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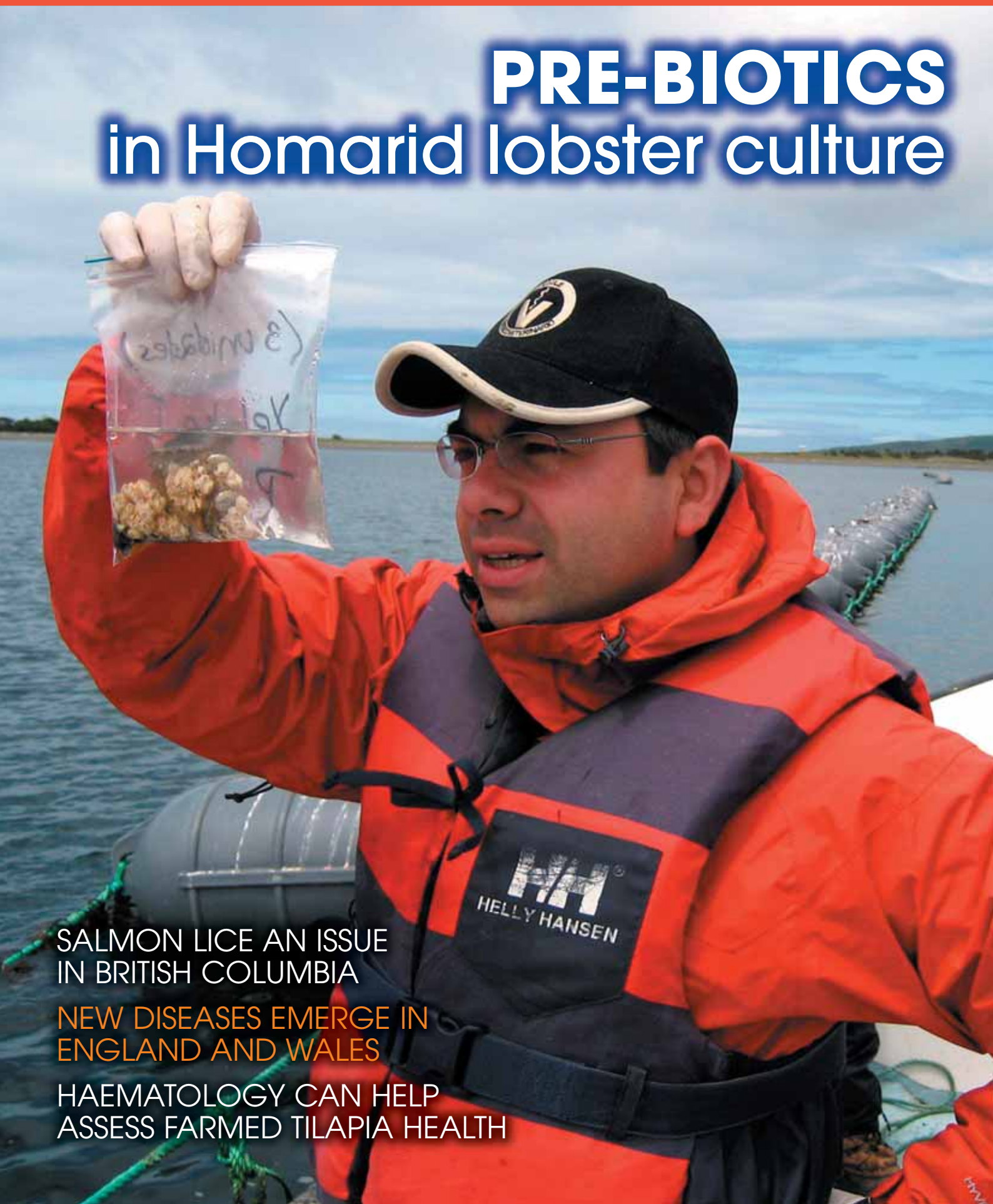
PRE-BIOTICS in Homarid lobster culture



SALMON LICE AN ISSUE IN BRITISH COLUMBIA

NEW DISEASES EMERGE IN ENGLAND AND WALES

HAEMATOLOGY CAN HELP ASSESS FARMED TILAPIA HEALTH

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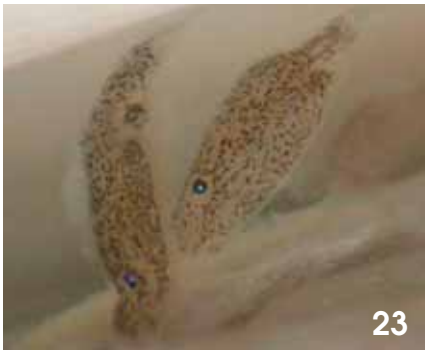

ON THE COVER

Aquagestión staff take field samples
See page 6.

PHOTO BY: Aquagestión

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AQUATIC ANIMAL DISEASE AND BIRD FLU ARE NO RESPECTER OF BOUNDARIES

DR SCOTT PEDDIE, EDITORIAL DIRECTOR

Few of us residing in the United Kingdom will have missed the recent media coverage of the H5N1 bird flu outbreak at a turkey farm in Suffolk.

At the time of writing, the BBC was reporting that the virus that killed the turkeys at the UK plant was 99.96 percent similar to the one that recently infected geese in Hungary. The European Union was investigating how bird flu could have spread from Hungary to England amid claim and counter-claim from the UK farming company involved and the Hungarian state veterinary authorities. Hopefully the issue will be resolved in the very near future and the route and mode of transmission will be elucidated.

Although the specifics of the UK outbreak are perhaps best left for poultry specialists to comment on, the general issues raised by this incident and its trans-boundary overtones are of course of interest to those working in the fish health arena.

It is against such a backdrop that I'm delighted to include a very timely and comprehensive article in this issue of AHI by Rohana Subasinghe and Melba Reantaso of the FAO. The topic of their article is "Pathogen risk analysis".

How bird flu could have spread from Hungary to England

As Rohana and Melba correctly point out, in the aquaculture arena globalisation of trade has played a pivotal role in the spread of a number of pathogens across geographical and political boundaries. And here is where Pathogen Risk Analysis, or PRA, comes into play as a decision-support tool. Rohana and Melba make the point that more widespread usage of PRA will help to reduce the risks associated with trans-boundary aquatic animal diseases, or TAADs. Pro-active risk analysis, used in concert with the whole suite of disease transfer mitigation strategies, can help to reduce the threat posed by TAADs.

Trans-boundary movement of disease is a vital issue for all of us to focus on, whether we have a professional interest in terrestrial or aquatic animal health. The consequences of getting it wrong can have wide-ranging consequences, not least for farmers' livelihoods.

THE COARSE FISH SECTOR IN ENGLAND AND KHV

BY BERNICE BREWSTER (AQUATIC CONSULTANCY SERVICES, UNITED KINGDOM)

In July 1996, in my capacity as an independent fish health consultant, I was asked to attend an ornamental retail outlet that was suffering a disease problem among its koi stocks.

The affected koi had sunken eyes and eroding gills, and had been treated for a range of different parasites, and a large number had already died. Initially it could be assumed that the management and catalogue of treatment meted out was responsible for the state of these unfortunate fish, except the problem was confined to just the koi, although the systems held mixed species and it was apparently spreading to ghost and other carp variants held on the premises.

Koi that had been sold in good faith to customers were causing mortalities to koi and carp in these ponds, while other species of fish remained unaffected. At least 20 hobbyists were affected in a local area, and the koi were not all sourced from the retailer I visited.

What was becoming clear was this was a disease which just affected carp. Samples of the affected koi from the retailer were taken by the Ministry of Agriculture, Fisheries and Food, the then government body in the United Kingdom responsible for fish disease, and tested for SVC and unknown viruses. These results were negative.

This mystery ailment appeared to behave like a viral disease, but in the absence of any positive identification it was delegated as "an infectious agent".

In 2000 the virus was finally isolated and identified as koi herpes virus, and since this date techniques for isolating and identifying the virus have improved. My 1996 samples taken from the koi retailer have been re-submitted for histology, and the slides show every indication of being positive for KHV.

THE UK SITUATION

The koi sector of the ornamental industry flourished in the UK 10 years ago, and it was commonplace for wholesalers to sell koi in the summer and carp for re-stocking fishing lakes in the winter. Similarly, many coarse fish suppliers dabbled in koi sales through the summer months.

There are also strict laws and regulations under the Salmon and Freshwater Fish Act governing the movement of freshwater fish to lakes, rivers and canals in the UK. Under this act, fish may not be stocked into any fresh water without the approval of the Environment Agency, which is designed to protect native fish species from the introduction of alien species or disease.

Regrettably, stocking without the authority of the agency does take place. Sometimes owners of garden ponds, in ignorance of the law, release ornamentals, including koi, into the freshwater environment. More commonly, a member of an angling club who decides to no longer keep pet fish "donates" the koi or their black offspring, which in captivity have achieved weights of 9-13kg, a very desirable size for many carp anglers.

In the winter months, excess carp stocks are routinely cropped and sold on to other fisheries, or used to stock new sites that are going to be opened for fishing. Sometimes carp mortalities had occurred on the site being cropped, but then historically this has occurred in the UK for many years. So once the carp population had recovered in subsequent years these fish would be moved, regardless. This country had the perfect mechanism in place for the insidious spread of KHV.

CARP MORTALITIES REQUIRE IN-DEPTH INVESTIGATION

KHV IDENTIFIED

Sporadic outbreaks of KHV in coarse fisheries have been identified between the period 2000 to 2005. Last summer, however, proved quite decisive in confirming that KHV is in the UK. Through June and July we experienced a period of exceptionally intense heat, and water temperatures on lakes and still waters comfortably exceeded 20° C, the ideal temperature for the virus to replicate. As a consequence some 23 coarse fisheries have suffered serious mortalities of carp and KHV has been identified as the cause.

These are the fisheries where mortalities were investigated by The Environment Agency or the Centre for Fisheries and Aquaculture Science (CEFAS), but there are other sites where significant carp mortalities have occurred but which were not reported to either of the government agencies, as under existing legislation there is no requirement to do so. As from this year, KHV will be included as a notifiable disease, but how this will be regulated is still subject to discussion and will not be finalised until spring 2007. It is my belief that any regulation is now too little and too late, and KHV is already endemic in the UK.

The koi herpes virus is without doubt a serious emerging disease, and much research remains to be done to determine the effects of latency, and whether other cyprinid species are vectors of the disease.

While it is my opinion that KHV is endemic in the UK, it requires a study into the epidemiology in this country. Without ascertaining the spread of the disease in the UK it is difficult to imagine how we might plot a way forward in learning to live with KHV.

Carp vaccinated against KHV using an attenuated vaccine have been imported and stocked in the UK, a move that the coarse fish sector as a whole has widely criticised. Certainly, such antibody-positive carp have the potential to complicate identification of fish that have been naturally exposed to the virus.

Ultimately, a vaccine may be the only method of controlling KHV in the future but, more worryingly, there seems to be a wave of resistance throughout the UK to the use of vaccines, whether for human or veterinary medicine and despite the health benefits achieved through their use.

SERIOUS IMPACT

During the outbreak of 2006 the popular angling press lost sight of KHV as a disease and those affected who were victims of the disease, but turned the incident into an opportunity to curiously berate an industry on which it relies and to instruct readers not to buy fish for re-stocking angling waters and fisheries. Needless to say, this has impacted badly on the coarse fish farmers, for whom sales are very poor this year and whose fish are a reliable source of stock, free from KHV.

The summer of 2007 is forecast to be one of the hottest on record, due to a combination of climate change and the effects of El Nino, in which case we can anticipate further outbreaks of KHV in the UK.

Given the very negative press associated with the 2006 outbreak of KHV and despite the notifiable status imposed on the disease, one has to wonder whether carp mortalities will be reported if temperatures rise as predicted, or whether those involved will try to deal with it and without notifying the authorities. The consequences of the latter are very disturbing. ■

DIAGNOSTIC LABORATORY SERIES

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

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

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

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

This objective services review, in combination with subjective input on management philosophy, will provide readers with a balanced description of the laboratory, and will ultimately help aquaculture veterinary professionals to make informed decisions on selecting appropriate diagnostic service laboratories, aquatic health training and research programmes.

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

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

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

FEATURED DIAGNOSTIC LABORATORY:
Aquagestión SA (Santiago and Puerto Montt, Chile)

AUTHORS: DR GERARDO MUÑOZ AND DR FABIÁN AVILÉS

Aquagestión SA is a subsidiary of Fundación Chile (See *www.fundacionchile.cl*), a privately owned, non-profit institution created in 1976 by the government of Chile and ITT Corporation of the United States. Its mission is to increase the competitiveness of human resources and of the productive services sector by promoting and developing high-impact innovations, technology transfer and administration in Chile.

AQUAGESTIÓN'S FACILITIES

Aquagestión SA, the aquatic animal health division, was created in order to provide an integral service to the national aquaculture sector and to food companies. The company has focused on providing technical-health assistance and diagnosis to the salmon culture industry, and today Aquagestión is one of the primary "*go-to*" industry partners, providing a wide diversity of diagnostic, research and development services to the aquaculture industry in Chile.

Aquagestión currently employs 28 staff and two students. The staff is divided into administrative support (10 people), technical support (five people) and professionals, including five veterinarians, three marine biologists, two biochemists, two general biologists and one food engineer. With this diverse compliment of professional and technical staff, the company is well positioned to satisfy the heavy demand in the southern zone of Chile (Regions X, XI and XII) for

- aquaculture production and health services
- control and certification of aquaculture products
- personnel training and project development in aquaculture health, sanitation and nutrition and
- assistance with quality and environmental management systems.

SERVICES PROVIDED

Aquatic animal health services provided by Aquagestión currently operate out the cities of Santiago and Puerto Montt in Chile, and recently from Guayaquil in Ecuador. The company has provided diagnostic services to the aquaculture sector in Chile for more than 15 years, including diagnostic pathology, parasitology, bacteriology and virology analyses. During the past few years, Aquagestión has developed specialized aquatic health services devoted to mollusk culture, primarily abalone and mussels. The laboratory recently opened in Ecuador is dedicated to the diagnosis of shrimp and tilapia diseases.

The suite of services provided by the diagnostic division of Aquagestión have been officially certified by the Chilean national fisheries office, SERNAPESCA. The procedures used for analysing the samples are based on protocols of the International Sanitary Code for the Aquatic Animals of the OIE. The company comprises specialised and multi-disciplinary sections, including field veterinarians, biochemists, marine biologists, food engineers, medical technologists, microbiologists and general laboratory technicians. The staff has extensive experience in aquatic animal health, and have been trained in all aspects of laboratory quality assurance (QA). Aquagestión provides services to clients in the following areas of expertise:

PATHOLOGY AND PARASITOLOGY:

- Gross pathology and necropsy (fin-fish, mollusk, crustacean)
- Detection of *Myxobolus cerebralis*
- Detection of *Kudoa sp*. (by enzymatic digestion)

AQUAGÉSTION VETERINARIAN GERARDO MUÑOZ TAKING SAMPLES IN THE FIELD

- Post-vaccination evaluation (Speilberg scale for Adhesions)
- Post-vaccination evaluation (Aquaculture Veterinary Society scale for Adhesions)
- Detection of *Diphyllobothrium sp*.

BACTERIOLOGY:

- Basic stains - Gram, Giemsa, Acridine orange, Methylene blue, etc
- Direct and indirect Inmunofluorescence for *Piscirickettsia salmonis*

Aquatic health diagnostic services evaluation checklist

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

CATEGORY	CLINICAL VISITS	WATER QUALITY	PHYCOLOGY	P M	CLINICAL CHEMISTRY	HAEMATOLOGY	CYTOLOGY	HISTOPATHOLOGY	ELECTRON MICROSCOPY	BACTERIOLOGY	MYCOLOGY	VIROLOGY	PARASITOLOGY	TOXICOLOGY	SEROLOGY	ENDOCRINOLOGY
Finfish	F	F	S	F	S	S	S	F	S	F	S	F	F	S	S	S
Molluscs	F	F	S	F	NA	NA	S	F	S	F	S	S	F	S	NA	NA
Crustaceans	F	F	S	F	NA	NA	S	F	S	F	S	F	F	S	NA	NA
Agent ID			M					M		M,C,I, G	M,C	M,C,I,G	M			
Quality assurance	IN	IN	IN	IN	IN	IN		IN		IN	IN	IN	IN		IN	
Referral			A		A	A	A	A	A	A	A	A	A	A	A	A
Reporting	P,F,E	P,F,E	P,F, E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E	P,F,E
Client base	R,N	R,N	R,N	R,N	R,N	R,N		R,N		R,N	R,N	R,N	R,N		R,N	R,N
Cost ($)	$	$	$	$	$	$	$	$	$	$	$	$	$	$	$	$

KEY

Scope of testing	Full testing available = F Selective testing = S
Pathogen/agent ID	By morphology = M By culture = C By immunology/serology = I By genomic / molecular = G By analytical chemistry = AC
Quality assurance scheme	Internal quality control = IN External quality assurance/ring testing = EX Certification = ISO
Referral testing	Available = A Not available = NA
Reporting options	By post = P By fax = F By email = E By website = W
Client base	Regional = R National = N International = I
Cost of services	Full cost recovery = $ Partial subsidy = P$ Full subsidy = F

FEATURED DIAGNOSTIC LABORATORY: AQUAGESTIÓN SA

AT WORK IN ONE OF THE AQUAGESTIÓN LABORATORIES

FRANCISELLA IN SALMON

A DISEASED ABALONE

- Indirect Inmunofluorescence BKD, *Vibrio ordalli, Vibrio anguillarum I and II, Moritella viscosus, Photobacterium damsella, Flavobacterium psycrophilum, Tenacibaculum maritimum, Aeromonas salmonicida atípica* and others
- Isolation of pathogens in specific culture media
- Bacterial counts in water (eg *Flavobacterium sp, Vibrio sp, Aeromonas sp, Pseudomonas sp*)
- Bacterial identifications using biochemical conventional tests, commercial tests (API), serological tests, etc

VIROLOGY AND CELL CULTURE:

- Diagnosis of IPNV (Cell culture, cell culture + IFAT IPNV)
- Diagnosis of IHN, VHS, EHN, OMV (Active Surveillance Programme of Sernapesca: PVA, cell culture with neutralising IPNV)
- Isolation of *Piscirickettsia salmonis*
- Minimum inhibitory concentration (MIC) for *P salmonis*
- Diagnosis of other virus in sensible cellular lines: CCO

MOLECULAR DIAGNOSTICS:

- RT-PCR IPNV
- RT-PCR ISAV
- RT- PCR NORWALK
- RT-PCR MULTIPLE IHN-VHS-EHN
- PCR BKD
- PCR SRS
- PCR *Flavobacterium psychrophilum*
- PCR *Tenacibaculum maritimum*
- PCR *Vibrio anguillarum*
- PCR *Aeromonas salmonicida* atypical

HISTOPATHOLOGY AND CLINICAL PATHOLOGY:

- Sampling and fixation of fish, shellfish and crustacean for histopathological diagnosis
- Histology slide preparation and staining
- Inmuno-histhochemistry for the diagnosis of IPNV, SRS, atypical Furunculosis, Vibriosis, Flavobacteriosis, BKD
- *In situ* hybridisation for the diagnosis of the Withering Syndrome of abalone

- Imaging laboratory
- Storage of histology blocks and slides for retrospective studies
- Serum metabolites analyses, including AST, ALT, alkaline phosphatase, creatinine, cholesterol, triglycerides, total proteins, albumin, inorganic phosphorus, amylase, magnesium, calcium, total bilirubin, glucose, urea and others
- Blood analyses, including haematocrit, haemoglobin, total counts and differential counts of erythrocytes and leukocytes
- Plasmatic electrolytes analysis
- Aquagestión offers clients a range of QA programmes based on specific client needs and goals. Currently Aquagestión - Fundación Chile participates in a quality assurance level that allows the company to complete work to GLP (Good Laboratory Practices) standards where and when necessary. The company has targeted ISO 17025 as a final quality assurance goal.

RESEARCH COLLABORATIONS AND LINKAGES

The company has long been dedicated to aquatic animal health research and development investigation (r and d) with industry partners. For example:

- Development of new techniques for the prevention and control of infectious and non-infectious diseases in abalone culture
- Development in Chile of techniques for diagnosis and control of the virus causing the coho salmon *icteric-syndrome* (jaundice)
- Comparison of diagnostic techniques for detection of the virus IPN in Atlantic salmon broodstock (cellular culture, RT-PCR-ELISA, RT-PCR-Electrophoresis)
- Comparison of diagnostic techniques for the detection of bacterial kidney disease and Piscirickettsiosis in Atlantic salmon broodstock
- Comparison of three methodologies of extraction and diagnosis of Virus IPN by means of electrophoresis in gels and ELISA in carriers of Atlantic salmon
- Comparison of IFAT and FAT *Piscirickettsia salmonis* for the diagnosis of carriers in Atlantic salmon
- Determination and characterisation of the prevalence of bacteria of the *Flavobacterium* genus in the ovarian fluid in broodstocks of Atlantic salmon and rainbow trout:

Several noteworthy achievements have resulted from these collaborative projects, including,

- the developed methodologies that allow isolation of *Piscirickettsia salmonis*
- Genotyping of infectious pancreatic necrosis virus (IPNV) by means of RLFP (Restriction Fragment Length Polymorphisms), and the
- Development of PCR assays for the diagnosis and confirmation of infectious salmon anaemia virus (ISAV), *Nucleospora salmonis, Streptococcus phocae, Aeromonas salmonicida, Piscirickettsia salmonis* and *Renibacterium salmoninarum*.

During the past year Aquagestión has increased efforts to develop integrated molecular testing of aquatic animal pathogens, employing both conventional PCR and qPCR (Real-Time PCR). Currently, conventional PCR is used for identifying viral and bacterial agents. Serotyping/genotyping of IPNV isolates is further accomplished using the RFLP technique. The Real-Time RCR is employed for rapid identification of IPNV and BKD in reproductive sample screening programmes.

