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PARASITIC DISEASES OF TILAPIA

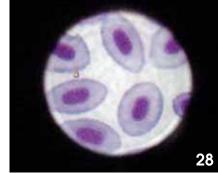
FOCUSING IN ON THE AQUANOR FORUM

ADVANCES IN SAPROLEGNIA TREATMENT

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Sporangia (main photo) and signs of clinical symptoms in *Saprolegnia*



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ENHANCED STAKEHOLDER Participation is key to success

SCOTT PEDDIE, EDITORIAL DIRECTOR

Those of you who read my editorials on a regular basis will by now have perceived that I have an interest in fish welfare and communication. Scientists can at times be rather poor at communicating the importance of their work to a wider audience; in many ways this is engrained at an early stage in a scientific career given that all our training is focused on communicating with other scientists via peer-reviewed journals and the like. Likewise, scientific conferences can be dry affairs where it almost seems to be the case that the more obscure the topic and the more convoluted the presentation, then the more academic gravitas the topic exudes (I am of course exaggerating for effect here, but hyperbole is sometimes a useful rhetorical tool!).

So, it is against such a backdrop that it is refreshing to publish an account of the AquaNor forum Welfare as a Driver for Technological Development in Aquaculture by Alistair Lane, Kjell-Inge Reitan and Kari Attramadal in this issue of *Aquaculture Health International*. Held at the AquaNor conference in Trondheim, Norway on August 15 and 16, the forum was a clear success judging by attendance and participant feedback. The organisers came up with the "forum" concept after recognising that too many workshops lack interaction and stakeholder/participant input. This is indeed true, but there are numerous models from outwith the 'aquaculture' research community that attest to the effectiveness of such an approach. For example, in a previous role I had as an animal health economist, I took part in a sheep welfare forum involving large numbers of participants from a wide range of stakeholder groups that included scientists, veterinarians, non-governmental organisations, policy-makers, retailers, farmers

and consumers. From my perspective, the event, and indeed the concept, was a success because we managed to integrate scientific knowledge with farmer experience and consumer perception to develop a consensus on the way (or at least a way) forward for the industry. Using some pretty sophisticated statistical techniques (which are normally



used by market researchers ... but then that's another story ... I won't bore you with the details here!) we came up with some interesting conclusions and points for further investigation.

It seems to me that at times scientists are restrained by their training and are often reluctant to look "outside the box" and borrow innovative concepts and techniques from other disciplines, especially those that deal primarily with qualitative data. Involving all stakeholders in the fish welfare debate, which is after all a perfect topic for such treatment, seems to me to be an eminently sensible way of enhancing fish welfare in aquaculture. I therefore look forward to the next AquaNor forum event, in addition to publishing the remaining three articles in the AquaNor Forum report series in this magazine.

A FINAL NOTE

Our subscribers may note that this issue of *Aquaculture Health International* is reaching you later than planned. Issue 11 has been delayed by a month as a result of the editorial director being ill. We apologise for any inconvenience this delay has caused.

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NUTRIZYMES IDEAL NUTRACEUTICALS IN AQUAFEED: **POTENTIAL AND LIMITATIONS**

BY AMIT KUMAR SINHA¹, KARTIK BARUAH¹, DIPESH DEBNATH² AND AK PAL²

This article is reproduced (with permission) from Aquaculture Europe magazine and has been modified.

The increasing cost and low availability of fish meal, the major component (protein source) of aquafeed, is directing researchers as well as feed manufacturers to search for cheaper alternative sources of protein.

Plant ingredients are found to be rich in protein content and also other nutrients. The quality of aquafeed however, not only depends on the nutrient composition and nutrient balance, but also on the effective utilisation of nutrients by the animal. Fish/shellfish fed plant-based diets show lower nutrient digestibility, due to the high level of carbohydrate and various antinutritional factors (ANFs).

Moreover, the metabolic activity of fish/shellfish during the juvenile stage is quite high, and consequently requires a nutrient dense (mainly high protein) diet in order to optimise survival and growth. However, the digestive system is not well developed enough to digest the nutrient-dense diet. Improper digestion and malabsorption of nutrients often have far-reaching effects that include reduced growth, impaired immunity, allergic reaction and poor wound healing.

Nutrizymes are viable solutions for these problems. Nutrizymes are nutraceuticals from exogenous enzymes that increase the digestibility of feed ingredients, thereby reducing nutrient excretion into the environment and ultimately enhancing growth. They facilitate a considerable saving in production costs and infrastructure, and offer nutritional consistency and off-the-shelf convenience.

Nutrizymes provide additional commanding tools that can inactivate ANFs and enhance the nutritional value of a plant-based diet. They also provide a natural way to transform complex feed components into absorbable nutrients.

This article covers the various nutrizymes that have been or can be used in aquafeed to increase nutrient digestibility with a view to developing eco-friendly and low-cost feed, in addition to improving the health status of cultured aquatic organisms.

DIGESTIVE FLUIDS AND ENZYMES

The breakdown of ingested food into its constituent parts requires the presence of a number of different digestive fluids and enzymes. In fish with a stomach, hydrochloric acid is secreted to reduce the gut pH and convert the inactive pepsinogen into active pepsin, which breaks down the protein component of the diet.

Other enzymes secreted by the stomach work on lipids and carbohydrates. These include gastric lipase, amylase and gelatinase. Chitinase specifically breaks down chitin, the main component of crustacean and molluscan shells. In contrast, in agastric fishes where there is no true stomach, neither hydrochloric acid nor pepsin is formed in the gut.

The pancreas secretes a large number of digestive enzymes which



are stored in inactive forms, known as zymogens, to prevent selfdigestion. Activation results from the presence of food in the gut, which causes the intestine to produce proteases. These convert trypsinogen into trypsin, which in turn activates other enzymes specific to protein, lipid, carbohydrate and chitin breakdown. Several enzymes are secreted by the intestine itself and aid in the final breakdown of food into molecules small enough to be absorbed across the intestinal wall and into the bloodstream.

ENZYME CRISIS

During ontogenesis of fish larvae, certain important changes occur in the digestive tract. At hatching, the digestive tract is a straight tube closed at the mouth and histologically undifferentiated along its length. It remains quite unchanged until the completion of yolk absorption, and then becomes segmented into a buccopharynx, foregut, midgut and hindgut. The larval period ends with the development of a stomach with gastric glands and pyloric caeca.

