling and fixation of fish, shellfish and crustaceans for histopathological diagnosis
Histology slide preparation and staining, including storage for retrospective analysis

Microbiology
Bacterial identification using primary and secondary identification techniques, as well as serological tests such as ELISA
Tests for the residues of antimicrobials in fish muscle tissues

Virology
Identification of viral pathogens using CHSE, BF-2, EPC, TO, SHK, KF-1 cell lines
Isolation of VHSV, IHNV and SVCV under active surveillance programmes

CONTINUED ON PAGE 29

FIGURE 5: THE FHU HAS DEVELOPED CLOSE LINKS WITH THE IRISH AQUACULTURE INDUSTRY

NEW GLOBAL AQUATIC VETERINARY ASSOCIATION

Emerging from the needs of a large number of veterinarians already involved in aquatic animal medicine, a new veterinary organisation has been formed, initially doing business under the name Aquatic Veterinary Association. The need for the organisation is compounded by the demands from owners of companion aquatic animals, aquatic food species producers, industries such as aquaculture and governments, for veterinary assistance in aquatic animal health and welfare, public health and seafood safety.

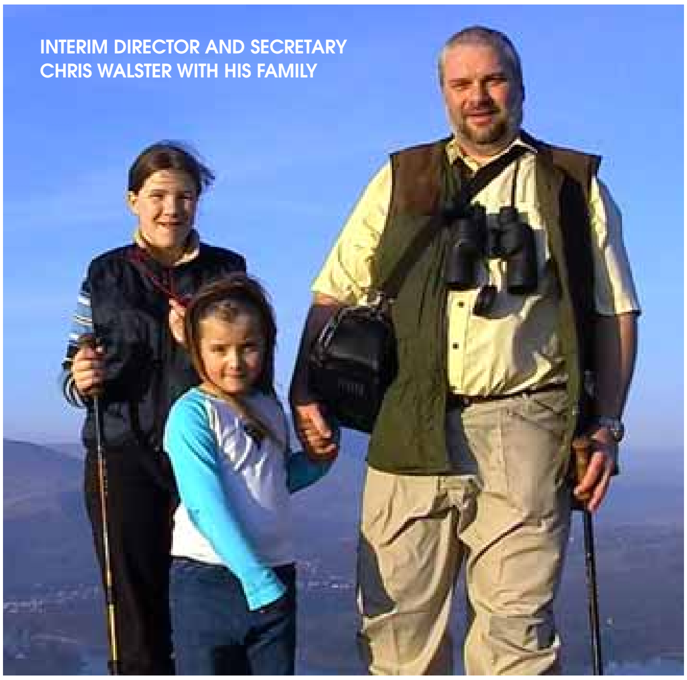
"Formalising a group to address the concerns of aquatic veterinary medical practitioners under an organised, incorporated and registered non-profit professional association will help elevate aquatic veterinary medicine from a niche area of the veterinary sciences to a well-recognised discipline within the profession," says Dr Peter Merrill, who is serving as the association's interim president. "We hope this organisation will cater to the needs of an estimated 5000 to 10,000 aquatic veterinarians world-wide, and indirectly to those who seek their expertise," says Dr Merrill.

After receiving input over the past year from aquatic veterinarians in the United Kingdom, France, Germany, the United States, Canada, Australia, New Zealand and other countries, a group of six interim directors stepped forward to consolidate the ideas they heard into formalised preliminary bylaws and a structure for the association. These and other official documents will be ratified by the founding members at the first annual general meeting, to be held in conjunction with the American Veterinary Medical Association's convention in Washington, DC in July.

Dr Chris Walster, who is serving as the interim secretary, says, "We are endeavouring to cater to the professional needs of all veterinarians and veterinary surgeons throughout the world who are involved with all disciplines of aquatic veterinary medicine and all aquatic species."

With outbreaks of disease in the fastest growing global sector of animal production and animal ownership - aquatic animals - the demand for veterinarians involved with traditional farmed finfish, crustaceans and molluscs (eg, farmed shrimp, oysters and clams) has grown dramatically over the past decade.