Aquagestión also provides environmental and food safety testing services, primarily satisfying the demands of the food processing sector of the aquaculture industry in southern Chile. This sector of the industry is expanding, and the company expects demand for food safety and environmental testing to expand in the coming years. These services are being developed primarily to assist aquaculture companies to meet the ever more stringent environmental regulations in Chile.

DODECACERIA FROM ABALONE

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VACCINATION PROGRAMME OFFERS *STREPTOCOCCUS* CONTROL AND BETTER RETURNS

BY CHRIS HAACKE
(SCHERING-PLOUGH ANIMAL HEALTH, SAFFRON WALDEN, UNITED KINGDOM)

Extensive trials at tilapia farms in Latin America have indicated that the Garvetil Immersion Prime and Oral boost vaccination programme limits the costly impact of *Streptococcus* infection. The trials have demonstrated a reduction in the costly effects of mortality and fillet damage at harvest.

These are the key findings of a 15-month trial using a vaccination programme developed by Schering-Plough Animal Health Aquaculture. This involved immersion vaccination of over 140,000 fry with AquaVac™ Garvetil™ vaccine, followed by an in-feed vaccination booster, AquaVac Garvetil Oral. The oral booster was given 11 weeks after the immersion vaccination to selected groups, and was shown to provide significant protection against *Streptococcus iniae* infection.

SITE ASSESSMENT AND TRIAL PREPARATION

Before the commercial trials began, a comprehensive survey of the prevailing pathogenic profile of the operation was completed. Meninges and kidneys from tilapia taken from the fattening cages were analysed. Lesions and melanisation typical of infection were observed. The results confirmed a significant level of *Streptococcus* iniae and other *Streptococcus* species present in the operation.

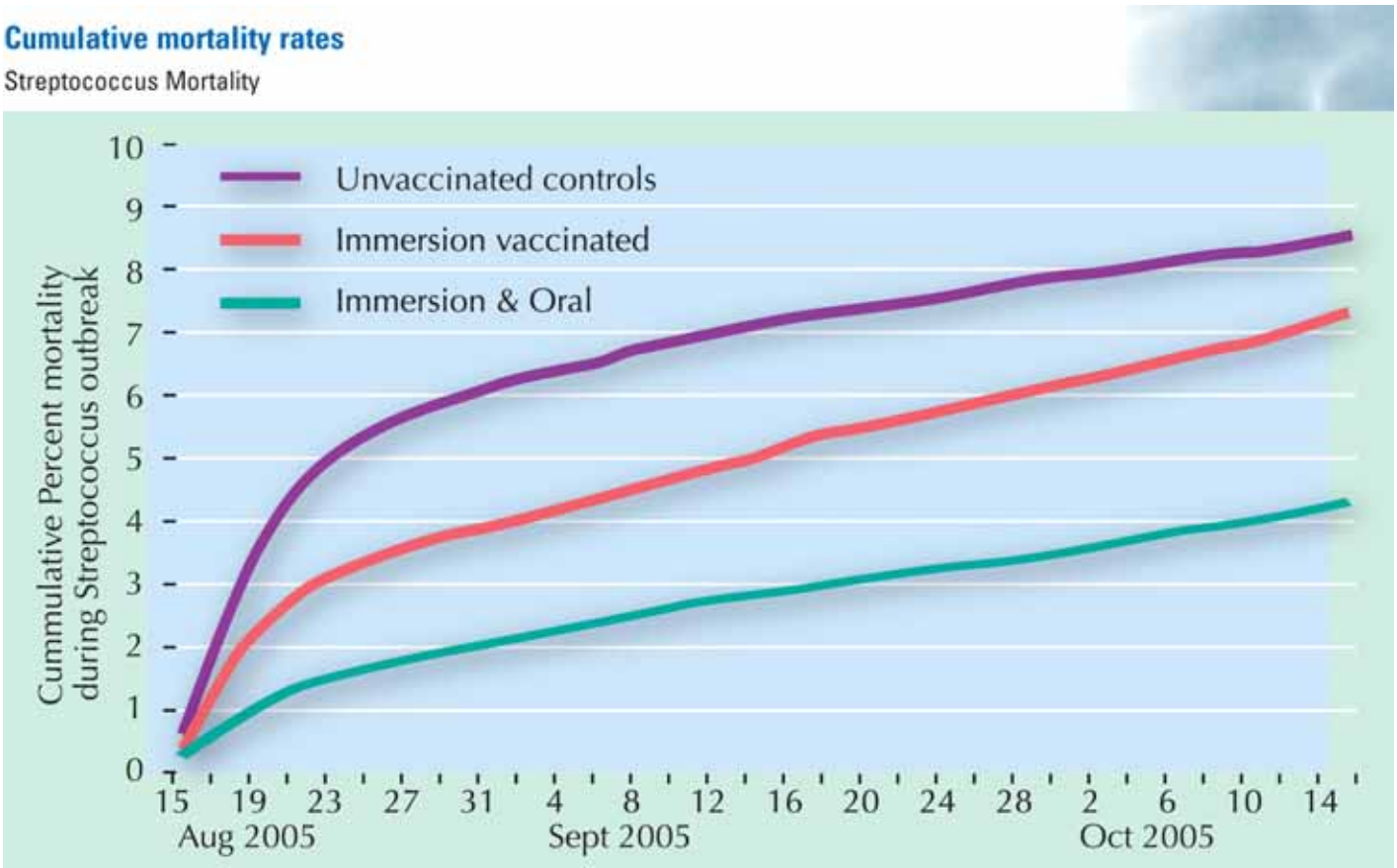
An assessment of the historic disease profile, seasonal variations and operating routines was also explored. The information gathered provided the necessary background to establish the vaccination routine. The sample groupings for the trial comprised:

- Control group - (not vaccinated)
- Immersion group
- Immersion and oral booster group (taken from group 2)

> The mortality profile showed that losses in tilapia that had immersion and booster vaccination cover were significantly lower than the other trial groups

IMMERSION

On day one, fry from the hatchery were immersion-vaccinated for 60 seconds with AquaVac Garvetil. The tilapia fry averaged 1.3g weight. Fry were then returned to the hatchery and held ▶



VACCINATION PROGRAMME OFFERS STREPTOCOCCUS CONTROL AND BETTER RETURNS

Vaccination	Mortality	RPS
Unvaccinated controls	8.6%	N/A
Garvetil Immersion Prime only	7.28%	15%
Garvetil Immersion and Garvetil Oral Booster	4.28%	50%

Fish from group 3 displayed only moderate myositis in a clear process of healing and scar tissue formation

for a week. This time allowed for the onset and development of immunity post-vaccination.

One week later and at 2.2g the fry were transferred to nursery units. The routine was in accordance with the recommendations from Schering-Plough Animal Health Aquaculture. This also helps to ensure effective vaccination by highlighting the importance of limiting stress before, during and after the immersion.

Fish were not handled for 60 days post-vaccination, allowing for optimal development of immunity. At the end of the period the group's recorded average weights were 126g and 141g respectively. Survivability levels were recorded at 85 percent, which were consistent with previous results. The mortality profile showed no difference between the vaccinated and control fry. The figures clearly indicated successful management of the immersion vaccination.

There were no reported instances of disease during the nursery phase. However, bacterial sampling indicated that *Streptococcus spp* and *Streptococcus agalactia* were present.

BOOSTER

Eleven weeks later, AquaVac Garvetil Oral was administered to the booster group of 62,000 fish (including replicates). The in-feed booster routine was carried out in two stages over a two-week period.

STAGE 1
Day 1-5, oral booster included in the feed
Day 6-10, normal feed routine

STAGE 2
Day 11-15 oral booster included in the feed

Vaccine uptake is assisted by Schering-Plough's antigen protection vehicle, APV. This is a unique technology the company has developed for in-feed vaccination. It protects the antigens through the acidic stomach environment, and delivers them intact to the hindgut, where an immune response is initiated.

As with the initial immersion, mortality levels were monitored and no noticeable changes were reported post-vaccination.

TRANSFER TO GROW-OUT CAGES

Three weeks later the tilapia were transferred to grow-out cages in their groups. Losses reported post-transfer amounted to six percent. Two weeks later a classic outbreak of *Streptococcus* disease was observed. The mortality profile showed that losses in tilapia that had immersion and booster vaccination cover were significantly lower than the other trial groups.

The mortality rate of the Immersion-primed and orally boosted group was only half that of the unvaccinated controls (Relative Percentage Survival (RPS) 50 percent, P=0.032). This confirms that the protection provided had the desired effect at the key challenge point (See associated figure and table).

HARVEST AND HISTOPATHOLOGY

At harvest, signs of *Streptococcus* infection are easily identifiable in fish that have been challenged. The classic presentation of fillet lesions is clearly visible in fish that have experienced a *Streptococcus* challenge. As the original trial groups had been maintained during the study, detailed analysis of the response to *Streptococcus* challenge was possible.

Respected fish pathologist Dr Gina Conroy reviewed the histopathology on fish from the trial. The results indicated clear differences in the degree and progression of infection.

In the unvaccinated control group there was clear evidence of severe and phlegmatose myositis consistent with significant challenge.

Group 2, which had received only the immersion vaccine, exhibited severe pyogranulomatous myositis with melanisation. Fish from group 3, which had received immersion followed by booster vaccination, displayed only moderate myositis in a clear process of healing and scar tissue formation.

This fillet examination provided further confirmation of the levels of protection provided by the two-stage vaccination programme. Mario Aguirre from Schering-Plough Animal Health Aquaculture believes that tilapia farmers have a significant opportunity to protect their operations from *Streptococcus* challenges.

"These results and other field-based experiences clearly demonstrate the benefits of using the Garvetil vaccination programme to protect against this highly damaging disease," Aguirre said.

Importantly for operations with a *Streptococcus* disease burden, two-stage vaccination offers real financial benefits. "In this situation, to have a programme that reduces production losses at the same time as protecting fillet values is a real opportunity," he said.

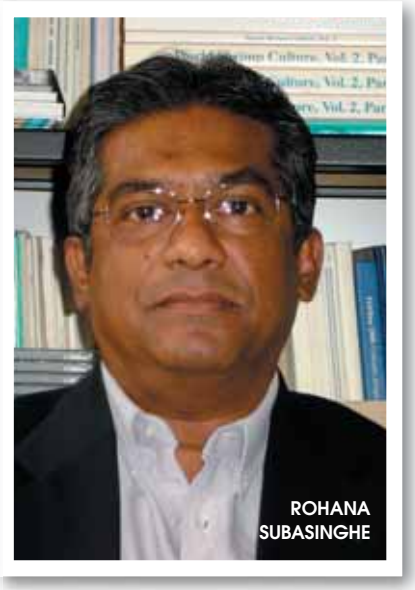
AquaVac Garvetil vaccines are now registered in Honduras, Venezuela, Ecuador, Philippines and Indonesia, and are in the process of registration in other key tilapia-producing countries.

For more information please contact your local Schering Plough Animal Health representative.

PATHOGEN RISK ANALYSIS – CAN IT MINIMISE THE IMPACTS OF TRANS-BOUNDARY AQUATIC ANIMAL DISEASES?

BY DR MELBA G BONDAD-REANTASO AND DR ROHANA P SUBASINGHE
(AQUACULTURE MANAGEMENT AND CONSERVATION SERVICE, DEPARTMENT OF FISHERIES AND AQUACULTURE, FOOD AND AGRICULTURE ORGANISATION OF THE UNITED NATIONS, ITALY)


MELBA REANTASO


ROHANA SUBASINGHE

The General Agreement on Tariffs and Trade (GATT) and the creation of the World Trade Organisation (WTO) in 1995 resulted in the liberalisation of international trade which, while creating new market opportunities for farmed aquatic animals and their products, also facilitated the global spread of pathogens and diseases associated with host movements. Consequently, importing countries are required to develop mechanisms to protect themselves against exotic diseases, while assuring trading partners that any disease concerns are justified and are not disguised barriers to trade.

The Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) requires WTO member countries to use the risk analysis process as a means to justify any restriction on international trade based on risk to human, animal or plant health. As a result, risk analysis has become an internationally accepted standard method for deciding whether trade in a particular commodity (a live aquatic animal or its product) poses a significant risk to human animal or plant health, and, if so, what measures could be applied to reduce that risk to an acceptable level.

Let us look at the history of risk analysis and its application to aquatic animal health.

WHAT IS A RISK AND WHAT IS A HAZARD?

If you went to work this morning by bicycle, car or train, if you put your money in a bank or in stocks or under a mattress, if you bought a lottery ticket at a newsstand or gambled at a casino, and if you engaged in activities that involve an element of chance you were taking a risk. Because chance is something that is intimately connected with risk.

Risk originated from the French word risqué, which means "danger, in which there is an element of chance", while hazard comes from a game of chance (a type of dice game) invented in a castle named Hasart or Asart in Palestine while it was under siege.

In this modern age, risk is defined as a combination of the likelihood (or possibility) of occurrence of undesired outcomes and the severity (or magnitude) of consequences, while hazard is defined as the presence of a material or condition that has the potential to cause loss of harm. No matter how well managed a system is, there will always be associated risks and hazards.

Driven by multiple objectives for resource protection (human, animal and plant health; aquaculture, wild fisheries and the general environment) as embodied in international agreements and responsibilities that include the WTO's SPS Agreement (WTO 1994), UNEP's Convention on Biological Diversity and the supplementary agreement Cartagena Protocol on Biosafety (CDB 1992) and the Codex Alimentarius Commission), risk analysis is now considered as an important decision-making tool.

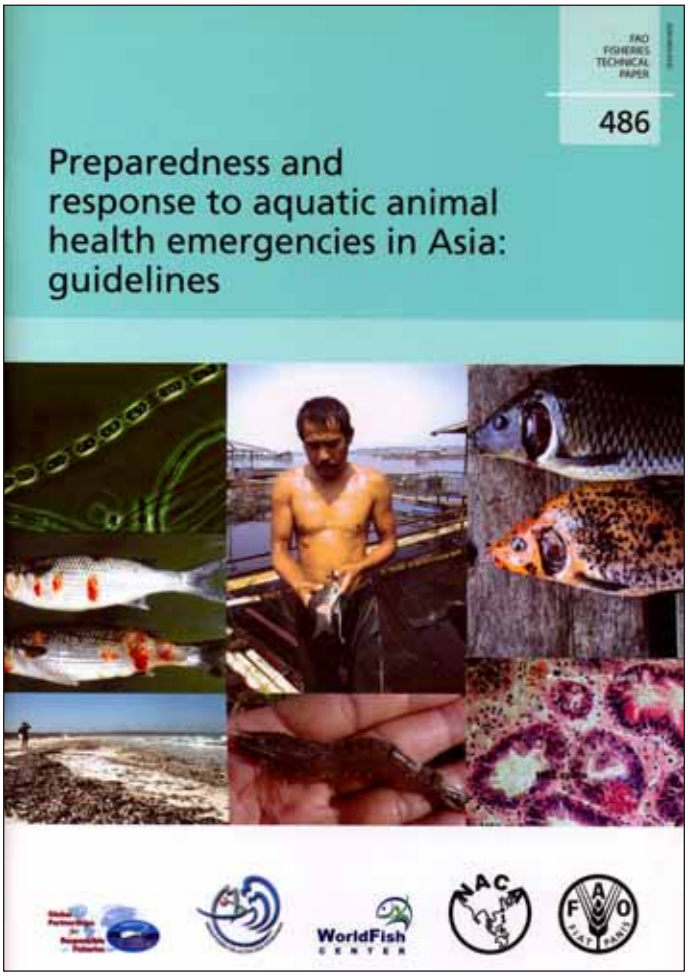
In the context of aquatic animal health, risk analysis (also termed "import risk analysis") is a structured process for analysing the disease risks associated with the international and domestic movements of live aquatic animals and their products. Although formal risk analysis is used primarily to address international trade issues, the risk analysis process can also be applied to assessing disease risks at the regional, local and farm level.

The World Animal Health Organization (OIE) made the pioneering initiative in applying risk analysis to aquatic animal health when it organised the International Conference on Risk Analysis in Aquatic Animal Health in 2000 (OIE 2001) in Paris, France. This conference successfully engaged an international dialogue and provide information on the subject to scientists, academics and regulators responsible for developing, evaluating and implementing import measures in aquatic animal health.

The Asia-Pacific Economic Cooperation (APEC), in cooperation with the Network of Aquaculture Centres in Asia-Pacific (NACA) and FAO, jointly implemented a project in 2002 which raised awareness and built capacity (Arthur and Bondad-Reantaso 2004) and produced a manual on pathogen risk analysis (Arthur *et al* 2004) in the Asia-Pacific and Latin America and the Caribbean regions.

Other examples of implemented risk analysis include that of the Secretariat of the Pacific Community's Pathogen and Ecological ▶

FAO PUBLICATION - STATE OF WORLD AQUACULTURE 2006



FAO PUBLICATION - PREPAREDNESS AND RESPONSE TO AQUATIC ANIMAL HEALTH EMERGENCIES IN ASIA: GUIDELINES

Risk Analysis for the introduction of blue shrimp and giant river prawn from Brunei Darussalam to Fiji, and from Fiji to the Cook Islands, respectively. Some countries have quite strong biosecurity programmes, and routinely conduct generic and specific IRAs for the movement of aquatic animals.

UNCERTAINTIES IN RISK ANALYSIS

Uncertainty is a central feature of risk analysis for aquatic animals. This is due to the wide variety of species traded, the various life cycle stages, production systems, etc, and the fact that essential information on aspects of pathogen biology is often lacking. Nonetheless, governments are obliged to make practical decisions based on risk analysis within these constraints.

The lack of scientific information and capacity to undertake risk analysis will continue to be major challenges. When data is lacking and evidence of serious risks exists, an important approach that needs to be considered is the precautionary approach (Garcia 2006).

It must be applied responsibly and should be used as a temporary measure until such time as a more objective risk analysis (supported by scientific information) can possibly be undertaken. Deciding on the *appropriate level of protection*, or ALOP (a societal value judgement about how much a community is willing to pay for protection against incursions, in forgone trade versus the benefits of trade) or answering the question "What is an acceptable risk", is another major challenge. This will need to take into consideration the economic and social value of aquaculture and capture fisheries, the perceived value of natural biodiversity, the likely economic and social benefits of trade in aquaculture animals and their products, as well as the diverse interests of all the relevant stakeholders.

Another great hurdle is how to protect the people at risk (those most vulnerable) and their livelihoods. How they could be the focus of the "first mile" of protection is another challenge that needs to be addressed. Risk communication plays a critical role and is essential to maintain the integrity of the risk analysis process.

FAO INITIATIVES

The FAO's Department of Fisheries and Aquaculture strongly supports responsible and safe trans-boundary movement of live aquatic animals where the application of risk analysis is a key element in the process. A number of the FAO's Technical Cooperation Projects have, for example, components on developing national strategies on aquatic animal health, capacity building on pathogen risk analysis and surveillance, emergency preparedness and strengthening the biosecurity framework.

The FAO also recently published FAO Fisheries Technical Paper 486 (Arthur *et al* 2005) entitled Preparedness and response to aquatic animal emergencies in Asia: guidelines, and is currently finalising the global Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals, the 15th in the series of FAO Technical Guidelines for Responsible Fisheries.

These guidelines will assist member governments to reduce the risk of introducing and spreading serious trans-boundary aquatic animal diseases (TAADs) via the international and domestic movement of live aquatic animals, as well as to provide guidance on health management at the farm and farm-cluster level to the extent that these local production units are involved in the spread of TAADs.

The FAO has also broadened its interest in the risk analysis process within the overall framework of biosecurity, and is currently preparing a desk-top review on the application of risk analysis in

aquaculture. The objective of this review is to

- better understand the risks, hazards and vulnerabilities
- develop methods to assess them, as well as study the connections between the different risk events and patterns, and
- identify integrated approaches to risk management.

Part of this work will lead to an expert consultation that will initiate the development of Technical Guidelines on the Application of Risk Analysis in Aquaculture.

COULD PATHOGEN RISK ANALYSIS (PRA) PREVENT FUTURE OUTBREAKS, MINIMISE THE IMPACTS OF POTENTIAL TAADS AND CONTRIBUTE TO AQUACULTURE SUSTAINABILITY?

Epizootic ulcerative syndrome, white spot syndrome virus, viral nervous necrosis, Taura syndrome virus, *Haplosporidium nelsoni* (MSX disease) and koi herpes virus are some classical examples of trans-boundary aquatic animal diseases.

Some of them continue to spread, other diseases remain unresolved, and there are newly emerging diseases (in shrimp, eg infectious myonecrosis virus, Monodon slow growth agent). The list goes on. As indicated at the beginning, globalisation of trade facilitated by more efficient transportation played pivotal roles in the introduction and spread of most of these pathogens across aquaculture regions of the world.

Could PRA prevent future outbreaks, minimise the impacts of potential TAADs and contribute to aquaculture sustainability? We leave it to the concerned individuals to judge.

Pathogen risk analysis will not prevent or reduce TAADs, but the application of PRA in making decisions on movements will certainly help in reducing the risks on TAADs.

Risk analysis is simply one part of the health management process, and a key element of national strategies on aquatic animal health and biosecurity. It cannot function effectively unless other components of the national strategy have also been developed, ie in addition to appropriate legislation and policy and the means to implement them, capacity in areas such as diagnostics, quarantine and inspection services, disease surveillance, monitoring and reporting, national pathogen lists, contingency planning, emergency preparedness and research.

Although we have listed a number of constraints, these should not deter any country or organisation with strong interests in resource protection from striving to address such challenges so that the risk analysis process will truly be an effective decision-making tool.

Developing countries, in particular, which are always faced with limited manpower and competence and other resources, should pro-actively embrace the risk analysis process at the level possible under existing national expertise and capacity. The biggest challenge would be to convince live aquatic traders that the application of risk analysis is not an overly complicated process, and good PRA prior to movement would certainly create long-term financial incentives.

Preventing the entry of diseases into aquaculture systems (pro-active risk analysis), better husbandry and management practices, maintaining a healthy environment and responsible trading are the key to safe and healthy aquatic production. Otherwise, with the current trend of intensification, the aquaculture sector will be continuously challenged with lost revenue due to production losses and efforts to contain and eradicate diseases.