The liver and the pancreas are formed at hatching and at first feeding; they are functional although the digestive system is not fully functional. In Asian seabass, *Lates calcarifer* and in most marine fish larvae, prior to the formation of the pyloric sphincter, the intestinorectal valve is the only constriction along the digestive tube before the anal opening.

At such stage, digestion of ingested food (in the absence of a stomach) mainly takes place in the larval intestine, where the pH remains alkaline, and trypsin-like enzyme activity accounts for the proteolytic activity. Pancreatic and intestinal enzyme activities were generally low at first feeding. In some species, trypsin, aminopeptidases and alkaline phosphatase have not been observed, while in other species such as gilthead sea bream, *Sparus aurata* and Japanese flounder, *Paralichthys species*, phosphatase, trypsin and ATPase activities have been reported three to four days after hatching, whereas aminopeptidases are present before hatching (Kurokawa and Suzuki 1998).

In most fish species, pepsin-like enzyme activity is not found prior to the formation of the gastric glands. However, unsynchronised development of gastric glands and the presence of pepsin in sea bass and coregonid larvae have been reported.

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EXOGENOUS PROTEASES

Although fish larvae have sufficient levels of digestive enzymes to digest live food organisms at first feeding, there may not be enough for the digestion of microparticulate diet. The microparticulate diets are rich in nutrients (mainly protein) as well as other antinutritional factors that larvae find hard to digest. Binders used for microbound diets as well as proteins and synthetic polymers used for crossbinding with encapsulation methods etc. have been found to be difficult to digest by fish larvae. Moreover, microparticulate diet contains 60 percent to 90 percent dry matter, compared with only 10 percent in zooplankton. This may lead to insufficient digestibility, as it is much harder to digest dry, hard particles than live organisms.

Dietary supplementation of proteolytic enzymes can overcome this problem. Common carp fed diet supplemented with bovine trypsin showed increased proteolytic activity, and the increase in the activity was correlated with the bovine trypsin proportion (Dabrowski and Glogowski 1977). Likewise, dietary incorporation of porcine pancreatic extract (pancreatin) significantly improved the feed assimilation and growth rate of gilthead sea bream larvae by 30 percent and 200 percent respectively (Kolkovski *et al* 1993).

EXOGENOUS CARBOHYDRASES

Dietary protein can be replaced by carbohydrate or lipid to minimise the production cost and nitrogen effluent. Although lipid constitutes an important non-protein energy source for fish, it is more expensive compared with carbohydrate. Carbohydrate-rich ingredients, apart from being the most economical source of energy, are also abundantly available throughout the world. However, the systems for carbohydrate digestion in many fish are ill-developed.

Reduced growth and feed efficiency in many fish fed carbohydrate-rich diets have already been documented. However, dietary modification of carbohydrate may improve its utilisation in fish. Chemical modification of starch done by adding exogenous enzyme, α -amylase (Kumar *et al* 2006) showed that low-cost raw ingredients or even less processed materials can be used with equal and even better performance than more expensive materials, thereby increasing the wide choice and flexibility for the ingredients by the feed manufacturer.

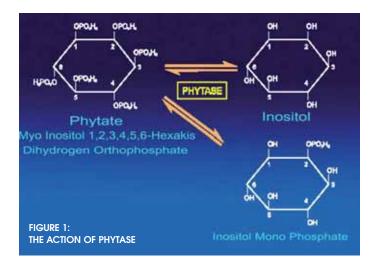
The positive effect of adding exogenous carbohydrases to aquafeed in enhancing the utilisation of unavailable dietary carbohydrate by many fish like *Salmo salar*, *Sparus aurata*, *Labeo rohita*, *Catla catla* and *Penaeus monodon* has already been shown. However, the utilisation efficiency varies among different species, due to different endogenous carbohydrases activity. Dietary carbohydrases have a more promising manoeuvre in carp because of their herbivore and omnivore feeding habit than carnivorous fishes.

EXOGENOUS LIPASE

Several types of lipases have been recognised in the digestive tract of juvenile and adult fish. Among them, non-specific bile salt-activated lipase (BAL) activity has been found to play an important role in digestion of neutral lipids in some fish species such as anchovies, striped bass, pink salmon, leopard sharks, rainbow trout, cod and red sea bream.

Exogenous BAL, purified from the pyloric caeca of cod, when incorporated into aquafeed along with sodium cholate and sodium taurocholate, showed very reflective response in lipid utilisation. This type of enzyme hydrolyses the carboxyl ester bonds, not only of acyl-glycerols, but also of minor dietary fats, including cholesterol esters and vitamin esters.

There is also evidence for the presence of a mammalian-type mammalian pancreatic lipase in several species, for instance rainbow trout. This enzyme is specific for triacylglycerides. Phospholipase A2 (PLA2) activity is also found in several fish species. It functions by hydrolysing the fatty acid ester bond at the sn-2 position of phosphoacylglycerides, and produces free fatty



acids and lysophospholipids. Improved feed conversion efficiency and growth was observed in broilers fed diets supplemented with PLA2. However, there are only very limited reports detailing the use of PLA2 in aquafeed.

EXOGENOUS CELLULASE

The majority of cultured species are incapable of digesting cellulose and starch. Most fish species, with the exception of herbivores like grass carp, are deficient in cellulase enzymes. As a result they cannot utilise plant-based diets efficiently. The administration of exogenous cellulase is very well reported in ruminants.

Dietary addition of this enzyme has a marked effect on increasing the total microbial population in the rumen and increased microbial protein synthesis. It also enhances digestibility as a result of the increase in total microbial population, or stimulation of its activity. Considering the increasing trend of intensive fish culture and the high cost of a fish meal-based diet, this method may provide a promising alternative.

EXOGENOUS CHITINASE

Chitin is a structural polysaccharide. Chitinase hydrolyses chitin into oligosaccharides. It is present in the digestive system of many fish species, regardless of their dietary habits. The primary function of chitinase enzymes is still debatable. They are primarily associated with the stomach, where they disrupt exoskeletons, allowing other digestive enzymes access to nutrient-rich inner tissues. Chitinases in the intestine may aid in removing fragment blockage. Demersal marine fish are very rich in chitinase and can therefore serve as a potential source of exogenous chitinase.

PHYTIC ACID AND PHYTASE

Phytic acid is one of the most powerful ANFs in plant ingredients. It chelates various nutrients like phosphorus, calcium, magnesium, iron and zinc, protein, amino acids and even lipids. Most fish do not possess phytase enzymes to break down the phytate, and thus excrete the bound nutrients as such into the environment, causing eutrophication.

