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Aquaculture without Frontiers seeks help

STREPTOCOCCUS IN WARM-WATER FISH

PROBIOTIC BACTERIA'S EFFECT ON SHRIMP SURVIVAL

FEATURED DIAGNOSTIC LABORATORY

CANNIBALISM in juvenile Atlantic cod

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Ph +64 9 533 4336, Fax +64 9 533 4337
Email keith@aquaculturehealth.com
www.aquaculturehealth.com

EDITORIAL DIRECTOR: Dr Scott Peddie

PUBLISHER: Keith Ingram

MANAGER: Vivienne Ingram

ASSISTANT EDITOR: Mark Barratt-Boyes

DESIGNER: Rachel Walker

WEBSITE: Web4U

CONTRIBUTORS:
Dr Franke Berthe, Dr Bernice Brewster, Dr Mathieu Castex, Professor David A Conroy, Dr Gina Conroy, Dr Joyce Evans, Dr David Groman, Professor Hugh Ferguson, Dr Phillip H Klesius, Dr Scott E LaPatra, Dr Scott Peddie, Sylvie Roquefeuil-Dedieu, Dr Craig A Shoemaker, Dr Jim Treasurer, Vincent Usache

ARE MERGERS & ACQUISITIONS GOOD FOR FISH HEALTH?

DR SCOTT PEDDIE, EDITORIAL DIRECTOR

The news this week emanating from the United Kingdom's competition authority, the Competition Commission, is that provisional clearance has been granted for Pan Fish's proposed acquisition of Marine Harvest.

In a summary of provisional findings released on November 7, the commission indicated that it had reached the conclusion that the acquisition would not result in a substantial lessening of competition.

Pan Fish's acquisition of Marine Harvest, together with Fjord Seafood, now makes it the largest salmon farming company in the world by far, accounting for approximately 25 percent of global harvest volume (using 2006 figures).

Of course acquisitions and mergers are nothing new to a global salmon farming industry where consolidation is a by-word for survivability in an increasingly competitive marketplace. Economies of scale make larger and larger farming units, and hence larger and larger companies, inevitable. Such consolidation ensures that the industry remains viable and continues to employ large numbers of people in economically fragile and often geographically isolated areas.

But what about fish health? Recent changes in the salmon farming industry have led me to ponder on the knock-on effects of consolidation on this important aspect of production management. Thankfully, it seems to me that in the fish welfare and health arena, the effects are overwhelmingly positive.

Larger companies typically invest more money in fish health programmes and expertise and related research and development. They also tend to lead the way in developing best practices in fish health management at a very practical level.

Not only that, it would seem logical to assume that the increasing prominence of Area Management Agreements and the like will be aided by a reduction in the number of companies operating in particular localities. More consistent strategic management in this respect can only be a good thing.

I wonder also how the pharmaceutical companies will view an increasingly consolidated global industry? Competition in this market is very tight indeed, and is therefore dominated by a small number of key players. One can only assume that competition will increase in the relatively small market segments where companies have products that compete directly for market share.

So, as always, changes in industry structures result in numerous opportunities for positive developments, and in this respect the fish health arena is no different. One thing's for sure - industry consolidation should make for interesting developments in the months and years ahead!

THE RESEARCH AND DEVELOPMENT CENTRE IS NEXT TO OUR RESEARCH HATCHERY AND THE JESS O "TED" EASTMAN VISITOR CENTRE IN THE SNAKE RIVER CANYON IN SOUTHERN IDAHO

RESEARCH AND DEVELOPMENT AT CLEAR SPRINGS FOODS

BY DR SCOTT E LAPATRA
RESEARCH DIVISION, CLEAR SPRINGS FOODS, INC, BUHL, IDAHO, UNITED STATES

Clear Springs Foods Inc is an employee-owned, vertically integrated company producing high quality rainbow trout for human consumption. Clear Springs Foods is composed of several divisions, including operations (broodstock, feed mill, fish production, processing and distribution), marketing, sales, administration, and research and development.

The research and development centre is located next to our research hatchery and the Jess O "Ted" Eastman Visitor Centre in the Snake River Canyon in southern Idaho, USA.

The research and development division conducts primarily applied research focused on optimising fish production and ensuring consistent, high quality trout are produced economically and in an environmentally responsible manner.

WATER QUALITY ANALYSES ARE CONDUCTED ACCORDING TO GOOD LABORATORY PROCEDURES, INCLUDING A QUALITY ASSURANCE PROGRAMME

Work areas include environmental science, fish health management, fish culture, fish production and breeding, and fish nutrition. As a resource-based industry we are committed to responsible environmental stewardship.

Our rainbow trout are produced in the high quality water of the Snake Plain aquifer. This water quality and volume is routinely monitored. Effluent water quality is continually being characterised, and techniques to further minimise fish production impact on the environment developed. Two "dry" water quality laboratories are devoted to this effort. All analyses are conducted according to good laboratory procedures, including a quality assurance programme.

Maintenance of fish health is essential for optimal rainbow trout production. We do this by identifying potential problems and developing strategies to minimise any impact they may have. These strategies range from formulating effective vaccines, to improvements in fish culture techniques such as biosecurity.

The basic strategy is to first diagnose a problem, then develop a solution and test the solution under controlled environmental conditions. After successful laboratory evaluation, solutions are field tested and fine-tuned. We are constantly striving to refine our production programme through research on photo-period control, nutrition and selective breeding.

SELECTIVE BREEDING

Selective breeding of rainbow trout is an important component of our r and d programme. The current goals of the selective breeding programme are to improve growth and disease resistance. To improve these traits, data is recorded on thousands of individuals each year. Growth data is collected at various ages to determine

THE SPECIFIC-PATHOGEN-FREE LABORATORY, WHERE SELECTED RAINBOW TROUT FAMILIES ARE INCUBATED AND HATCHED

which families and which individuals within each family have the best growth. To improve disease resistance, a portion of the progeny from each family is exposed to specific pathogens in a standardised challenge test. Currently, each family is tested for survivability to infectious hematopoietic necrosis virus and *Flavobacterium psychrophilum*, the causative agent of bacterial coldwater disease and rainbow trout fry syndrome.

Selection to improve growth began when the breeding programme was initiated. The average weight of the odd-year generation group increased from 660g at 328 days of age in 1991 to 921g at 301 days in 2003. The average weight of the even-year group increased from 620g at 328 days in 1992 to 866g at 301 days in 2004.

Selection to improve IHN resistance started with the 1994 generation. Using a standardised challenge test, IHN mortality decreased by 25.8 percent in the odd-year generation group and 29.7 percent in the even-year group. Growth is a moderately heritable trait that can be changed rapidly and economically with traditional quantitative genetic techniques. (See photo above.)

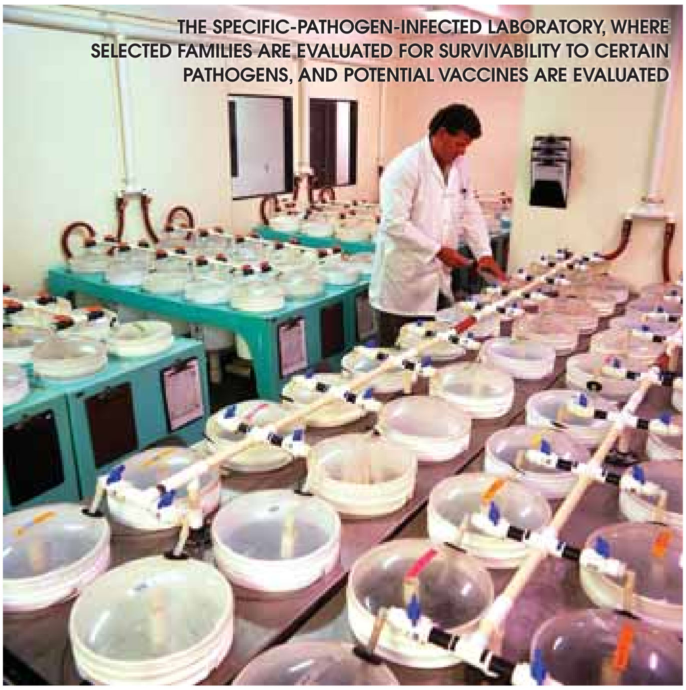
GENE EXPRESSION

Studies involving fish growth and disease resistance rely heavily on monitoring physical parameters such as overall weight gain and fillet weight, or mortality after exposure and immunological activity. These measurements do provide valuable information, in a general manner, on variation between control and treatment groups.

The recent substantial increase in genome sequence information for trout and other species now allows for the generation of assays for the analysis of expression for genes of interest. These assays can be used to study the effects of immunological stimulus on genes of known function, or to develop assays that correlate expression changes with known changes in animal physiology.

In collaboration with Dr Ken Overturf (United States Department of Agriculture-Agricultural Research Service, Hagerman, Idaho), we have developed a number of real-time quantitative PCR assays for examining the expression of genes involved with muscle development, immunology and metabolism.

In some experiments there is a high correlation between the level of gene expression and treatment. We are now using these assays to study how immune stimulation affects muscle growth in aquaculture species. Specifically, we are examining what immune genes are stimulated by specific pathogens and how these, and immuno-stimulants added into diets, affect muscle growth.



THE SPECIFIC-PATHOGEN-INFECTED LABORATORY, WHERE SELECTED FAMILIES ARE EVALUATED FOR SURVIVABILITY TO CERTAIN PATHOGENS, AND POTENTIAL VACCINES ARE EVALUATED

DNA VACCINATION

Dr Gael Kurath's research group (United States Geological Survey, Seattle, Washington) has developed a highly efficacious experimental DNA vaccine against IHNV, based on the viral glycoprotein (G) gene, and we have collaborated extensively on characterising this vaccine. (See photo above.)

Rainbow trout fry injected intra-muscularly (i.m.) with small quantities of vaccine plasmid DNA (0.1µg) are protected against virulent IHNV challenge for up to two years post-vaccination. The vaccine induces three distinct phases of response: an early anti-viral response (EAVR), a specific anti-viral response (SAVR) and a long-term anti-viral response (LAVR).

The three phases differ in the specificity of protection, the magnitude of relative percent survival of vaccinated animals in a virulent IHNV challenge, and neutralising antibody prevalence and titer.

Dr Jim Winton's research group (USGS, Seattle, Washington) used a combination of micro-array and quantitative reverse transcriptase PCR (qRT-PCR) methodology to define the host responses during the EAVR phase.

Increased expression of a large number of immune-related genes was observed at the primary site of vaccine delivery, while only the type I interferon-related genes were up-regulated in secondary systemic sites. These studies emphasise the potent nature of the IHNV viral glycoprotein to stimulate innate anti-viral immunity.

Many questions still remain regarding immune mechanism(s) involved in the EAVR, SAVR and LAVR, and research strategies are being developed to address these questions. One of these studies examined whether administering exogenous antigen in the form of a whole, killed IHN virus vaccine could elicit an EAVR in rainbow trout similar to that elicited by the DNA vaccine.

The killed IHN vaccines were prepared by Drs Eric Anderson and Sharon Clouthier (Maine BioTek Inc, Winterport, Maine) by the inactivation of IHN virus with formaldehyde, binary ethylenimine (BEI) or propiolactone. We immunised rainbow trout by intraperitoneal injection with propiolactone inactivated IHN virus, and were able to elicit a potent EAVR 105 degree days after vaccination, with relative percent survival (RPS) values of between 80 and 100 percent.

The formaldehyde and BEI inactivated IHN virus vaccines also elicited an early antiviral response in immunised rainbow trout, though the RPS values were 50 to 80 percent. These results indicate that vaccination of rainbow trout with exogenous IHN virus ▶

THE CLEAR SPRINGS FOODS RESEARCH AND DEVELOPMENT TEAM

antigen(s) can elicit an EAVR comparable to that elicited by the DNA vaccine, and that the inactivating agent used to prepare the antigen can alter the efficacy of the response.

As DNA vaccination is a relatively new technology, various theoretical and long-term safety issues related to the environment and the consumer remain to be fully addressed, although inherently the risks should not be any greater than with the commercial fish vaccines that are currently used.

Present classification systems lack clarity in distinguishing DNA-vaccinated animals from genetically modified organisms, which could raise issues in terms of licensing and public acceptance of the technology. A study recently examined the effect on vaccine efficacy of using alternative i.m. injection sites that would ultimately be removed from the fish during processing before the product goes to market. The results indicate that alternative sites are available that do not effect the efficacy of the vaccine.

FISH PERFORMANCE

It has also been hypothesised that vaccination with a potent biologic could negatively impact fish growth and performance. The IHNV DNA vaccine was used to test this hypothesis.

Triplicate 30-fish groups of rainbow trout with a mean weight of 50g were either injected i.m. with 10µg of the DNA vaccine, or mock-vaccinated and fed approximately 1.8 percent body weight per day ration. The fish were bulk weighed, counted and their percentage daily gain and food conversion ratios calculated and compared every month for three months.

The results indicated that there was no significant difference in fish growth and performance due to the DNA vaccine. Another study was also conducted that compared the i.m-delivered IHNV DNA vaccine when it was administered either jointly or separately with an i.p-injected polyvalent bacterial oil-adjuvanted vaccine using a similar experimental design.

This study also examined the concept of antigenic competition by evaluating the immune response against IHNV when four different bacterial antigens in an adjuvant were simultaneously delivered. The results again indicated that there was no significant difference in fish growth and performance due to the DNA vaccine, polyvalent bacterial oil-adjuvanted vaccine, or when the two vaccines were used together.

BACTERIAL PATHOGENS

Flavobacterium psychrophilum, the aetiological agent of coldwater disease and rainbow trout fry syndrome, has emerged as one of the most significant bacterial pathogens in salmonid aquaculture worldwide. Previous studies with Dr Ken Cain and his graduate student, Ben LaFrentz (University of Idaho, Moscow, Idaho), have suggested that the O-polysaccharide (O-PS) component of the lipopolysaccharide (LPS) of *F psychrophilum* is highly immunogenic, and may be involved in eliciting a protective immune response in rainbow trout.

Further characterisation of the carbohydrate antigens of *F psychrophilum* by SDS-PAGE and western blotting revealed the presence of two distinct carbohydrate banding patterns. One banding pattern corresponds with the LPS, and we hypothesise that the other carbohydrate banding pattern is the loosely associated slime layer, or glycocalyx, of *F psychrophilum*.

Electron microscopy of *F psychrophilum* cells immuno-gold labeled with a monoclonal antibody specific for this banding pattern supports this hypothesis, as the outermost layer of the bacterium was heavily labeled.

This is a significant finding, because the antigens that have been referred to as the O-PS of LPS (and implicated as a potential vaccine candidate antigen) appear to be components of the glycocalyx of *F psychrophilum* and not LPS.