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REFERENCES

Arthur JR and Bondad-Reantaso MG (eds) 2004. Capacity and awareness building on import risk analysis (IRA) for aquatic animals. Proceedings of the workshops held April 1-6, 2002 in Bangkok, Thailand and August 12-17, 2002 in Mazatlan, Mexico. APEC/FWG 01/2002. NACA, Bangkok. pp203

Arthur JR, Bondad-Reantaso MG, Baldock FC, Rodgers CJ and Edgerton BF 2004. Manual on pathogen risk analysis for the safe movement of aquatic animals (FWG/01/2001). APEC/DoF/NACA/FAO. pp59

Arthur JR, Baldock FC, Subasinghe RP and McGladdery SE 2005. Preparedness and response to aquatic animal health emergencies in Asia: guidelines. FAO Fisheries Technical Paper No. 486. Rome, FAO. pp40

CBD 1992. Convention on Biological Diversity. June 5, 1992. pp29 (See http://www.biodiv.org/convention/articles.asp).

Garcia S 1996. The precautionary approach to fisheries and its implications for fishery research, technology and management: an updated review. In: Precautionary approach to fisheries. Part 2: scientific papers. Prepared for the Technical Consultation on the Precautionary Approach to Capture Fisheries (including species introductions). Lysekil, Sweden, June 6-13, 1995. FAO Fisheries Technical Paper No. 350, Part 2. pp210

OIE 2001. Proceedings of the OIE International Conference on Risk Analysis in Aquatic Animal Health. C Rodgers (ed). OIE, Paris, France. pp346

WTO 1994. Agreement on the Application of Sanitary and Phytosanitary Measures. P. 69-84. In: The results of the Uruguay round of multilateral trade negotiations: the legal texts. General Agreement on Tariffs and Trade (GATT), World Trade Organisation, Geneva.

INTER-CALIBRATION OF WHITE SPOT SYNDROME VIRUS PCR LABORATORIES IN INDIA

DR CV MOHAN

BY DR CV MOHAN (NACA, THAILAND)
AND PROF PETER WALKER (CSIRO, AUSTRALIA)

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PROF PETER WALKER

The elimination of infected seed prior to stocking is arguably the most important single factor in reducing the risks of diseases in shrimp farming within India and throughout the region. This can be achieved by the proficient PCR testing of broodstock and/or seed. In fact in many countries around the region PCR is now used to screen shrimp seed for white spot syndrome virus or WSSV prior to stocking.

However, white spot disease (WSD) continues to seriously impact shrimp production, and it is suspected that variations in the reliability of screening results, compounded by on-farm factors, may result in the outbreak of disease even when seed has been properly screened. One of the main reasons for this has been a lack of harmonisation or an inter-calibration of the PCR testing capabilities of different service-providing laboratories. This variance in the quality of testing and accuracy of results has contributed toward an erosion of the confidence shrimp farmers have in laboratory PCR testing.

PACKING SAMPLES FOR RING TESTING

FIRST PCR TRAINING WORKSHOP

Under the ACIAR-funded Regional Project - Application of PCR for improved shrimp health management in the Asian region - being implemented since January 2005, a five-day PCR training workshop was jointly organised by MPEDA, CIBA-ICAR, CSIRO and NACA from October 17 to 21, 2005, at CIBA, Chennai, India.

A total of 28 participants representing mainly the private and government PCR service-providing laboratories were trained. The training workshop had lectures and practical components. The course curriculum followed a format successfully implemented by ACIAR and CSIRO in several Asian countries and further developed at Mahidol University, Thailand. The participants of the I PCR training workshop also agreed on the modalities for the conduct of I PCR inter-calibration exercises.

FIRST PCR INTER-CALIBRATION

Under the framework of the ACIAR-funded regional project, a voluntary PCR inter-calibration programme was developed and executed in June 2006. The technique, which is also known as ring testing, aimed to provide an overview of the current quality of WSSV PCR testing in participating laboratories in India.

The approach would identify which laboratories might require more assistance in improving their testing procedures, and offer individual laboratories the opportunity to compare their results with other laboratories undertaking PCR testing for WSSV. In effect, inter-calibration not only provided a step towards accreditation but also gave participants an opportunity to assess their own performance.

Briefly, 100 sets of ten samples (1000 samples) were prepared and coded by scientists of CSIRO and CIBA. These included 100 sets of five DNA samples and 100 sets of five tissue samples. This preparation and coding of samples took considerable planning and required two weeks of meticulous work by scientists from CSIRO Australia and CIBA Chennai.

The DNA samples were derived from WSSV experimental infection in adult *Fenneropenaeus indicus* using infected material from a hatchery near Chennai. Batches were sent to 49 laboratories throughout India, with five batches sent to the CSIRO in Australia and six batches in CIBA, India.

Participation in the inter-calibration exercise was voluntary, and the results of all testing remained strictly confidential, a key to the success of the exercise. A code number identified each participating laboratory. Throughout the trials the code numbers remained private, with participating laboratories only informed of their own identification number.

Once completed, the laboratories returned their results, which were collated into a summary table. Individual laboratories could then view their own results and compare these with results from other facilities. However, the confidential nature of the test results meant that the names of the laboratories would never be identified.

The overall performance of the participating laboratories was rated as good, with over half of the participating laboratories returning either excellent or acceptable results. This is very reassuring, and should help to boost the confidence of the farmers and hatcheries in the seed testing process implemented in India.

However, some of the laboratories reported positive reactions in negative samples (indicative of problems with test contamination), while some failed to detect positive samples (indicative of a problem with test sensitivity).

This is a cause for concern and needs to be addressed on a case-by-case basis through assistance with resourcing, technical advice and training. The participating labs have been requested to review their individual results, compare the performance with other labs and contact relevant institutions for technical assistance. ▶

BASIC ATLAS OF ATLANTIC SALMON (*Salmo salar* L) BLOOD CELLS

By DA Conroy and G Conroy 2006 (Patterson Peddie Consulting, Carrickfergus, UK. CD-ROM. ISBN: 978-0-9553926-0-3)

The expansion of salmon culture has brought in its train the development of other needs, such as health and nutritional problems that commonly occur in this type of activity. In general the prevention, and more particularly the anticipation, of these problems obliges one to be in permanent contact with the animals.

For that reason, stricter control requires the use of auxiliary methodology, such as occurs with haematology, as a means of assessing the health status of the animal in its culture conditions. The authors (Dr David Conroy and Mrs Gina Conroy MSc) accept this need, and with their ample experience of the subject they offer a wide vision on the use of different blood cell elements that frequently occur in disease problems affecting salmonids.

In this book we find a precise explanation of the presence of members of the red blood cell series and of the white blood cell series in Atlantic salmon. It also complements the Manual of Haematology which the authors are currently preparing.

In conclusion, this is a magnificent aid that will be required in productive Atlantic salmon farming activities, and serves to help interpret many phenomena which occur in practice. It has very clear photographs, which could only be obtained with great difficulty in fieldwork.

Dr Manuel M Fukushima-Nagaoka, Profesor Emérito, Departamento de Pesquería, Facultad de Ciencias Biológicas, Universidad Nacional de Trujillo, Trujillo, Departamento de La Libertad, Perú

The photographs are excellent, and the descriptions of the blood cells are very clear. These descriptions are very concise, and well related to the figures. The atlas is very clear and didactic. No further comments are necessary.

Dr Gustavo A Alvis-Hernández, Director Nacional de Acuicultura, Solla SA, Buga, Departamento del Valle, Colombia

It is with great pleasure that I recommend the *Basic Atlas of Atlantic Salmon (Salmo salar L) Blood Cells*, written by the well-known specialists David and Gina Conroy. This atlas contains excellent colour photographs of the blood cells, and a clear and detailed description of each blood cell type. It is very detailed and didactic, and excellent for use in classrooms and laboratories. It really recommends itself.

Dr Lucio Galaviz-Silva, Patología Molecular y Experimental, Centro Nacional de Sanidad Acuícola (CENASA), Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Monterrey, Estado Nuevo León, México

For further information on how to order this CD-ROM, which is available in both English and Spanish language versions, see www.pattersonpeddie.com/cdbooks.html, or email info@pattersonpeddie.com

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2ND PCR TRAINING WORKSHOP

The second PCR training workshop was held in Chennai from October 23 to 26, 2006. Twenty-six participants (23 from India and one each from Bangladesh, Myanmar and Sri Lanka) attended the workshop. The same set of participants had attended the first PCR training workshop completed in the previous year.

The workshop provided an opportunity to participants through hands-on training to further improve their skills in performing PCR. To ensure effective learning and uptake, practical exercises were conducted in five small batches of five participants, and each batch was provided with one expert demonstrator.

All the participants were provided with individual operator ID's. The participants ran a total of 1056 PCR reactions over four days, and each participant had an opportunity to run over 40 PCR reactions with different types of samples and primers.

2ND PCR INTER-CALIBRATION

A half-day workshop on PCR laboratory accreditation attended by training workshop participants was held on October 27, 2006 at CIBA, Chennai. The workshop provided an opportunity to discuss the results of the first PCR inter-calibration exercise, and also to agree on the modalities for running the second PCR inter-calibration exercise.

The second PCR calibration was scheduled for January, 2007. The PCR laboratory workshop participants agreed that participation in the second PCR calibration exercise would be on a voluntary basis, and the identity of the labs should be kept confidential.

WAY FORWARD

MPEDA is keen to develop and implement a PCR laboratory accreditation programme in India and is likely to become operational before the end of 2007. The purpose of the two PCR training workshops and two PCR inter-calibration exercises, under the umbrella of the ACIAR regional project, is to prepare the PCR laboratories for participation in a future PCR laboratory accreditation programme.

For further information, please contact Dr CV Mohan at mohan@enaca.org or Prof Peter Walker at Peter.Walker@csiro.au

VIETNAM: TESTING OF PIONEERING SHRIMP PRODUCT BEGINS

The biotechnology company Aqua Bounty Technologies Inc has been granted approval to begin large-scale testing of its pioneering immunostimulant Shrimp IMS ("IMS") in Vietnam, one of the world's four largest shrimp-producing countries. IMS is a feed additive administered through the entire shrimp life cycle to protect it against a range of diseases.

This endorsement by Vietnam's National Aquaculture and Fisheries Quality Assurance Veterinary Directorate (NAFIQAVED) builds upon the contract awarded to the company earlier in 2006 to supply shrimp-disease diagnostic kits to government laboratories throughout the country.

NAFIQAVED is establishing comprehensive measures to assist farmers after disease has considerably reduced Vietnam's shrimp harvests in recent years.

Additionally, Aqua Bounty Technologies, which focuses on enhancing productivity in the aquaculture market, continues to make substantial progress in securing regulatory approval for Shrimp IMS in its other key target markets in Asia and Latin America. Certificates of Exportability issued by the United States Food and Drug Administration, which confirm that the product complies with all US regulations and can be exported from the US, have been submitted to 14 countries, including China, Thailand and Indonesia, the three leading shrimp-producing countries in the world.

Aqua Bounty continues to consolidate its commercial position in the Asian and Latin American shrimp markets, and says it expects to add several new distributors over the coming months.

There are currently 15 active trials with potential customers in Latin America, and Asian trials were reported to have begun. Before the sales process can begin, trials of the product are required to demonstrate its effectiveness and to secure local regulatory approval.

The commercial effectiveness of Shrimp IMS has been proven in Mexico and Ecuador, as well as in numerous laboratory and field trials. Results, on average, have shown a 30 percent increase in the survival rates of IMS-treated shrimp compared to untreated shrimp, and a return of investment of up to $2.50 for every dollar shrimp farmers spent on the product.

The company says it is confident that regulatory approval will be granted in the majority of locations in the first half of 2007.

"I am delighted with the commercial progress of our Shrimp IMS product, and look forward to working with the NAFIQAVED on the roll-out of the product throughout Vietnam," said the chief executive officer of Aqua Bounty, Elliot Entis.

"This is an important milestone for us in delivering against our strategy that we communicated on flotation. There continues to be strong demand for our products, and a visible pipeline of development and opportunities in the Asian and Latin American shrimp markets that the company continues to target."

AUSTRALIA: DRAFT RISK ANALYSIS FOR PRAWNS RELEASED

Biosecurity Australia issued a revised draft import risk analysis (IRA) report at the end of November that assesses the quarantine risks associated with importing prawns and prawn products into Australia.

Australia currently allows prawns and prawn products to be imported for human consumption, subject to compliance with quarantine conditions intended to manage the risks of yellowhead virus, white spot syndrome virus, or WSSV, and Taura syndrome virus.

These conditions, which include health certification, on-arrival inspection and testing for WSSV, were introduced progressively from 2000 on an interim basis while the comprehensive risk analysis was being completed.

The draft IRA report concludes that five of nine disease agents of potential quarantine concern require quarantine risk management measures if imports are to continue. These are:

- white spot syndrome virus
- yellowhead virus (YHV)
- infectious hypodermal and haematopoietic necrosis virus (IHHNV)
- Taura syndrome virus (TSV), and
- necrotising hepatopancreatitis bacterium (NHPB).

The report proposes stronger risk management measures to address these quarantine risks, requiring either

- country or zone disease freedom, or
- removal of the head and shell and testing for WSSV, YHV and IHHNV
- a high level of processing for uncooked prawns, for example crumbed prawns, or pastries or dim sum-type products, or
- cooking product offshore with acceptable certification or cooking on-shore under quarantine control.

Subject to considering stakeholder comments, Biosecurity Australia says it intends to recommend strengthening the current interim quarantine measures soon after the end of the comment period, consistent with the measures proposed in the draft report, pending finalisation of the IRA.

The draft IRA report is available on Biosecurity Australia's website.

See www.biosecurityaustralia.gov.au

IRAN: NACA BUILDS AQUATIC ANIMAL HEALTH CAPACITY

(Source: www.enaca.org)

The recent outbreak of white spot disease which affected Bushehr, the largest shrimp-producing province of Iran, has prompted the Iranian government to tackle capacity building on aquatic animal health management.

Through its Aquatic Animal Health Programme, NACA, the Network of Aquaculture Centres in Asia-Pacific has been supporting the development of strategies to effectively control the impact of aquatic animal diseases. As part of these efforts, a training course on aquatic epidemiology was conducted in Bushehr province from September 16 to 19, 2006.

The course was supported by the Iranian Fisheries Research Organisation and delivered by Dr Flavio Corsin of NACA, who is also a regional resource expert in aquatic epidemiology.

Fourteen participants attended the course, comprising staff from seven IFRO offices located across Iran, and two offices of the Iranian Veterinary Organisation (IVO) in Tehran and Bushehr.

After a brief introduction on what epidemiology is and how it can help to answer important questions for managing aquatic animal diseases, the trainees used real-life examples to learn how to design, implement and analyse epidemiological studies. They were also given a chance to strengthen their knowledge and put it into practice by working as a group. As part of this activity the preliminary design of two epidemiological studies was conducted. These studies were aimed at

- identifying the prevalence of monodon baculovirus in shrimp hatcheries in Iran, and

PARTICIPANTS IN THE TRAINING COURSE ON AQUATIC ANIMAL EPIDEMIOLOGY

- identifying the effect of introducing mechanisms to lower water temperature and increase dissolved oxygen on the occurrence of mortality and streptococcosis in rainbow trout farms.

The active participation and enthusiasm of the trainees made the training course a real success, leading the IFRO and IVO to request NACA for several follow-up activities, including a second, more advanced, training course on aquatic epidemiology, and support for the development of epidemiological studies aimed at controlling diseases in shrimp and marine, cold water and warm water fish.

CHILE: UA2 CLASSIFIED AS A *FRANCISELLA*

According to the Pharmaq website, UA2 and Harry Birkbeck at the Universities Marine Biological Station Millport recently characterised the agent termed UA2 isolated from salmon parr in Chile as a new member of the *Francisella* family.

The bacteria were isolated from kidney and spleen and grown in Pharmaq laboratories at different temperatures. The optimum temperature for cultivation was slightly higher than the reported growth optimum for *Francisella* isolated from Atlantic cod.

This may reflect an adaptation to a warmer water temperature. For identification of the growing agent, PCR of the 16S rRNA and the 16S - 23S internal transcribed sequence (ITS) was performed, followed by sequencing (Genebank accession number AM403242 and XXX).

Phenotypic characterisations were also performed by Western blotting of bacterial antigens and by enzymatic tests to allow further identification. The antigen pattern in the immunoblot was identical for the *Francisella* isolates originating from Chilean salmon and Norwegian cod.

All analysis showed that the new *Francisella* species has a lot in common with *Francisella* isolated from other fish species like Atlantic cod and tilapia, both genetically and phenotypically.

However, genetic differences in the 23S rRNA region were found, and the ITS region had between 97.2 and 98 percent similarity with other *Francisella* species from fish. It is possible that other *Francisella* isolates originating from different locations in Chile will have slightly different phenotypic and genetic characteristics.

It is also possible that *Francisella* sp. will be isolated from saltwater sites in region X or XI in Chile, or other locations in the world. This diseases agent has earlier been isolated from fresh water in Chile and been described as UA2 (2).

Pharmaq says it will continue to study the *Francisella* isolates from salmon, and investigate if it is possible to prevent disease by vaccination.

USA: MARICAL BOARD HAS TWO NEW DIRECTORS

The past chief executive officers of Alpharma's Animal Health Division and Purina Mills have joined the board of directors of MariCal. Bruce I Andrews and Edward L McMillan were elected to the board at its November quarterly meeting, said the chairman of MariCal, Steve Morrell.

The private aquatic life science biotechnology company is expanding its intellectual property applications into the estimated US$230.4 billion worldwide animal health and nutrition industries that serve the food and companion animal markets.

Bruce Andrews is a former president and chief executive officer of the Animal Health Division of Alpharma, Inc, a leading global manufacturer and marketer of pharmaceutical and feed additive products for livestock, poultry and fish.

Edward McMillan is a former president and chief executive officer of Purina Mills, which was the largest manufacturer and distributor of animal nutrition products in the United States.

"The MariCal board is significantly strengthened by the addition of Bruce Andrews and Ed McMillan," said Morrell. "These gentlemen are premier talent, being extremely well respected and known by the industry. They will help provide the key strategic guidance MariCal needs to exploit the nutrition and health segments of agribusiness that will return value to our stockholders."

MariCal's key discovery is of a class of molecular extracellular ion receptor proteins, called calcium receptors, which serve as the biological sensors or "master switches" that enable aquatic organisms to sense and respond to changes in water salinity and nutrients in their aquatic environment.

This patented technology also has applications in food and companion animals by providing specialised dietary formulations that enhance the nutritional and growth performance of livestock and pets.

"I am very excited about MariCal's calcium receptor technology, which utilises a natural process. I believe it will have as big an impact on the livestock and poultry industries to improve feed utilisation and overall health as feed antibiotics had in the early 1950s," Andrews said.

Said McMillan, "I look forward to assisting MariCal in the pursuit of the successful application of their calcium receptor technology into numerous food producing and companion animal species worldwide."

MariCal's senior vice president, William Thomas, said Bruce Andrews and Ed McMillan were exceptional leaders of the animal health and nutrition industry. "Their joining MariCal's board is validation of the scientific strength of our technology and the promising future our product development efforts will bring to food and companion animals, as well as to our core aquaculture business."

Thomas indicated that FountainAgriCounsel, a leading management consulting and strategic advisor firm to the agribusiness industry, was instrumental in facilitating the introduction of the two board members to MariCal.

McMillan is currently an independent business consultant focusing on food and agribusiness, and Andrews serves as the chief operating officer of BioValve Technologies, Inc. a specialty pharmaceutical company. Both men have extensive involvement with the animal health and nutrition industry, and also serve on numerous corporate boards.

See www.marical.biz

USA: MAJOR NEW PRODUCT APPROVED

The USA approved a new product, 35 percent PEROX-AID® (hydrogen peroxide) on January 11 for controlling mortality in:

- freshwater-reared finfish eggs due to saprolegniasis
- freshwater-reared salmonids due to bacterial gill disease, and
- freshwater-reared cool water finfish and channel catfish due to external columnar is disease.

Eka Chemicals, Inc, based in Marietta, Georgia, is the sponsor of 35 percent PEROX-AID®. According to Roz Schnick, the national coordinator for aquaculture new animal drug applications at Michigan State University, this is a very important approval, because it is:

- the first new waterborne drug approved for a disease claim for any aquatic species in more than 20 years
- the second aquaculture drug to gain designation under the Minor Use and Minor Species Animal Health Act, which entitles Eka Chemicals to seven years of exclusivity for marketing rights for the approved label claims, and
- the first new aquaculture drug with an original approval covering multiple label claims for use in a variety of finfish species.

Various entities played a role in this achievement. The Upper Midwest Environmental Sciences Centre (UMESC; US Geological Survey, La Crosse, Wisconsin) developed the data that resulted in the approval for these label claims, with financial support through base funds and the Federal-State Aquaculture Drug Approval Partnership Project.

UMESC

- wrote the environmental assessment that completed the environmental safety requirements
- performed target animal safety studies on representative species and their eggs so that all freshwater-reared finfish and their eggs could be placed on this or future labels, and
- conducted laboratory and field effectiveness studies that resulted in these label claims being approved.

Eka Chemicals completed the requirements for manufacturing, and worked together with the national coordinator for Aquaculture New Animal Drug Applications to complete the requirements for human food safety, labelling, and all other information on safety and effectiveness, and also write the original new animal drug application.

35 percent PEROX-AID® is approved with over-the-counter marketing status, and has no requirement for an acceptable daily intake, tolerance, withdrawal time or regulatory method. Eka Chemicals Inc. has licensed Western Chemical Inc as the sole distributor of 35 percent PEROX-AID®.

The FDA Centre for Veterinary Medicine has indicated that the low regulatory priority drug status for hydrogen peroxide is rescinded. Formerly, facilities could purchase and use almost any brand of hydrogen peroxide that was consistent with the FDA's policy.

This has changed, and 35 percent PEROX-AID® is the only hydrogen peroxide product that can legally be purchased and used for the approved label claims. Licensed veterinarians may be able to prescribe a legal extra-label use to use 35 percent PEROX-AID® to treat additional diseases or additional species not covered on the current label.