Dietary phytase hydrolyses the phytate (Figure 1) and releases the various bound nutrients, paving the way for their maximum utilisation and less reduction in faecal matter (Baruah *et al* 2007). So far, the positive effect of this enzyme on nutrient digestibility and growth has been observed in various fish (Baruah *et al* 2007).

ADVANTAGES OF PHYTASE

The inclusion of inorganic phosphorus, an expensive nutrient, can be drastically reduced.

The release of phytase-phosphorus into the environment can be reduced by making the bound phosphorus available to the fish for growth.

Phytase added to the diet improves protein and amino acid digestibility in fishes. It improves the metabolisable energy of feeds by breaking down the phytate-lipid complex.

Cheaper, plant-based protein sources can be substituted for fish meal, thus lowering feed costs.

SOURCES OF ENZYMES

a) Microbial sources

Fungi are an ideal source for various enzymes, although enzymes from other micro-organism-like bacteria have also been reported. The specific strain of fungus used for enzyme production has to be extensively screened to determine if the organism is capable of producing mycotoxins under the conditions of fermentation. Only those organisms that do not produce any toxins are selected for use in the fermentation process.

Even after an organism is determined to be "safe" and is used in fermentation, every second generation is again checked to verify that mutations have not occurred that might enable the organism to produce mycotoxins.

Enzyme derived from *Aspergillus* fermentation was first used in food production. It is important to use only fungal enzymes derived from the fermentation of non-toxigenic strains of *Aspergillus niger* and *Aspergillus oryzae*. The food and pharmaceutical industries have studied these organisms extensively to establish their safe use in the production of amino acids, enzymes, antibiotics and other beneficial compounds. These enzymes, which are produced by fermentation process, have been successfully used in poultry and pig diets, but they have not been much used in commercial scale in aquafeed. Therefore, more research needs to be carried out in different species to optimise their doses to use on a commercial scale.

b) Plant sources

Plants are a good source of enzymes, and include bromelain from pineapples, papain from papaya, nanokinase from soy fermentation and others. They are protein-digesting enzymes common in commercial and industrial use. Bromelain is utilised in the meat industry as a meat tenderiser, and papain is used in the tanning industry to soften leather. There are reports on the use of papain in the feed of few fish. Besides, phytase has also been reported in rice, wheat, maize, soybeans, corn seeds and other legumes or oil seeds. The use of enzymes from plant sources in fish is still a great challenge for nutritionists.

c) Animal sources

Of the enzymes being used industrially, over half are from fungi and yeast, and over a third are from bacteria, with the remainder divided between animal (eight percent) and plant (four percent) sources. Microbes are preferred to plants and animals as sources of enzymes because of low price, easy availability and purity. Some of the enzymes of animal sources used industrially are catalase, chymotrypsin, lipase and trypsin. Although some enzymes isolated and purified from animals have been used in aquafeed, as mentioned above, to see their effect on growth performance, their use commercially has yet to take place.

MICROBIAL ENZYMES VERSUS ANIMAL ENZYMES

There are many advantages of using exogenous microbial enzymes rather than animal-derived enzymes. Fermented by microorganisms, microbial enzymes can be specially selected on the basis of each enzyme's unique characteristics. They exhibit broad ranges of pH, temperature and substrate specificities. They are chosen on their ability to work within the gastrointestinal system of the organisms.

Unlike animal origin enzymes, microbial enzymes work at the pH found in the upper stomach. Food sits in the upper portion of the stomach for as long as an hour before gastric secretions begin to act. Studies have shown that microbial enzymes are active in the pH

range of three to nine, and can facilitate the utilisation of a much larger amount of protein, carbohydrate and fat before hydrochloride is secreted in sufficient amounts to neutralise their activity.

In contrast, animal enzymes are destroyed by the low pH within the stomach unless they are enterically coated. Furthermore, animalbased enzymes function only at the narrow pH ranges found at specific anatomical sites.

LIMITATIONS

Despite the aforementioned utility of enzymes in aquafeed, there are various drawbacks. These include:

- Enzymes are thermolabile and are denatured when exposed to high temperature. It therefore restricts their application on extruded pellets.
- Each enzyme has its own optimal pH range for utmost activity, which is difficult to monitor inside the fish's body.
- Enzymes are substrate specific. A single enzyme cannot function for all nutrients.
- Screening the mixture of enzymes to be integrated in a single feed is a complicated task.
- There is a possibility that the target animal may not respond to exogenous enzyme.

CONCLUSION AND FUTURE RESEARCH

In essence, feeding a diet fortified with enzymes offers the possibility of enhancing nutrient digestibility and can facilitate a better growth rate, although more research is required in this area. Many of the concepts borrowed from animal nutrition may have applications in fish nutrition also.

Some suggestions for further research in this area are summarised below:

- The species specificity of enzymes should be tested for different fish/shellfish.
- The optimal enzyme dose should be standardised for gastric and agastric species.
- The interaction of different enzymes (rather than individual enzymes) should be investigated/tested in aquafeed.
- Greater numbers of enzymes from diverse sources should be identified for potential use in aquafeed.
- The negative effect of dietary enzymes should be thoroughly studied.

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PISCICULTURA RURAL: UNA EXPERIENCIA DE DESARROLLO EN LA AMAZONÍA BOLIVIANA

EDITED BY ELISA CANAL 2007. CENTRO DE ESTUDIOS AMAZÓNICOS (CEAM), SANTA CRUZ DE LA SIERRA, BOLIVIA. PP303 REVIEWED BY DR DAVID A CONROY

This extremely well written and welldocumented publication is divided into four principal chapters, or sections, to which eight different authors have contributed. The final book, printed and published in Spanish, has been carefully edited by Dr Elisa Canal of the CEAM. Its contents provide a detailed and interesting insight into the development and growth of rural and semiintensive fish farming as a productive activity in various localities in the Amazonian region of Bolivia.

The contributors have made an in-depth evaluation of the data accumulated over the years that has been generated by several departmental, national and international technical assistance programmes and projects with respect to the possibilities of productive fish farming in the region. The information is presented as a factual analysis of the situation,

and provides details ranging from the construction of ponds, the application *in situ* of induced spawning and larval rearing techniques, feeds and feeding (including the types of raw materials and pelleted feeds available locally) to the capture, harvesting, processing and transportation of the final products to the markets.