Passive immunisation experiments utilising the monoclonal antibody specific for the glycocalyx are underway to determine if antibodies to the glycocalyx of *F psychrophilum* provide protection to rainbow trout following experimental CWD challenge.

If protection is conferred, future research will focus on purification and further characterisation of the glycocalyx as a potential vaccine candidate antigen for CWD and RTFS.

CONCLUSION

In summary, Clear Springs Foods maintains an active in-house service and research programme, along with collaborating with a number of different research groups on a wide variety of projects. Increased market competitiveness will only occur through reducing production costs. Research in the areas of feed and ingredients is required to enhance fish performance and identify acceptable and least-cost fish meal and fish oil replacements.

Tremendous gains in production efficiencies could be realised through rainbow trout strain improvement for growth and disease resistance. Biosecurity will continue to be a critical feature in any fish health management strategy. Because antibiotics will be relied upon less and less, there is a huge need for vaccines and vaccine delivery strategies, along with identifying potential feed additives that could enhance innate immune defense mechanisms. These are our challenges for the future. ■

BACTERIAL HAEMORRHAGIC SEPTICAEMIA IN TILAPIAS

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

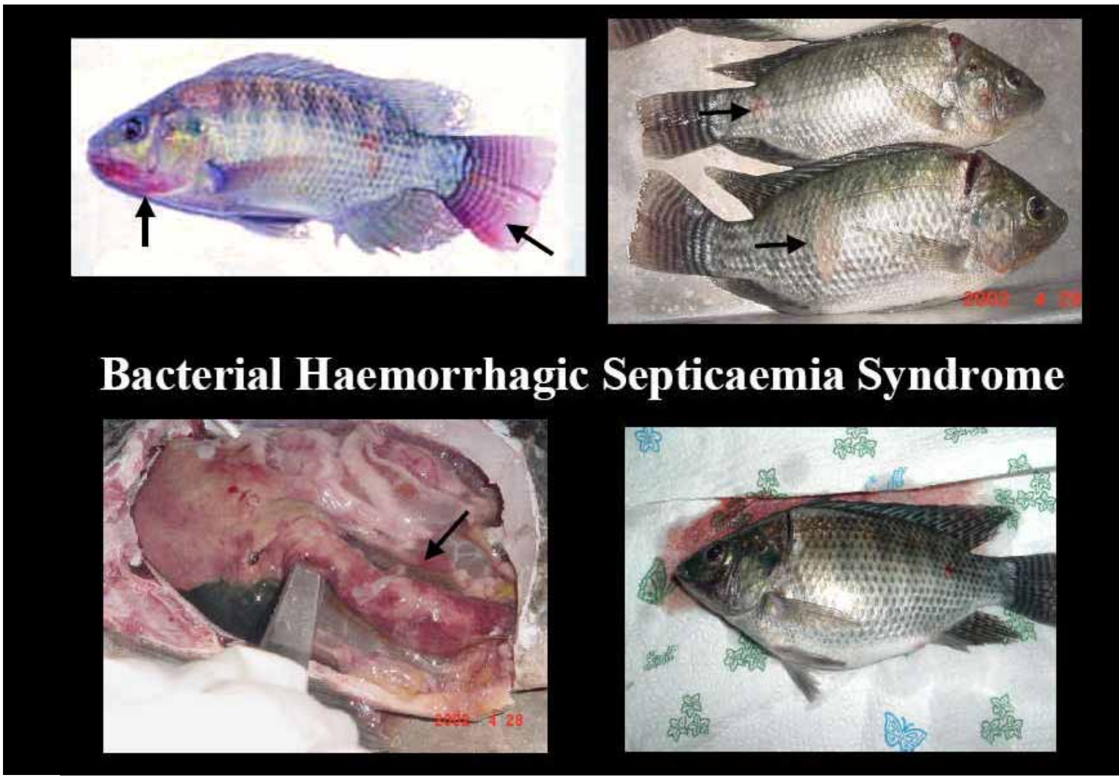


FIGURE 1: EXTERNAL ULCERATION AND NECROSIS IS EVIDENT IN THESE EXAMPLES OF BHS-AFFECTED FISH. NOTE ALSO THE NECROSIS AND HAEMORRHAGIC FOCI PRESENT IN THE INTERNAL ORGANS OF A TILAPIA

Bacterial haemorrhagic septicaemia, or BHS, has become recognised as being one of the most common disease problems that occur in tilapia farming operations on a worldwide basis.

This condition can be associated with the presence of several different types of bacteria that produce similar clinical signs in fish, and for this reason it is preferable to speak of the problem as the bacterial haemorrhagic septicaemia syndrome.

Tilapias affected by BHS generally show quite characteristic external and internal clinical signs. The fish tend to be darker in colour, lethargic and anorexic. One or both eyes may be swollen, a condition known as exophthalmos.

The most common external signs include areas of haemorrhage, frequently accompanied by varying degrees of ulceration and necrosis on the body surface, at the bases of the pectoral and ventral fins, and in the eye region. (See Figure 1)

Internally, haemorrhagic foci are commonly observed in the heart, liver, kidney and spleen, and also on the surface of the viscera and the abdominal cavity. Necrosis is usually present in the heart, liver, spleen and skeletal muscle tissue, and particularly in the haematopoietic tissue of the head kidney (see Figure 1). As the name of the syndrome suggests, outbreaks are always characterised by the presence of septicaemia, and both free and macrophage-ingested bacteria can be detected in stained blood films obtained from diseased tilapias.

BHS is capable of producing mortalities ranging from five to 100 percent in tilapias farmed in fresh and salt water environments. Bacterial types isolated from outbreaks of BHS in tilapias include the following Gram-negative rods: *Aeromonas hydrophila* and other motile aeromonads (= the "*Aeromonas hydrophila* complex", causing motile *Aeromonas* septicaemia, MAS), *Edwardsiella tarda, Pasteurella multocida, Pseudomonas fluorescens* and *Vibrio* spp.

The example of BHS associated with *Vibrio* spp is of particular interest, as cases of vibriosis can occur in both fresh and salt water. Plumb (1997) reported that *Vibrio anguillarum*, *V parahaemolyticus* and *V vulnificus* are more frequently isolated from outbreaks occurring in salt water, whereas *V cholerae, V mimicus* and *V parahaemolyticus* are more common in outbreaks in fresh water.

Edwardsiella tarda is of considerable practical importance, as this organism is capable of producing cavities in the muscle tissue which become filled with hydrogen sulphide gas, thereby seriously affecting the appearance of the fillets, and constituting a problem which may require the processing plant to be temporarily shut down for thorough cleansing and disinfection.

Certain of the Gram-positive streptococci (eg *Streptococcus agalactiae, S iniae*) are also capable of producing clinical signs of BHS in cases of acute infections.

It is of the greatest importance that outbreaks of BHS should be correctly diagnosed as soon as the first cases are detected on site. The diagnostic procedures must include isolation and identification of the possible aetiological agent in a bacteriology laboratory. For this, representative samples must be taken from the affected organs - using suitable bacteriological techniques - and streaked onto plates of trypticase soy agar or blood agar containing five percent sheep blood cells.

Once pure cultures have been obtained, these must be identified to generic and specific levels on a basis of suitable phenotypical typing methods in the laboratory. Table 1 outlines certain basic characteristics that differentiate the various types of Gram-negative organisms most frequently associated with outbreaks ▶

BACTERIAL HAEMORRHAGIC SEPTICAEMIA IN TILAPIAS

TABLE 1. BASIC DIFFERENTIAL CHARACTERISTICS OF IMPORTANT GRAM NEGATIVE BACTERIA ASSOCIATED WITH BHS IN TILAPIAS.

TEST	REACTION			
	AEROMONAS HYDROPHILA	EDWARDSIELLA TARDA	PSEUDOMONAS FLUORESCENS	VIBRIO SPP.
GRAM STAIN	—	—	—	—
MORPHOLOGY	ROD	ROD	ROD	ROD
MOTILITY	+	+	+	+
CYTOCHROME OXIDASE	+	—	+	+
O/F OF GLUCOSE IN HUGH & LEIFSON MEDIUM	F(+ GAS)	F	O	F(NO GAS)
H2S PRODUCTION	.	+	—	—
SENSITIVITY TO O/129 VIBRIOSTAT (150 U/DISC)	—	—	—	+
PRODUCTION OF FLUORESCENT PIGMENT	—	—	+	—

of BHS in tilapias.

Most of the bacteria involved in cases of BHS are normal components of the bacterial flora of the fish themselves, and of the aquatic environment in which they are being farmed. It follows from this that prevention must be based on good management practices throughout the entire production process.

The water and soil quality must be constantly monitored and maintained at optimum levels (eg with particular reference to dissolved oxygen, temperature, pH, hardness, ammonia, nitrite and other physico-chemical parameters in the case of the water).

Care must be exercised to ensure that stocking densities are kept well within the limits established for the corresponding stages of growth of the fish, and great attention must be paid to the use of suitable feeds and correct feeding practices.

Information has become available on the successful use of suitable vaccination techniques as a means of preventing massive outbreaks of edwardsiellosis and *Aeromonas hydrophila*-associated BHS in tilapia farming activities.

The application of antimicrobial compounds in the treatment of clinical cases of BHS must only be contemplated in cases where the aetiological agent has been duly isolated and correctly identified in the laboratory, and the minimum inhibitory concentration of the antimicrobial compound whose use is contemplated has been duly established and pre-determined on an individual basis.

The administration of any type of antimicrobial compound should only be undertaken on the basis of the considered professional advice of the fish health specialist responsible for the disease status of the tilapia farm concerned, and the treatment schedule must be carried out under the direct supervision of that specialist.

The *ad libitum* use of antimicrobials without such professional advice is not recommended, as this practice could lead to the development of antimicrobial-resistant strains of the bacteria, and would complicate matters further in the event that future outbreaks of BHS involving antimicrobial-resistant strains of the same types of bacteria should occur.

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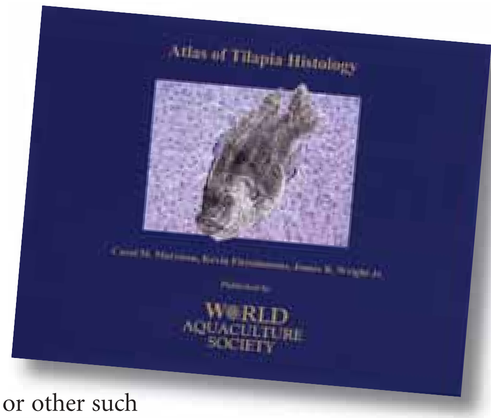

BY PROFESSOR DAVID A CONROY, PHARMA-FISH SRL, VENEZUELA

Tilapia farming is currently one of the most widespread and successful types of warm-water aquaculture in the world. In spite of the significant advances made with respect to several aspects of the biology and culture of tilapias, a few "gaps" still remain in relation to our knowledge of these fish.

The recent appearance of the Atlas of Tilapia Histology, authored by Drs Carol Morrison, Kevin Fitzsimmons and James Wright Jr, and published by the World Aquaculture Society, has very successfully filled one such "gap", the normal histology of the tilapias.

In the 96-page document, with abundant full-colour illustrations and detailed explanatory notes, the authors describe the anatomy and histology of the tilapias, making special reference to the Nile tilapia (*Oreochromis niloticus*).

The histological descriptions are extremely detailed and clearly illustrated, making this document an important, original and authoritative source of reference on the normal histology of tilapias for biologists, veterinarians and other fish health specialists, in addition to other workers in the field of tilapia farming. The individual organs and systems are dealt with in a methodical manner, and will enable possible "pathological or other such alterations" to be interpreted in the light of what is "normal". This publication should be at the fingertips of all those who have any need to examine and interpret histological sections obtained from tilapia material.

The authors, as the publishers, are to be congratulated on making this important document available. It addresses a definite need in respect to the biology and culture of tilapias in general, and can be fully recommended.

STREPTOCOCCUS IN WARM-WATER FISH

DR JOYCE J EVANS, DR PHILLIP H KLESIUS, DR CRAIG A SHOEMAKER
USDA, ARS, AQUATIC ANIMAL HEALTH RESEARCH LABORATORY, USA

This overview will focus on the epidemiological aspects of *Streptococcus iniae* and *S agalactiae* in warm-water fish, including global distribution, fish host susceptibility, disease signs, sample collection, transport and storage for successful survival and isolation, identification by conventional, automated and molecular diagnostic techniques, antibiotic sensitivities, and vaccination and environmental influences on disease susceptibility. Overall, this article will chronicle the road from the emergence of these piscine pathogens to detection, understanding, treatment and prevention.

Despite being known mainly as mammalian disease agents, *Streptococcus agalactiae* and *S iniae* have become recognised as important pathogens of wild and cultured fish, causing severe economic losses to the aquaculture and fisheries industries. Although *S agalactiae* has its origins in terrestrial mammals, whereas *S iniae* originated from aquatic mammals, their zoonotic potential has not been adequately explored. *Streptococcus agalactiae*, Lehmann and Neumann 1896, or Lancefield's group B streptococcus GBS has long been recognised as causing bovine mastitis and human neonatal infections, Elliott et al 1990.

More recently, Evans et al 2006a reported the isolation of *S agalactiae* from an aquatic mammal. The first report of GBS in freshwater fish, golden shiners *Notemigonus crysoleucas*, Robinson and Meyer 1966, preceded that of *S iniae* by 10 years. *Streptococcus agalactiae* is the only streptococcal species that has a group B antigen.