INTERIM DIRECTOR AND TREASURER DR DUSAN PALIC, AND A DOLPHIN!



INTERIM DIRECTOR AND SECRETARY CHRIS WALSTER WITH HIS FAMILY

"Three of five recent animal disease national emergency declarations in the US have been for aquatic animal diseases - the other two being for Newcastle's disease and avian influenza in poultry. The problems in the UK, Europe and Canada are similar," says Dr Walster. Dr Colin Johnston, another interim AqVA director, who covers Australasia, states that, "a similar situation exists in Australia, New Zealand and Asia."

National and international responses from governments and industries have opened up huge demands for aquatic veterinarians. National plans and programmes, such as those of the US and Canadian governments, Australia's Aquaplan and Aquavetplan, and European Union directives and regulations that are being developed and implemented place a huge demand on an aquatic veterinary workforce. The World Animal Health Organisation's codified standards and its biosecurity initiatives for protecting aquatic animal industries likewise require an organised aquatic veterinary approach.

"We are also seeing a similar demand for aquatic veterinarians in the private practice sector who deal with pet or ornamental aquatic animals owned by clients," said Dr Julius Tepper, another of the organisation's interim directors, who owns a private practice in New York.

Dr Tim Miller-Morgan, a director who is organising the association's upcoming meeting, says, "a similar situation is being felt in the aquarium industry, with marine mammals and in wild managed fisheries and hatcheries."

The final interim director, Dr Dusan Palic of Iowa State University, who is serving as the association's treasurer, says that, "this association has significant potential to represent the voices of aquatic veterinary practitioners around the world, and permit aquatic practitioners to become better identified through public recognition of their role in all aspects of aquatic animal medicine.

"The solution to demand for aquatic veterinarians in all spheres of practice and with all aquatic animals is in organised veterinary medicine, which requires the formation of a professional society that represents aquatic veterinarians," says Dr Merrill.

"If we structure this organisation correctly it will substantially contribute to all veterinary medicine through strong liaisons with existing large and small veterinary organisations, as well as industries, governments and the public which have a need for our member's expertise."

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See www.AquaVets.org

The current mission of the association (formed in 2007 and doing initial business as the Aquatic Veterinary Association) is to serve the discipline of aquatic veterinary medicine in enhancing and advancing the science and art of aquatic veterinary medicine, aquatic animal health and welfare, public health, and seafood safety in support of the veterinary profession, aquatic animal industries and other stakeholders.
For more information on this new aquatic veterinary organisation, to provide input into its development, or to become a founding member, contact any of the Interim directors, or visit the association's website at www.AquaVets.org

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FIGURE 6: THE MARINE INSTITUTE

Isolation of IPNV and *Salmonid alphavirus* (SAV, causing pancreas disease)
Serological identification of SAV in fish serum
Ability to identify the exotic virus ISAV

Molecular diagnosis
Identification of all listed pathogens by PCR or RT-PCR
In situ hybridisation for the diagnosis of *B ostreae*

RESEARCH COLLABORATIONS

As the Marine Institute was set up to undertake, coordinate and promote marine research and development in Ireland, all sections of the institute are heavily involved in applied research. The institute has recently launched Sea Change: A marine knowledge, research and innovation strategy for Ireland 2007-2013, which outlines the marine research strategy for Ireland in the coming years, including fish and shellfish health.

The FHU is currently involved in a number of large and small-scale projects in collaboration with national and international third-level institutes and the Irish aquaculture industry, including

- field investigations on pancreas disease in farmed Atlantic salmon
- involvement in the Tri-Nation (Ireland, Scotland and Norway) working group on pancreas disease and related pathologies (cardiomyopathy syndrome, heart and skeletal muscle inflammation)
- epidemiology of disease outbreaks in Ireland (PD, IPN, *Bonamia*)
- development and comparison of diagnostic techniques for new and emerging disease pathogens, with particular emphasis on the development of molecular diagnostic tools, *in situ* hybridisation and immuno-histochemistry
- screening of wild fish for pathogens, and
- determination of epidemiological cut-off values for the determination of antimicrobial resistance of *Aeromonas salmonicida*.