For further information, contact:
Rosalie (Roz) Schnick, phone 608 781 2205, fax 608 783 3507 or email RozSchnick@centurytel.net.
See http://aquanic.org/jsa/aquadrugs/index.htm

SCOTLAND: SALMON DISEASE CONTROL PLAN PUBLISHED

A contingency plan to tackle a disease that could wipe out 2000 jobs if it contaminated Scottish salmon stocks was published by the Scottish government in early December.

Gyrodactylus salaris (*Gs*), a deadly parasite of Atlantic salmon, is not present in Scotland but is found in Scandinavia. The contingency plan sets out steps that would be taken if an outbreak were to occur in Scotland.

Launching the action plan, the then Deputy Environment and Rural Development Minister, Rhona Brankin, said *Gs* presented a significant risk to Scotland's salmon fisheries and freshwater aquaculture. "I am grateful to all those who have contributed to this important piece of work. We now have a contingency plan, but under existing legislation we could not attempt eradication of the parasite, should it arrive.

"We are seeking these powers through the Aquaculture and Fisheries (Scotland) Bill, and I very much hope that the Scottish Parliament will give us the tools we need."

An exercise to test the robustness of the plan was planned with major stakeholders early in 2007.

See www.scotland.gov.uk/Topics/Fisheries/Fish-Shellfish/18610/13929. This page links to the report of the chairman of the task force, including the recommendations of the task force sub-groups.

The Scottish Executive Environment and Rural Affairs Department is evaluating these, and will take into account any views of the Scottish Parliament on the Gs provisions of the bill.

INTERNATIONAL: DIPNET PROJECT REVIEW PUBLISHED

The main outcome of the European Union-funded DIPNET research project is the work package one scientific review, which is now available for downloading. The scientific review document comprises 82 disease chapters with bibliographies. Fifty-two authors have contributed to the written report, and over 100 people have been involved in the work.

A non-technical summary of the scientific review is now available on the project website. Aimed at stakeholders and non-expert public, the non-technical summary follows an ecosystem approach and is divided into four sections.

- The North Atlantic Scenario: disease interactions between coastal net-pen aquaculture and migrating wild fish populations
- The Continental European Scenario: disease interaction between freshwater resident wild populations and traditional (pond) aquaculture
- The Mediterranean Scenario: disease interactions between wild marine fish populations and Mediterranean sea-cage aquaculture, and
- The Shellfish and Crustacean Scenario: disease interactions between wild and farmed shellfish and crustaceans.

See www.dipnet.info/documents

INTERNATIONAL: OIE LAUNCHES GLOBAL ANIMAL HEALTH DATABASE

The World Animal Health Information Database interface is now available on the OIE website. OIE, the world organisation for animal health, says the new, extensive database is a milestone in its efforts to improve the transparency, efficiency

and speed for disseminating animal health information throughout the world.

WAHID offers all available data on animal diseases, including zoonoses, per country, region, month and year. The database also compiles country animal populations, exceptional epidemiological event maps, global animal diseases distribution maps or comparative disease status between two countries. The last application can help define health hazards linked to the trade of live animals and animal products between countries.

The database is complementary to the on-line notification made by OIE member countries through the World Animal Health Information System, or WAHIS, launched last April.

"WAHID is designed to provide high quality animal disease information to all stakeholders, including veterinary services, international organisations, trading partners, academics, the media and the public. All can access and monitor with us the evolution of animal diseases in one or several countries or regions of the world," said Dr Karim Ben Jebara, the head of the OIE Animal Health Information Department.

The new web interface will replace Handistatus II, which compiles data from 1996 to 2004. Handistatus II will disappear when all this data is transferred into the more user-friendly WAHID.

WAHIS and WAHID are unique worldwide in animal health information. *See www.oie.intenthome page or www.oie.int/wahid.*

INTERNATIONAL: RECENT REPORTS FROM ASIA-PACIFIC AQUACULTURE CENTRES

The report of the fifth meeting of the Asia Regional Advisory Group on Aquatic Animal Health has been released.

The 10-member high-level regional advisory group constituted by NACA, in cooperation with OIE and the Food and Agriculture Organisation, provides advice to NACA and Asian governments on aquatic animal health management.

During the three-day meeting, the AG addressed key global and regional aquatic animal health issues, and provided a number of useful recommendations on aquatic animal disease control in Asia-Pacific. Important topics covered in the report include:

- regional aquatic animal health programmes of NACA
- outcomes of the OIE General Session in May, and the AAHSC meeting in October
- emerging crustacean, finfish and mollusc diseases in the region
- OIE list of diseases in the 9th edition 2006 of the Aquatic Code
- Regional QAAD quarterly aquatic animal disease reporting system and QAAD list for 2007
- implementation of the Asia Regional Technical Guidelines
- regional and international cooperation in Asian aquatic animal health management, and
- updates on the three-tier regional resource base on aquatic animal health.

The Quarterly Aquatic Animal Disease Report Q3 2006 includes disease reports from 17 countries in the Asia-Pacific region. The report also provides a summary of emerging diseases in the region. In addition to all the OIE-listed diseases, QAAD reports cover diseases of regional importance and hence serve as an early warning system for some of the emerging diseases in the region.

See the NACA website: www.enaca.org ■

testimonials

"Certainly international in scope with a focus on aquatic animal health, Aquaculture Health International *has something of interest for everyone. Superbly illustrated this magazine reports on current events, new research and profiles laboratories and people involved in aquatic animal health. I personally look forward to each new issue."*

DR SCOTT LAPATRA, RESEARCH DIRECTOR,
CLEAR SPRINGS FOODS INC., USA

"Having read each and every issue since its inception, I believe Aquaculture Health International *has developed into an indispensable source of information that will benefit many thousands of veterinarians throughout the world that are engaged with aquatic veterinary medicine."*

DR A DAVID SCARFE PHD, DVM, MRSSA.
ASSISTANT DIRECTOR, SCIENTIFIC ACTIVITIES DIVISION,
AMERICAN VETERINARY MEDICAL ASSOCIATION, USA

"Good communication and good publications rely on two crucial factors: content and layout. Aquaculture Health International *combines both in a way that appeals to "informed" readers and "generalists", such as myself. It is an excellent publication and one of the models on which the new format of the* EAS *magazine, Aquaculture Europe, is based."*

ALISTAIR LANE, EXECUTIVE DIRECTOR,
EUROPEAN AQUACULTURE SOCIETY, BELGIUM

"I have read the successive numbers of Aquaculture Health International *with much interest, and consider this to be an up-to-date and comprehensive publication which makes the reader fully aware of what is going on within the general subject area, and with a wide international coverage of topics. The articles are authoritatively written and well illustrated. Dr Peddie is to be complimented on his excellent editorial direction of this important publication."*

DR DA CONROY, TITULAR PROFESSOR (RETIRED) OF
FISH PATHOLOGY/AQUATIC PATHOBIOLOGY, FACULTY OF
VETERINARY SCIENCES, CENTRAL UNIVERSITY OF VENEZUELA,
MARACAY, VENEZUELA

"It is good to see a publication devoted to topical fish heath issues that has an attractive presentation without the normal defined journal format. Aquaculture Health International *is readable and presents a wide range of health topics and with international coverage. It is also interesting to hear what specific laboratories are working on and what services they provide."*

DR JIM TREASURER, RESEARCH MANAGER,
ARDTOE MARINE LABORATORY, UK

A SYNOPSIS OF THE **SALMON LICE ISSUE** IN BRITISH COLUMBIA

BY DR KEVIN G BUTTERWORTH, DR K FIONA CUBITT AND DR R SCOTT MCKINLEY
(CENTRE FOR AQUACULTURE AND ENVIRONMENTAL RESEARCH, UNIVERSITY OF BRITISH COLUMBIA, CANADA)

L salmonis has been a severe problem for both farmed and wild salmonids in the North Atlantic, with an estimated cost of between €23.9 million and €59.7 million in Norway alone (Boxaspen and Næss 2000, Fast *et al* 2002, Stone *et al* 2002, Glover *et al* 2003, 2004, Heuch *et al* 2003, Wagner *et al* 2003, 2004).

However, the situation in the North Pacific along British Columbia's coast is rather different. For example, instead of one species of migrating salmon there are five species, each of which has a different level of susceptibility to *L salmonis*.

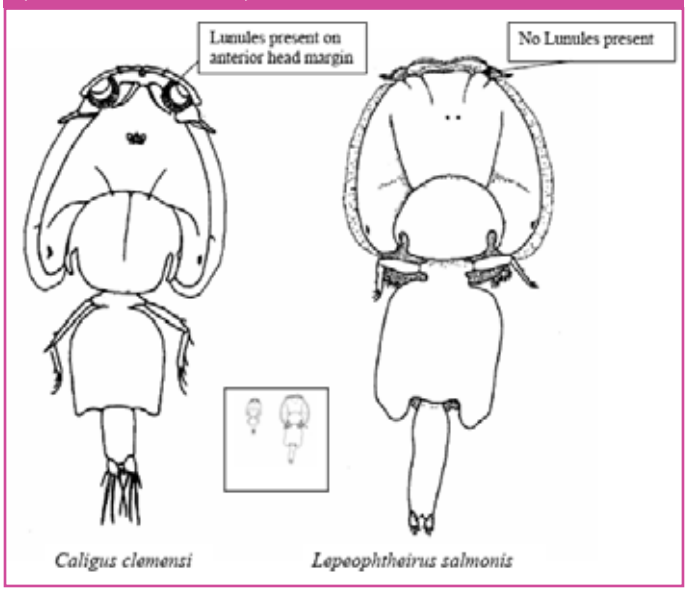
The sea lice that naturally affect salmon and trout in the marine environment belong to the family *Caligidae* and to the genera *Caligus* and *Lepeophtheirus*. In British Columbia, 14 species (two species of *Caligus* and 12 of *Lepeophtheirus*) of sea lice parasitise many different species of marine fish (Kabata 1973).

These 14 species of sea lice have a similar body shape (Figure 1). Additionally, differences within species of each of these two genera are small, and they are difficult to identify without the assistance of a magnifying glass and some taxonomic training. However, only two species, *Lepeoptheirus salmonis* and *Caligus clemensi*, pose a potential threat to both farmed and wild salmon.

In the context of salmon aquaculture and wild salmon in British Columbia, *Lepeophtheirus salmonis* is the important species. Also commonly known as the salmon louse, *L salmonis* is a parasitic caligid copepod (Johnson and Albright 1991, Butterworth *et al* 2004). Ubiquitous in the North Pacific and Atlantic Oceans, *L salmonis* is a parasite on both farmed and wild salmon and on sea-run trout (Kabata 1973, Pike 1989, Johnson *et al* 1996, Bjørn and Finstad 1998).

On salmon, high infection intensities lead to stress, impaired performance, reduced physiological ability, and in extreme cases, death through either primary lesions or secondary infections (Bjørn and Finstad 1997, Pike and Wadsworth 1999, Bowers *et al* 2000; Finstad *et al* 2000, Wagner *et al* 2003)

FIGURE 1. EXTERNAL ANATOMY OF THE DISTINGUISHING FEATURES BETWEEN THE GENUS *CALIGUS* AND THE GENUS *LEPEOPHTHEIRUS*. DORSAL VIEW SHOWN. SMALL INSET SHOWS RELATIVE SIZE DIFFERENCE (BUTTERWORTH *ET AL* 2005)



LIFE CYCLE

Overall, *L salmonis* has 11 life stages, starting from the egg through to the adult stage (Figure 2). The first three stages are free-swimming and non-parasitic. The larval sea lice then finds a host and progresses through seven parasitic life stages before the next generation of eggs is produced.

INFLUENCING FACTORS

Environmental factors that have the greatest impact on the development and success of the infective (copepodid) larval stage are temperature and salinity. Depending on the combination, these two factors can either promote swift growth and survival of salmon lice, or retard their development and severely reduce their survival.

TEMPERATURE

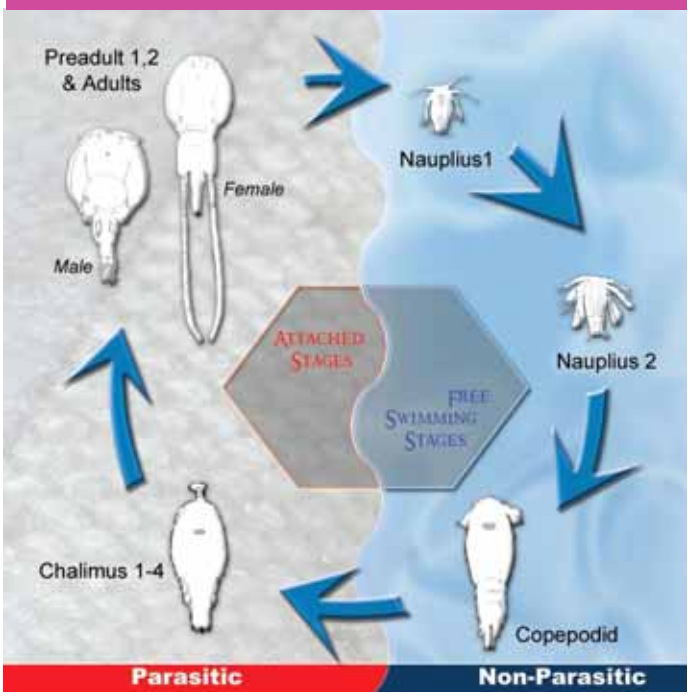
At temperatures below 7° C, salmon lice in the free-swimming copepodid stage are less able to take the next step and settle onto a host (Figure 3), than when the water temperature is warmer (Tucker *et al* 2000)). The impact of temperature on the overall generation time for salmon lice is equally pronounced. At 7.5° C, the generation time is 106 days, but at 14° C, this decreases to 36 days (Tully 1992).

Such temperature-dependent growth rates can significantly impact population densities of the copepodid life stage (settlement stage) of *L salmonis* in shallow, coastal waters, which are prone to warming. As a result, warmer summers are likely to result in an increased number of sea lice copepods in these waters.

SALINITY

Salinity plays a very important part in the life cycle of *L salmonis*.

FIGURE 2. LIFE CYCLE OF *L SALMONIS* SHOWING NAUPLII, COPEPODID, CHALIMUS, PRE-ADULT AND ADULT LIFE STAGES. SOURCE: CATHERINA MURPHY, AQUANET CANADA. REPRINTED WITH PERMISSION



IN SEARCH OF SALMON LICE. LOOKING SOUTH OVER THE QUEEN CHARLOTTE STRAIT. THE NORTHEAST OF VANCOUVER ISLAND, WHICH CAN BE SEEN ON THE RIGHT HORIZON OF THE PHOTOGRAPH, PROTECTS THE QUEEN CHARLOTTE STRAIT AND THE BROUGHTON ARCHIPELAGO (CENTRAL HORIZON) FROM THE WORST EFFECTS OF STORMS ORIGINATING OVER THE PACIFIC OCEAN. PHOTOGRAPH BY KG BUTTERWORTH

Successful development of the copepodid stage has been reported to occur only at salinities above 30‰ (Pike and Wadsworth 1999). However, in British Columbia, successful copepodid development and subsequent host settlement have been achieved at salinities as low as 28‰ (Butterworth 2005, personal observation).

Once at this stage, copepodids actively avoid seawater with salinities below 20‰ (Heuch 1995) and their optimal survival is at 30‰ (Johnson and Albright 1991). Hence, lower salinities such as those commonly recorded in BC inshore waters could have a significant damping effect on *L salmonis*' distribution and population sizes.

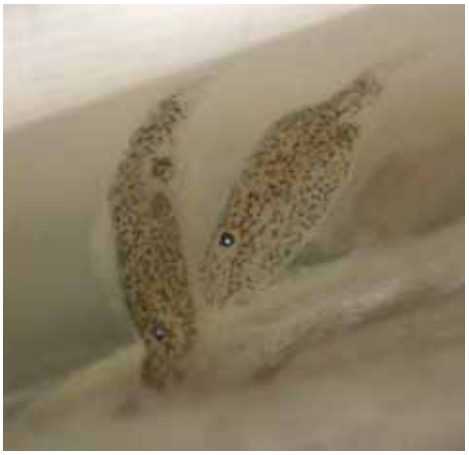
DIFFERENCES BETWEEN SALMON LICE POPULATIONS

In the North Atlantic, *L salmonis* has been a severe problem for both farmed and wild salmonids (Fast *et al* 2002, Stone *et al* 2002, Glover *et al* 2003 2004, Heuch *et al* 2003, Wagner *et al* 2003 2004). Atlantic salmon make up the bulk of finfish aquaculture in the North Atlantic, and *L salmonis* have had a large impact on wild Atlantic salmon and sea-run brown trout (*Salmo trutta*).

The population of wild salmon stocks in the North Atlantic has and continues to decrease (Hiscock *et al* 2005). This decrease, alongside the poor ability of Atlantic salmon to re-establish populations in traditional spawning streams (Cubitt *et al* 2006) has led to farmed Atlantic salmon outnumbering wild Atlantic salmon in some locations.

However, the situation in the North Pacific along the coast of British Columbia is rather different. First, instead of one species of migrating salmon, there are five that undertake long migrations. To complicate the picture further, *L salmonis* shows differential levels of prevalence on different species of Pacific salmon.

In the Pacific Ocean, the highest levels of infection have been reported on pink salmon (*Oncorhynchus gorbuscha*) and rainbow trout (*O mykiss*) (Nagasawa 2001). Lower infection levels were found on coho salmon (*O kisutch*), chum salmon (*O keta*) and chinook salmon (*O tshawytscha*). The sockeye salmon (*O nerka*) was found to have the lowest infection levels, but this relationship is poorly understood, as subsequent surveys found high levels of salmon lice on sockeye salmon in excess of those found on pink salmon (Beamish *et al* 2005, Nagasawa 2001).



SUCCESSFULLY SETTLED AND MOULTED AND FEEDING; CLOSE-UP PHOTOGRAPH OF CHALIMUS STAGES OF *L SALMONIS* ATTACHED TO A JUVENILE PINK SALMON SMOLT. PHOTOGRAPH BY KG BUTTERWORTH

In BC, wild salmon vastly outnumber farmed salmon. There are 128 salmon farm tenures in BC, compared to more than 9600 distinct stocks of wild Pacific salmon identified on the BC coast. All of these distinct stocks are considered to be separate populations, subject to unique environmental and anthropogenic pressures.

Additionally, over-wintering wild coho and chinook salmon (Healey 1991, Sandercock 1992) and schools of wild sticklebacks (Jones *et al* 2006) in coastal waters provide an ideal stock of potential hosts upon which the salmon lice can over-winter, ready to infect out-migrating wild smolts in the spring. Hence, there is a large potential reservoir of salmon lice associated with wild fish. This is the opposite of the North Atlantic where, due to the severe depletion of wild fish stocks, salmon farms contain the largest pool of potential hosts upon which salmon lice can over-winter. ▶

A SYNOPSIS OF THE SALMON LICE ISSUE IN BRITISH COLUMBIA

ADULT MALE AND FEMALE *L SALMONIS*. THE FEMALES ARE SLIGHTLY LARGER, WITH ELONGATED GENITAL SEGMENTS. INSIDE THE BLACK OVAL ON THE TOP LEFT OF THE PHOTOGRAPH, DEVELOPING EGG STRINGS ARE VISIBLE ON A FEMALE. PHOTOGRAPH BY KG BUTTERWORTH

HYDROGRAPHICAL TRENDS IN BC

The vegetation on Canada's western coast is predominately temperate rainforest. Locally known as the Raincoast, these forests are some of the rarest intact inland and island ecosystems in the world, and receive an average annual rainfall of 1.8m (74in).

Precipitation is heaviest in the winter and drops off during the spring to a low in summer, before increasing swiftly in the fall (autumn). High precipitation causes a large influx of fresh water into the marine environment from rivers, via the inlets, and into coastal waters. As fresh water is less dense than salt water, it sits on top of the seawater and a strong, vertical salinity gradient (a halocline) forms between the two bodies of water.

Large influxes of fresh water can dramatically affect the surface salinities found in coastal waters. This effect is exacerbated by the addition of glacial melt in the spring, when the new fry first start their migration to the sea. As discussed previously, salmon lice are inhibited by low salinities such as those caused by large freshwater inputs.

Correspondingly, Heuch *et al* 2002 hypothesised that lower salinities are of paramount importance in restraining the growth of salmon lice populations. However, it is possible that the salmon lice larvae simply avoid this layer by moving below it in the water column. Therefore, the less saline layer on top of the water may slow the development of viable settlement stages of the salmon lice, and additionally provide a "safe corridor" for migrating smolts moving through inshore waters.

As discussed above, salmon lice are only able to develop to the copepodid stage at salinities greater than 28 to 30‰. Hence, we would hypothesise that it is the difference in salinity in near-shore coastal waters in BC that prevents the high infection intensities of salmon lice on salmon in the North Atlantic, as reported by the news media. However, at this time, we do now know whether migrating Pacific salmon favour the low salinity surface waters or deeper waters with higher salinities.

IMPACT OF SALMON LICE

To date, the bulk of research has focussed on the impact of salmon lice infestations on Atlantic species of salmonid (Stone *et al* 2002, Glover *et al* 2003 2004, Heuch *et al* 2003, Wagner *et al* 2003 2004) and not on Pacific salmon. Research on Atlantic salmon is very useful to scientists studying Pacific salmon, as it provides core methodologies and insights on some of the physiological mechanisms that are affected by salmon lice infestation.

This is called the August Krogh principle, and is the underlying ethos behind the field of comparative physiology and biochemistry. However, although the mechanisms may be the same, the levels of susceptibility and response vary between species. Hence, the number of salmon lice that cause mortality in Atlantic salmon cannot be assumed to cause mortality in Pacific salmon. Each species needs to be examined individually.

Research has shown that while Atlantic salmon have little resistance to salmon lice infestation, this resistance can be strengthened by selective breeding (Kolstad *et al* 2005). Atlantic salmon and sea trout develop lesions when infected with salmon lice, and appear to have very little defence against the infestation, aside from turning away from the sea and heading back into freshwater streams. This causes the lice, which are intolerant to low salinities, to drop off the afflicted salmon.