Table 1 Dolphin and fish hosts naturally or experimentally infected with *Streptococcus iniae, S. agalactiae and both *S. iniae* and *S.agalactiae* ‡* from freshwater or estuarine and marine environments.**

Freshwater	Estuarine/Marine	
Amazon dolphin (*Inia geoffrensis*)	Bottlenose dolphin (*Tursiops truncatus*)*	
Cichlidae	**Ariidae**	**Sciaendiae**
Blue tilapia (*Oreochromis aureus*)	Sea catfish (*Arius felis*)*	Red drum (*Sciaenops ocellatus*)
Mossambique tilapia	**Carangidae**	Spot (*Leiostomus xanthurus*)*
(*Oreochromis mossambicus*)	Yellowtail (*Seriola quinqueradita*)‡*	Atlantic croaker
Tilapia hybrid	**Clupeidae**	(*Micropogon undulatus*)*
(*Oreochromis niloticus* x *O. mossambicus*)‡*	Gulf menhaden *(Brevoortia patronus)**	Silver trout (*Cynoscion nothus*)*
Red tilapia (*Oreochromis niloticus* x *O. aureus*)‡*	**Fundulidae**	Sea trout (*Cynoscion regalis*)*
	Gulf killifish (Fundulus grandis)*	Common mackerel (*Scomber japonicus*)
Nile tilapia *(Oreochromis niloticus*) ‡*	**Dasyatidae**	Jack Mackerel (*Trachurus japonicus*)
Tilapia spp. unspecified (*Oreochromis spp.*)*	Stingray (*Dasyatis* sp.)*	**Serranidae**
Red Tilapia tetrahybrids (*Oreochromis mossambicus x O. urolepsis x O. niloticus x O. aureus*)*	**Haemulidae**	Brown spot grouper (*Epinephelus coioides*)
	Grunt (*Haemulidae* sp.)	European sea bass (*Dicentrarchus labrax*)
	Black margate (*Anisotremus* sp.)	
Centrarchidae	Lined piggy (*Pomadasys stridens*)	**Siganidae**
Bluegill (*Lepomis macrochirus*)*	**Kyphosidae**	Marbled spinefoot (*Siganus rivulatus*)
Green sunfish (*Lepomis cyanellus*)*	Bermuda sea chub (*Kyphosus sectatrix*)	Dusky spinefoot (*Siganus fuscescens*)
Cyprinidae	**Latidae**	Whitespotted spinefoot (*S. canaliculatus*)
Sheepshead minnow (*Cyprinodon variegatus*)*	Barramundi (*Lates calcarifer*)	
Red-tail Black shark (*Epalzeorhynchos bicolor*)	**Lutjanidae**	**Sparidae**
Rainbow shark (*Epalzeorhynchos erythrurus*)	Snapper (*Ocyurus chrysurus*)	Gilthead sea bream (*Sparus auratus*) ‡*
Golden shiners (*Notemigonus crysoleucas*)*		Pinfish (*Lagodon rhomboids*)*
Zebrafish (*Danio rerio*)	**Moronidae**	**Stromateidae**
Moronidae	Striped bass (*Morone saxatilis*)*	Silver pomfret (*Pampus argenteus*)*
Hybrid striped bass/ Sunshine bass	**Mugilidae**	**Synodonttidae**
(*Morone chrysops x M. saxatilis*)	Borneo grouper (*Liza macrolepis*)	Lizard fish (*Synodus variegates*)
Mugilidae	Klunzingeri mullet (*Liza klunzingeri*)*	
Gray mullet (*Mugil cephalus*)	Striped mullet (*Mugil cephalus)**	
Plecoglossidae	**Paralichthyidae**	
Ayu (*Plecoglossus altivelis*)	Japanese/ Olive flounder	
Salmonidae	(*Paralicthys olivaceus*)	
Amago salmon	**Pomatomidae**	
(*Oncorhynchus rhodurus* var. *macrostomus*)	Bluefish (*Pomatomus saltatrix*)*	
Rainbow trout (*Oncorhynchus mykiss*)‡*	**Scaridae**	
Coho salmon (*Oncorhynchus kisutch*)	Parrot fish (*Sparisoma aurofrenatum*)/	
Terapontidae	(*S. viride*)	
Jade Perch/Barcoo grunter (*Scortum barcoo*)		
Ictaluridae		
Channel catfish (*Ictalurus punctatus*) ‡*		

Despite this, most of the non-haemolytic GBS fish isolates were originally unspeciated, Robinson and Meyer 1966, Plumb et al 1974, Baya et al 1990, or misidentified as *Streptococcus difficile*, Eldar et al 1994.

Many of these isolates have now been characterised as Group B *S agalactiae,* Wilkinson et al 1973, Elliott et al 1990, Vandamme et al 1997. Kawamura et al 2005 have proposed a reclassification of *S difficilis* to unify *S difficilis* and *S agalactiae*, with *S difficilis* regarded as the junior synonym of *S agalactiae*. Twelve years lapsed before *S agalactiae* (haemolytic) was again reported in fish kills, Evans et al 2002a.

Pier and Madin 1976 and Pier et al 1978 first described *Streptococcus iniae* from a warm-blooded captive vertebrate, the freshwater Amazon dolphin, *Inia geoffrensis*. These *S iniae* isolates are the American Type Culture Collection ATTC, Rockville, MD type strains 29178 and 29177, and serve as the standard identification of *S iniae* phenotypic characteristics. Eighteen years after its initial characterisation, *S iniae* and the erroneous *S shiloi*, a junior synonym of *S iniae*, was reported as the causative agent responsible for fish kills in cultured hybrid tilapia *Oreochrimis niloticus* x *O aureus* in the United States and Israel, and in cultured trout in Israel, Perera et al 1994, Eldar et al 1994, 1995a.

Synonyms for *S iniae* disease derived from the literature include hemolytic streptococcal disease, bacterial meningoencephalitis, mad fish disease, golf ball disease of freshwater dolphin and *S shiloi*.

GLOBAL DISTRIBUTION

The geographical distribution of *Streptococcus iniae* and *S agalactiae* GBS is primarily confined to temperate and tropical regions that culture warm water fish. These bacterial species have been reported from six continents and 23 countries. Reports of *S iniae* have been more numerous, occurring in more countries and fish hosts than *S agalactiae*.

Group B *Streptococcus* has been reported from six countries on three continents: United States (North America), Israel, Japan, Kuwait, Thailand (Asia) and Brazil (South America). Countries in which both *S iniae* and *S agalactiae* have been reported include the United States (North America), Israel, Japan and

Thailand (Asia). (Figure 1)

Kitao 1993 indicated that 22 species of wild or cultured fish were susceptible to *S iniae*. Since that time, the number of species reported to be susceptible has doubled. More than 50 species in 29 families of freshwater, estuarine and marine fish have been reported to be susceptible to either *S iniae* or *S agalactiae*, indicating a broad host range and environmental adaptability of these organisms. (Table 1)

Both *S iniae* and *S agalactiae* are more than likely under-represented geographically and in numbers of fish hosts, due to the absence of formally published reports or inadequate identification and speciation.

Streptococcus iniae has been reported in several tilapia species *Oreochromis* spp from 14 countries. Three species of tilapia have been naturally infected with Group B *Streptococcus agalactiae* in Israel, Thailand and Brazil. Natural *S agalactiae* infections in tilapia cultured in the USA have not been reported. Despite the initial reports of non-haemolytic GBS causing fish kills in cultured freshwater golden shiners in the USA and later rainbow trout *Oncorhychus mykiss* in Israel, the majority of natural infections of *S agalactiae* in the USA, Japan, Eldar et al 1994, and Kuwait, Evans et al 2002a, Duremdez et al 2004 have involved non-tilapine estuarine and marine fish. The reader is referred to Evans et al 2006b for a review of *S iniae* and *S agalactiae* in tilapia species.

DISEASE SIGNS

Fish infected with either *Streptococcus iniae* or *S agalactiae* show similar acute neurotropic disease signs. The behavioral manifestations of these infections include abnormal, spiral or erratic swimming, swimming and whirling at the surface, and head-up or tail-up swimming. The hallmark of streptococcal infection is a C or comma-shaped body curvature. Common ocular abnormalities include peri-orbital and intraocular haemorrhage, opacity and exophthalmia in the chronic stage of infection.

Reddening and haemorrhage are often noted in the integumental and muscoskeletal system, particularly cranially and around the mouth, snout, operculum and fins. Darkened coloration is also commonly noted in infected fish. Anomalies that have not been reported from *S iniae*-infected fish but from *S agalactiae* infections include an unusual opercular clearing or "window to the gill", Evans et al 2002a, and long mucoid faecal cast, Pasnik et al 2005.

SAMPLE COLLECTION

Due to the sudden occurrence of streptococcal disease outbreaks in remote locations worldwide and the absence of trained fish diagnosticians or adequate facilities in these areas, fish collection, storage and transport techniques that can be performed by aquaculturists are needed.

The determination of proper collection, storage, transport and isolation techniques for a streptococcal pathogen can enhance the recovery, characterisation and identification of the pathogen. One of the first steps in the collection process should be deciding where the cultures are obtained from the fish.

The brain and anterior kidney are common sites of bacteriologic culture of *Streptococcus iniae*, Shoemaker et al 2001. However, non-lethal bacteriological sampling of the nares may also be useful during the early stages of *S iniae* infection, Evans et al 2001.

Evans et al 2002ab demonstrated the survival of *S agalactiae* collected from naturally infected fish using dry swabs and in transport for four days, and survival of *S iniae* and *S agalactiae* for up to 11 days following dry swab sampling and enrichment in experimentally infected fish.

The length of time tissues can remain frozen and still yield viable *Streptococcus* sp has been determined from frozen fish, both naturally and experimentally infected with *S agalactiae*, Evans et al 2004a. *Streptococcus agalactiae* could be recovered from fish tissues frozen at -20° C for nine months. The use of frozen fish may prove to be a useful archival or retrospective alternative to fresh fish for recovering pathogenic streptococci in instances when diagnostics are unavailable or impractical.

FIGURE 1

Global Distribution of *Streptococcus iniae* and *S. agalactiae*

Countries where *Streptococcus iniae* has been reported
Countries where *Streptococcus agalactiae* has been reported
Countries where both *Streptococcus iniae* and *Streptococcus agalactiae* have been reported

IDENTIFICATION

There has been significant confusion in the published literature with respect to which streptococcal species is the agent responsible for causing disease signs, mortality and pathology in fish. Identification of *S iniae* and *S agalactiae* is hampered by modifications in the taxonomy of the *Streptococcus* genus, the source of infection and familiarisation of the diagnostician with other Gram-positive bacteria that infect fish.

Accurate comparison of an isolates' phenotypic characteristics to Type strains and recognised mammalian bacterial species, ▶

Table 2. Conventional tests to confirm *Streptococcus* genus and discriminate between *Streptococcus iniae* and *Streptococcus agalactiae*.

Conventional Tests	*S. iniae*	*S. agalactiae*
Gram stain	+	+
Catalase	-	-
Hemolysis on 5% sheep blood agar	+	+/-
Motility	-	-
Growth @ 10°C	+	-
@ 45°C *	-	-
in 6.5% NaCl broth	-	-
Reaction on Bile -Esculin media	-	-
Production of :		
Pyrrolidonyl arylamidase (PYR)	+	-
Leucine aminopeptidase (LAP)	+	+
Gas from glucose in MRS broth	-	-
Acid in sorbitol	-	-
CAMP test	+	+
Voges-Proskauer (VP) reaction ‡	-	-/+
Susceptibility to Vancomycin (30μg)	-	-
Hydrolysis of:		
Hippurate	-	+
Starch	+	-
Lancefield group	None	B

* Perera et al. (1994) reported atypical *S. iniae* growth at 45°C; ‡ Commercial multitest API systems give a positive VP reaction for *S. agalactiae*.

STREPTOCOCCUS INIAE AND *S AGALACTIAE* DISEASE SIGNS IN NILE TILAPIA: OCULAR DISEASE

selection of appropriate microbiological media and laboratory tests and correct interpretation of laboratory tests, are important.

Alterations in the taxonomy and nomenclature of the *Streptococcus* genus have occurred as a result of the advent of molecular techniques employed to help delineate differences in bacterial genera and species. In addition, similarity among the phenotypic characteristics of catalase negative, Gram-positive coccus genera such as *Lactococcus* and *Enterococcus* and haemolytic *Streptococcus* spp can cause confusion and misidentification.

The genus *Streptococcus* formerly contained both *Lactococcus* and *Enterococcus*. The *Streptococcus* genus has since been split into three distinct genera: *Streptococcus, Enterococcus* and *Lactococcus,* Facklam 2002.

Numerous earlier reports of unspeciated *Streptococcus* spp are not *Streptococcus* species at all, based on the current classification system. For example, in pathogenesis studies conducted in yellowtail with the Japanese YT-3 strain, the isolates were initially referred to as a *Streptococcus* sp, Kimura and Kusuda 1982, but are now recognised as *Lactococcus garvieae,* formerly *Enterococcus seriolicida*.

Likewise, the *Streptococcus* sp in rainbow trout described by Boomker et al 1979, has been identified as a Lancefield's group D *Enterococcus*. Therefore, researchers should heed the taxonomic changes in the *Streptococcus* genus, and be aware that references to *Streptococcus* spp in older literature use outdated nomenclature.

The investigator should perform the conventional laboratory tests in Table 2 to determine the phenotypic characteristics of the bacteria, confirm the isolate as a member of the *Streptococcus* genera, and differentiate between *S agalactiae* and *S iniae*.

These include the Gram stain, catalase test, haemolysis on trypticase soy agar containing five percent sheep blood, motility, growth at 10° C and 45° C, growth in broth containing 6.5 percent NaCL, reaction on bile-esculin BE medium, production of leucine aminopeptidase LAP, pyrrolydonyl arylamidase PYR, gas from glucose in Mann, Rogosa, Sharp MRS *Lactobacillus* broth and acid in sorbitol broth, CAMP test for enhanced beta haemolysis production by *Streptococcus* using *Staphylococcus aureus*, Voges-Proskauer reaction VP for production of the enzyme acetymethyl carbinol, hydrolysis of hippurate and starch, susceptibility to vancomycin 30μg and Lancefield group.

As part of the *Streptococcus* genus, *S iniae* and *S agalactiae* are Gram-positive, catalase-negative cocci in pairs or chains. Both are non-motile, susceptible to vancomycin, negative in the VP reaction, and do not react on BE media, produce acid in sorbitol, gas in MRS broth, or grow at 45° C or in broth containing 6.5 percent NaCl, although some strains may show weak growth.

Both *S iniae* and *S agalactiae* are positive for LAP and CAMP. To confirm the diagnosis of *S iniae* and differentiate between *S agalactiae*, *S iniae*, unlike *S agalactiae*, usually grows at 10° C, does not hydrolyse hippurate but hydrolyses starch, and does not possess a Lancefield group.

S iniae is always haemolytic on blood agar plates, whereas *S agalactiae* can be either haemolytic or nonhaemolytic. Starch hydrolysis testing is essential for identification of *S iniae* and differentiation from other non-starch hydrolysing streptococcal organisms, Evans et al 2004a. Lancefield serological grouping and starch hydrolysis testing, however, is lacking from many *S iniae* characterisations.

COMMERCIAL TESTS

Commercial multi-test system kits API 20 Strep, Rapid Strept 32, API CH 50 and Vitek often used in the rapid presumptive identification of streptococci should be validated by conventional tests. These commercial multi-test systems cannot identify *S iniae*, as this organism is not in these systems' databases, Evans et al 2004a.

Furthermore, the biochemical reaction for starch in multi-test systems API 20 Strep and API CH 50 is acidification, not hydrolysis. Fish researchers began and continue utilising API multi-test systems to identify *S iniae*, and many substitute acidification of starch for starch hydrolysis in publications. Variability in phenotypic characteristics of *Streptococcus* species has been reported using these test systems and may reflect artifacts of the assays. Vandamme et al 1997 reported differences in hippurate hydrolysis between GBS using API test systems incubated at different temperatures.