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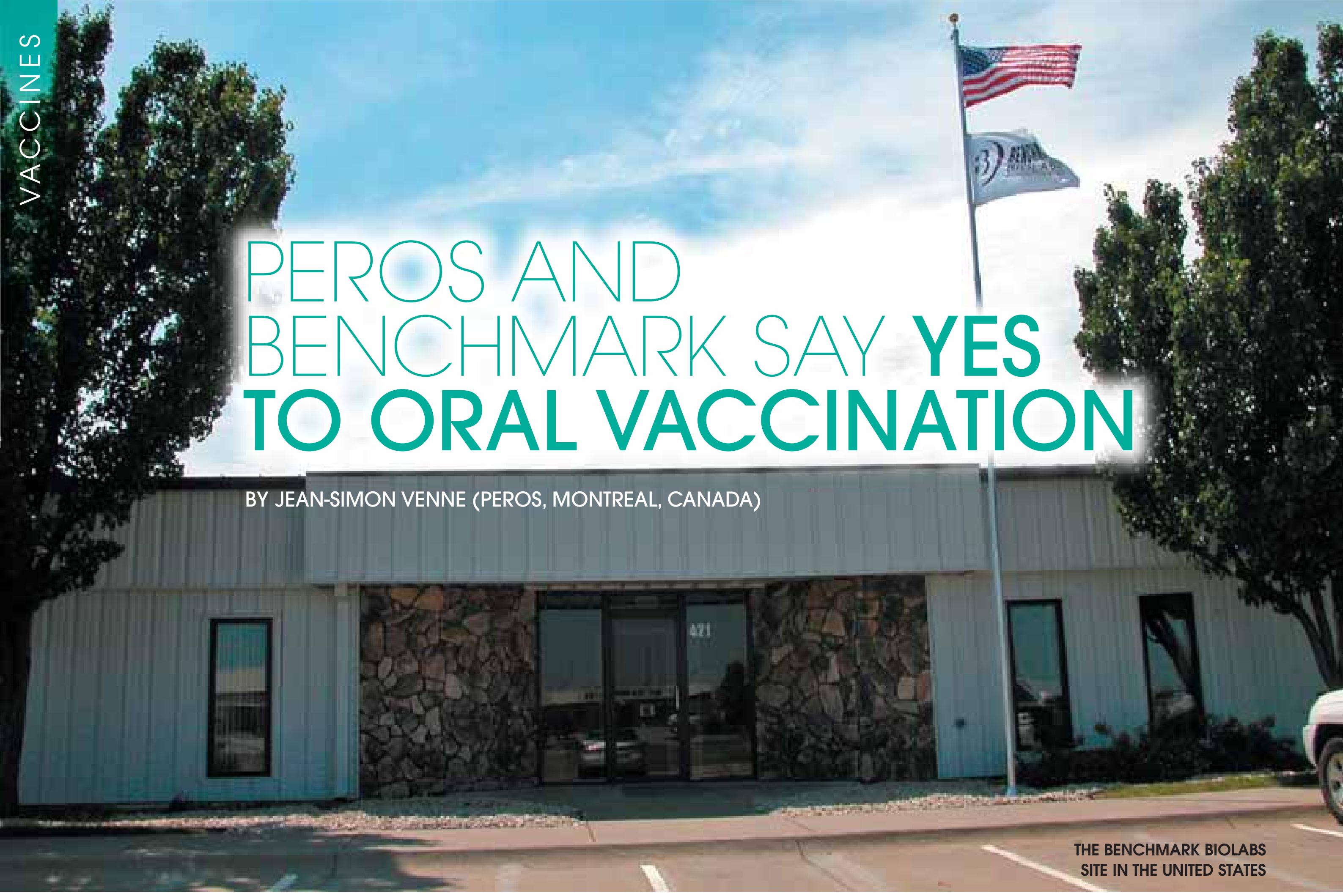