In July and August of 2003 and 2004, The Department of Fisheries and Oceans Canada conducted a study of skin damage caused by salmon lice to returning wild Pacific salmon (Beamish *et al* 2004 2005). Of 1046 wild Pacific salmon found infected with salmon lice, the authors reported that there were a small number of sockeye and pink salmon with lesions present (skin removed and muscle exposed, or skin partially removed exposing necrotic tissue) and haemorrhaging at margins of lesions (Beamish *et al* 2004 2005). However, the authors stressed that this was a rare occurrence.

The majority of the pink salmon and some of the sockeye salmon had subcutaneous haemorrhaging ranging from mild red discolouration to moderate over the area only half the size of the anal fin. Although this data would suggest that Pacific salmon appear to be more resistant to skin damage from salmon lice than their Atlantic counterparts, there are reported cases where Pacific salmon have had severe lesions from salmon lice infestation (Kabata 1970, Johnson *et al* 1996).

Perceived differences in the severity of skin damage between Pacific and Atlantic species may be partly due to the lack of epithelial hyperplasias and the inflammatory response of Atlantic salmon to an infestation of salmon lice (Johnson and Albright 1992, Johnson 1993). It is important to note that even though the Pacific salmon species appear to be more resistant to salmon lice infestation, there is as yet no scientific assessment of the impact of infestation intensity on the general health of these salmon. Thus, there is no scientific data available on the number of sea lice needed to have a detrimental effect on Pacific salmon.

SALMON INTERACTION

The alleged role of commercial salmon farms as a possible source of salmon lice infections in passing wild salmon has received much attention from both the scientific community and the news media. The debate in British Columbia has focussed on pink salmon stocks in the Broughton Archipelago. For a comprehensive review of this issue, see Brooks 2005. The controversy is based on evidence that correlates higher salmon lice infestation intensities in areas of BC with salmon farms, as opposed to areas without salmon farms (Morton *et al* 2004, 2005, Morton and Routledge, 2006). The concept of correlation compares two variables (proximity to salmon farms and the number of salmon lice on wild salmon) that are changing in a similar manner and therefore appear to be linked. However, there is actually no evidence of a link (cause and effect) between the two variables. Hence, the variables, in this case proximity to salmon farms and the number of salmon lice on wild salmon, may or may not be related.

An increase in sea lice with proximity to farms has also been reported in the Atlantic Ocean (Costelloe *et al* 1996 1998, Bjørn et al 2001, Penston *et al* 2002, McKibben and Hay 2002). However, while higher salmon lice infestations tend to occur in areas of BC with salmon farms, this correlation cannot be used to conclude that salmon farms are, in fact, the cause of the more intense infestations.

Although it has not been possible to date to establish a direct causal link between the decline of wild salmon stocks and the expansion of the salmon aquaculture industry in British Columbia (Butterworth *et al* 2004), it is obviously important to establish whether salmon farms are actually significantly contributing to

salmon lice prevalence among wild Pacific salmon (Butterworth *et al* 2004).

If the farms are not contributing significantly to the problem, then salmon lice on farmed salmon is a farm management problem, not a potential interaction issue between wild and farmed salmon. Were this separation to be proven, it would facilitate the development of more specific management policies for effective controlling salmon lice on salmon farms.

More recent research suggests that it is possible for farmed and wild salmon to co-exist in a sustainable manner in the same habitat (Beamish *et al* 2006). In the Pacific, salmon lice monitoring programmes have reported the occurrence of salmon lice on 91 to 100 percent of salmon sampled in areas with and without salmon farms (Nagasawa 2001, Beamish *et al* 2004 2005).

Complicating the picture is the occurrence of *L salmonis* on the three-spine stickleback *Gasterosteus aculeatus*, a species common to inshore waters in British Columbia (Jones *et al* 2006). Additionally, given the rates of dispersal of the lice in their larval stages (O'Donoghue *et al* 1998) by dynamic flow fields caused by changing tides, currents and local shifts in wind direction, there is a huge potential for larval dispersal (Asplin *et al* 1999 2004). Therefore, more conclusive evidence is needed before a cause and effect relationship can be demonstrated between salmon lice present on salmon farms, and infection levels among wild Pacific salmon in British Columbia.

SUMMARY

- Temperature and salinity have a large impact on *L salmonis* growth rates and survival.
- Free-swimming sea lice larval stages are intolerant of low salinities such as those found in BC's inshore waters.
- In BC there is evidence that wild salmon and sticklebacks provide a potential host reservoir for sea lice.
- Much of the information currently available on the impact of sea lice on health is from Atlantic salmon, not Pacific salmon.
- It is not known at this time what intensity (concentration) of sea lice has on the health of Pacific salmon, nor what level would cause mortality.

REFERENCES

Asplin L, Boxaspen K and Sandvik AD 2004. Modelled distribution of sea lice in a Norwegian fjord, *ICES CM 2004/P*: 11. pp12

Asplin L, Salvanes AGV and Kristoffersen JB 1999. Non-local wind-driven fjord-coast advection and its potential effect on plankton and fish recruitment. *Fisheries Oceanography* **8**. pp255-263

Beamish RJ, Jones S, Dawe S, Gordon E, Sweeting RM, Neville CM, Johnson S, Trudel M, MacDonald T and Ambers N 2004. Prevalence, intensity and life history strategy of sea lice on adult Pacific salmon returning to the spawning areas in the Central Coast of British Columbia. *Fisheries and Oceans Canada*.

Beamish R, Neville CM, Sweeting RM and Ambers N 2005. Sea lice on adult Pacific salmon in the coastal waters of British Columbia, Canada. *Fisheries Research* **76**. 1987-208

Beamish R, Jones S, Neville CM, Sweeting RM, Karreman G, Saksida S and Gordon E 2006. Exceptional marine survival of pink salmon that entered the marine environment in 2003 suggests that farmed Atlantic salmon and Pacific salmon can coexist successfully in a marine ecosystem on the Pacific Coast of Canada. *ICES Journal of Marine Science* **63** (7). pp1326-1337

Bjørn PA and Finstad B 1997. The physiological effects of salmon lice infection on sea trout post smolts. Nord J Freshw. Res **73**. pp60-72

Bjørn PA and Finstad B 1998. The development of salmon lice *Lepeophtheirus salmonis* on artificially infected post smolts of sea trout *Salmo trutta*. *Canadian Journal of Zoology* **76**. pp970-977

Bjørn PA, Finstad B and Kristoffersen R 2001. Salmon lice infection of wild sea trout and Arctic char in marine and freshwaters: the effects of salmon farms. *Aquacult. Res*. **32**. pp947-962

Bowers JM, Mustafa A, Speere DJ, Conby GA, Brimacombe M, Sims DE and Burka JF 2000. The physiological response of Atlantic salmon, *Salmo salar L*, to a single experimental challenge with sea lice, *Lepeophtheirus salmonis. Journal of Fish Disease* **23**. pp165-172

Boxaspen K and Næss T 2000. Development of eggs and the planktonic stages of salmon lice *Lepeophtheirus salmonis* at low temperatures. *Contributions to Zoology* **69**(1/2).

Brooks KM 2005. The effects of water temperature, salinity and currents on the survival and distribution of the infective copepodid stage of sea lice *Lepeophtheirus salmonis* originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada. *Reviews in Fisheries Science* **13**. pp177-204

Butterworth KG, Li W and McKinley RS 2004. Carbon and nitrogen stable isotopes: a tool to differentiate between *Lepeophtheirus salmonis* and different salmonid host species? *Aquaculture* **241** (1-4). pp529-538

Butterworth KG, Ronquillo JD and McKinley RS 2005. Simplified illustrated sea lice identification guide for *Lepeophtheirus salmonis* and *Caligus clemensi* in British Columbia, Canada. ACC Spec. Publ. 9. pp101-103

Butterworth KG 2005. Personal communication with K Butterworth (January). Vancouver: Centre for Aquaculture and Environmental Research, University of British Columbia.

Costelloe MJ, Costelloe G and Roche N 1996. Planktonic dispersal of larval salmon lice, *Lepeophtheirus salmonis*, associated with cultured salmon, *Salmo salar*, in Western Ireland. *The Journal of the Marine Biological Association of the United Kingdom* **76**. pp141-149

Costelloe M, Costelloe J, O'Donohoe G, Coghlan NJ, Oonk M and Van der Heijden Y 1998. Planktonic distribution of sea lice larvae *Lepeophtheirus salmonis* in Killary Harbour, West Coast of Ireland. *The Journal of the Marine Biological Association of the United Kingdom* **78**. pp853-874

Cubitt KF, Butterworth KG, Finstad B, Huntingford F and McKinley RS 2006. Escaped farmed salmon: a threat to BC's wild salmon? *Fraser Institute Alert Series.* Vancouver, Canada.

Fast MD, Ross NW, Mustafa A, Sims DE, Johnson SC and Conboy GA 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon, *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis. Diseases of Aquatic Organisms* **52** (1). pp57-68

Finstad B, Grimnes A, Bjørn PA and Hvidsten NA 2000. Laboratory and field investigations of salmon lice *Lepeophtheirus salmonis* (Krøyer) infestation on Atlantic salmon *Salmo salar L* post smolts. *Aquacult*. Res. **31**. pp795-803

Glover KA, Hamre LA, Skaala O and Nilsen F 2004. A comparison of sea louse *Lepeophtheirus salmonis* infection levels in farmed and wild Atlantic salmon *Salmo salar L* stocks. *Aquaculture* **232** (1-4). pp41-52

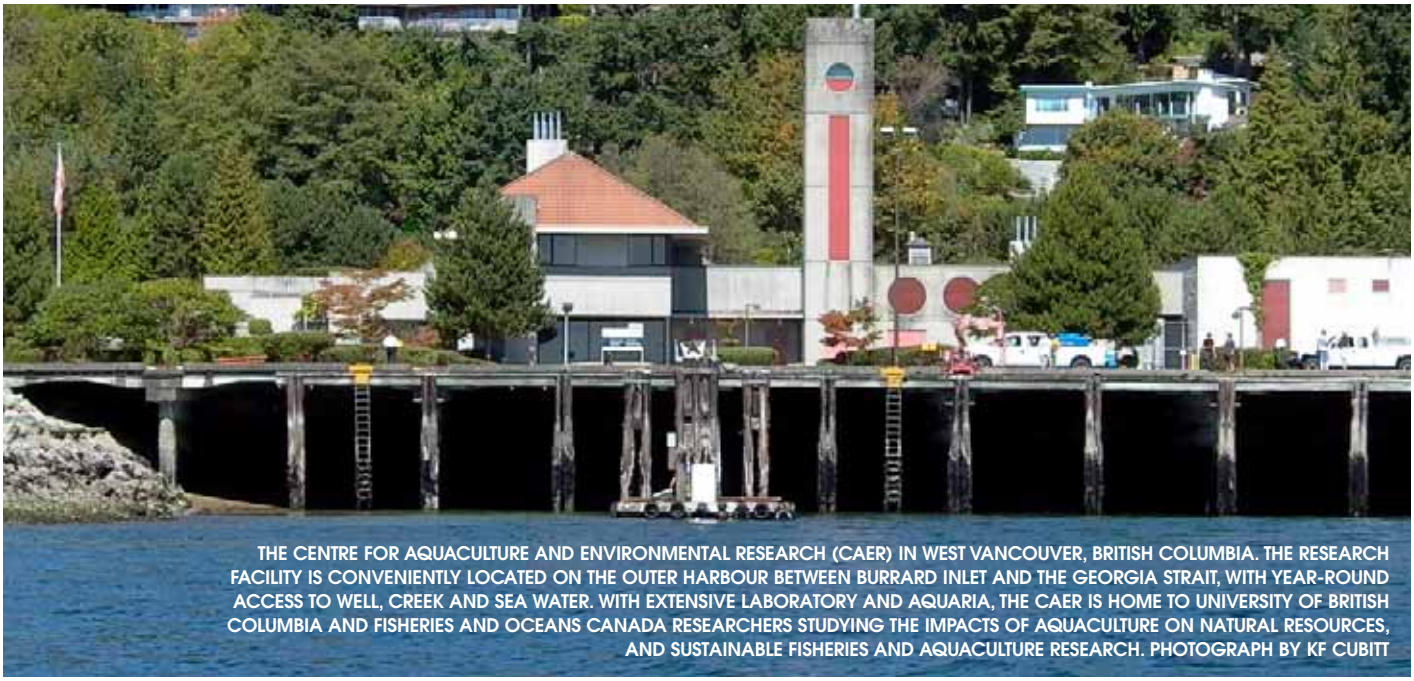
Glover KA, Skaala O, Nilsen F, Olsen R, Teale AJ and Taggart JB 2003. Differing susceptibility of anadromous brown trout *Salmo trutta L* populations to salmon louse *Lepeophtheirus salmonis* (Kroyer 1837)) infection. *ICES Journal of Marine Science* **60** (5). pp1139-1148

Healey MC 1991. The life history of chinook salmon. In: C Groot and L Margolis (eds.) *Pacific Salmon Life Histories*. UBC Press. pp311-393

Heuch PA 1995. Experimental evidence for aggregation of salmon louse copepodids *Lepeophtheirus salmonis* in step salinity gradients. *Journal of the Marine Biological Association of the United Kingdom* **75**(4). pp927-939

Heuch PA, Knutsen JA, Knutsen H and Schram TA 2002. Salinity and temperature effects on sea lice over-wintering on sea trout *Salmo trutta* in coastal areas of the Skagerrak. *The Journal of the Marine Biological Association of the UK* **82**. pp887-892

Heuch PA, Revie CW and Gettinby G 2003. A comparison of epidemiological patterns of salmon lice *Lepeophtheirus salmonis*, infections on farmed Atlantic salmon *Salmo salar L* in Norway and Scotland. *Journal of Fish Diseases* **26** (9). pp539-551



THE CENTRE FOR AQUACULTURE AND ENVIRONMENTAL RESEARCH (CAER) IN WEST VANCOUVER, BRITISH COLUMBIA. THE RESEARCH FACILITY IS CONVENIENTLY LOCATED ON THE OUTER HARBOUR BETWEEN BURRARD INLET AND THE GEORGIA STRAIT, WITH YEAR-ROUND ACCESS TO WELL, CREEK AND SEA WATER. WITH EXTENSIVE LABORATORY AND AQUARIA, THE CAER IS HOME TO UNIVERSITY OF BRITISH COLUMBIA AND FISHERIES AND OCEANS CANADA RESEARCHERS STUDYING THE IMPACTS OF AQUACULTURE ON NATURAL RESOURCES, AND SUSTAINABLE FISHERIES AND AQUACULTURE RESEARCH. PHOTOGRAPH BY KF CUBITT

Hiscock K, Sewell J and Oakley J 2005. Marine Health Check 2005. Marine Life Information Network, Marine Biological Association of the United Kingdom. World Wildlife Fund - United Kingdom.

Johnson SC and Albright LJ 1991. The development stages of *Lepeophtheirus salmonis* (Krøyer 1837) (Copepoda: Caligidae). *Canadian Journal of Zoology* **69**. pp929-950

Johnson SC and Albright LJ 1992. Comparative susceptibility and histopathology of the response of naive Atlantic, chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Diseases of Aquatic Organisms* **14** (3). pp179-193

Johnson SC 1993. A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naïve Atlantic salmon *Salmo salar* and chinook salmon *Oncorhynchus tshawytscha*. In: Boxshall GA and Defaye D (eds). *Pathogens of wild and farmed fish: Sea lice*, Chichester, Ellis Horwood. pp68-82

Johnson SC, Blaylock RB, Elphick J, Hyatt KD 1996. Disease induced by the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) in wild sockeye salmon *Oncorhynchus nerka* stocks of Alberni Inlet, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **53**. pp2888-2897

Jones SRM, Prosperi-Porta G, Kim E, Callow P and Hargreaves B 2006. The occurrence of *Lepeophtheirus salmonis* and *Caligus clemensi* (Copepoda: Caligidae) on three-spine stickleback *Gasterosteus aculeatus* in Coastal British Columbia. *Journal of Parasitology* **92** (3). pp473-480

Kabata Z 1970. Diseases of Fishes. In: Snieszko SF and HR Axelrod (eds). *Book 1: Crustacea as Enemies of Fishes.* New Jersey: TFH Publications.

Kabata Z 1973. The species of *Lepeophtheirus* (Copepoda:Caligidae) from fishes of British Columbia. *Journal Fisheries Research Board of Canada* **30** (6). pp729-759

Kabata Z 1988. Part II - Crustacea. In: Margolis L and Kabata Z (eds). *Guide to the parasites of fishes of Canada*, Canadian Special Publication of Fisheries and Aquatic Sciences 3. pp127

Kolstad K, Grisdale-Heiland B, Meuwissen THE and Gjerde B 2005. Family differences in feed efficiency of Atlantic salmon *Salmo salar*: a pilot study. *Aquaculture* **247** (1-4). pp21

McKibben MA and Hay DW 2002. Planktonic distribution of sea lice *Lepeophtheirus salmonis* larvae in intertidal plankton samples in Loch Shieldaig, Western Scotland in relation to local salmon farm production cycles. *ICES CM 2002/T:06.*

Morton A and Routledge R 2006. Fulton's condition factor: Is it a valid measure of sea lice impact on juvenile salmon? *North American Journal of Fisheries Management* **26**. pp56-62

Morton A, Routledge R, Peet C and Ladwig A 2004. Sea lice *Lepeophtheirus salmonis* infection rates on juvenile pink *Oncorhynchus gorbusha* and chum *Oncorhynchus keta* salmon in the near-shore environment of the British Columbia Coast, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **61**. pp147-157

Morton A, Routledge RD and Williams R 2005. Temporal patterns of sea louse infestation on wild Pacific salmon in relation to the fallowing of Atlantic salmon farms. *North American Journal of Fisheries Management* **25** (3). pp811-821

Nagasawa K 2001. Annual changes in the population size of the salmon louse *Lepeophtheirus salmonis* (Copepoda:Caligidae) in a high-seas Pacific salmon *Oncorhynchus spp*. and relationship to host abundance. *Hydrobiologia* **453/454**. pp411-416

O'Donoghue G, Costello M and Costello J 1998. Development of a management strategy for the reduction/elimination of sea lice larvae *Lepeophtheirus salmonis* parasites of salmon and trout. *Marine Resource Series* No. 6. pp1-51. The Marine Institute, Dublin, Ireland

Penston MJ, McKibben M, Hay DW and Gillibrand PA 2002. Observations of sea lice larvae distributions in Loch Shieldaig, Western Scotland. *ICES CM2002/T:09.*

Pike AW and Wadsworth SL 1999. Sea lice in salmonids: their biology and control. *Advances in Parasitology* **44**. pp233-337

Pike AW 1989. Sea lice - major pathogens of farmed Atlantic salmon. *Parasitology Today* **5**. pp291-297

Sandercock FK 1992. The life history of coho salmon. In: C Groot and L Margolis (eds.) *Pacific Salmon Life Histories*. UBC. Press. pp395-445

Stone J, Roy WJ, Sutherland IH, Ferguson HW, Sommerville C and Endris R 2002. Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon, *Salmo salar L*, smolts in fresh water as a preventative treatment against infestations of sea lice *Lepeophtheirus salmonis* (Krøyer). *Aquaculture* **210** (1-4). pp21-34

Tucker CS, Sommerville C, Wootten R 2000. The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Krøyer 1837) on Atlantic salmon *Salmo salar L. Journal of Fish Diseases* **23**. pp309-320

Tully O 1992. Predicting infestation parameters and impacts of caligid copepods in wild and cultured fish populations. *Invertebrate Reproduction and Development* **22**. pp9 -102

Wagner GN and McKinley RS 2004. Anaemia and salmonid swimming performance: The potential effects of sub-lethal sea lice infection. *Journal of Fish Biology* **64** (4). pp1027-1038

Wagner GN, McKinley RS, Bjorn PA and Finstad B 2003. Physiological impact of sea lice on swimming performance of Atlantic salmon. *Journal of Fish Biology*, **62** (5). pp1000-1009

PRACTICAL FISHKEEPING ONLINE

BY MATT CLARKE WWW.PRACTICALFISHKEEPING.CO.UK

Practical Fishkeeping has been the market-leading fishkeeping magazine in the United Kingdom for decades. It now has a massive online presence in the form of a busy and highly interactive 50,000-page website.

Like its print companion, the website aims to cater for those in every area of fishkeeping, from tropical and marine aquarium owners to pond hobbyists and specialists in obscure groups of fishes. However, while space in the magazine is tight and typically given over to the most popular fish species, online space is essentially unlimited, so the website content tends to cater for a different, often much more specialist reader.

The *Practical Fishkeeping* site is well known for its fish news, and caters for all areas and academic levels of the hobby. Coverage ranges from reports on topical stories on fish in the news, to investigative articles on trade issues and the low-down on recently published scientific papers that are of interest to the fishkeeping community.

Practical Fishkeeping writers scour dozens of journals for topical stories and have covered a range of fish health issues in recent months, including developments with the koi herpes virus, or KHV, and details on an iridovirus believed to be responsible for current problems with some popular aquarium species.

New fish species are also very popular with aquarists, so details on new species form the bulk of the website's news coverage. In 2006 the site covered the descriptions of 164 species.

Using Really Simple Syndication, or RSS, readers can receive up-to-the-minute updates on fish news in their email client, web browser or mobile phone, or syndicate news headlines on their website or personalised Google home page. There's also a monthly newsletter for registered members which includes a digest of the month's top stories, as well as details on what's in the latest issue of the print magazine. Those who have an opinion on a story can leave their comments below.

A selection of around 500 articles from older issues of *Practical Fishkeeping* is also archived on the site. These span tropicals, marines, coldwater and pond fish, aquarium plants, frequently asked questions, basic fishkeeping advice and Interesting Imports, a column on new and unusual fish species on sale in the UK aquarium trade. A wide range of in-depth product reviews and trials is also archived in the Reviews section, and readers can also review their own equipment online.