Evans et al 2002a reported positive VP reactions for GBS isolates in API systems and negative VP reactions by conventional methods. Excellent results, however, have been obtained using the Biolog system Hayward CA, USA for characterisation and identification of *S iniae* and *S agalactiae*, although atypical *S agalactiae* isolates are not always identified correctly.

MOLECULAR TECHNIQUES

Klesius et al 2006a developed a rapid and sensitive monoclonal-antibody-based technique for detecting *S iniae*. Several molecular techniques have been developed as rapid, sensitive and specific complements to conventional diagnostic and taxonomic protocols for *S iniae* and *S agalactiae*. The molecular techniques include randomly amplified polymorphic DNA RAPD, restriction fragment length polymorphism RFLP, amplified fragment length polymorphism AFLP and whole genome DNA-DNA hybridisation, though polymerase chain reaction PCR has been the most widely studied.

Specific PCR primer sequences obtained from genes or DNA fragments have been successfully used to identify *S iniae* and *S agalactiae* infections in fish, Berridge et al 2001, Mata et al 2004, Kawamura et al 2005.

PCR targets in these studies have included the 16S rRNA, 23S rRNA, 16S-23S RNA intergenic spacer, DNA gyrase, superoxide dismutase, and lactate oxidase genes. PCR and whole-genome DNA-DNA hybridisation were used to identify *S difficile* as a group B *Streptococcus* sp, *S agalactiae*, based on genetic homogeneity, Vandamme et al 1997, Berridge et al 2001, Kawamura et al 2005. However, molecular techniques have not always successfully distinguished between strains that exhibited biochemical differences, Dodson et al 1999.

ANTIBIOTIC SENSITIVITIES

Streptococcus agalactiae and *S iniae* are sensitive to numerous antibiotics including oxytetracycline, ciprofloxacin, amoxicillin-clavulanic acid, penicillin, chloramphenicol, tetracycline, rifampin, sulfamethoxazole trimethoprim, erythromycin and vancomycin. *Streptococcus iniae* isolates are resistant to streptomycin, amikacin, nalidixic acid and colistin, and sensitivities vary to gentamicin and ampicillin, Pier and Madin 1976, Eldar et al 1994, Perera et al 1994.

The sensitivities of GBS fish isolates to bacitracin, gentamicin, streptomycin and novobiocin have varied, Baya et al 1990, Wilkinson et al 1973, Evans et al 2002a. Israeli GBS isolates were resistant to gentamicin, amikacin, nalidixic acid, colistin, novobiocin and optochin, Eldar et al 1994, Vandamme et al 1997.

DISEASE SUSCEPTIBILITY

Vaccination is an alternative to chemotherapeutics. The reader is referred to Klesius et al 2006b for a review of streptococcal vaccinology in warm-water fish. Currently, efficacious *S agalactiae* and *S iniae* extracellular product ECP vaccines have been developed and patented. (US patent # 0208077 A1 and # 6,379,677 B1.)

These vaccines produce antibody-mediated acquired immunity and therapeutic effects, Shelby et al 2002, Pasnik et al 2005, Evans et al 2006c. The potential of stress and vaccination, Evans et al 2004c, and poor water quality, such as sub-lethal dissolved oxygen, Evans et al 2003, for causing increased susceptibility to *S agalactiae* disease has been experimentally evaluated in Nile tilapia using a simple blood glucose monitor. This research has demonstrated the link between disease and low dissolved oxygen stress, and the protective effect of the vaccination process on disease resistance. In addition, this technology may be possibly amenable to field assessment of fish stress at aquaculture facilities and in the wild, and is of practical application to fish farmers to mitigate streptococcal disease outbreaks.

Conversely, research has demonstrated that exposure to toxic unionised ammonia may not increase *S agalactiae* disease susceptibility as previously believed, Evans et al 2006d. Chang and Plumb 1996 observed increased susceptibility of injured Nile tilapia to *S agalactiae* at elevated salinities at 25° C and 30° C. Shoemaker et al 2000 evaluated the practical considerations of cultured Nile tilapia density and mortality rates following immersion or cohabitation exposure to *S iniae*. Tilapia densities of 11.2 g/l and above had a significant affect on *S iniae* mortality.

SUMMARY

Streptococcus agalactiae and *S iniae* are clearly important fish pathogens. It is unclear if their emergence as fish pathogens is associated with heightened recognition of their occurrence in recent fish kills. It is likely that their existence has been previously under-reported by the fish health community. The advent of increased awareness and visibility of these pathogens and adequate confirmatory diagnostic techniques may enhance their frequency of detection worldwide. Epidemiological considerations as to the source, origin, potential for zoonotic infection by streptococcal organisms, and effective treatment and prevention against these organisms emphasise the importance of accurate identification.

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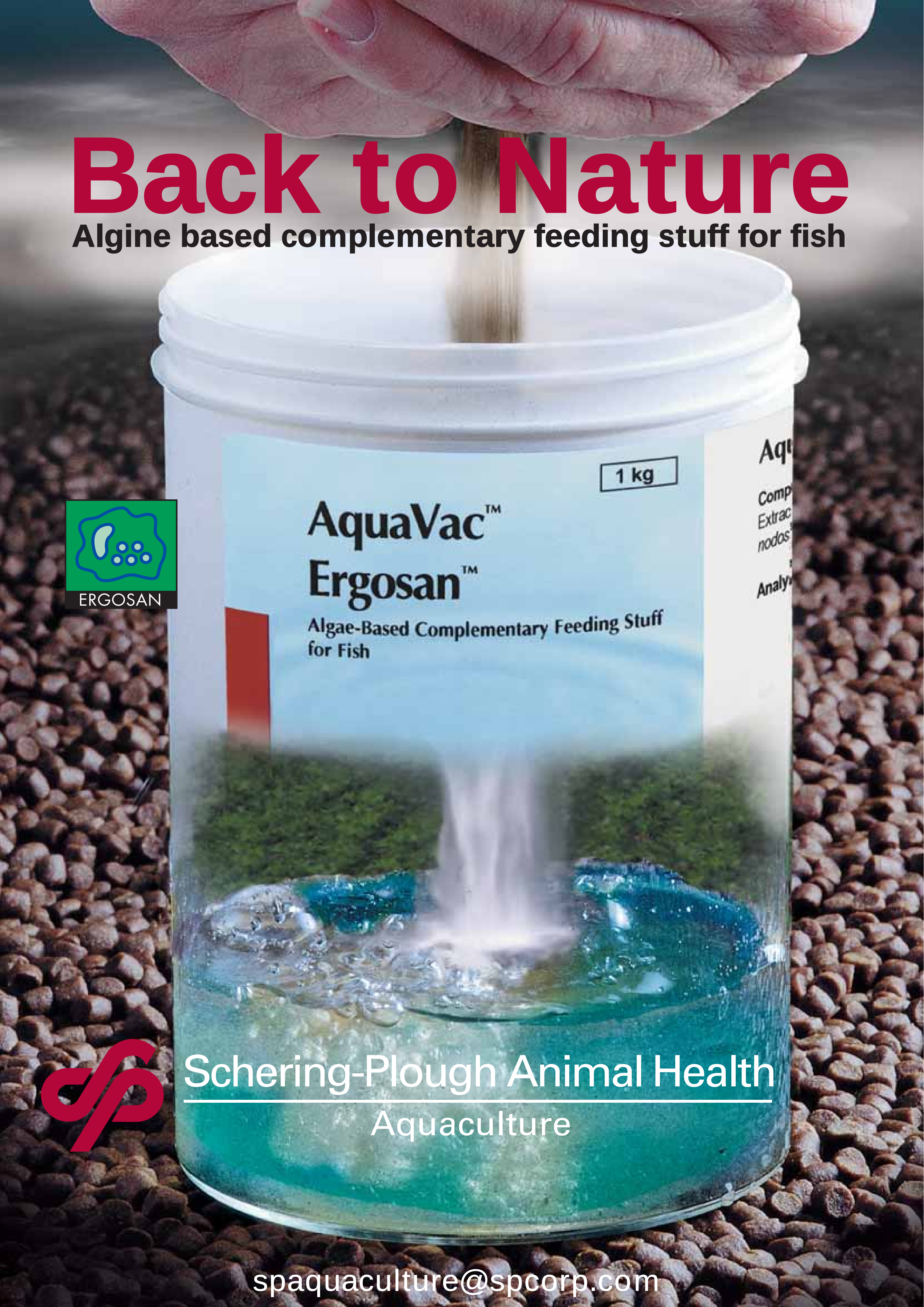
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news

NODAVIRUS FOUND IN NORWEGIAN FARMED COD

Jan Fredrik Frantzen, Fiskeriforskning, Norway

Earlier this year, the National Veterinary Institute and the Norwegian Food Safety Authority reported that nodavirus, which has previously affected both halibut and turbot fry in Norway, has been detected for the first time in Norwegian farmed cod. The virus can cause the disease viral nervous necrosis (VNN) in fish that become infected. The result can be reduced appetite and high mortality.

Senior scientist Ann-Inger Sommer, engineer Saskia Mennen and others have spent several years studying infection by nodavirus in halibut, spotted wolf fish and cod. Sommer says that in cod, as in the other fish species, fry are most vulnerable to VNN after infection through the water.

Tests have shown that the smallest cod fry become sick after infection with a nodavirus from Norwegian farmed cod. During the two-month test period, 56 percent of the cod fry died that weighed 0.5g when infected via the water. Conversely, larger fry that weighed 5g did not die of infection via the water. In this group, 35 percent died of VNN when they were infected through injection of the virus into the fish.

The last tests also show that when the temperature in the water rises, there is a pronounced increase in mortality in fish infected with the virus. Fry weighing 5g infected by injection of the virus suffered 60 percent mortality in water kept at around 15°C. The mortality rate was much lower in cooler water. "The tests show that infected cod are far more likely to die of nodavirus if the water temperature rises", says Sommer.

The research results at Fiskeriforskning are the beginning of a challenge model for the disease VNN in cod, on a level with the model scientists at the institute have created for the viral disease IPN in salmon. For example, such a challenge model can help scientists create a vaccine against VNN in cod, or used in the cod-breeding programme to select cod families that are most resistant to the disease. "When complete, the model will also help us learn how cod thrives best in farming conditions," says Sommer. "A fish that that thrives also has a better immune defence."

The Research Council of Norway, Innovation Norway and Fiskeriforskning financed the research.

Contact the following Fiskeriforskning staff:
Senior scientist Ann-Inger Sommer (+47) 77 62 92 01
Director of Research, Erling Sandsdalen (+47) 77 62 92 17
Photo caption: Atlantic cod (Courtesy of Frank Gregersen, Fiskeriforskning)

AN ATLANTIC COD (PICTURE COURTESY OF FISKERIFORSKNING)

FISH AND DNA CHIPS

(Source: Tracey Duncombe, BBSRC and Professor Alan Teale, University of Stirling)

Salmon farmers and wildlife biologists share a common dilemma: How do you assess well-being in an animal that often gives few indications that things are badly wrong before being found dead?

Research by the Exploiting Genomics Initiative at the Biotechnology and Biological Sciences Research Council is being used to develop a genetic tool that will make it easy to monitor the health and performance of Atlantic salmon. The work is set to benefit the salmon farming industry, and could also help conserve and restock wild salmon populations.

Collaborative research between scientists at the Universities of Stirling, Aberdeen and Cardiff, together with ARK Genomics at the Roslin Institute and researchers at the Norwegian School of Veterinary Science is developing a DNA chip to monitor indicators of health and performance.

The Atlantic salmon *Salmon salar* is the most important farmed fish species in the United Kingdom, and a disappearing species in the wild. The idea behind the DNA chip is to take a "snapshot" of indicator biological processes within the fish, from small tissue samples. It is the culmination of a four-year, multi-centre study to identify the genetic basis of commercially important traits. The project, known as Salmon TRAITS (TRanscriptome Analysis of Important Traits in Salmon), involves a number of industrial partners: Operon Biotechnologies Gmbh, Marine Harvest and Scottish Quality Salmon, an organisation that represents the producers of 65 percent of Scottish farmed salmon.

"The four most important constraints on commercial production are the supply of contaminant-free highly unsaturated oils, including omega-3s, for the salmon diet; the long and complex lifecycle; infectious disease; and protein growth efficiency," says Professor Alan Teale, lead researcher at the Institute of Aquaculture at the University of Stirling.

"We have identified genes involved in polyunsaturated fatty acid metabolism, protein metabolism, bacterial and viral infection, and freshwater-to-seawater adaptation. Greater knowledge of these processes will help us monitor the health, development and productivity of farmed and wild Atlantic salmon populations with a depth and precision that has not been possible previously," Prof Teale said.

The life cycle of the Atlantic salmon includes a number of dramatic physical and body chemistry changes that prepare the fish for the shift from a freshwater to a saltwater environment. In nature, this metamorphosis, known as smoltification, occurs during spring, and marks the start of a period of intense growth.

However, as little is known about the genetic control of smoltification, salmon farmers have had to rely on their own experience to predict the best time to move fish into saltwater cages. Get it wrong and the consequences can be serious.

It is difficult to tell when fish are ready to be moved. Farmers have to make a judgement based mainly on the appearance of the fish. "Move them too early and the fish may not thrive, becoming stunted and eventually dying, while stress associated with premature transfer may leave them more vulnerable to infection," says Dr Glen Sweeney of the University of Cardiff.

The Cardiff team has identified a number of genes that appear to be up-regulated during smoltification in tissues such as the gill, brain, pituitary and kidney. "The identification of these markers could be useful in determining the readiness of fish for saltwater transfer, and would also provide a means of studying the effects of environmental pollutants on smoltification," says Dr Sweeney.

Highly unsaturated fatty acids (HUFA), including omega-3s, are essential nutrients, important for growth and development in all animals, particularly the normal functioning of cell membranes of the central nervous system. However, many species cannot produce active forms of these oils from plant oil sources effectively. They need ready-made supplies.

For man, this is why oily marine fish are an important part of the diet, as wild salmon get most of their omega-3s through the marine food chain. "The problem is that feeding fish to salmon in order to supply them with omega-3s is no longer sustainable, and farmed salmon must become vegetarian for at least part of the production cycle," says Prof Teale.

Research in Stirling is focussed on the genes controlling synthesis of HUFA in salmon. "Being able to monitor the function of these genes in fish stocks will enable precision in dietary formulation and optimisation of feeding levels," says Prof Teale. "It will also enable breeders to select for fish in which these genes are more active than in the majority of the population."

Studies of wild salmon populations may also prove revealing, as HUFAs help nerve cells function across a wide temperature range. "With climate change, freshwater streams where salmon parr live are getting warmer," he says. "But after smolting, when much of the UK's wild salmon population moves into the North Atlantic and out to the Labrador Sea, they are faced with increasingly lower temperatures. We will not know until we do the research, but it is possible that these opposing natural selecting forces partly explain why wild stocks are in serious decline."