PEROS AND BENCHMARK SAY **YES** TO ORAL VACCINATION

BY JEAN-SIMON VENNE (PEROS, MONTREAL, CANADA)

THE BENCHMARK BIOLABS SITE IN THE UNITED STATES

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

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

The emerging biotechnology company PerOs Systems Technologies Inc has developed Oraljectᵀᴹ, which it says is a unique and proprietary oral drug delivery platform. Benchmark Biolabs Inc, a United States bio-pharmaceutical product development firm focused on the validation, development, optimisation and commercialisation of biologics "from concept to commercialisation," has teamed up with PerOs to make oral vaccination a reality. The rapidly growing aquaculture industry is their first commercial target.

Benchmark's proven track record over its 10-year history, such as co-developing the world's first plant-derived vaccine approved by the USDA, including the design, licensure and management of the associated manufacturing facility, make them the perfect partner to complete the development effort for PerOs, and design and manage a fully licensed facility to incorporate the desired bioactive components into the Oraject platform.

The core of Benchmark's business over the last decade has been to apply creative science to support regulatory approval for both conventional and innovative product development. Benchmark's customer base represents a broad composite of firms seeking to develop or manufacture best-in-class products in the biological sector.

PerOs will focus the initial commercial effort of its Oraject technology to meet the demands of the rapidly growing aquaculture industry.

AT WORK IN THE BENCHMARK BIOLABS LABORATORY

The intensification of fish farming has resulted in an increased need to control disease to decrease sickness and improve growth performance. Much effort has been dedicated to vaccine development, and the use of vaccines has become an essential component of disease management in fish farming.

Until now, the limitation on vaccine use has been the costs and stress of physical constraints associated with the vaccine administration process. Each fish must be manually injected numerous times during the production cycle in order to obtain protection from bacterial and viral diseases. During each treatment, the producers experience a loss of three to five percent.

Other problems include tissue damage at the injection site, internal adhesions and suppression of growth, which further reduces the value. Inoculation by injection is also limited, as only fish large enough to tolerate the injection (approximately 20g) may

CELL CULTURE AT BENCHMARK BIOLABS

BENCHMARK BIOLABS STAFF WORKING UNDER STERILE CONDITIONS

be treated, which leaves fish unprotected for several months.

PerOs has delivered oral vaccines to fish as small as 0.5g. Additionally, Oralject can be used to give vaccine boosters in a variety of production stages, further helping producers to protect their stock against disease. Oralject will eliminate the problems associated with injection, and provide producers with the flexibility to build a vaccination strategy to address disease challenges throughout the life cycle of the fish.

PerOs expects that the introduction of Oralject could increase the effectiveness of vaccine usage and its applications, reduce the use of antibiotics, expand current vaccine markets and increase the development of the industry as a whole.

Oralject offers a number of powerful advantages over current therapies. Oralject does not require any specialists or specific skills, as delivery consists of simply replacing a single meal with Oralject. With no needles and a simple administration procedure, Oralject technology provides a straightforward, cost-effective solution that requires only minimal human intervention, leading to important labour savings. It is thus ideal for rapid mass vaccination of large populations of animals.

Benchmark's proven team of product development scientists and manufacturing specialists plans to enable a broad spectrum of bioactive compounds and expand the scope of Oralject applications. Benchmark Biolabs has already completed constructing a licensed Oralject manufacturing plant on its campus in Lincoln, Nebraska, and plans to complete the final phase by the fall of 2007.

PerOs' plans to sell and supply its proprietary Oralject delivery system to regional and multinational pharmaceutical companies, veterinary service organisations and certain fully integrated producers that manufacture custom vaccines for their own use.

Photo courtesy of Dr. Dave Little, University of Stirling