TOOLS

The Tools section of the *Practical Fishkeeping* site includes over 40 web applications for working out anything from the amount of water

an aquarium holds to the toxicity of ammonia at different pH and temperature levels. Besides 25 calculators and 11 convertors, there is also a Treatment Finder for finding the correct disease medication for your fishes' illness, and the UK Tapwater Quality Map.

This is a community project to map the data in freely available drinking water reports so fishkeepers know the basics on their local water chemistry, including whether it has been chlorinated. Readers have already submitted over 100 reports, with much of the UK covered.

In 2006 the site launched a new taxonomic database of fishes containing details of over 25,000 fish species. The Fish Database is to form the skeleton of another larger project, in which on-line readers will be involved in expanding the database themselves by adding contributed images and reports on aquarium care.

The Fish Database is also connected to the site's popular Fish Mapper tool. This innovative gadget takes a fish's scientific name, fetches distribution data on the species held by museums and plots the coordinates on an interactive satellite map. It allows fishkeepers to visualise the distribution of over 16,000 fish.

In many cases you can zoom in so far on satellite maps that you can get a rough idea of what the terrain is like where a species is found. The maps generated are linked to those of other species, so you can compare the distributions of related species, which can help with the identification of some aquarium fish species.

Several writers write popular blogs on the site where readers can converse on a range of topics and give their own opinions on fishkeeping. There is also a popular monthly poll, and regular surveys, the results of which appear in later issues of the print magazine. Most of the content is freely available to all users, but you do need to register to gain free access to some areas, such as the Tools section and other interactive areas.

See www.practicalfishkeeping.co.uk ■

HAEMATOLOGY AS A TOOL TO ASSESS FARMED TILAPIA HEALTH

BY GINA CONROY AND PROFESSOR DAVID A CONROY (PHARMA-FISH SRL, MARACAY, VENEZUELA)

The blood of tilapias and other fish, as in the case of other vertebrates, can be defined in general terms as a liquid in which the principal cellular elements (erythrocytes and leucocytes) are suspended in an isotonic plasma.

One of the principal functions of the blood is respiratory, and involves transporting oxygen from the gills to the tissues, and transporting carbon dioxide from the tissues to the gills. In addition, the blood also serves as a vehicle in absorption and transporting nutrients, vitamins and hormones, as well as removing waste products and protecting the body against certain infectious agents.

BLOOD SAMPLE ANALYSIS

From the point of view of the fish health specialist, one of the great practical advantages of the blood is that small samples can be taken for analysis without necessarily having to kill the animal itself, so that many of the haematological changes detected can often be related to particular diseases and disorders, including certain infectious processes, unfavourable environmental conditions and nutritional deficiencies.

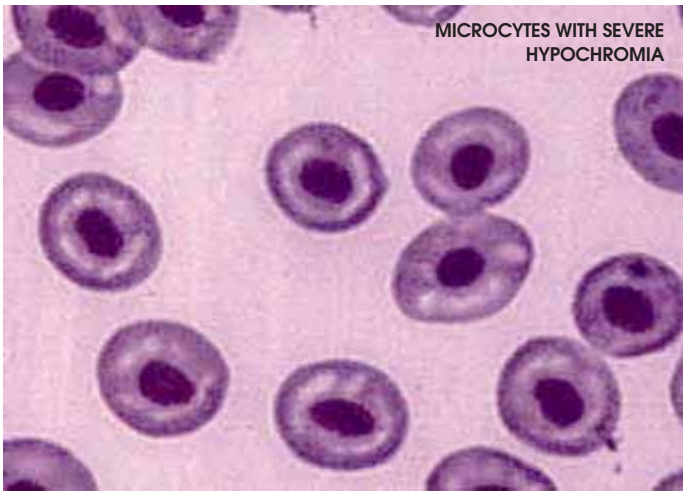
The blood parameters that are most frequently evaluated during routine haematological examinations of fish populations include the haemoglobin concentration, the haematocrit level, the red blood cell count, and aspects of the morphology and distribution of the formed cellular elements.

BLOOD FILM PREPARATION

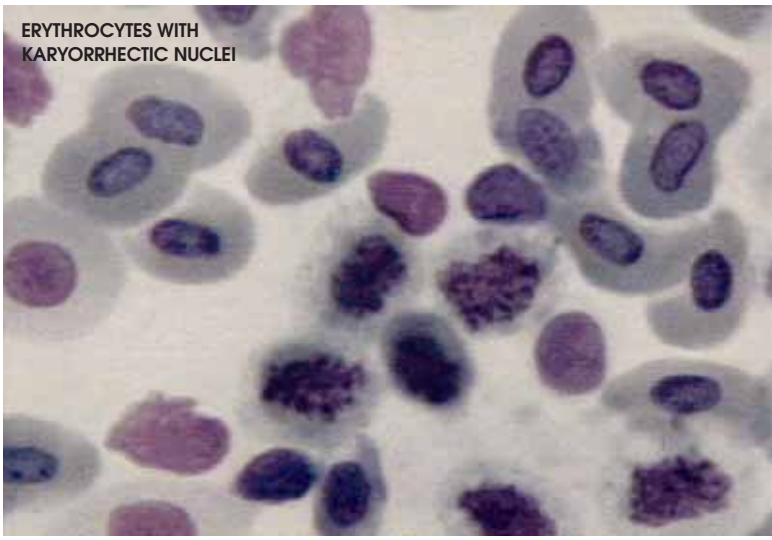
It is always convenient to prepare two thin blood films from each fish, one of which is stained by Leishman´s or Giemsa´s techniques, with the duplicate held apart in the event that it should be necessary to effect a Gram stain in order to detect the presence of bacteria in the blood associated with bacteraemia.

In such cases, the results of the Gram stain could indicate, for example, possible bacterial haemorrhagic septicaemia on a basis of the detection of Gram-negative rods or bacilli (which stain red), or possible chronic streptococcosis in the event that Gram-positive cocci (which stain deep bluish-purple) should be detected free or phagocytosed within circulating macrophages in the peripheral blood films.

With specific reference to the tilapias, a fully illustrated and explanatory CD-ROM has been prepared (Conroy & Conroy 2007) to enable tilapia farmers and their technical personnel and advisers to recognise the various normal and abnormal cells that might be present in stained smears of the peripheral blood.



MICROCYTES WITH SEVERE HYPOCHROMIA



ERYTHROCYTES WITH KARYORRHECTIC NUCLEI

It is important that every tilapia farm or production centre should establish its own "normal haematological parameters"

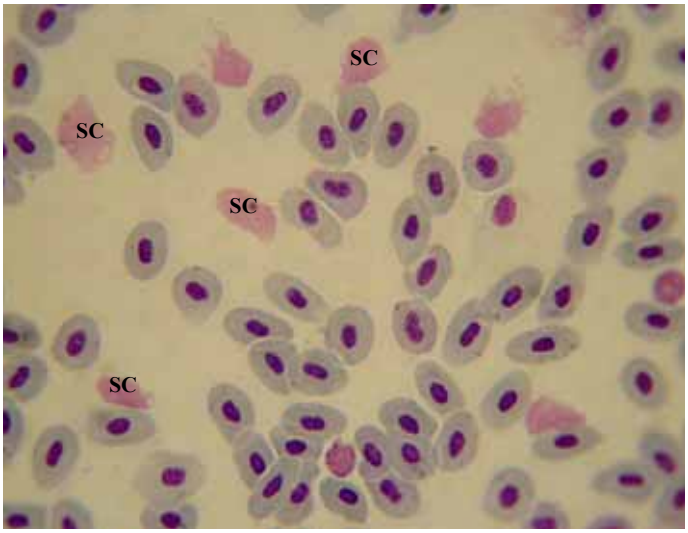
ANAEMIA

Ferguson 1989 has provided an extremely useful description of the various types of anaemia that may be detected in fish. In the first place, he established that these anaemias can be classified in accordance with the numbers, size and haemoglobin concentration of the erythrocytes.

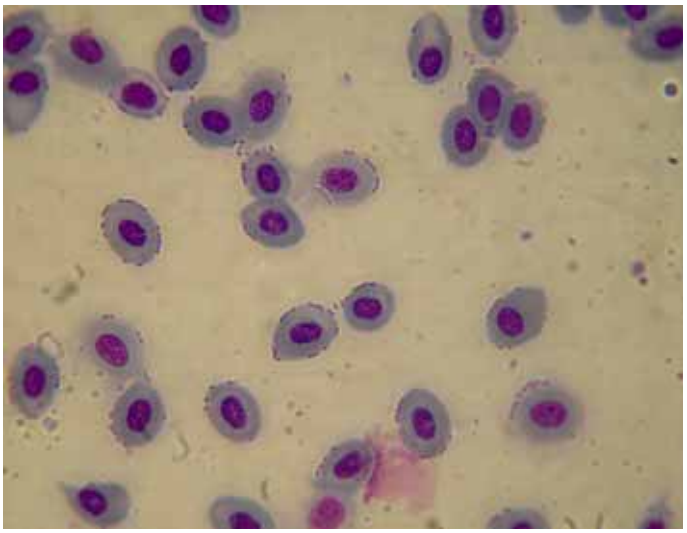
On this basis, the anaemias can be classified as responsive (or regenerative) or non-responsive. In addition, they can be further classified as haemorrhagic (in cases where loss of blood occurs), haemolytic (where there is an increased rate of cellular breakdown) or hypoplastic (where the rate of cell production is reduced).

An increase in the production of polychromatocytes, for example, is characteristic of a responsive (= regenerative) anaemia. In cases of haemolytic anaemia, haemosiderin will accumulate in the spleen, and increasing numbers of polychromatocytes will appear in the circulating blood (unless, of course, haematopoietic activity has been partially or completely suppressed by destruction of the haematopoietic tissues themselves).

Anaemic conditions associated with nutritional deficiencies and other such causes are becoming more common in farmed tilapias. One example is hepatic lipoidosis (fatty liver), a condition in which marked haematological changes occur. When pelleted feeds contain high levels of oil and polyunsaturated fats, these can easily become rancid or oxidised, a process which gives rise to the presence of peroxides and other potentially toxic substances. These in turn ▶



SMUDGE CELLS (NOTE – SOME OF THE SMUDGE CELLS ARE HIGHLIGHTED AS SC)



STREPTOCOCCI ADHERING TO THE SURFACE OF ERYTHROCYTES

produce necrosis of the renal haematopoietic tissue, thus exercising a negative impact on the process of haematopoiesis *per se*.

As a result, large numbers of erythrocytes, polychromatocytes and even erythroblasts are released into the peripheral blood, giving rise to a characteristic type of anaemia in which these immature erythrocytic elements prevail. The haemoglobin and the haematocrit values can also be diminished as a result of this process.

Oxidation of dietary fats can be avoided by incorporating vitamin E (tocopherol) into the feed, where it functions as an antioxidant. However, when oxidation does occur, the reserves of vitamin E are used up and, as a result of this, signs of vitamin E deficiency are produced.

This latter is usually characterised by increased erythrocytic fragility, which results in their breakdown, and in the formation of abundant smudge cells. The process is further complicated by a reduction in the number of circulating polychromatocytes, and possibly by a marked thrombocytopaenia, which prevents normal coagulation of the blood from taking place.

Any suspicion as to the occurrence of hepatic lipoidosis and/or vitamin E deficiency should always include haematological examinations as part of the overall diagnostic procedures.

Although detailed information is not always available on the precise nature or type of anaemia produced, it is known that a lack of dietary vitamin K3 (menadione) tends to reduce the haematocrit level and prolongs the clotting time of the blood. Several reports of vitamin deficiency signs in tilapias and their hybrids make mention of "low haematocrits" (vitamin B1, or thiamine), "anaemia" (vitamin C, or ascorbic acid), "low haemoglobin" (vitamin D, or colecalciferol) etc., without providing any more specific details on the condition.

Thiamine deficiency anaemia, however, is known to produce a microcytic anaemia in which numerous dividing polychromatocytes can be detected in the blood. Iron deficiency anaemia is also characterised by its hypochromic and microcytic nature.

The case of dietary vitamin B12 (cyanocobalamin) and folate (folic acid) requirements in tilapias is something which needs to be carefully considered and evaluated. In nutritional terms these have complementary roles, so that a deficiency of one of them is likely to cause problems. *Tilapias* with vitamin B12 deficiencies develop a characteristic hypochromic anaemia, frequently associated with the presence of erythrocytes with signs of nuclear division, segmentation and/or karyorrexis.

Folate deficiency anaemia tends to be characterised by the presence of normochromic macrocytes, together with dividing erythrocytes displaying an "hour-glass" type of division.

It is interesting to mention that, under natural conditions, the Nile tilapia *Oreochromis niloticus* has been shown to possess an intestinal microflora capable of synthesising vitamin B12 in sufficient quantities to meet its nutritional requirements for vitamin B12 (Lovell & Limsuwan 1982, Sugita *et al* 1990 1991.) These latter workers encountered a very close relationship between the amounts of vitamin B12 and the viable bacterial counts of "*Bacterioides* Type A" in the intestine. Lovell & Limsuwan (*op cit*) also found that additional vitamin B12 is not always necessary in Nile tilapias that receive adequate amounts of choline in their feed.

It is highly likely, however, that any increase in the intensity of the type of culture could lead to a need for dietary supplements containing vitamin B12, as it might not be possible to maintain a stable and active intestinal microflora capable of producing this vitamin under more intensive culture conditions.

The minimal dietary vitamin supplement levels for farmed tilapias, as elucidated by the United States' National Research Council (NRC 1993), is presented in Table 1.

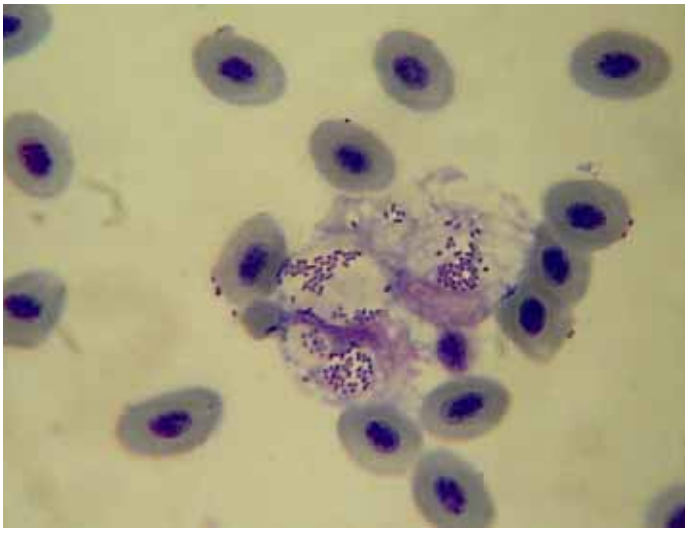
INFECTIOUS DISEASE AND HAEMATOLOGY

With respect to haematological changes brought about by infectious diseases in tilapias, Ranzani-Paiva *et al* 2004, undertook an interesting study of the responses in the blood picture of Nile tilapias *Oreochromis niloticus* induced by experimental *Mycobacterium marinum* infection (= mycobacteriosis).

They established that the infection had a pronounced effect on the haematopoietic tissues of the kidney and spleen, and produced a discrete hypochromic and microcytic anaemia. On the first

TABLE 1. RECOMMENDED INCLUSION LEVELS OF VITAMIN SUPPLEMENTS (AFTER NRC, 1993).

VITAMIN SUPPLEMENT	TILAPIA SPECIES	MINIMUM INCLUSION LEVEL (MG/KG FEED)
VITAMIN E (TOCOPHEROL)	OREOCHROMIS AUREUS O. NILOTICUS	25 50 – 100
VITAMIN B2 (RIBOFLAVINE)	O. AUREUS	6
VITAMIN C (ASCORBIC ACID)	O. AUREUS	50
VITAMIN B12 (CYANOCOBALAMIN)	O. NILOTICUS	NO REQUIREMENT DETERMINED



MONOCYTES/MACROPHAGES ENGORGED WITH STREPTOCOCCI

day following initial infection, there was a transient leucocytosis associated with neutrophilia and a modest lymphocytosis.

On the third day, there was neutropenia and a marked lymphocytosis. The numbers of circulating monocytes increased after two weeks, and these cells showed evidence of cytoplasmatic vacuolisation as the infection progressed. These changes in the differential leucocyte counts were interpreted as indicative of an initial acute inflammatory reaction, which subsequently became transformed into a chronic infectious condition in the tilapias.

AND FINALLY…

Although advances are constantly being made, it is obvious that current knowledge of the nutritional requirements, (particularly with respect to the vitamins, minerals, oligoelements etc) of tilapias, has not yet reached the corresponding level for farmed salmonids. Tilapia farmers should therefore ensure that commercial manufacturers who supply them with feed products can guarantee acceptable levels of vitamins and other essential nutrients, together with the absence of oxidised fats, aflatoxins etc.

It is important that every tilapia farm or production centre should establish its own "normal haematological parameters" for the species and/or hybrids of tilapias that are under culture in the corresponding establishment. (Hrubec *et al* 2000) has published some haematological reference intervals for hybrid tilapias (*Oreochromis niloticus* X O *mossambicus* X O *aureus*), mean weight 240g, mean length 220mm, under conditions of intensive culture where the fish were fed with a commercial feed. The values reported are presented in Table 2.

Haematological examinations, and the correct interpretation of the results, are becoming of increasing importance in tilapia farming activities, and their routine utilisation is highly recommended as a practical tool to "get to grips" with implementing standard diagnostic procedures in investigating problems associated with tilapia diseases and parasites.

TABLE 2: HAEMATOLOGICAL REFERENCE INTERVALS FOR HYBRID TILAPIAS AFTER HRUBEC *ET AL.*, 2000).

PARAMETER	VALUE
HAEMOGLOBIN (G/100 ML)	8.2 (7.0 – 9.8)
HAEMATOCRIT (%)	33 (27 – 37)
RED BLOOD CELL COUNT (X 106/MM^3)	2.31 (1.91 – 2.83)
WHITE BLOOD CELL COUNT (X 103/MM^3)	7.56 (2.15 – 15.47)
MCV (U3)	135.7 (115 – 183)
MHC (UUG)	34.9 (28.3 – 42.3)
MCHC (%)	25.7 (22 – 29)

REFERENCES

Conroy DA and Conroy G 2007. Basic atlas of normal and abnormal blood cells in farmed tilapias/Atlas básico de las células sanguíneas normales y anormales en tilapias cultivadas. Bilingual (English-Spanish) CD-ROM. Patterson Peddie Consulting Ltd, Carrickfergus, UK.

Ferguson HW 1989. Systemic pathology of fish: a text and atlas of comparative tissue responses in diseases of teleosts. Iowa State University Press, Ames, US.A. 1st edition. pp263

Hrubec TC, Cardinale JL and Smith SA 2000. Haematology and plasma chemistry reference intervals for cultured tilapia *Oreochromis hybrid*. *Vet. Clin. Pathol.* **29**. pp7-12.

Lovell RT and Limsuwan T 1982. Intestinal synthesis and dietary nonessentiality of vitamin B12 for *Tilapia nilotica. Trans. Am. Fish. Soc.* **111**. pp485-490

NRC 1993. Nutrient requirements of fish. National Academy Press, Washington DC, USA. pp114

Ranzani-Paiva NJT, Ishikawa CM, Cocuzza das Eiras A and Risaffi da Silveira V 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia *Oreochromis niloticus* (Linnaeus 1757). *Braz. Arch. Biol. Technol.* **47** pp1-11

Sugita H, Miyajima C and Deguchi Y 1990. The vitamin B12-producing ability of intestinal bacteria isolated from tilapia and channel catfish. *Nippon Suisan Gakkaishi* **56**. pp701

Sugita H, Miyajima C and Deguchi Y 1991. The vitamin B12-producing ability of the intestinal microflora of freshwater fish. *Aquaculture* **92**. pp267-276

DEVELOPING & UNDERSTANDING THE USE OF PRE-BIOTICS IN HOMARID LOBSTER CULTURE

BY C DANIELS, D BOOTHROYD, S DAVIES, R PRYOR AND C WELLS
(THE NATIONAL LOBSTER HATCHERY, CORNWALL, UNITED KINGDOM)

Limited use of antibiotics in culture environments has led to the search for alternative substances that can not only protect against disease but also enhance performance. Immunostimulants have been shown in recent years to act as excellent alternative substances for antibiotics, especially in agriculture. This has opened a market for their potential use in aquaculture situations where high mortalities during larval phases cause high levels of loss in stock production.

The application of antibiotics in both aquaculture and agriculture has become limited in recent years, due to usage restrictions bought about by concerns of antibiotic resistance and food quality. With the use of antibiotics having diversified from not only disease control but to growth promotion (Waldroup *et al* 2003) there is huge pressure on the industries to find suitable alternatives. These alternatives must therefore offer both protection from disease and performance enhancement.

Natural immunostimulants (both pre and pro-biotic substances) have been shown, as dietary supplements in agriculture, to reduce the risk of disease via activation of an organism's innate immune response, as well as improving digestibility of various dietary substances. Similar results have also been found in a small number of aquacultural species (Daniels *et al* 2006), however research is limited.

Examples of such alternative substances include oligosaccharides such as mannan-oligosaccharide and fructo-oligosaccharide (Lji *et al* 2001). Theses have shown the potential to reduce the common diseases such as those caused by Vibrio spp. which cause huge problems in all cultured species. There is therefore a clear calling for alternative products such as this in aquaculture.

Pre and pro-biotics have been shown to possess immunostimulant properties. Pre-biotics are indigestible carbohydrates which stimulate the growth and activity of beneficial bacteria of the intestine, and can activate the innate immune responses of cultured organisms when used as a dietary supplementation. Pre-biotics have also increased the efficiency of the digestive tract in many organisms.

This is done by increasing the regularity, height and integrity of the gut villi (Hooge 2004) and acting as an alternative binding site for pathogenic growth inhibiting microbes (bacteria) inhabiting the gut (Lji *et al* 2001). Examples of pre-biotic immunostimulants include Mannan Oligosaccharide, which is derived from the cell wall of the yeast *Saccharomyces cerevisiae* (Miguel *et al* 2002, Fritts and Waldroup 2003), and various forms of Fructo-oligosaccharides. Pro-biotic immunostimulants are cultures of living beneficial bacteria which, with oral application, have improved the host's health by inhibiting the colonisation and growth of some pathenogenic micro-organisms, and compete with other pathenogenic micro-organisms for resources such as nutrients and space within the digestive tract (Vine *et al* 2006).