Given the environment in which farmed fish are maintained, together with high stocking densities and handling issues, bacterial and viral pathogens, including a number of emerging diseases, pose a difficult challenge for aquaculture. Poor health can often be hard to see. The first indication of a problem may be large numbers of dead fish.

Professor Secombes' group at the University of Aberdeen has analysed gene expression in fish tissue samples, following infection with different pathogens. Several hundred genes were found to be increased or decreased following a bacterial infection in salmon.

"These genes have different roles in the immune response, including bactericidal activity, acute phase response proteins and cytokines," says Prof Secombes. "We have also studied the relationship between nutritional status and immune response, and there are indications that the progression of an immune response may be affected by nutritional status."

With the large-scale gene expression analysis completed and performance indicators selected, the final stage of the project is to construct the DNA chip and confirm its suitability for use in the aquaculture industry and for wild salmon stocks.

Contact:
Prof Alan Teale, email a.j.teale@stir.ac.uk
Prof Chris Secombes, c.secombes@abdn.ac.uk
Dr Glen Sweeney, SweeneyGE@Cardiff.ac.uk

WINTER ULCER DISEASE APPEARING IN FRY IN NORWAY

Irene Andreassen (Fiskeriforskning, Norway)

Adding seawater to salmon hatcheries before the fish are physiologically ready may be contributing to an increase in the bacterial disease winter ulcer in Norwegian salmon, where the disease has caused substantial economic losses.


WINTER ULCER DISEASE IN SALMON (COURTESY OF FISKERIFORSKNING)

The winter ulcer bacteria, *Moritella viscose*, is well adapted to low temperatures both in seawater and brackish water. If the water is cold, ie below 10°C, there is an increased probability of a winter ulcer outbreak.

Tests show that salmon smolts introduced to brackish water before they are ready will become weakened and their health compromised. They also exhibit impaired appetite and show minimal weight gain.

Salmon hatcheries with intensive production which have low water exchange and add too much seawater will have favourable environmental conditions for the bacteria. However, these conditions are unfavourable for the smolts, and can reduce their resistance to diseases.

"We examined what effect the water quality in the tanks has for the susceptibility of the smolt to infection with the winter ulcers bacteria after it is introduced into the sea," says a scientist with Fiskeriforskning, Dr Helene Mikkelsen. "We tested different water qualities such as high and low water exchange and fresh versus brackish water with two percent salinity.

"Our results show that mortality after infection was highest in smolts that had been exposed to brackish water with two percent salinity. In contrast, fish that had received optimal water quality had the lowest mortality," Dr Mikkelsen said. "Unfortunately, there is no obligation to report this disease, and thus it isn't possible to know the extent of the problem."

In Norway, all seawater brought into salmon hatcheries must be UV-treated to reduce the level of bacteria in the water, but the treatment is probably not effective enough. The bacteria that cause winter ulcers most likely has a greater ability to survive UV radiation than other bacteria naturally found in seawater.

Fish with winter ulcers are treated by administering in-feed antibiotics. Indeed, a large part of today's use of antibiotics in Norway is due to winter ulcers. "The efficacy of the antibiotic treatment varies because sick fish have lower appetites and don't eat enough of the antibiotic feed. Better control of the incidence of the disease will thereby also result in less use of antibiotics," says Mikkelsen.

The experimental work is part of a larger joint project between Fiskeriforskning, NIVA, the Norwegian University of Life Sciences at Ås, the University of Bergen, Marine Harvest and AquaNet. The project is financed by the Research Council of Norway, Marine Harvest, AquaNet and Fiskeriforskning.

Contact Dr Helene Mikkelsen (+47) 77 62 90 55

INTERNATIONAL: CONFERENCE RAISES AWARENESS

The first Global Conference on Aquatic Animal Health was dedicated to reinforcing the commitment of OIE-member countries to their rights and obligations regarding disease notification and implementation of OIE standards. The conference was organised by the World Organisation for Animal Health (OIE), in collaboration with the Norwegian government,

The OIE director-general, Dr Bernard Vallat, said about half global fish production came from farming, and that bad animal health conditions were one of the major constraints to its development.

"The conference applied itself to raising awareness and defining roles and responsibilities of national veterinary services, other competent authorities possibly involved and the private sector in the prevention and control of aquatic animal diseases," Dr Vallat said.

The conference, held in Bergen, Norway from October 9 to 12, established a set of recommendations to create a framework for improved cooperation among stakeholders to improve global aquatic animal health monitoring, information sharing and control of aquatic animal health and welfare issues. Delegates from the OIE member countries, representatives of national veterinary services, other competent authorities and aquaculture producers made up the 150 participants.

The market for ornamental fish is significant, with an estimated 3.5 million homes in the United Kingdom

The conference served to underpin the efforts of the OIE and those of the OIE Aquatic Animals Commission chaired by Dr Eva-Maria Bernoth in promoting training at farm level up to the veterinary network and authorities level in early detection and appropriate rapid response to aquatic animal outbreaks.

It emphasised the need for enhanced information sharing and official notification in the field of aquatic animal health, based on the success of the OIE World Animal Health Information System, or WAHIS, in monitoring and controlling the occurrence and spread of terrestrial animal diseases.

The OIE also updated the advancement of the elaboration of scientifically based guidelines on aquatic animal welfare to be prepared for discussion with all OIE member countries. These include chapters on live transport of fish, killing for human consumption and sanitary killing of fish. Similar chapters concerning terrestrial animals already exist in the OIE Terrestrial Animal Health Code for Terrestrial Animals.

The OIE, with the support of the Aquatic Animals Commission, will respond to the outcomes of this first global conference, and implement these newly outlined recommendations using its network, including its regional and sub-regional offices.

USA/CANADA: DISTRIBUTION AGREEMENT FOR NORTH AMERICA

Eka Chemicals Inc. and Aquatic Life Sciences Inc have appointed the Aquatic Life Sciences companies Western Chemical Inc and Syndel Laboratories Ltd as exclusive distributors for 35 percent PEROX-AID throughout the United States and Canada.

Eka Chemicals Inc, a leading hydrogen peroxide manufacturer, has been pursuing FDA approval for 35 percent PEROX-AID (35 percent aquatic-grade hydrogen peroxide) for use on freshwater-reared finfish and their eggs, in collaboration with scientists at the Upper Midwest Environmental Sciences Centre and the National Aquaculture NADA Coordinator. FDA approval is expected in the coming months for three new label claims. Once approved, the current low regulatory priority status of hydrogen peroxide will be rescinded, and 35 percent PEROX-AID will be the only FDA-approved hydrogen peroxide product for use on freshwater-reared finfish and their eggs.

"With the pending approval of 35 percent PEROX-AID(r) we needed a partner with the expertise and distribution channels to rapidly and effectively distribute our product," said the director of Business Development at Eka Chemicals, Anthony R Colasurdo. "The Aquatic Life Sciences companies of Western Chemical and Syndel Laboratories are the logical choice, as they are the leaders in providing fish health products in the United States, Canada and the rest of the world."

Aquatic Life Sciences said it welcomed the opportunity to incorporate 35 percent PEROX-AID into its line of approved fish health products, said the chief executive officer, Steven J Becker. The product would be a valuable and much-needed tool for its aquaculture customers. "We applaud the work that Eka Chemicals has put into this approval, and we look forward to working with them."

Contact
In the USA: Western Chemical,
Ferndale WA 98248 USA, (+1) 800-283-5292
In Canada: Syndel Laboratories Ltd,
Vancouver BC (+1) 800-663-2282

UK/USA: KHV VACCINE WORK PROGRESSES

Henderson Morley says it is ready to start field trials of a candidate vaccine against the koi carp herpes virus. The trials follow 10 months of work in collaboration with the Hagerman Aquaculture Research Institute, based in Idaho, USA, under the supervision of the institute's director, Professor Ron Hardy.

The directors of Henderson Morley believe that these initial immunogenicity studies will produce results within six months. That should enable Henderson Morley scientists to optimise the vaccine formulation with a view to securing a product licence from the veterinary authorities as soon as practicable.

The board says it is aware that the time taken to commercialise human products may be significant, so products in the Henderson Morley portfolio targeted at animal health may offer the prospect of nearer-term revenue opportunities.

The market for ornamental fish is significant, with an estimated 3.5 million homes in the United Kingdom and 139 million fish in 13 million homes in the United States, with koi carp species being among the most popular and most valuable.

In 1998, widespread outbreaks of mortality occurred in both fish farms and ornamental ponds, when 85 percent to 100 percent of infected fish died within a few days. It was subsequently discovered that this disease was caused by a newly isolated virus - koi herpes virus, or KHV. This virus has now been isolated in at least 28 countries, including the USA, Japan and the UK, and has become a significant problem for koi breeders and enthusiasts. Once infected, a pond will lose the majority of its fish within days, and no treatment or vaccine is currently licensed in the UK.

The impact of KHV is very significant in the koi keeping and breeding community. The National Fisheries Laboratory, in its notes on koi herpes virus provided by the UK Environment Agency, says that, "Due to the pathogenicity of the virus and difficulties with detection, the Environment Agency is very concerned about the potential impact of KHV to carp fisheries within England and Wales."

The company has appointed Professor Ronald John Roberts, Professor Emeritus at the University of Sterling, to its Scientific Advisory Board to help develop the KHV vaccine.

"We are very pleased to welcome Professor Roberts to the board," said the chairman of Henderson Morley, Andrew Knight. "As we concentrate on our animal health research programme, his vast experience will be of enormous benefit to the company and its scientific advisory board."

US LIMITS FISH MOVEMENTS

The United States government issued a federal order on October 24 immediately prohibiting the interstate transport of about 37 live fish species from eight states surrounding the Great Lakes region and two Canadian provinces.

Its purpose was to prevent an outbreak of viral haemorrhagic septicemia, or VHS, from spreading into aquaculture facilities.

The order applied to Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania and Wisconsin, and the Canadian provinces of Ontario and Quebec. It was distributed by the deputy administrator of the United States' Department of Agriculture's Animal Plant Health Inspection Service, Veterinary Services. The order was issued pursuant to the Animal Health Protection Act.

The list may be amended if other species are identified as being susceptible to VHS.

See www.aphis.usda.gov/vs/aqua

VACINE GAINS PROVISIONAL AUTHORISATION

Schering-Plough Animal Health has gained a provisional marketing authorisation for the introduction of AquaVac™ Relera™, a new bivalent vaccine providing further protection against enteric redmouth disease (ERM). The immersion vaccination allows farmers to enhance existing vaccination programmes by combining the typical endemic Hagerman Type 1 ERM-causing strain of *Yersinia ruckeri* with a new biogroup called EX5.

EX5 has been responsible for widespread losses in the UK rainbow trout industry and appears to be becoming increasingly prevalent. During the past five years the industry has become aware of this new biogroup and the potential for it to be as equally destructive as the widespread Hagerman Type 1 strain of ERM.

Highlighting the potential for trout farmers, Dr Chris Gould, global technical services manager at Schering-Plough Animal Health says, "Existing licensed vaccines do not confer sufficient protection against this new biogroup, and it has been necessary to develop a new vaccine which offers full protection. Through collaboration with the UK trout industry, Schering-Plough Animal Health has been able to quickly respond to industry needs, isolate the new biogroup and develop this new vaccine."

AquaVac™ Relera™ will be available as a POM-V veterinary product in the UK. The company is supporting the announcement with a series of communications designed to inform fish vets on the development of AquaVac™ Relera™ and the most appropriate vaccine programmes for clients with ERM.

CHARITY: AQUACULTURE WITHOUT FRONTIERS SEEKS HELP

Dr Scott Peddie (Patterson Peddie Consulting Ltd, UK)

AQUACULTURE IS A SMALL-SCALE FAMILY AFFAIR IN THE AREAS IN WHICH AWF OPERATES

TILAPIA GROW WELL IN AN AWF-VOSD PROJECT IN BANGLADESH

As many readers of *Aquaculture Health International* may be aware, the independent non-profit organisation Aquaculture without Frontiers promotes and supports responsible and sustainable aquaculture initiatives.

This in turn helps to alleviate poverty by improving rural livelihoods in developing and transition countries. Formed in 2004, AwF is registered as a charity in the United Kingdom and as a non-profit organisation in the United States of America. The organisation draws on the experience of respected professionals from every relevant discipline, and already has a database of more than 100 volunteers located across the globe.

AwF is said to be unique in devoting all of its resources and attention to aquaculture. It does not seek to promote aquaculture in isolation, but as a component of integrated rural and coastal development plans, and of strategies to alleviate poverty.

For example, AwF is currently teaching very poor families in India and Bangladesh how to raise fish for food and income, while new projects have been commissioned in Thailand and Kenya. Its volunteers have also been assisting tsunami victims in Aceh, Indonesia, to re-start growing fish, shrimp and seaweed after the loss of their facilities and, in many cases, members of their families.

See www.aquaculturewithoutfrontiers.org. Please consider supporting AwF in any way you can. Instructions are on the website.

INTERNATIONAL: INTERVET SUPPORTS AQUACULTURE WITHOUT FRONTIERS AND OTHER NGO'S

Intervet International B.V. (www.intervet.com) is dedicating its 2006 Christmas donation (in lieu of sending out Christmas and New Year cards) to four non-governmental organisations (NGOs). Four initiatives from different parts of the world have been selected.

news

news

The first initiative, Aquaculture without Frontiers (AwF), is an independent non-profit organization that promotes and supports responsible and sustainable aquaculture and alleviation of poverty by improving livelihoods in developing countries. AwF does not seek to promote aquaculture in isolation, but as a component of integrated rural and coastal development plans, and of strategies to alleviate poverty (see www.aquaculturewithoutfrontiers.org for more details).

The other three NGOs aim to support disadvantaged communities in Africa or to take care of orphaned animals. A South African-based non-profit NGO initiative, Animal Aid for Africa (AAA), aims to uplift disadvantaged communities across sub-Saharan Africa. Focus is on delivering sustainable solutions to malnourishment and the spread of zoonotic diseases, by exchanging skills and knowledge between veterinarians. (www.animalaidafrica.co.za).

The other two organisations are Sophianum explores South Africa, supported by the Dutch Ministry of foreign affairs (www.ZA.DEBIOLOOG.nl and www.africanorphanage.com), and Global Vets (www.ovc.uoguelph.ca/associations/globalvets), a programme at the Ontario Veterinary College that offers students a unique opportunity to investigate animal health care in developing countries. Global Vets was formed to promote international collaboration on animal health and welfare, agricultural development and ecosystem health.