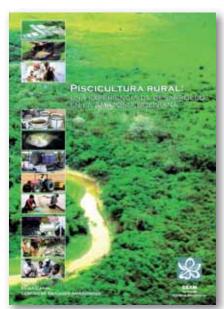
The methodology applied in collecting and evaluating the corresponding information is clearly indicated, as also are aspects of the costs and benefits related to the investments made.

The publication makes a concrete and up-dated contribution to our understanding of the current status and future perspectives for fish culture in the Amazonian region of Bolivia and, in that respect, serves to adequately address a number of gaps in our knowledge of the realities of the situation.

As may be expected, particular attention has been paid to native species of characins such as the pacú (*Colossoma macropomum*), the tambaquí (*Piaractus brachypomus*), the sábalo (*Prochilodus nigricans*) and the boga (*Schizodon fasciatus*) which, along with certain other related species, are recognised as having a great potential for fish farming operations in many tropical and subtropical freshwater environments in South America.

The contributors to this book have provided a wealth of original information which may come as quite a surprise to many readers. Two typical examples of this are:

- the current ready availability in local markets of fresh farmed pacú and tambaquí is now replacing other farmed species such as carp and tilapia, which were formerly more popular, and
- photographs of the list of prices of fresh products available on a fish market in Santa Cruz, and of prices of the dishes available on the menu of a restaurant in Cochabamba, are simply fascinating. This information includes the following items:
- 1. Prices per kilo in a fish market in Santa Cruz (prices quoted in



Bolivian bolivianos: (US\$1 = Bol Bs 7.64, I euro = Bs 11.12)

surubí (*Pseudoplatystoma fasciatum*, a South American pimelodid catfish): Bs 35

pacú 35

pejerrey (Odontesthes (Basilichthys) bonariensis *) 35

dorado (Salminus sp) 25

piranha** 20

tucunaré (Cichla ocellaris) 20

rainbow trout (*Oncorhynchus mykiss****): 30 sábalo - heads and bony parts available for making soup: no price tag given.

* originally introduced into Lake Titicaca and elsewhere, currently the object of commercial fishing activities.

** very much wild-caught rather than farmed. After heading and gutting, the piranha makes a very tasty dish when fried as fillets or boiled in a soup (personal comment by this

reviewer!).

*** introduced into Lake Titicaca and its environs in the Andes, and currently the object of commercial capture and of culture in cages and in land-based race ways.

2. Prices per serving as listed on the menu of a restaurant in Cochabamba:

surubí (grilled)	Bs 30
surubí (nuggets)	29
pacú (grilled)	30
pacú (nuggets)	29
rainbow trout (grilled)	30
rainbow trout (nuggets)	29
carp (according to size)	35 to 45
chicken (nuggets)	20

The editor and the contributors to this publication are to be complimented on their dedication to the fine detail in their various presentations. The resulting document has made available an impressive amount of information which has been generally lacking in the public domain.

This book and its conclusions will serve as a valuable impetus to the development of farming of native Amazonian fish species, not only within Bolivia but also in other South American countries that share the Amazon Basin and its watershed.

For further information, contact ceam@ceam-ong.org or hoyam. mojos@gmail.com

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THE HUNT FOR AN ALTERNATIVE TREATMENT FOR SAPROLEGNIASIS

RAMA FALK¹, SIMON TINMAN², NIR FROYMAN² AND ITZHACK POLACHECK¹



FISH PONDS IN NORTHERN ISRAEL (COURTESY OF DAVID YANZEN)

Saprolegnia parasitica and other members of the genus Saprolegnia cause saprolegniasis, one of the most economically damaging fish diseases. Saprolegniasis causes losses of millions of dollars annually worldwide, particularly in the salmon and trout markets.

However, it affects almost every freshwater fish, including a number of other teleosts, especially those in aquaculture such as tilapia, carp, red drum, striped bass, rainbow trout, elver, barramundi and mullet (Bruno and Wood 1994).

Saprolegnia exhibits many fungal characteristics, but is not a true fungi, and it is classified in the kingdom protista. Like true fungi, it feeds on decaying matter and grows as branching filaments with non-septate hyphae. However, its cell wall is composed mainly of cellulosic compounds as is the case in plants, and not chitin, as found in true fungi (Torto-Alalibo 2005). Furthermore, it has several defined developmental stages that are lacking in true fungi, displayed by a complex life cycle. Its asexual reproduction includes production of nonseptate hyphae, which delimit to form sporangia. The sporangia then releases motile primary zoospores that are the main infecting agent. This process is repeated several times.

The secondary spores can settle on the fish skin and germinate to hyphae, penetrating the skin and causing tissue destruction and loss of epithelial integrity. If untreated, *Saprolegnia* leads to death by haemodilution and osmoregulation imbalance. *Saprolegnia spp* can also infect fish eggs by adhering and penetrating the egg membrane (Dick 1990).

SAPROLEGNIA CONTROL

Until recently, *Saprolegnia* infections were successfully treated and controlled by malachite green, the most effective fungicide agent

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THE RESULTS OF A HEAVY SAPROLEGNIASIS INFECTION, ALSO KNOWN AS WINTER-KILL SYNDROME, IN A TILAPIA POND IN A FARM IN THE BEIT-SHEAN VALLEY

for saprolegniasis. However, its use has been prohibited in many countries, including Israel, due to cumulative evidence relating to its carcinogenic, mutagenic and teratogenic properties.

As yet there is no alternative treatment for this infection that is suitable for the diverse needs of aquaculture, and the result is continued significant economic losses. Consequently, outbreaks of saprolegniasis have become common in Israel, particularly among farmed tilapia during the winter, when pond water temperature falls below 15°C.

Concomitant with a low water temperature are husbandry practices that result in the fish being exposed to the harsh conditions of handling and crowding (up to 80 tonnes per hectare in tilapia farms) in winter storage ponds. Consequently, there is an urgent need to find an alternative compound to malachite green.

This compound should be highly water-soluble, safe to both living organisms and the environment, biodegradable and financially sustainable. For this purpose collaboration between the Central Fish Health Laboratory and the Laboratory of Medical Mycology was established. The research project was fully supported by the chief scientist of the Ministry of Agriculture and Rural Development (MOARD).

This research is directed by Professor Itzhack Polacheck, the head of the Laboratory of Medical Mycology in the Department of Clinical Microbiology and Infectious Diseases in the Hadassah-Hebrew University Medical Centre, Jerusalem. Professor Polacheck has wide-ranging experience in a variety of different areas, including biochemistry, diagnosis, the development of an antimycotic drugs, mutant isolation and analysis, virulence studies, the study of the course of infection in animal models, and the development of an antifungal drug and molecular biology.