Unlike agricultural studies, which used pre-biotics as a direct dietary supplementation in dry feed, the inclusion of immunostimulants in larval diets for aquaculture must occur via indirect bio-encapsulation. Live *Artemia* shrimp are non-selective obligate filter feeders, so it is possible to manipulate them during rearing, through enrichment or bio-encapsulation, to replicate the specific nutritional requirements of the species being cultured (Dhont and Stappen 2003). This trait allows for the indirect inclusion of immunostimulants into larval diets.

Initial dietary supplement studies, using Mannan Oligosaccharide pre-biotics at a recommended dosage for dry feed, showed increased growth and survival during the larval stages of European lobster *Homarus gammarus* culture (Taylor 2005). This demonstrated the potential of pre-biotics to reduce the effects of bacterial diseases which cause low larval survivability within crustacean culture, and also to improve larval growth by increased food breakdown and so nutrient uptake.

Research was conducted on *H gammarus* at the National Lobster Hatchery (NLH) in 2005 to determine the effect of various dietary concentrations of Mannan Oligosaccharide (Bio-Mos® aquagrade (supplied by Alltech, Lexington, Kentucky, US) on their growth and survival. The results showed short-term effects of increased larval survival to stage IV, with concentrations of 2ppt and 20ppt, in comparison to larvae fed the control diet excluding Mannan Oligosaccharide (Daniels *et al* 2006).

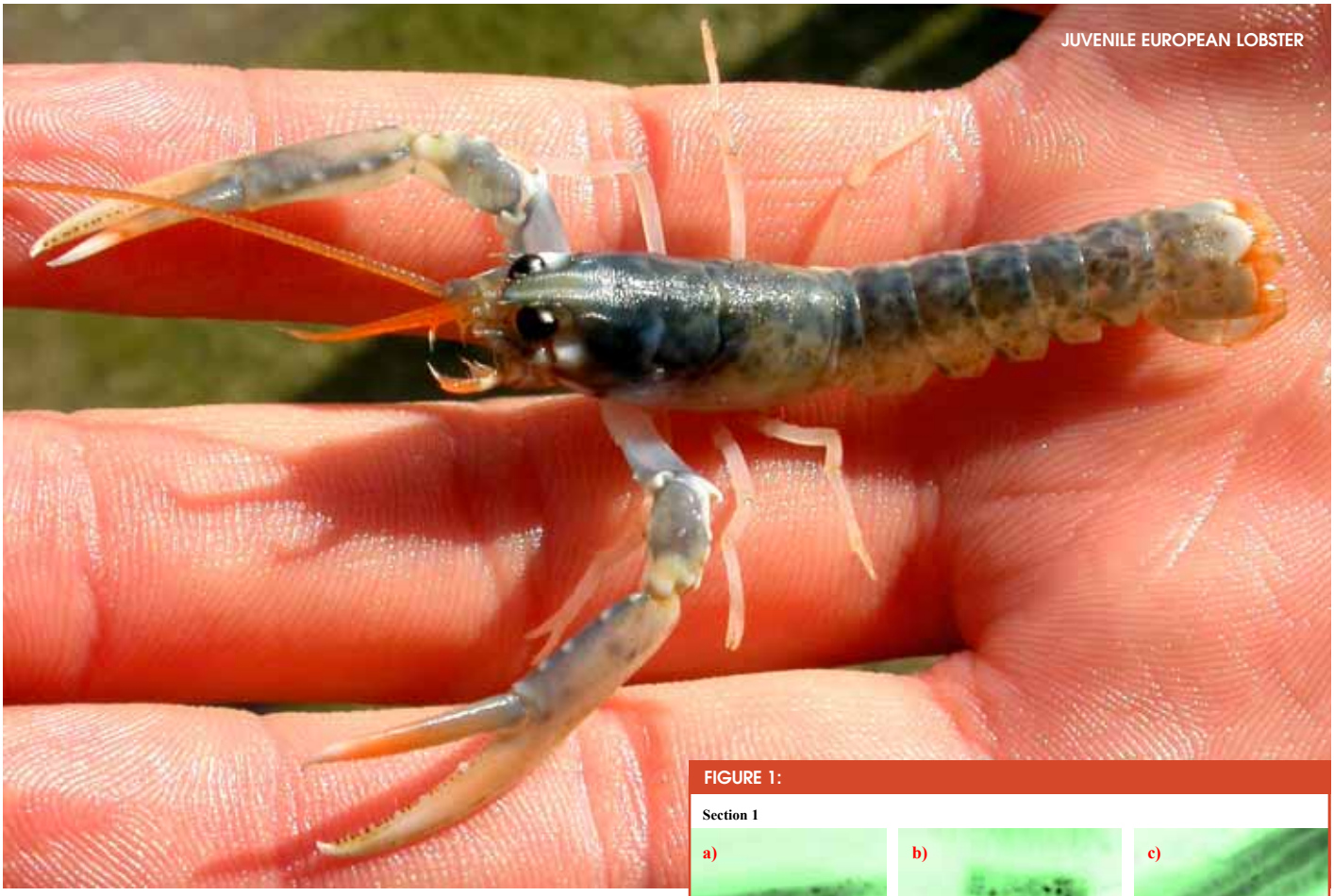
However, the results also identified high concentrations of Mannan Oligosaccharide to have negative effects on the survival of lobster larvae. This indicated that only certain dietary concentrations of immunostimulants positively effect the survival of *H gammarus* throughout their larval stages of development. Trials were therefore conducted at the NLH in 2006 to further study and understand the use of immunostimulats throughout the crustacean culture process.

FLUORESCENT LABELLING TRIALS

Research was conducted to determine how, and in what concentration, immunostimulants were taken up by *Artemia*. The indirect inclusion of enrichments through *Artemia* makes it difficult to determine at what concentration the given substance is reaching the cultured organism. Fluorescent dye was chosen to label Bio-Mos® in order to trace its route and presence through *Artemia* and subsequently lobster larvae.

Artemia were enriched with fluorescently labelled Bio-Mos® over a 24-hour period, and the process was analysed under fluorescence microscope at 0, 1, 5, 16, 20 and 24 hours.

Fluorescently labelled Bio-Mos® particles appeared present in the



Artemia guts from as early as one hour, with evidence of excretion of Bio-Mos® seen at around 16 hours. As enrichment time progresses the particle size of Bio-Mos® appears reduced (see Figure 1). This is supported by particle size analysis where, with elapsing time, a reduction in particle size occurs with the presence of *Artemia*, as shown in Figure 2. The control solution, lacking *Artemia*, does not show this pattern, so removing suspicion that reduction in particle size may be due to Bio-Mos® dissolving.

SOLUTION FLUORESCENCE

Artemia proved to increase the background fluorescence of a given solution containing fluorescently labelled Bio-Mos® (see Figure 3) indicating potential breakdown of Bio-Mos® by *Artemia*. Fluorescently labelled Bio-Mos® was found to be present through the gut of the *Artemia* and in the excretion, as shown in Figure 1. The indication of increased solution fluorescence caused by Bio-Mos® breakdown by *Artemia* is also supported by the clear reduction in particle size.

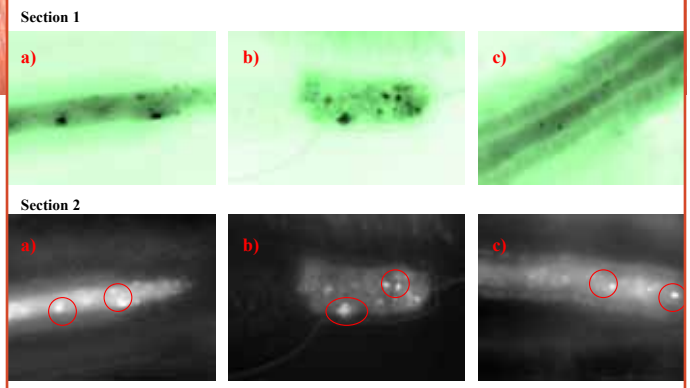
Bio-Mos® is undoubtedly orally ingested by *Artemia* and slowly broken down over a 24-hour enrichment period. With Bio-Mos® appearing to pass through *Artemia* within 16 hours of enrichment, it may therefore be suggested that an optimum enrichment time may be lower than previously used in the culture situation. However, further work would be needed to determine optimum enrichment time of other substances in the enrichment media, such as Selco™.

GUT MORPHOLOGY

Lobster larvae were then fed with *Artemia* containing fluorescently labelled Bio-Mos® for 10 days. Throughout this period samples were taken for analysis to evaluate the presence of Bio-Mos® in the gut. Larval tails were set in wax and sections of 10µm were cut at the University of Plymouth, United Kingdom. Sections were mounted onto microscope slides and the wax removed.

These unstained sections were analysed for the presence of ▶

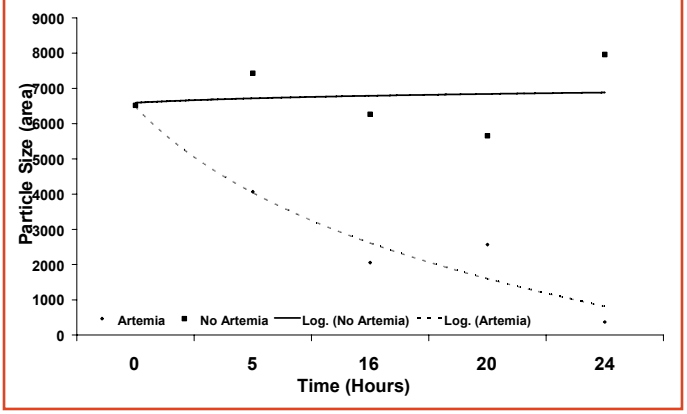
FIGURE 1:



SECTION 1 **Negative fluorescent microscope photos in the green spectrum depicting the internal gut sections and external views of *Artemia* at various stages through a 24-hour enrichment period.**

SECTION 2 **Fluorescence microscope photos depicting the internal gut sections and external views of *Artemia* at various stages through a 24h enrichment period. Red circles depict the fluorescently labelled particles of Bio-Mos®. a) Photo taken 1h into the enrichment period b) Photo taken 16h into the enrichment period depicting an external view of *Artemia* faeces at the hind end of the organism c) Photo taken at the end of the enrichment period (24h).**

FIGURE 2: EFFECTS OF THE PRESENCE OF *ARTEMIA* ON THE PARTICLE SIZE OF FLUORESCENTLY LABELLED BIO-MOSIN A SOLUTION OVER 24 HOURS.



DEVELOPING & UNDERSTANDING THE USE OF PRE-BIOTICS IN HOMARID LOBSTER CULTURE

FIGURE 3. EFFECTS OF THE PRESENCE OF *ARTEMIA* ON THE BACKGROUND FLUORESCENCE OF A SOLUTION CONTAINING FLUORESCENTLY LABELLED BIO-MOS® OVER A 24 HOUR PERIOD.

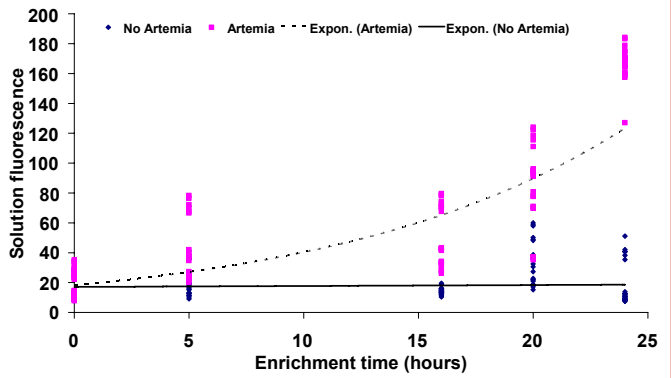


FIGURE 4. LASER SCANNING CONFOCAL MICROSCOPY GUT SECTION PHOTOS NEGATIVE COLOURS FROM LARVAL LOBSTERS TAIL SECTIONS A) FED ON A CONTROL DIET B) FED A FLUORESCENTLY LABELLED BIO-MOS® DIET. RED CIRCLES DENOTE AREAS OF BIO-MOS®.

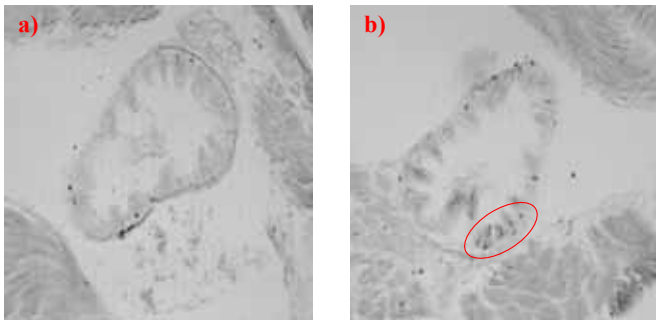
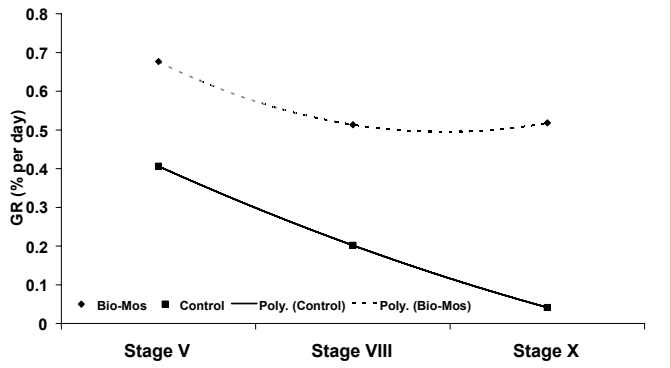


FIGURE 5. EFFECTS OF DIETARY PRESENCE OF BIO-MOS® ON THE GR RESPONSE OF CLAWED EUROPEAN LOBSTERS FED DIETS WITH AND WITHOUT THE ADDITION OF BIO-MOS® FOR 40 DAYS.



fluorescently labelled Bio-Mos® and also for physical properties of gut wall lining, using laser scanning confocal microscopy (carried out at the Marine Biological Association, Plymouth, UK).

From initial viewing of gut section photographs there appears to be darker patches in the epithelial folds, indicating fluorescence (see Figure 4). These darkened patches are not visible in the control-fed samples. It may be that the Bio-Mos® particle size is too small for the specified magnification and so appears as patches of dark where many particles are present. To validate such conclusions, further research is needed to analyse gut villi sections in the epithelial folds.

SYNERGISTIC USE OF IMMUNOSTIMULANTS

Bio-Mos® is one of numerous immunostimulants which have shown the ability to activate innate immune responses when used as a dietary supplementation. It was therefore logical to further study the additional effects of b-glucans and other pre-biotics. These could potentially, as a supplement to Bio-Mos®, have greater positive effects on the growth and survival of European lobsters during high stress larval periods where resistance to pathogens appears innately low.

Four larval lobster diets were trialled over a two-week period. Mixed origin larvae were reared in a 12 Kreisel cone recirculation system with a maximum of 2500 larvae per cone. At the moult to stage IV (approximately two weeks of growth) the miniature lobsters were separated into individual rearing pens to prevent canabalism.

Larvae were fed daily from hatching through to stage IV of growth on five *Artemia* per ml of the specified enrichment. *Artemia* were harvested and enriched over a 48h protocol (de-capsulated cysts re-hydrated and hatched for 24h, then hatched *Artemia nauplii* enriched for a further 24h). Four different enrichments were used, as described in Table 1.

Data collected from this trial showed no significant effect on larval survival and growth with the simultaneous dietary use of pre-biotics and ß-Glucans. However, the potential simultaneous use of pre and pro-biotics is a top priority for investigation in future studies. Pre-biotics decrease the number of harmful pathenogenic bacteria in the digestive tract, whereas pro-biotics increase the number of beneficial bacteria in the digestive tract. Therefore, simultaneous use could potentially have greater positive effects on the growth and survival of cultured crustaceans during high stress larval periods where resistance to pathogens appears innately low.

IMMUNOSTIMULANT INCLUSION INTO JUVENILE DIETS

In order to understand the use of immunostimulants in aquaculture it is important to consider and examine their exploitation at all stages throughout a culture period. The consideration of artificial diets for juvenile stages of growth which require larger food sources to satisfy nutritional and physical dietary requirements was essential, especially when considering the inclusion of immunostimulants.

Frozen natural foods that have proved very successful as juvenile diets, however, are not practical when the inclusion of dietary additive is taken into account. A pellet was therefore formulated based on previous formulations trialled in tropical spiny lobster *Panulirus ornatus* culture (Smith *et al* 2003, Barclay et al 2006).

Four lobster diets were initially trialled, a dry and a wet formulation of each of the two experimental pellets (control and Bio-Mos®). These preliminary trials showed the non-suitability of the wet pellet due to lack of physical stability during the feeding process. A dry pellet feeding experiment was therefore set up and run for eight weeks.

A total of 730 juvenile lobsters were placed into individual Orkney pots to prevent cannibalism, and sorted by age into three groups, stage V, VIII and X. These were then separated evenly between two raceways, one for each experimental pellet type. The raceways were provided with flow-through filtered seawater at 19° C with salinity at 35 g L^{-1}. Prior to the experiment the juveniles were fed on a diet of frozen adult brine shrimp for between eight and 24 weeks, depending on age.

During the experimental period juveniles were then fed once every two days with one or two pellets, depending on size. The response of the juveniles in terms of survival and growth was monitored between the two experimental pellets over a 30-day period. Microscopy photographs were taken at day one, 10 and 30 in order to determine growth rates over the 30 days.

DIET FORMULATION

Two 1mm extruded pellet diets were made up with the inclusion of Bio-Mos® manipulated at the expense of starch (cornstarch). Diets were prepared by mixing the dry ingredients, after which warm, distilled water was added to form a soft dough. The dough was thoroughly mixed and pressed through a 1mm die attached to an electro-hydraulic sausage filler. The spaghetti-like strands were

Table 1. Daily Enrichment Solution for *Artemia* of Four Experimental Diets.

Diet	Enrichment Quantities					
	Selco (g)[1]	Distilled water (g)	Bio-Mos[2] (g) 20ppt	Beta MAK C85[3] 5mg/g (g)	Aquacite[4] 0.25mg/g (g)	Macroguard[5] 0.5mg/g (5% solution) (g)
Control	6	3	0.072	0	0	0
1	6	3	0.072	0.03	0	0
2	6	3	0.072	0	0.015	0
3	6	3	0.072	0	0	10.26

[1] Bio-Mos® Aquagrade, Alltech, Lexington, Kentucky US
[2] Beta MAK C85™, James. A. Mackie (Agricultural), Clackmannanshire, UK.
[3] Aquacite™, James. A. Mackie (Agricultural), Clackmannanshire, UK.
[4] Macroguard, Biotec Pharmacon ASA, Tromsø, Norway.

Table 2. Growth and Survival Responses of Juvenile *H. gammarus*

Lobster Diet and Age	Development Response				
	Initial Length (mm)	End Length (mm)	Total Growth (mm)	Growth Rate (GR) (% day)	Survival (%)
Control-S5	6.12	6.87	0.75	0.41	94.32
Bio-Mos-S5	5.99	7.20	1.22	0.68	93.18
Control-S8	8.04	8.53	0.49*	0.20*	100
Bio-Mos-S8	7.76	8.96	1.20*	0.51*	100
Control-S10	10.04	10.17	0.13*	0.04*	98.75
Bio-Mos-S10	9.69	11.19	1.51*	0.52*	98.13

S = Stage of growth
* denotes significance differences

subsequently reduced to 5mm in length while wet.

This is noted to be an appropriate size for juvenile lobsters due to observed ingestion with minimal wastage and fragmentation occurring (Smith *et al* 2003). Preliminary trials on juvenile *H gammarus* also showed this to be the case. The pellets were separated while wet, then dried overnight in a slow-cook oven and stored at room temperature until used.

In this experiment the survival rate was high but unaffected by the diet fed (see Table 2). Despite this, the potential for effects on survival must not be discounted. The scope for a longer trial, which was not feasible in the time scale of this study, would provide the ability to monitor survival over a more realistic period, probably up to a year.

Lobster growth did exhibit a clear response to dietary inclusion of Bio-Mos® during juvenile stages of development studied, with the benefit of increased size being apparent. Maximal growth responses occurred with the inclusion of Bio-Mos® at later stages of juvenile lobster development (VIII-X), with juveniles growing, on average, nearly twice (1.5 x) the rate of the control feed at stage V, and a huge 12.5 times the rate at stage X of development (Figure 5).

The growth rates obtained in this study are much higher (0.68 percent/day) compared to that reported by other studies (Jones *et al* 2001, Smith *et al* 2003) trialling extruded feeds on lobsters (0.15-0.2 percent/day).

However, there is a dearth of research pellet feeds and European lobsters, and these studies consider different species, so may not be directly comparable. Even so, there are still definite increases in growth with the inclusion of Bio-Mos® into juvenile lobster diets in this study which are directly comparable.