FISKERIFORSKNING INVESTIGATES THE LINK BETWEEN SALINITY AND SMOLT HEALTH

Irene Andreassen (Fiskeriforskning, Norway)

Normally, juvenile salmon grow up in fresh water, but in today's salmon smolt production, increasingly more seawater is used. Scientists have now found that use of seawater before the salmon smolt is ready for it can be detrimental.

Salt water is used in smolt production for several reasons. Among other things, it can help increase production capacity and improve water quality in some cases. However, incorrect use of seawater can weaken the fish's health and might introduce pathogenic bacteria. Our recent studies show that too high doses and too early exposure of seawater during smolt production can result in reduced growth and additional problems with winter ulcers. Such outbreaks can impact fish welfare and have a negative influence on the company's financial return.

DR HILDE TOFTEN, SENIOR RESEARCH SCIENTIST AT FISKERIFORSKNING

SEAWATER - NOT JUST "A PIECE OF CAKE"

It has been asserted that a gradual adaptation to saline water before the salmon is fully smoltified is favourable, but the research in this area has shown contradictory results.

In a Fiskeriforskning experiment, scientists chose to use 2 % salinity before the fish was fully smoltified, which is not uncommon with smolt producers. "There is evidence that such a high salinity isn't particularly favourable for the fish", says Senior Scientist Hilde Toften at Fiskeriforskning. Toften continued, "the fish ate and grew more poorly, and became clearly more susceptible to winter ulcers. Our results also show that these problems are aggravated when we combine them with a very intensive production, which has become relatively widespread in the industry in recent years. So far, we haven't known enough about this, but we're hoping that several factors will be clarified when the project is completed."

The fish's welfare is also very important for the industry, and the Fiskeriforskning's scientists are interested in studying whether the practice of intermixing seawater affects the salmon's well-being.

"Of course, we can't say anything with complete certainty about how the fish experiences these problems, but we can find the optimal conditions that ensure that the farmed fish thrives, and which prevent the fish from becoming stressed and sick. Winter ulcers are a serious disease which causes large and deep wounds on the fish. If we can manage to prevent outbreaks, we can help improve the welfare of the smolt", says Hilde Toften in closing.

The tests are part of a larger joint project amongst Fiskeriforskning, NIVA, Norwegian University of Life Sciences at Ås, University of Bergen, Marine Harvest and AquaNet. The project is financed by the Research Council of Norway, Marine Harvest, AquaNet and Fiskeriforskning.

For more information contact Senior Scientist Dr Hilde Toften on phone +47 77 62 90 53.

INTERNATIONAL: FROM BOXMEER TO DUBLIN!

Dr William Enright, the international marketing & pharmaceutical manager for the Aquatic Animal Health (AAH) Division, has decided to return to his native country of Ireland. After nearly 11 years in Boxmeer, The Netherlands, Dr Enright has accepted the position of marketing manager for the companion animal & equine business unit at Intervet Ireland. The move allows him to return to Ireland and yet stay within Intervet. The change in species focus and the more commercial environment will offer him new and exciting challenges and learning opportunities.

Dr Enright has been closely associated with the Intervet AAH Newsletters. The first issue was published in October 2000 and it has appeared at six-month intervals since. However, perhaps unknown to the aquaculture community, his background includes research on a variety of animal species and he has in excess of 250 publications to his name. "It's no wonder then that he has been an enormous help to us here at *Aquaculture Health International* in providing high quality articles and news items of interest to our diverse readership," says the editorial director, Dr Scott Peddie.

"We thank William not only for his contributions to *Aquaculture Health International*, but indeed to promoting aquaculture in general. We will miss his expertise and valued guidance, but wish him well for his continued success within the Intervet family." ■

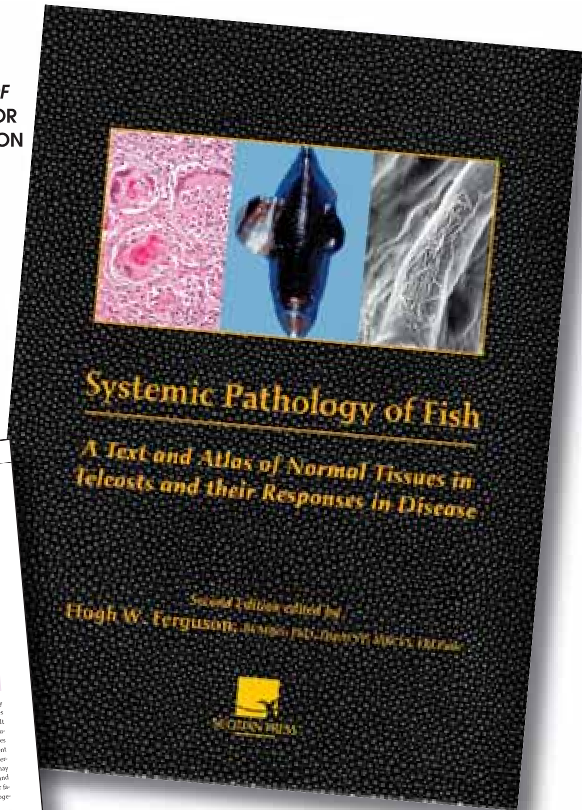
WILLIAM ENRIGHT PROUDLY DISPLAYING THE FIRST TEN ISSUES OF INTERVET'S AAH NEWSLETTER

SYSTEMIC PATHOLOGY OF FISH

A text and atlas of normal tissues in teleosts and their responses in disease. Edited by HW Ferguson. Scotian Press 2006. 2nd edition

BY DR SCOTT PEDDIE. PATERSON PEDDIE CONSULTING LTD/AQUACULTURE HEALTH INTERNATIONAL UK

THE EDITOR OF *SYSTEMIC PATHOLOGY OF FISH*, PROFESSOR HUGH FERGUSON



HIGH QUALITY PHOTOGRAPHS ARE A MAJOR FEATURE OF THIS BOOK

The first edition of Systemic Pathology of Fish was published in 1989 to much acclaim. Nearly two decades on, the much anticipated revised and expanded second edition has finally reached the bookshops.

Edited by Professor Hugh Ferguson of the University of Stirling, the contributor's list reads like a "Who's who" in the fish pathology world. With a combined professional experience of what must equate to several hundred years, the book is certainly not lacking in authorial gravitas.

At just under 400 pages in length, this publication is substantial. It is packed with almost 700 extremely high quality figures or pictures that include light, scanning and electron micrographs. Indeed, it is no exaggeration to say that the quality of the photography throughout is second-to-none. A hardback binding and a relatively large page size further enhance the visual appeal.

The book consists of 14 chapters organised according to organ systems. The last chapter deals with physiological and clinical pathology and is a new, and very welcome, addition to the second edition. A functional and aesthetically pleasing full-page colour photograph separates each chapter.

The introduction sets the scene nicely for the rest of the book by summarising post-mortem techniques and general pathology. The writing style is very fluid and makes for an easy read, without compromising any of the detail required in a textbook format.

Subsequent chapters deal with

- gills and pseudobranchs
- skin
- kidney
- spleen, thymus, reticuloendothelial system, blood
- cardiovascular system
- gastrointestinal tract, swim bladder, pancreas and peritoneum
- liver
- nervous system
- the eye
- endocrine and reproductive system
- neoplasia, and as mentioned previously,
- physiological and clinical pathology.

Each of the above chapters contains a plethora of informative illustrations of both gross and microscopic pathology. The diagrams in the last chapter are also worthy of particular note, given their "user friendly" and informative nature and their ability to dovetail with the text (which incidentally represents one of the best overviews on the subject I've read to date).

Most chapters start out with a description and a graphic illustration of normal organ morphology and function. Thereafter, specific abnormalities are discussed in depth and richly illustrated.

The chapter on the kidneys, written by Renate Reimschuessel and Hugh Ferguson, is a case in point. It opens with an illustrated section on general anatomy, and then looks in some depth at the response of the nephron and interstitium to disease, and closes with a discussion on the effect of parasitic infection on kidney function and pathology.

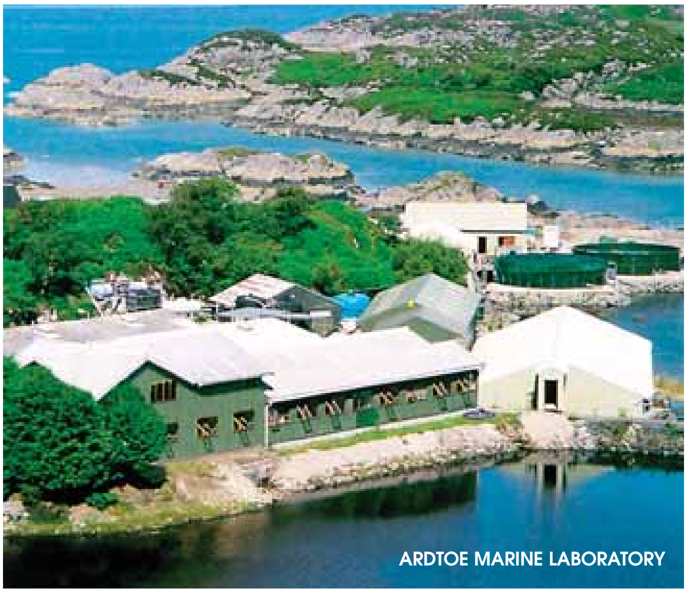
Hugh Ferguson's book will appeal to a wide range of researchers, diagnosticians and students. It is readily accessible to those familiar with fish biology, but not necessarily experienced in pathology. That said, it will also be an invaluable reference tool for pathologists and diagnosticians more used to working with other species.

All in all, this book is an excellent, highly informative and well-balanced addition to the somewhat limited range of fish pathology textbooks currently available. It is one of those rare publications that can only be described as a "must have" for any serious fish health professional.

For sample pages, and ordering information, see www.scotianpress.com

FISH HEALTH INVESTIGATIONS AT ARDTOE MARINE LABORATORY, SCOTLAND

BY DR JIM TREASURER



ARDTOE MARINE LABORATORY

LARVAL REARING TANKS

KTP ASSOCIATE YOLANDA CORIPIO STUDIES THE DEVELOPMENT OF THE HADDOCK IMMUNE SYSTEM

Although ownership of Ardtoe has changed recently and the company trades as Ardtoe Marine Laboratory, research on fish health continues. The site was well known as the Marine Farming Unit, with a remit to develop methods for cultivating marine finfish following its inception in 1965 by the White Fish Authority, later the Seafish Industry Authority.

Work was initially on plaice in an extensive lagoon system, but later on turbot. From 1983 Ardtoe pioneered the culture of halibut in the United Kingdom, and this was followed by wrasse culture and then demonstration projects with Marks and Spencer and other partners on cod from 1998 and haddock from 2001.

The site currently has a wide range of fish broodstocks, including cod, haddock, halibut, dover sole, turbot, whiting, pollock, saithe and wrasse.

FISH HEALTH

Survival of marine larvae in culture has been low, for various reasons, and much of the research at Ardtoe has focussed on fish health and welfare issues.

Vibrio anguillarum is recognised as the main threat to cod and haddock farming. Work at Ardtoe, in association with KTP fellow Yolanda Corripio of Aberdeen University, has looked at the immune system of gadoids and the development of suitable vaccines.

The optimal route to challenge haddock was investigated with maximum effect by immersion. Five vaccination regimes were used and efficacy tested by bacterial challenge. There was a significant decrease in mortalities compared with unvaccinated controls. As in previous cod studies, vaccination of haddock did not induce a specific antibody response.

DISINFECTANT STUDIES

Larval rearing systems have a low exchange rate to reduce the loss of live feed from the system and this, together with the use of live feed such as rotifers and Artemia, can introduce a range of bacterial species, including Vibrios.

Disinfection of eggs and reducing the risk of transferring bacteria to the rearing tanks has been examined using short-contact immersion in Pyceze (50 percent w/v bronopol) (Figure 4), commonly used in salmonid rearing.

Static immersion in the rearing incubators for 30 minutes and exposure to 50mg l^{-1} bronopol also gave improved survival compared with controls and significantly reduced bacterial numbers.

Pyceze was also compared with ultraviolet in the disinfection of live feed by addition during the enrichment process of live feed. A

FIGURE 4: SURVIVAL OF COD EGGS FOLLOWING CONTACT DISINFECTION WITH BRONOPOL FOR 45 SECONDS

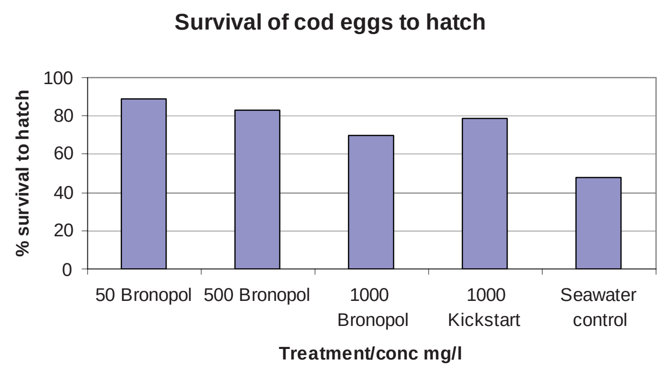
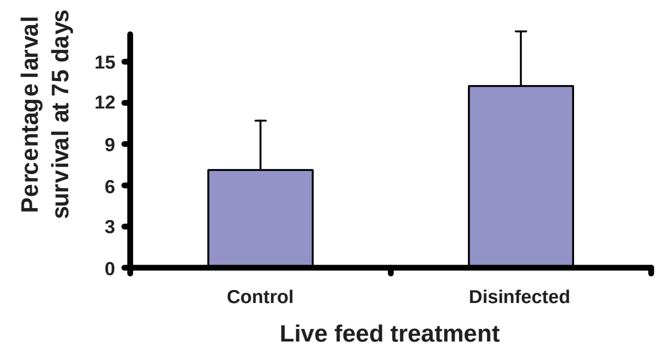


FIGURE 5: SURVIVAL OF COD LARVAE TO HATCH IN THREE TANKS SUPPLIED WITH LIVE FEED TREATED WITH PYCEZE, COMPARED WITH THREE REARING UNITS WITH UNDISINFECTED FEED



suitable concentration and contact time to disinfect live feed during the enrichment process was established as 30mg l^{-1} active Pyceze for six and 12 hours. This was tested on a commercial scale in six 1300 litre larval rearing tanks with higher survival in larvae fed disinfected feed, with an average of 13 percent from egg to weaning in three experimental tanks, compared with seven percent in control tanks. (Figure 5)

Following this work, Pyceze is now routinely used for disinfecting live feed in many marine hatcheries worldwide, and work continues in looking at other applications of Pyceze in marine hatcheries.

COD AND HADDOCK WELFARE

As cod and haddock are naturally cannibals, large losses and fin damage can occur in hatcheries if grading practices are not rigorous. A research student from Glasgow University, Heather Forbes, is working at Ardtoe, and has focussed on how the size of prey items can affect the development of the jaw apparatus, with large prey items inducing larger gape size, encouraging the development of cannibalism. Results will be used to recommend feeding and management practices that reduce cannibalism.