Dr Rama Falk, a post-doctoral fellow in this laboratory, is the principal investigator in this project. She is experienced in developing novel drug delivery systems and prodrugs for antifungals. The main role of the Laboratory of Medical Mycology is the diagnosis of





One of the objectives of this study was to identify saprolegniasis of tilapia to species level using molecular tools

pathogenic fungi (both yeasts and moulds).

The laboratory serves the Hadassah hospitals and clinics and also provides services to laboratories and clinicians outside Hadassah. The laboratory works to very high standards and is internationally recognised as a leader in service and research in medical mycology. It uses the most recent novel state-of-the-art molecular methods, supplemented by classical methods in identifying and characterising fungi and antifungal agent susceptibility testing. All such work is carried out according to high international standards and utilises advanced techniques.

Clinical data emerging from the laboratory is constantly scrutinised and evaluated by experts in the field of medical mycology, including clinical microbiologists and clinician specialists in infectious diseases.

The initiator and co-principal investigator of this project is Dr Simon Tinman, a veterinarian specialising in fish disease. Simon has experience with carp, ornamental fish and tilapia in the realms of both research and routine clinical diagnosis and treatment



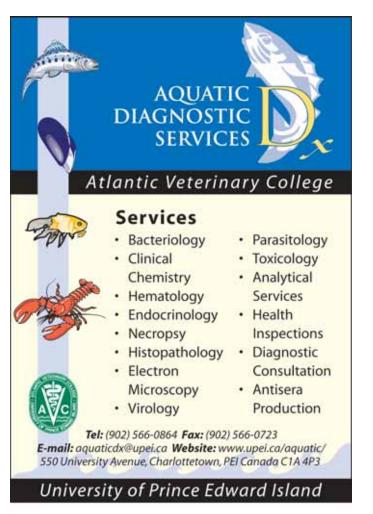
VARIOUS FISH EXHIBITING CLINICAL SYMPTOMS OF SAPROLEGNIASIS



THE HUNT FOR AN ALTERNATIVE TREATMENT TO SAPROLEGNIASIS



ASEXUAL FRUITING BODIES, CALLED SPORANGIA, OF *SAPROLEGNIA PARASITICA* ISOLATED FROM TILAPIA FISH IN THE CENTRAL FISH HEALTH LABORATORY, AND ZOOSPORES RELEASED FROM THE FRUITING BODIES. (X100)



trials of fish diseases. Dr Tinman is part of the Central Fish Health Laboratory, supervised by Nir Froyman, whose expertise is in ornamental fish genetics. The Central Fish Health Laboratory is part of the Department of Fisheries and Aquaculture within the Israeli Ministry of Agriculture.

This governmental facility specialises in aquatic animal health, and provides disease surveillance and diagnostic services, export certification, research and policy advice for edible and ornamental fish growers. The laboratory was founded at 1941, when aquaculture in Israel was initiated, and was responsible for addressing the basic requirements for a functioning fish diseases laboratory, with respect to methods of sampling fish and water, bacteriology, parasitology, pathology and water chemistry.

It is located in Beit-Shean Valley, in the northern part of the Jordan River basin, within a rural area where almost 70 percent of Israeli aquaculture is located. Israel's ornamental industry has expanded significantly in recent years. At present it constitutes eight percent of the imports of freshwater fish into the European Union, and comprises three percent of the world market for ornamental fish. As a consequence, the scope of the laboratory has expanded to include export certification and diagnosis of exotic diseases.

The Central Fish Health Laboratory provides not only specific expertise and experience in parasitic, viral and bacterial diseases of aquatic animals, but also other specialist facilities and skills, including histopathology, molecular biology, immunology, bacteriology, involvement in vaccine development and pathogenicity studies.

The infective agent causing saprolegniasis of tilapia in Israel has never previously been studied, and one of the objectives of this study was to identify it to species level using molecular tools, and to characterise it morphologically, physiologically and also to study it from an ecological point of view. Such knowledge concerning the local isolates of *Saprolegnia* would obviously be of significant value in developing disease control strategies.

During our research, a screening assay system was developed for evaluating various compounds as alternative treatments to saprolegniasis. This includes *in-vitro* susceptibility and fish toxicity testing, environmental impact assessment and evaluation of therapeutic effectiveness in *Saprolegnia* infection in a unique fish model.

We have found that the use of fluorescent whitening agents can prevent and treat saprolegniasis on fish and fish eggs without adversely affecting organisms in the environment such as algae and zooplankton. The new treatment product was found to be very effective and safe to use in tilapia and other fish. We believe that this product has the potential to herald a new era in *Saprolegnia parasitica* control. These results are protected by a patent, and in addition will be published soon in a comprehensive scientific article including all of our results.

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PARASITIC DISEASES OF TILAPIA

BY CEDRIC KOMAR AND NEIL WENDOVER (INTERVET NORBIO SINGAPORE)

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n the last three issues of the Intervet Aquatic Animal Health Newsletter we presented a series of articles on tilapia diseases: a general introduction of tilapia diseases was in Newsletter 11, followed by an article on Streptococcosis (Newsletter 12) and an overview of *Columnaris* disease (Newsletter 13). The severity of parasitic infections in tilapia is increasing with the intensification of culture systems. Therefore, the focus of this present article will be parasitic diseases of tilapia and possible control measures. The full article, including epidemiology and much more, can be found on our website http://aqua.intervet.com

PARASITE SCREENING AND ETIOLOGY OF MAJOR PARASITES AFFECTING TILAPIA

Understanding the etiology of parasitic disease is of crucial importance as it determines the choice of a potential treatment. Unlike bacterial or viral diseases for which it is necessary to identify the micro-organism to the species level before implementing an adapted vaccination strategy, the identification of parasites to the genus level is generally sufficient to implement an effective therapeutic or prophylactic strategy. Therefore, the only elements needed for an effective parasite diagnosis at the farm level are a