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REFERENCES

Barclay MC, Irvin SJ, Williams KC and Smith DM 2006. Comparison of diets for the tropical spiny lobster *Panulirus ornatus*: astaxanthin-supplemented feeds and muscle flesh. *Aquaculture Nutrition* **12**. pp117-125

Daniels C, Boothroyd D, Davies S, Pryor R, Taylor D and Wells C 2006. Bio-Mos® improves growth and survival of cultured lobsters. *Shellfish News* **21**. pp23-25

Dhont J and Stappen GV 2003. Biology, tank production and nutritional value of *Artemia*. In: Live Feeds in Marine Aquaculture. Blackwell Science. pp65-121

Fitzsimmons S, Saravana S, Walden J and Arthur G 2004. Effects of delay of onset of feeding and *Artemia* concentrations on the survival and growth of Stage I European lobsters, *Homarus gammarus* (Linnaeus). *Aquaculture Research* **34**. pp605-607

Fritts CA and Waldroup PW 2003. Evaluation of Bio-Mos® Mannan Oligosaccharide as a replacement for growth-promoting antibiotics in diets for turkeys. *International Journal of Poultry Science* **2**. pp19-22

Hooge D 2004. Meta-analysis of broiler chicken pen trials evaluating dietary Mannan Oligosaccharide, 1993- 2003. *Poultry Science* **3**. pp163-174

Jones CM, Linton L, Horton D and Bowman W 2001. Effects of density on growth and survival of ornate rock lobster *Panulirus ornatus* (Fabricius 1798), in a flow-through raceway system. *Marine Freshwater Research* **52**. pp1425-1429

Jones CM, Linton L, Horton D and Bowman W 2001. Effect of density on growth and survival of ornate rock lobster *Panulirus ornatus* in a flow-through raceway system. *Marine Freshwater Research* **52**. pp1425-1429

Lji PA, Saki AA and Tivey DR 2001. Intestinal structure and function of broiler ckickens on diets supplemented with Mannan Oligosaccharide. *Journal of the Science of Food and Agriculture* **81**. pp1186-1192

Miguel JC, Rodriguez-Zas SL and Pettigrew JE 2002. Practical effects of Bio-Mos® in nursery pig diets: a meta-analysis. In: *Nutritional Biotechnology in the Feed and Food Industries, from Niche Markets to Mainstream.* Proceedings of Alltech's 18th Annual Symposium TP Lyons and KA Jacques, (eds). Nottingham University Press. pp425-433

Smith DM, Williams KC, Irvin S, Barclay M and Tabrett S 2003. Development of a pellet feed for juvenile tropical spiny lobster *Panulirus ornatus*: response to dietary protein and lipid. *Aquaculture Nutrition* **9**. pp231-237

Taylor D 2005. Refinement and research lead to better rearing results at the UK's National Lobster Hatchery. *Hatchery International.* pp17-19

Vine NG, Winston DL and Kaiser H 2006. Probiotics in marine larviculture. *FEMS Microbiology Reviews* **30**. pp404-427

Waldroup PW, Edgar O, Oviedo-Rondon and Fritts CA 2003. Comparison of Bio-Mos® and antibiotic feeding programmes in broiler diets containing copper sulphate. *International Journal of Poultry Science* **2**. pp28-31

NEW AND EMERGING DISEASES IN ENGLAND AND WALES

BY DRS STEPHEN IRVING, STEPHEN FEIST, DAVID STONE, DAVID VERNER-JEFFREYS, KELLY BATEMAN, PAUL MARTIN, MARK THRUSH, MATT LONGSHAW, EDWARD ROBERTS AND GEORGINA RIMMER (CEFAS WEYMOUTH LABORATORY, WEYMOUTH, UNITED KINGDOM)

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Cefas (Centre for the Environment, Fisheries and Aquaculture Science) Weymouth Laboratory has a responsibility (under project FC1166 from Defra, the United Kingdom's Department for Environment Food and Rural Affairs) to investigate and assess the risk to cultured and wild fish stocks of new and emerging diseases. Cefas Weymouth is ideally suited for this purpose, as the laboratory houses the Fish Health Inspectorate (FHI), as well as several diagnostic and research groups. In this article we describe the processes used for such investigations, and give examples from some recent disease outbreaks.

INTELLIGENCE

The FHI visits all salmonid and coarse fisheries in England and Wales. Any reports of unusual occurrences or intelligence are therefore reported back to the laboratory. Scientific staff attend the first part of every monthly FHI meeting in order that this flow of information (reports from the field, reports from the laboratory of relevance to the field) can be exchanged.

Members of the FHI also attend the monthly research project meeting where new data is discussed, plans made and priorities agreed. The epidemiology group at Weymouth conducts a surveillance of significant new fish disease developments reported in scientific and "grey" literature throughout the world (including Internet newsletters, alerting services and news agency releases). Information on the occurrence of known diseases in new locations or species, new presentations caused by known pathogens and the appearance of new diseases is held on a database, and regular updates are provided to government laboratories in Scotland and Northern Ireland, the Environment Agency (EA) and the Fish Veterinarian Society (FVS).

In this way the threat from new diseases occurring in other countries can be assessed before they occur in England and Wales. In addition, the FVS has been approached to see if a mechanism whereby information from their case investigations can be included without compromising their requirement for client confidentiality.

FIGURE 1. VIEW OF REGISTRY OF AQUATIC PATHOLOGY WEBSITE (HTTP://WWW.AQUATICPATHOLOGY.CO.UK)

Disease outbreaks in wild stocks are an important priority for the project. Reports of disease in wild stocks received by the EA are passed to Cefas Weymouth for investigation. In addition, samples are received from the EA's routine programme of population monitoring of wild stocks.

Health status in marine fish is provided from the fishery liaison officers and these are added to the database. In this way, information on diseases in cultured, wild, freshwater and marine fish can be used in the project.

REGISTRY OF AQUATIC PATHOLOGY

Under this project the Registry of Aquatic Pathology (Figure 1) has been made available on-line. *See www.aquaticpathology.co.uk.* This comprehensive archive of fish and shellfish pathology contains over 1000 specimens of histological slides and parasites, and is constantly being expanded as material is generated from this project and others, as well as from sources around the world.

PLANNING AND RESOURCES

By its very nature, work on new and emerging diseases is unpredictable and can change on a weekly basis. In order to manage such a project, the decision-making and information exchange processes need to be nimble and flexible.

The members of the FC1166 project meet each month with the Defra programme manager in order to discuss results and assign priorities. The ultimate aim of this rapid-response approach is to collate new and emerging disease threats, identify the causative agent and its aetiology, enabling a risk assessment to be made of the risk it poses to cultured and wild stocks.

Diseases that represent a threat can then be passed to other current projects for more extensive research, while work is terminated on those that do not represent a threat. The following examples indicate the approaches adopted by the project team.

ROSETTE LIKE AGENT (RLA)

This work was initiated following the discovery of an intracellular parasite termed the Rosette-like agent in sunbleak and top mouth gudgeon (TMG) as part of a scientific collaboration (Gozlan *et al* 2005).

Analogy with Rosette Agent *Sphaerothecum destruens* in the United States indicated that this parasite was a potential risk to wild stocks. The publication of the Gozlan article triggered considerable media interest, and briefing statements between the collaborating partners (Cefas, Defra and the Centre for Ecology and Hydrology) were prepared. Considerable molecular biology and pathology effort has been expended on the analysis of sunbleak and TMG populations to identify the parasite (Figure 2). Following the analysis of presumed RLA-free and RLA-exposed populations, present data indicates that the

original hypothesis (TMG carrying RLA and thereby infecting sunbleak causing their decline) appears unlikely.

RLA appears to be present in sunbleak populations that have had no contact with TMG, and so far the RLA has only been detected in populations of TMG exposed experimentally to sunbleak in recirculating systems. Further, the RLA was not consistently detected in sunbleak, showing the classical wasting symptoms following exposure to TMG.

In an attempt to identify similar organisms which might also be present in United Kingdom waters, a *Dermocystidium* sp. from bullhead (obtained by monitoring wild stocks) was sequenced, and results indicated that the organism present was *D salmonis*, a recognised parasite of salmonids.

Archive material from previous reported outbreaks of *Dermocystidium* in Scottish salmon during the 1980s and 1990s was obtained. Histological and *in situ* hybridisation analysis of this archive material indicated that the organism from Scottish salmon *Dermocystidium* sp., the organism in sunbleak (RLA) and *S destruens* from US salmon were all related members of the Class Mesomycetozoa.

The RLA from sunbleak is indistinguishable from *S destruens* using currently available techniques, but the parasite from Scottish salmon was confirmed as a *Dermocystidium* species, probably *D salmonis*.

RA of US origin has been successfully cultured and attempts to culture RLA from sunbleak have been initiated. Present work centres on:

- identifying RLA in different populations in order to monitor its spread and assess its risk to wild UK stocks
- optimising a culture of RLA to compare with a culture of RA obtained from the US, and
- clarifying whether RLA is synonymous with S destruens.

Future work is in collaboration with CEH (Dorset), who will progress other aspects of work on this pathogen.

PSEUDAMPHISTOMUM TRUNCATUM

This parasite was detected in otter (initially) and mink (subsequently) road kills (Simpson *et al* 2005). The authors suggested that TMG or sunbleak, as invasive species, could have a role in the spread of this parasite, despite these fish species never having been reported as hosts of *P truncatum*.

Analysis of three samples of sunbleak and two of TMG from the area implicated as containing the parasite (the Somerset Levels) did not reveal the presence of the parasite. In fact the parasite has never been detected in fish in this country.

Subsequent meetings of the Defra Human Animal Infections and Risk Surveillance Group, which was set up to monitor public health risks from animal diseases, concluded that the parasite did not present a significant public health risk. The fish host and full life cycle of *P truncatum* remain unknown, and academic institutions are pursuing this aspect.

RED MARK SYNDROME

Red Mark Syndrome (RMS) is a transmissible condition of rainbow trout, characterised by the appearance of multiple ulcerated skin swellings of varying intensity on the flanks of affected fish (Verner-Jeffreys *et al* 2006). An example of the effects the disease can have on skin and muscle can be seen in Figure 3.

It shares some similarities with Strawberry Disease (SD), although there are epidemiological and pathological differences between RMS and SD. The condition causes losses to farmers in that affected fish are down-graded at harvest.

The condition was first noted in Scotland. 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 15° C). Epidemiological and laboratory studies strongly suggest that the condition is caused by a pathogenic agent of long latency, and that the spread of RMS can be caused by movement of infected fish.

FIGURE 2. DETECTION OF ROSETTE-LIKE AGENT IN THE LIVER OF SUNBLEAK USING IN SITU HYBRIDISATION. SPECIFIC LABELLING OF THE GENETIC MATERIAL OF THE ORGANISM IS CLEARLY DEMONSTRATED (→)

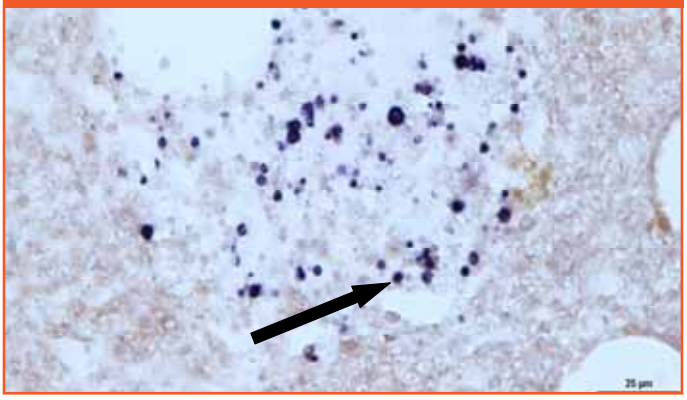
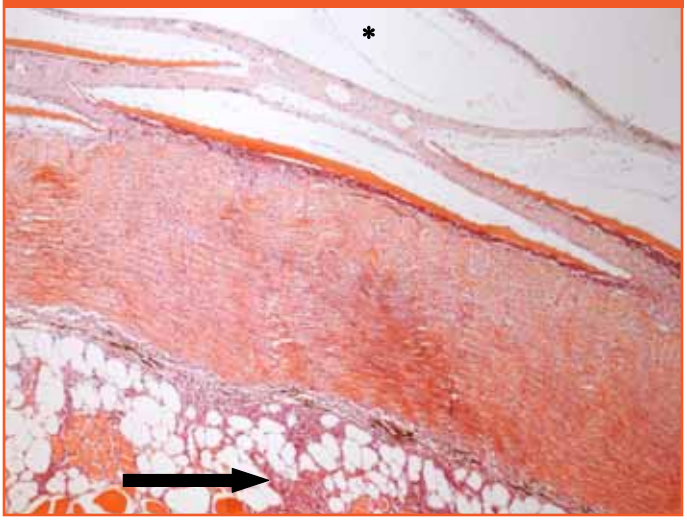


FIGURE 3. HISTOLOGICAL SECTION THROUGH THE SKIN AND MUSCLE OF A RAINBOW TROUT WITH MILD RMS. LIFTING OF SCALES (*) WITH INFILTRATION OF INFLAMMATORY CELLS INTO THE DERMIS AND UNDERLYING FAT AND MUSCLE TISSUE (→) CAN BE SEEN



There is as yet no evidence of an effect on wild fish adjacent to infected farms. RMS-infected fish have been used in co-habitation challenge studies during which transmission to naïve fish was achieved, although the causative agent could not be identified. Treatment with oxytetracycline has proved partially effective indicating that the condition may be of microbiological origin. However, it is also reported that if left untreated, affected fish will often spontaneously heal themselves. A sheet illustrating the syndrome is being shown to trout farmers by the Fish Health Inspectorate to better define the prevalence of the disease across the UK.

Work on attempting to identify the causative agent continues. Discussions (in the form of visits, meetings and video conferences) link Cefas with other workers on this novel syndrome at the University of Stirling, FRS Aberdeen and the British Trout Association.

Other areas of interest covered under the project are Cyprinid Herpes Virus 2 (= Goldfish Herpes Virus) and shellfish diseases. The project, now in the second of its four years, will continue to offer a flexible response to Defra as new disease threats to cultured and wild stocks emerge.

REFERENCES

Gozlan RE, St-Hilaire S, Feist SW, Martin P, Kent ML 2005. Disease threat to European fish. *Nature* **435**. pp23

Simpson VR, Gibbons LM, Khalil LF, Williams JLR 2005. Cholecystis in otters *Lutra lutra* and mink *Mustela vison* caused by the fluke *Pseudamphistomum truncatum*. *Veterinary Record* **157**. pp49-52

Verner-Jeffreys D, Algoet M, Feist S, Bateman K, Peeler E, Branson E 2006. Studies on Red Mark Syndrome. *Finfish News* **1**. pp19-22

AQUARIUM TRADE MAY HAVE SPREAD **GOURAMI VIRUS**

BY MATT CLARKE(PRACTICAL FISHKEEPING MAGAZINE, UNITED KINGDOM)

The ornamental fish trade is suspected of facilitating the spread of an emerging viral disease. The virus, which affects the dwarf gourami *Colisa lalia*, is so similar to one that infects farmed Murray cod *Maccullochella peelii peelii* that the two are believed to be a single species with a common geographic origin.

A team of Australian scientists headed by Professor Richard Whittington of the University of Sydney sequenced the genes of a virus that killed Murray cod in a disease outbreak in 2003, and compared the sequence to that of viral genes from imported Asian dwarf gouramies that had died in Australian aquarium shops.

When the sequences of the two viruses were compared, they had almost completed homology over 4527 base pairs, with 99.95 percent of the sequences being identical. The two viruses, which are known as Murray cod iridovirus (MCIV) and dwarf gourami iridovirus (DGIV) shared remarkable similarities - 99.9 percent - with a third virus known as infectious spleen and kidney necrosis virus (ISKNV). All three viruses are believed to represent a single species within the Megalocytivirus genus and are thought to have originated from the same location.

The study, published in the journal Molecular and Cellular Probes, also describes how a new PCR primer was developed for rapidly identifying the MCIV DGIV ISKNV virus. A test of this primer revealed that around 22 percent of dwarf gouramies in Australian aquarium retail stores were infected with the virus, raising fears that the ornamental fish trade may help spread the disease. The authors wrote: "The global trade in ornamental fish may facilitate the spread of Megalocytivirus and enable emergence of disease in new host species in distant bio-geographic regions". All of the gouramies examined had been imported from suppliers in Singapore.

SPREAD BY WATER

A follow-up study, which has just been published by Richard Whittington and Jeffrey Go in the journal Aquaculture, has shown that the dwarf gourami iridovirus can be spread from infected fish through the water. Whittington and Go injected Murray cod with filtered tissue homogenates from dwarf gourami and cohabited the fish in the same water. They found that the Murray cod subsequently tested positive for Megalocytivirus DNA through PCR analysis. The infection with the dwarf gourami virus resulted in 90 percent mortality.

"Other species may be susceptible to megalocytivirus infection and act as carriers," Whittington said. "For example, mosquito fish *Gambusia affinis* is widely distributed across Australia and is closely related to poeciliid aquarium species such as swordtails *X hellerii* and mollies *P latipinna*, which are known to be susceptible to infection by megalocytiviruses."

DWARF GOURAMI

A dwarf gourami iridovirus has been known about for several years, but it was not previously known that a single species was capable of apparently infecting other species. A separate study by scientists from the University of Florida in 2003 found that a dwarf gourami iridovirus caused clinical signs, including lethargy and the darkening of body colouration. The affected fish stopped eating, sometimes had a distended abdomen and, internally, an enlarged spleen, reddened intestine and a clear amber fluid in the body cavity.

"The global trade in ornamental fish may facilitate the spread of Megalocytivirus and enable emergence of disease in new host species in distant bio-geographic regions."

Practical Fishkeeping magazine has been aware of health issues in imported dwarf gouramies for many years. However, recent reports from readers have suggested a rise in mortalities and a decrease in lifespan. A number of major importers of fish from South East Asia confirmed to this magazine that dwarf gouramies have been of inferior quality for at least the past 10 years, but they had not noticed an unusual rise in mortalities.

Many of these companies had already switched to gouramies from non-Singapore suppliers due to health problems with imported livestock. Health problems in dwarf gouramies have historically been blamed on resistant bacterial infections, fish TB and Nocardia-like infections.

FURTHER INFORMATION

Go J, Lancaster M, Deece K, Dhungyel O and Whittington R 2006. The molecular epidemiology of iridovirus in Murray cod *Maccullochella peelii peelii* and dwarf gourami *Colisa lalia* from distant bio-geographical regions suggests a link between trade in ornamental fish and emerging iridoviral diseases. *Molecular and Cellular Probes* **20**. pp212-22

Go J and Whittington R 2006. Experimental transmission and virulence of a megalocytivirus (Family Iridoviridae) of dwarf gourami *Colisa lalia* from Asia in Murray cod *Maccullochella peelii peelii* in Australia. *Aquaculture* **258**. pp140-149

FORTHCOMING EVENTS

FISH IMMUNOLOGY WORKSHOP

Wageningen, The Netherlands
April 15-19

The Fish Workshops are set up for academic and company researchers, including PhD students, and are typically characterised by 30-minute to one-hour presentations by different but experienced lecturers, taking time to thoroughly introduce each subject.

The workshop includes two afternoons of practical training in fish immunology. The official language of the workshop is English.

The objective of this year's Fish Immunology Workshop is to provide participants with advanced knowledge, both theoretical and practical, of the fish immune system. Emphasis will be placed on innate and acquired immunity, immune modulation and immunity to infection.

The workshops will discuss the latest insights in the evolution of the immune system, but also related issues such as (experimental) animal welfare and the influence of stress on the immune response.

See www.cbi.wur.nl/uk

MOLLUSC HEALTH AND DISEASE MANAGEMENT WORKSHOP

Atlantic Veterinary College,
Charlottetown, Prince Edward Island, Canada
September 13-19

This advanced five-day training course targets diagnosticians, scientists, students and professionals of mollusc health management. The sessions will address major issues and challenges surrounding the most important mollusc species in wild and farmed situations.

Topics will include significant infectious diseases, disease causation, techniques for sampling for the presence/prevalence of disease, diagnostic techniques and test interpretation, and outbreak investigation. Laboratory sessions will involve the whole range of technical procedures for diagnosis, and demonstrations of significant diseases and conditions, including a field exercise.

This course is split into two blocks: the first runs on September 13-14 and the second from September 17-19. Participants can attend the International Shellfish Festival on September 15 and 16.

See the CAI website at www.upei.ca/cai.

HEALTH MANAGEMENT OF LABORATORY FISH

MDI Biological Laboratory,
Salisbury Cove, ME 04672,
United States
September 17-21

Health Management of Laboratory Fish is a novel short course to help technical staff, graduate students, postdoctoral fellows, junior faculty and investigators monitor the health of a colony of aquatic organisms.

This course is a one-week educational opportunity for individuals with maintenance, management or research responsibilities in which fish are used as laboratory animals.

The course is offered at the Mount Desert Island Biological Laboratory, Salisbury Cove in Maine. Topics to be discussed will include general system design and water quality management, anatomy and histology of fish, general fish diseases and disease management strategies.

Infectious and non-infectious diseases common to all fish, as well as specific diseases of importance to laboratory-maintained zebrafish, will be discussed.

The course will consist of lectures, laboratory exercises and discussions. During the course there will be an opportunity for students to discuss unusual and/or unsolved diagnostic case experiences from their home laboratories as problem-solving exercises.

See www.mdibl.org/courses/fishhealth07.shtml

6TH INTERNATIONAL ZOO AND WILDLIFE RESEARCH CONFERENCE ON BEHAVIOUR, PHYSIOLOGY AND GENETICS

Berlin, Germany. October 7-10

The Leibniz Institute for Zoo and Wildlife Research and the European Association of Zoos and Aquaria are organising the 6th International Zoo and Wildlife Research Conference on Behaviour, Physiology and Genetics, to be held in Berlin this autumn.

The conference aims to foster an exchange of ideas among international specialists from many disciplines working with free-ranging and captive animals, including fish.

The main topics include behavioural ecology, stress and disturbance, reproduction biology, conservation genetics and management of zoo, captive and small populations. The workshops will be diverse, and include nutrition and energetics, conservation genetics, animal welfare and conservation and behavioural rhythms.

See www.izw-berlin.de

Photo courtesy of Dr. Dave Little, University of Stirling

Working Together to Alleviate Poverty

Aquaculture without Frontiers

Requests Your Assistance

The independent non-profit organisation Aquaculture without Frontiers (AwF) promotes and supports sustainable aquaculture initiatives in developing countries around the world. AwF is currently teaching poor families in India and Bangladesh to raise fish for food and income. New projects have been commissioned in Thailand and Africa and AwF has been assisting tsunami-devastated fish farmers in Indonesia.

Your company can help AwF in achieving it's target of raising $360,000 by April 2007. Be a part of something special and give today. And it's simple to donate!

Please visit **www.aquaculturewithoutfrontiers.org** for instructions on how your company can donate.

Aquaculture without Frontiers - *be a part of something special.*