As various health issues of gadoids are encountered, investigation continues on subjects such as the control of atypical furunculosis to eye problems, the latter especially in halibut. Aggression can cause damage or eye loss, and cataracts may occur associated with diet and UV exposure, and these are also being investigated at Ardtoe.

DIET RESEARCH

Nucleotides are considered as semi-essential nutrients during food deficiency, rapid growth and immunological stress. In a study by Jose Vecino in association with SAMS, broodstock diets were enhanced with nucleotides in halibut and haddock. This was found to improve fecundity, egg quality and fertilisation rates in both species. The effect of diet enriched with nucleotides was also studied in cod larvae with Yolanda Corripio.

As the cost of marine ingredients and the availability of fish oil and meal will be potentially restrictive to the expansion of aquaculture, Ardtoe has been carrying on trials with IFFO and various feed companies on the effect of partial full-fat soya substitution on cod performance, and also looking at the anti-nutritional effects on cod growth and potential effects on the gut lining.

While the liver has not been affected, there have been minor effects such as a moderate inflammatory cell presence in the posterior gut, although these have been found to be mild compared with the effects of vegetable diets in salmonids. It also appears that after an initial induction period of several weeks, cod and haddock adapt to the addition of vegetable protein in the diet.

ONGOING WORK

Project work with ongrowing partners continues on the growth and health of cod and haddock in sea cages, including the potential importance of ectoparasites. Recent support from Seafish and a salmon company has primed a rearing programme for certified disease-free wrasse to stock with farmed salmon to control sea lice.

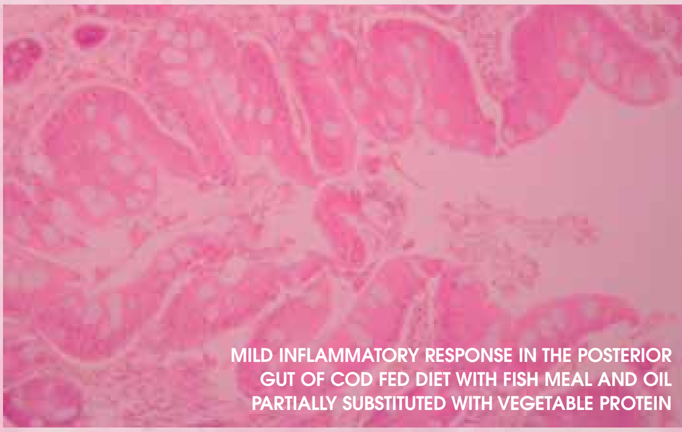
While cleaner fish have been used previously in salmon farming, there have been concerns in using wild-sourced fish, and the trend to organic production has awakened interest in the use of wrasse in sea lice control.

While Ardtoe increases the production of cod juveniles for diversification of marine finfish culture and to cover the core costs of operating the site, further work will be on key health issues affecting the health and welfare of marine finfish species. Dedicated trial facilities and an on-site residential accommodation unit have just been completed to encourage students and visiting researchers to enjoy the benefits of working in the relaxing environment of the spectacular Ardnamurchan Peninsula.

For further information, visit www.ardtoemarine.co.uk ■


A COD LARVA WITH INGESTED SIBLING (PHOTO COURTESY OF HEATHER FORBES)


HUSBANDRY IMPROVEMENTS ARE TACKLING EYE DAMAGE AND CATARACTS IN HALIBUT


MILD INFLAMMATORY RESPONSE IN THE POSTERIOR GUT OF COD FED DIET WITH FISH MEAL AND OIL PARTIALLY SUBSTITUTED WITH VEGETABLE PROTEIN

DIAGNOSTIC LABORATORY SERIES

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

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

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

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

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

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

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

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

FEATURED DIAGNOSTIC LABORATORY: Veterinary Diagnostic Service (VDS), Institute of Aquaculture, Stirling University, Scotland

BY PROFESSOR HUGH FERGUSON

The Veterinary Diagnostic Service (VDS) is located within the Institute of Aquaculture at Stirling University, Scotland. It was established in 1972 and is committed to providing a dedicated and leading role in the support of global aquaculture, fisheries and aquatic animal health in general.

We work with individuals, companies and organisations to develop and provide total health management, ensuring high standards of veterinary care and attention.

Staff at the VDS Stirling work mainly in a consultancy and referral basis on samples sent by veterinarians, fish farmers and companies (feed and pharmaceutical industries), and through other research organisations, to develop and provide advice and guidance on health management of research, farmed, wild and companion aquatic animals.

Definitions of new syndromes and pathogen identification continue to be the strengths of the VDS Stirling. Many new diseases have been identified over the years, and these have formed the basis for a large number of MSc and PhD research projects within the institute. Professor Hugh Ferguson, who joined the faculty in 1998 to head the diagnostic services, introduced a rapid 24-hour turnaround in disease diagnosis, much faster than most mammalian diagnostic facilities!

This rapid histopathological service includes provision of management strategies to improve the health status of a wide range of fish species, and is fully supported by active and experienced technical staff. Examples of some recent local diagnostic "firsts" include VHS in farmed trout in the United Kingdom, nodaviral infections in farmed cod and sole, and reoviral-like hepatitis in farmed halibut.

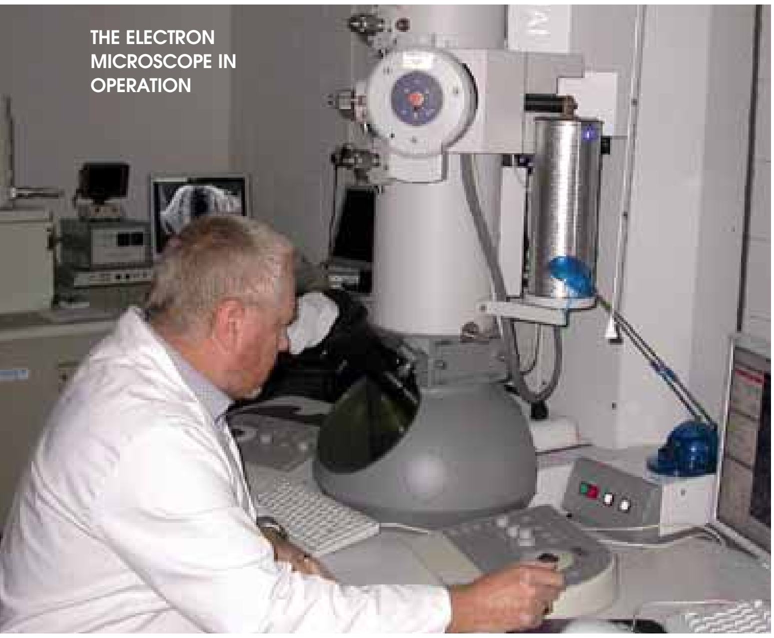

SAMPLING A SALMON IN THE PATHOLOGY LAB

The Veterinary Diagnostic Service has access to laboratory capabilities covering a wide range of disciplines. The service also has access to all the other expertise, information and facilities within the Institute of Aquaculture. Currently the VDS has two clinical veterinary officers, with Dr Ferguson as director and chief pathologist. The service is supported, either directly or through arranged consultation, by the Institute of Aquaculture staff, which includes five additional professors, 14 associate professionals, 30 technicians and 25 research fellows.

SERVICES PROVIDED

VDS at Stirling operates on a cost-recovery basis, and has supplied a diagnostic and referral service for decades, evolving over time to provide rapid diagnosis of aquatic animal diseases caused by a wide variety of pathogens affecting a diverse range of farmed aquatic animals. The full spectrum of diagnostic, research and consulting services is available, including:

- Histopathology
- Bacteriology
- Virology


THE ELECTRON MICROSCOPE IN OPERATION


ONE OF STIRLING'S WELL-EQUIPPED LABORATORIES

- Advanced molecular techniques
- Electron microscopy
- Parasitology
- Serology
- Immuno-histochemistry
- Health certification
- Veterinary investigations
- Health screening
- Specific pathogen testing
- Site visits
- Feed analysis
- Water chemistry
- Pathology referral service
- Expert reports
- Training
- Contract research, and
- Market research

For example, bacteriology workups include isolation and identification of fish pathogens, morphological, biochemical and molecular identification techniques and assess to the VDS reference bacterial fish pathogen collection.

Similarly, virology testing is done by a dedicated virology unit with cell culture and virus isolation facilities with access to a wide range of cell lines and molecular diagnostic techniques.

Some services are provided to a quality assurance level of good laboratory practices, a fact that vaccine and pharmaceutical companies find very useful for getting their products to market. Although the laboratory is based in the UK, the service provided is global. For example, the first description of a bacterial disease causing mass mortalities in farmed catfish in Vietnam, *Pangasius hypophthalmus,* was reported and the aetiological agent identified as *Edwardsiella ictaluri*. VDS include the following categories of clients:

- **Aquaculture companies**, where the VDS provides veterinary assistance and advice is provided to aquaculture companies through farm visits, regular fish stock screening via parasitology, bacteriology, histology, virology and specific pathogen certification. Integrated health management contracts are presently established in Scotland, England, Ireland and Greece.
- **Nutritional**, pharmaceutical and insurance companies, where veterinary investigation for nutrition and insurance companies is regularly provided, through either retained veterinary services or specific investigations. Expert reports, market research, field trials and contract investigation are provided for pharmaceutical companies.
- **Veterinary surgeons**, where referral work and laboratory service for veterinary surgeons in practice is provided and is also available to aquarists, fish wholesalers and public aquaria.

COLLABORATIONS AND LINKS

Staff in the VDS Stirling not only provide a diagnostic service, but also are also involved in research into new and emerging disease problems in farmed fish species. The VDS also participates, through the institute's Aquaculture Technology Centre, in commercial ▶

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THE STIRLING UNIVERSITY CAMPUS

enterprises on a worldwide basis, by offering a range of contract research, analytical and knowledge-based services that will help companies to innovate, improve efficiency and competitiveness, enhance product quality and ensure sustainability.

The merging of disease diagnosis and research is very strong within the VDS, and has enabled more sophisticated diagnostic techniques to be performed. Application of immuno-diagnostic and molecular tools (including sequencing) has enhanced identification and confirmation of viral and bacterial pathogens of significance within the aquaculture industry.

Combining molecular tools with routine histopathology is a particular aim of the VDS and, together with advanced pathogen identification techniques, these tools have conspired to markedly improve the provision of practical management strategies to alleviate aquatic health problems globally, thereby enabling the VDS Stirling to continue to provide high standards of veterinary care and attention.

The research focuses on identifying the aetiological agent(s), understanding the disease processes and associated influences on disease outbreaks within fish farming systems in the UK and Europe. Information from all research is widely disseminated to the fish farming industry through various media. Research tools are then applied within the diagnostic facility to strengthen pathogen identification and disease diagnosis capabilities to help promote sustainable aquaculture practices.

Research is currently being conducted into cardiomyopathy syndrome of farmed Atlantic salmon, as well as into two new syndromes affecting the rainbow trout industry: red mark syndrome/cold-water strawberry disease, and rainbow trout gastro-enteritis.

Contact Professor Hugh W Ferguson, email h.w.ferguson@stir.ac.uk, Richard Collins (r.o.collins@stir.ac.uk) and Dr Mags Crumlish (mc3@stir.ac.uk), or see www.aquaculture.stir.ac.uk/diagnostic/index.html

PROBIOTIC BACTERIA'S EFFECT ON SHRIMP SURVIVAL

BY MATHIEU CASTEX (IFREMER, NEW CALEDONIA), VINCENT USACHE AND SYLVIE ROQUEFEUIL-DEDIEU (LALLEMAND, FRANCE)
ALL PICURES COURTESY OF IFREMER

Shrimp production in New Caledonia is rapidly growing, with production aiming at 5000 tonnes per year by 2010. Production is essentially destined for export, mainly to France, Japan, Australia and the United States, and for this reason the sector must keep up with the demand for quality and high added value products, in line with international safety standards.

To attain these objectives, pharmaceutical solutions must be kept to a minimum and natural, scientifically proven preventive solutions must be sought. In this context, the use of probiotics seems a promising strategy, acceptable by consumers, and scientifically backed up to promote shrimp growth and protect them against infections.

This article presents the first results of a joint research project between probiotic specialist Lallemand and Ifremer (French Research Institute for the Exploitation of the Sea) which was initially presented at the WAS Aqua 2006 Conference.

This project shows the benefits of probiotic lactic acid bacteria *P acidilactici* MA 18/5M administration on shrimp zootechnical performance and survival following opportunistic infections, particularly "winter syndrome".

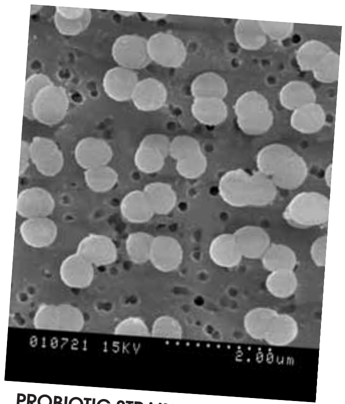
PROBIOTIC CONCEPT

Microorganisms are commonly used in aquaculture to control microbiological ecosystems, especially to treat the water in tanks and ponds. This is known as the bio-remediation concept.

More recently, the addition of live microorganisms through the feed (the probiotic approach) to balance the animals' gut ecosystems and improve digestion and sanitary conditions has been attracting growing interest. The advantage of probiotic over bio-remediation is a direct effect of the micro-organism in the gut, due to its ingestion, as well as a better control of the number of micro-organism delivered to the animal and the environment.

In particular, the probiotic strain *Pediococcus acidilactici* MA 18/5 M (Bactocell®, Lallemand, France), which is a lactic acid bacteria, has been specially selected for its ability to strengthen the gut microflora of monogastric species, with a positive impact on both pathogen control and feed digestibility.

It is characterised by its ability to produce exclusively and rapidly important amounts of lactic acid (homofermentative strain). It is also thought that *P acidilactici* MA 18/5 M prevents pathogens from attaching to the gut surface and colonising it, by competition.



PROBIOTIC STRAIN PEDIOCOCCUS ACIDILACTICI MA 18/5 M

Previous production trials have shown the zootechnical effects of Bactocell on marine shrimp. For example, a trial conducted at the Vung Tau Centre for Shrimp Research and Production, in Viet Nam (2002) showed that continuous Bactocell supplementation in feed of juvenile *Penaeus monodon*:

- improved growth rate by 52 percent after eight weeks
- improved size homogeneity, and
- increased shrimp length by 15 percent.

This effect on growth could be linked to improved feed digestibility.