ILLUSTRATION OF MAJOR PARASITES, ASSOCIATED CLINICAL SIGNS AND EPIDEMIOLOGY AND POSSIBLE TREATMENTS

	PARASITE	PICTURE	DISEASE SYMPTOMS	EPIDEMIOLOGY/ RISK FACTORS	POSSIBLE TREATMENTS
CILIATES	<i>Trichodina</i> spp. (<i>Trichodina</i> in gill wet mount)	40µm	Erratic swimming Opened operculum Scraping against walls Jumping out of water Erosion of fins, skin ulcers Gill hyperplasia	Massive mortality in hatchery and nursery phases	Salt bath Formalin bath H₂O₂ bath KMnO₄ bath
CILI	Ichthyophthirius multifilis (Ich on skin wet mount)	200µm	Appearance of white spots on skin Thick mucus on skin	Most severe in larval stages Problem in recirculation system	Repeated formalin bath Increased salinity
DINOFLAGELLATES	<i>Amyloodinium</i> spp. (Trophont in gill filament)	100µm	Decreased appetite Flashing Accumulation of mucus	Brackish water 10-15ppt Does not occur in freshwater	H ₂ O ₂ bath
TREMATODES	Monogenean <i>Dactyolgyrus</i> spp (Adult on skin wet mount)	125µm	Skin darkening, fin erosion, excessive mucus, Rapid movement of operculum emaciation in young fish	Juvenile and fingerling stage	Formalin bath H_2O_2 bath
	Digenena (<i>Clinostomum</i> spp) (Metacercariae from wet mount of muscle)	500µm	Grubs (yellow or white) on the skin. Skin hemorrhage and death if mass penetration of the parasite	Occur in pond farming when snails and birds are present	Remove or eradicate snails from the pond Prevent birds access to the farm
CRUSTACEANS COPEPODS	Argulus spp. (Adult from fish skin)	Imm	Skin irritation Loss of condition Associated secondary skin bacterial infection	Severe in larval stages and fingerlings	Organophosphate
CRUSTACEAN	<i>Lernea</i> spp. (Adult from fish skin)	3mm	Rub against sides of container. Whitish spots of curled up worms embedded in the skin.	Can affect mouth breeding	Organophosphate
HIRUDIDAE	Leeches (Adults on skin of fish)	lcm	High number of leeches on an adult fish induce anemia	Severe in early stages Affect fish already weakened by another disease	Organophosphate

light microscope and a basic knowledge of parasite taxonomy, size and morphology.

Parasite diagnosis should start by an external gross observation of the fish to check for the presence of larger parasites such as leeches and crustacean copepods. In addition, an infestation with a digenean trematode might be suspected when yellow or white grubs are seen on the skin. Then, parasite screening should be continued by observation of a skin and gill scrape by light microscopy at x40 and x100 magnification. At these magnifications, the typical shape and movements of protozoan ciliates such as *Trichodina* and *Ichthyophthirius multifiliis*, the presence of inert and opaque *Amyloodinium* trophonts, or the presence of monogenean parasites can be observed. Similarly, the presence of metacercariae of digenean trematodes can be demonstrated in a skin grub through observation of a fresh smear under light microscopy.

Once the identity of the parasitic is determined, the severity of a parasite infestation is assessed by the number of parasites affecting each fish. Generally, the presence of a single or small number of individual parasites per fish is not an alarming factor. However, the presence of a massive number of parasites on each fish might constitute a real threat to the fish population and should require immediate action.

Therefore, routine parasite screening is an important part of good health management and should be done on a regular basis to allow for rapid action if needed. Parasites affecting tilapia can be classified into several major groups: protozoan ciliates, dinoflagellates, monogenean and digenean trematodes, crustacean copepods and hirudidae.

The following table presents a short description of some of the most serious parasites in tilapia.

EPIDEMIOLOGY AND RISK FACTORS

Parasites are part of the normal environment. There are several factors that influence the prevalence and severity of a parasitic disease. These are biological factors directly related to the tilapia and environmental factors related to the water body and culture system. (This part of the article can be found at http://aqua.intervet.com

PREVENTIVE MEASURES AND POSSIBLE TREATMENT

We often say that prevention is better than cure for viral and bacterial diseases. This saying is also true when it comes to parasites. As these organisms are part of the normal fish environment, avoidance is impossible and we have to learn how to live with them. The nature of parasites may vary depending on farming systems and country. Therefore, routine parasite screening of healthy and sick fish is a major component of the prevention strategy in order to understand which parasites must be watched out for in a given farm.

Indeed, conducting weekly surveys over one entire year should give a complete overview of the parasitic fauna present in the farm and demonstrate variations in the incidence and prevalence due to seasonality, salinity and temperature. Once parasitic fauna have clearly been identified, it is important to adapt some of the farming practices based on the biology of parasites present in the farm.

For instance, it is common practice for tilapia hatcheries to keep water salinity between five and 10ppt in order to control the population of protozoan ciliates such as *Trichodina*. In extensive earthen pond systems, the biological control of snails and birds is the most effective strategy to break the cycle of digenean trematodes and avoid their presence in ponds.

Depending on the nature of parasites present in the farm and on chemicals legally authorised in the farming country, there are several potential chemicals that can be applied by bath, such as salt, formalin, H_2O_2 and $KMnO_4$. However, the dose and duration of treatment must be adapted to several parameters:

• *The age and physiological condition of the fish.* Juveniles are more sensitive than adults to chemical treatment and can't

tolerate a similar concentration of chemicals.

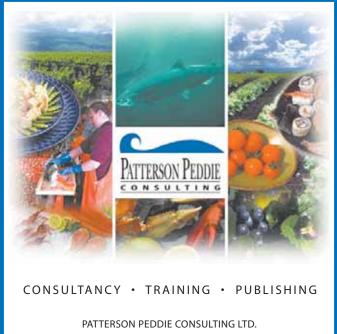
- *The availability of pure oxygen or aeration during the treatment.* During some of the chemical treatments, such as a formalin bath, the concentration of dissolved oxygen present in the water is reduced. Consequently, it is necessary to compensate that by artificial water oxygenation.
- *The type of culture system.* A highly concentrated treatment can be implemented by using a tarpaulin system on a cage, but this becomes impractical and not economical when applied to a large body of water, such as a pond. In such systems, lower doses of chemical for a longer duration of immersion should be tested.

Therefore, each treatment must be evaluated on a small scale for safety and efficacy before implementation at the farm level. In addition, it should be remembered that the application of any chemical treatment may have unwanted side-effects. For instance, salt water is an effective treatment for protozoan ciliates; however, pumping seawater without filtration into the farm can result in the concomitant introduction of unwanted bacterial pathogens. Moreover, formalin, another possible chemical treatment for protozoan ciliates, should be applied with care in ponds, as it might induce plankton die-offs.