WINTER SYNDROME

The winter syndrome, or syndrome 93, has had an important impact in New Caledonia, as it has been responsible for very high mortality rates (about 60 percent). As a result, most shrimp farmers have had to stop production during winter, resulting in significant economic losses.

The winter syndrome is caused by *Vibrio penaeicida*. When the water temperature decreases below 22° C, shrimp are subject to physiological perturbations and then become more vulnerable to the pathogen present in the water.

In the trial conducted at Ifremer in Nouméa, *Litopenaeus stylirostris* shrimp reared in tanks received Bactocell in the feed twice daily (two doses were tested: 106 and 107 CFU/g feed*) during the cold season. Mortality was recorded daily (Figure 1). The probiotic treatment significantly improved the shrimps' survival.

FIGURE 1: DAILY MORTALITY AND TEMPERATURE VARIATIONS IN TANKS

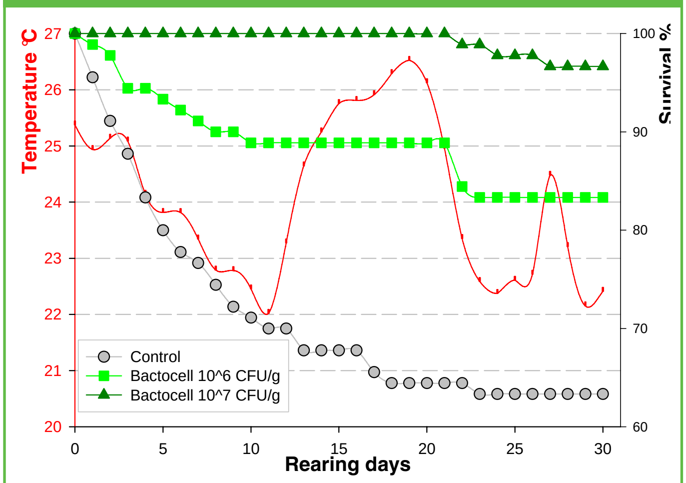
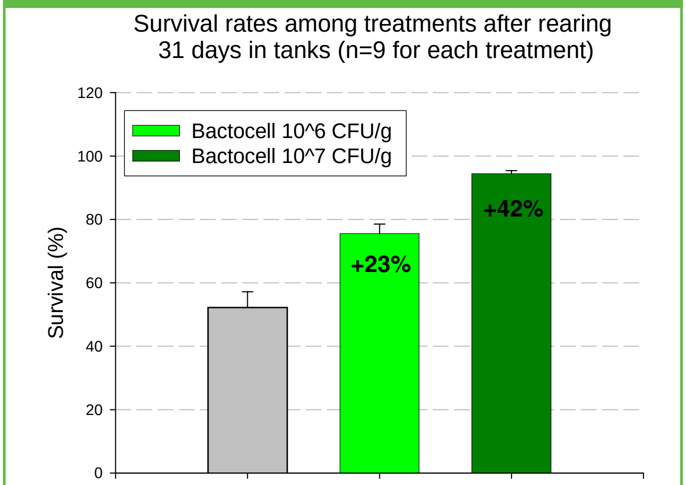


FIGURE 2: OVERALL SURVIVAL AFTER 31 DAYS REARING IN TANKS



THE EXPERIMENTAL TANKS WERE SUBMITTED TO TEMPERATURE VARIATIONS TO INDUCE WINTER SYNDROME IN SHRIMPS

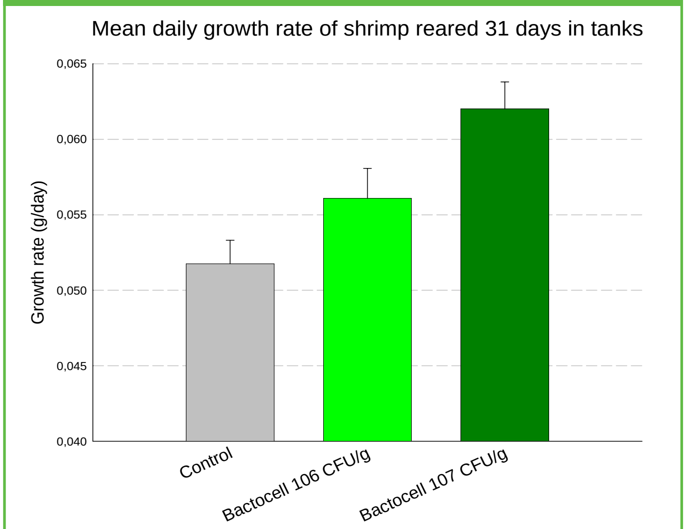
When decreased water temperature lead to nearly 50 percent mortality in control tanks, the survival for probiotic treated shrimps was on average 75 and 95 percent, with 106 and 107 CFU Bactocell/g feed, respectively. There was a significant dose effect. Globally, after 31 days treatment in tanks, the shrimp survival rate was increased by up to 42 percent with the higher dose of Bactocell in the feed.

GUT MICROBIOLOGICAL STATUS

Further microbiological analysis of the shrimps' gut was performed in order to estimate the effect of the probiotic on the bacterial gut microflora. The total number of bacteria and the total vibrio population were assessed in the gut of shrimps from the control group, and the group receiving the higher dose of Bactocell (106 and 107 CFU/g feed), at day 16 and 30.

It appeared that both the gut total bacteria and the vibrios populations were significantly inhibited with the probiotic treatment. These results indicated that Bactocell could improve shrimps' survival against pathogenic infection by acting on the gut microflora, in particular by reducing the number of vibrio pathogens in the gut.

FIGURE 3: SHRIMP GROWTH RATE IN TANKS IS IMPROVED WITH DAILY ADMINISTRATION OF BACTOCELL



Several mechanisms have been put forward to explain these beneficial effects of probiotic, although on-going studies are aimed at further elucidating the mechanisms involved in the case of Bactocell in shrimps. These include:

• the competition or exclusive competition for space at the gut

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COARSE FISHERIES AND FISH HEALTH IN ENGLAND

BY BERNICE BREWSTER (AQUATIC CONSULTANCY SERVICES, UK).

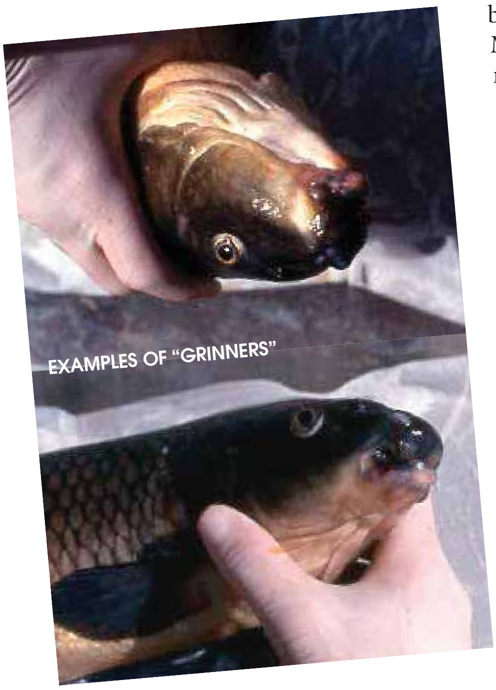
More people in the United Kingdom enjoy the pastime of angling than any other sporting activity. The tradition dates back many centuries as evidenced by Izaac Walton's treatise on British fishes in 1653. Freshwater angling is administered by the Environment Agency, a government agency responsible for the licensing of the sport, and sales of rod licences exceed 1.2 million annually. Licences are also sold for game fish, but these are not included in this figure. Coarse fishing currently attracts the greater interest.

In the middle of the last century, most coarse fishing was largely confined to rivers or canals, with a limited interest in the lakes or still waters. Carp (*Cyprinus carpio*) were and still are revered for their wily nature and difficulty to catch. The 1950s saw an upsurge in fishing still waters for carp, with the legendary angler Richard Walker setting the record with a specimen later named Clarissa, that weighed almost 20kg (44lb) in 1952.

A CARP SUFFERING FROM ERYTHRODERMATITIS

Subsequent to the publicity surrounding Clarissa's capture, the passion for carp fishing in the UK has increased year on year. These days it is probably the still waters that attract more attention to anglers than the abundance of beautiful rivers in this country. An increasing number of lakes are being excavated, and as agriculture and farming continues to suffer from poor returns, more farmers are diversifying by creating angling lakes.

It is worth bearing in mind that although there is some discrepancy as to the actual date when carp arrived in this country, they are not native. After the initial importation of carp they were indeed on the extreme of their range, being a native to the Mediterranean region, naturally occurring in slow-flowing rivers or lakes where the water temperature is a minimum of 18° C.



EXAMPLES OF "GRINNERS"

If indeed the favoured date for introduction is the mid-16th century, the climatic conditions were unfavourable for this species and perhaps it is surprising that it managed to survive. None-the-less, survive it did, although growth and reproduction would have been severely limited.

In the early 1950s, when Clarissa was caught, such a large carp would indeed have been unusual for this country because the weather conditions were not ideally suited for rapid growth.

Richard Walker's capture of Clarissa was the spark which ignited the passion for carp in the UK, with this species increasingly attracting more attention. In the latter part of the last century, a "double figure" (ie a specimen weighing more than 4.5kg or 10lb) carp and possibly a "twenty" (9kg) was regarded as a good catch.

Regrettably the trend to want to catch ever-larger carp continued, which resulted in the illegal importation of large, specimen carp from continental Europe, sometimes accompanied by outbreaks of the notifiable disease spring viraemia of carp virus, or SVC, into which these imports were stocked.

> Many carp are known as "grinners", where repeated catching has damaged their jaws and tissues, resulting in seriously deformed mouths

The laudable work of the Centre for Fisheries and Aquaculture Science, which managed to catch and prosecute many illegal carp importers, was later supported by the formation of the English Carp Heritage Organisation, which has achieved an excellent following, and the trade of illegal fish is slowing. Certainly, UK farms produce large numbers of carp for the angling market annually.

However, the popularity of carp is not waning and seems to go from strength to strength. As a result, this species has been stocked into almost every still water in the country. Certainly some 48 percent of my annual business is examining carp for movement consent.

Coupled with the desire to catch specimen fish is the onset of a society which demands instant gratification, and angling is no exception. As a result, many fisheries are heavily stocked, predominately with carp, but also bream (*Abramis brama*), roach (*Rutilus rutilus*), rudd (*Rutilus erythrophthalmus*) and smaller numbers of other species.

The numbers of fish stocked into fishing lakes that may be either commercial ventures or even those administered by angling clubs and societies can exceed 2000kg fish weight per hectare. Such intensive stocking of fish impacts on the aquatic environment, with little in the way of submergent or emergent plant life.

Fish stocks quickly destroy the submergent plants, which can never thrive because of the discolouration of the water. The knock-on effect is that there is little in the way of natural food for these fish, which become entirely reliant on anglers' bait for nutrition.

There are two issues with regard to this matter. Firstly, most angling takes place in the warmer summer months, but once temperatures cool, the numbers of fishermen and therefore sufficient food for the autumn and early winter when the fish are still feeding diminishes. Secondly, while anglers are keen to use baits with attractants and stimulants that draw the fish to the bait, there must be a question over the nutritional completeness of such a diet and its shelf life.

HEALTH IMPLICATIONS

Given that carp are the preferred species in these heavily stocked waters, the fish are subject to tremendous angling pressure. Many carp are known as "grinners", where repeated catching has damaged their jaws and tissues, resulting in seriously deformed mouths, and often you have to wonder how on earth they manage to feed.

Clearly such damaged fish show poor growth due to the restraints on feeding, which perhaps makes them ever more likely to be caught as the angler's bait becomes an easier meal. Over a period of years those that are the most severely deformed probably simply lose condition and die.

It is also common to find carp suffering from carp erythrodermatitis on heavily stocked or fished waters. These ulcers are often found on almost every fish that is caught.

Treatment is impossible because the underlying stressors of poor nutrition and repeat catching cannot be removed. These of course are just the physical manifestations of a heavily stocked fishery, and the stocks suffer from the same physiological effects of an intensive culture system.

The difference of course is that in intensive culture the fish are culled at a certain size, usually for the table. It is also worth remembering that intensive stocking of fish and its associated stress provides the best background for the emergence of disease.

Carp are traditionally regarded as being strong fish, possibly because they appear to tolerate handling, and they are certainly able to survive in polluted water and low oxygen. But they easily succumb to a range of diseases, some of which, such as spring mortality of carp syndrome, are of unknown aetiology. Perhaps they do not tolerate handling and poor conditions as readily as we assume.

PROBIOTIC BACTERIA'S EFFECT ON SHRIMP SURVIVAL

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surface, the production of molecules inhibiting the growth of pathogens, such as bacteriocins or antibiotics, or
- creating a hostile environment, such as low pH (eg lactic acid production).

Finally, the new trial also showed a significant improvement of shrimp growth performances, with a dose effect. In tanks, growth rate at 31 days was improved by 10 percent and 16.5 percent respectively (Figure 3). These results confirmed previous studies done in commercial rearing conditions, and demonstrated the economic relevance of using probiotics in shrimp production.

INDIVIDUAL SHRIMP MARKING ALLOWS INDIVIDUAL WEIGHT MONITORING FOR MAXIMUM STATISTICAL RELEVANCE

PROBIOTIC PATTERN

As seen before, the modes of action of probiotics such as Bactocell is linked to their activity, implying that in order to exert their effects, probiotics must remain alive until they reach the gut. For aquaculture applications, in particular, this means that the microorganisms must survive the stressful conditions of pellet feed processing (particularly harsh conditions in case of shrimp feed, with exposure to high temperature and pressure), feed storage, the aquatic environment and the digestive process.

For these reasons, extensive studies were conducted to determine the optimal way of incorporating Bactocell into shrimp feed to ensure its survival up to the animal gut and to guarantee the expected count of live cells. The method finally selected was a post-pelleting incorporation, in fish oil.

Bactocell showed good stability during the process, and the feed could be stored for up to 15 days at 25° C. The probiotic survival was still satisfying after 48 hours immersion in marine water. Finally, Bactocell resisted well the digestive process as shown by live bacterial count in the shrimps' digestive tract. The experiment also confirmed that Bactocell did not colonise the animals' gut. It is for this reason that it has been administered daily, with each meal, to ensure consistent effects.

CONCLUSION

Lactic acid bacteria is a natural solution to improve shrimp performance and to ensure producers receive a good return on investment. Most importantly, it represents a good approach to controlling gut microflora, keeping pathogens at bay and improving survival in conditions of stress.

**CFU: Colony forming units. The measuring unit used to count live microorganisms. Only microorganisms that are able to multiply on a Petri dish (and form a colony) are taken into account.*

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For further information, please contact Dr Scott Peddie via e-mail at s.peddie@pattersonpeddie.com, or by telephone +44 (0) 28 93351379

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