Finally, simple health management measures should be implemented as much as possible to reduce the introduction and spread of parasites at the farm level. Indeed, before introducing new fish in a farm or during transfer from one unit to another, fish should receive a prophylactic parasite treatment.

Moreover, the farming equipment should be kept separated between different units of the farm to avoid horizontal spreading of parasites. After each cycle, fish-holding systems should be properly cleaned and disinfected to limit the build-up of parasites from one cycle to the next.

Ultimately, as stressed fish are more susceptible to parasites, it should be the golden rule for every farmer to keep stress levels as low as possible for fish, especially during and after any event or procedure that causes stress, such as transportation, handling, a change in the season and/or a change in water quality.



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kiotechagil

INTERNATIONAL Kiotech and Agil rebrand

Following Kiotech International's acquisition of Agil at the end of 2006, the company has rebranded to become Kiotechagil. This reflects the bringing together of the expertise in both companies across the three key meat protein sources of fish, poultry and pigs. The agricultural experts of Agil were working closely with Kiotech's aquaculture team, allowing more funds to be directed into product development and providing a clear rationale for the new Kiotechagil name, said the chief executive, Richard Edwards.

"Our aim is for Kiotechagil to become recognised as the leading player in supplying high-performance natural feed additives which enhance health, growth and sustainability in aquaculture and agriculture," Edwards said. "Our drive to build closer ties with leading research organisations should also lead to an enhanced product portfolio."

To help achieve this goal, Kiotechagil has strengthened its management team by recruiting Ian Cockshott as technical sales manager and David Bullen as business development director.

"With our new Kiotechagil brand we now have a clear focus on becoming a leading supplier of natural feed additives to the aquaculture and agriculture industries," said Edwards. "In addition, with a strengthened management team we can concentrate on developing new products for our customers and opening up in new territories in addition to the 50 countries we currently serve."

2007 Blue Book now available





In 2003, the Fish Health Section of the Asian Fisheries Society released a new digital version of the FHS Blue Book. In that version were many new diagnostic chapters, hundreds of colour pictures, videos and a new inspection section written jointly with the United States Fish and Wildlife Service.

The latest version of the FHS Blue Book is ready for distribution and sale. It contains an updated Fish Health Inspection Manual co-authored with the

USFWS, many new diagnostic chapters, and (for the first time) QA/QC guidelines for fish disease laboratories.

The new diagnostic chapters include seven shellfish disease chapters written by east and west coast experts. These are all upto-date and lavishly illustrated. The new QA/QC document, the result of a multi-year, multi-agency international collaboration between Patricia Barbash, Marilyn Blair, Dave Groman, Hui-Min Hsu and Linda Vannest, provides detailed guidelines for fish health laboratories.

Those with subscriptions to the Blue Book will receive their new copies very soon. Those wishing to buy the Blue Book CD can follow the Bookstore links on the AFS website (http://www. fisheries.org) or see www.afsbooks.org.

Schering-Plough completes acquisition of Organon BioSciences

The Schering-Plough Corporation announced on November 19 that it had completed the acquisition of Organon BioSciences NV, creating a stronger combined company with broader human and animal health portfolios, an enhanced pipeline and increased research and development capabilities. The agreement was announced on March 12. Schering-Plough acquired Organon BioSciences from Akzo Nobel NV for approximately 11 billion euros in cash.

"By bringing together complementary businesses, we will be growing even stronger and even better in our people, products and science," said the chairman and chief executive officer of Schering-Plough Corporation, Fred Hassan. "The promise of this combination is profound. We will be working hard to realise that promise for our customers, for the patients, for our other stakeholders and for our shareowners."

Organon BioSciences is comprised primarily of Organon, a human health business, and Intervet, an animal health business. It also includes Nobilon, a human vaccine development unit, and Diosynth, a third-party manufacturing unit of Organon.

"Through this combination we create a powerful science and technology platform," Hassan said. "We acquire a robust biologics manufacturing capability that is an excellent match for our earlier-stage biologics projects."

The transaction also made Schering-Plough a global leader in animal health, he said. "This greatly increases the value we will bring to customers. We see this strong combined animal health unit as a key strategic part of our integrated business that will contribute to long-term high performance."

The combination brings together complementary pharmaceutical products and vaccines to help prevent and cure diseases and increase performance. He said Schering-Plough would be able to add additional vaccines for major animal species to its portfolio, and expand on its products such as Nuflor, a broad-spectrum antibiotic for fish, cattle and swine.

Schering-Plough's world headquarters will continue to be in Kenilworth, New Jersey. As previously reported, Boxmeer, the Netherlands, will become the headquarters of the global animal health business.

SCOTLAND

Greater co-operation between wild and farmed fish interests on Skye

Increased co-operation and collaboration between wild fish interests and farmed fish interests of the salmon industry on the Isle of Skye is now assured, following the signing of an Area Management Agreement. It encompasses all of Skye, including its 14 salmon rivers and coastal waters, including the southern isles. The signatories are the fish farming companies Marine Harvest, Pan Fish and Scot Trout, with wild fish interests represented by the Skye District Salmon Fishery Board and the West Highland Fisheries Company Ltd. A tripartite working group established in 1999 to address problems common to farmed and wild salmon, and to ensure the maintenance of healthy stock, was also involved.

The aims of the Skye AMA are to promote and implement measures to maintain healthy stocks of wild and farmed fish in the area, and restore wild salmon and sea trout stocks.

"We are proud to have been closely involved in this AMA initiative from the start," said the production manager for Pan Fish Scotland, Brian Floyd. "We are deeply conscious of the benefits of co-operation with wild fish interests, since we share the same environment and we all profit from ensuring that we maintain and improve it. The rural communities in which we farm benefit greatly from the income generated by angling tourism, as well as from the income which fish-farming brings."

The chairman of the Skye District Salmon Fishery Board, Peter Kinloch, said his board was delighted to have reached an accord on the terms of the AMA. "This is a real milestone in promoting the best in terms of health, welfare and good management of all the salmon in Skye waters, whether they be wild or in fish-farm cages. We all have the same interest at heart, which is healthy fish, and close co-operation between the wild fish and aquaculture sectors can only be an advantage in making improvements."

The agreements cover a range of objectives, including:

- single-year class management and synchronised production/ fallowing cycles
- synchronised lice treatments
- zero ovigerous salmon lice, particularly during the critical wild smolt migration period from February to June
- vaccination of smolts against furunculosis
- preparation of containment and contingency plans to minimise escapes
- adherence to industry codes of practice
- regular monitoring and information exchanges between AMA partners
- preparation of catchment management plans
- adherence to disease control mechanisms in wild fisheries, and
- the possibility of augmenting with biological control using wrasse cleaner fish.

For further information contact Julie Edgar at 0789 987 5151 or email julie@jecommunications.co.uk.

Top award for fish scientist

Professor Chris Secombes of the University of Aberdeen received a top award on September 28 in recognition of his research, which has increased our understanding of the immune system of fish.

The work of the world-leading fish biologist has also played a major role in helping to protect farmed fish against disease.

Professor Secombes received the Royal Society of Edinburgh's highly regarded Alexander Ninian Bruce Prize, which is awarded just once every four years for meritorious research. Just 20 years ago, our knowledge of the immune system of fish was rudimentary. However, work by Professor Secombes has been instrumental in showing that fish have a sophisticated immune system that can be manipulated.

These advances in our understanding have opened up major opportunities in the aquaculture industry for developing and refining vaccines for farmed fish.

Professor Secombes, who has published 220 peer-reviewed papers and is a Fellow of the Royal Society of Edinburgh, was the first to describe the actions of the molecules in fish that coordinate their immune responses, and he also discovered novel peptides which act like a natural antibiotic in fish.

The Royal Society of Edinburgh made the award for the professor's "outstanding contribution to our understanding of the immune system of fish, particularly salmonids."

"It is a great honour to be nominated for what is the third Alexander Ninian Bruce Prize presented by the Royal Society of Edinburgh," said Professor Secombes, who is head of the university's School of Biological Sciences, and also of the Scottish Fish Immunology Research Centre. "Fish farming is of major economic significance to Scotland, and furthering our understanding of the immune systems of fish so that we can develop and refine vaccines to protect them is of huge importance to the industry," he said. "So it is tremendous that research to help support this multi-million pound sector is being recognised in this way."

IRELAND

Pharmaq's MS 222 receives licence

MS 222, the only fully licensed fish anaesthetic compound in the EU, has received a marketing authorisation in Ireland.

The product, containing 100 percent active tricaine

methanesulphonate, is available by prescription in Ireland. It is indicated for use in fish, covering both the ornamental and food fish sectors.

Dr Lydia Brown, who is responsible for sales in the United Kingdom and Ireland, said that this registration in Pharmaq's name was a milestone in the history of the product, which stretches back to the original development by Sandoz and its initial UK launch in 1975 by Joe Joseph of Thomson and Joseph.

As a water-soluble product packaged in a number of pack sizes, it can benefit all sectors of the aquatic industry, from tranquilisation of ornamental fish during transport, to full anaesthesia for surgical procedures, as well as anaesthesia of food fish at vaccination and stripping of brood fish.

Pharmaq AS is a global pharmaceutical company with a primary focus on aquaculture. The company says it is the world's leading manufacturer of vaccines to farmed fish. Its vaccines are manufactured at Overhalla in mid-Norway, while its administration, research and development is in Oslo.

For further information, contact your veterinarian or email Dr Ben North, ben.north@pharmaq.no

UNITED KINGDOM Red vent syndrome in UK salmon

Since early June 2007 there have been reports from across Scotland of wild Atlantic salmon (*Salmo salar*) returning with inflamed vents. These symptoms have not been reported in farmed Atlantic salmon or sea trout, and seem to be restricted to wild Atlantic salmon populations.

Fisheries Research Services are investigating the cause and extent of the problem, and have produced an information leaflet on red vent syndrome in wild Atlantic salmon (*Salmo salar*) available for downloading from the FRS website: www. marlab.ac.uk. The leaflet provides detailed information on the investigations and images of external signs of the condition.

Anyone concerned about this issue, or who finds fish with these symptoms, should contact the Fish Health Inspectorate at FRS Marine Laboratory, phone 01224 876 544 and ask for the duty inspector, or email fishhealth@marlab.ac.uk

Soil Association meets organic deadline

The Soil Association says it has met its target of eliminating the use of synthetic antioxidants in its certified organic fish feeds. Only natural antioxidants are now permitted in these feeds and their ingredients.

This is the first time such a requirement has been placed on any sector of the fish farming industry. The association said all those involved in producing Soil Association-certified organic fish feeds and their ingredients had successfully switched to natural products.

Organic foods and animal feeds aim to avoid all synthetic ingredients, but while the terrestrial organic feed industry had already replaced synthetic antioxidants with natural alternatives, it had been more of a challenge to protect the unique, highly unsaturated fats found in fish meal and fish oil from spoilage by oxidation.

After establishing the availability of potential natural products, the association had set a deadline of July 1, 2007 for all antioxidants used in organic fish feeds and their raw materials to be of natural origin. It had met this target after working with fish meal and oil manufacturers, feed mills and other companies.

The Soil Association's aquaculture programme manager, Peter Bridson, said the project had required a lot of testing. "By using fish meal and oil made from the recycled filleting wastes of fish already caught for human consumption, we already have the most sustainable feeds in the industry, and it is great to know that we can also protect their unique omega-3 fatty acids with natural antioxidants."

UNITED STATES

Contingency funding for controlling viral haemorrhagic septicemia

The United States Department of Agriculture's Animal and Plant Health Inspection Service, known as APHIS, is making US\$1.5 million in contingency funds available for activities to control viral haemorrhagic septicemia. These activities, which include confirmatory testing, surveillance and compliance, and education and outreach efforts, will help prevent VHS from spreading into aquaculture facilities.

VHS is a destructive pathogen that causes internal haemorrhaging and death in a wide range of fish species. Dead and diseased wild fish have been reported in the St Lawrence River and in Lakes Erie, Huron, Michigan, Ontario and St Clair. Outbreaks of VHS have also been reported in inland lakes in Michigan, New York and Wisconsin.

The disease does not pose a risk to people, but it has been found to affect many different species of fish, including several commercially farm-raised species in the United States previously not known to be susceptible to the disease.

The contingency funding that APHIS is providing will be used for surveillance and compliance activities and other VHS-related efforts. These include laboratory upgrades to USDA's National Veterinary Services Laboratories to support confirmatory testing, and an educational campaign that promotes biosecurity efforts and addresses human-related activities which, though they are not easily regulated, could spread the disease.

Surveillance activities will be risk-based and focus on states in the Great Lakes watershed and surrounding watersheds. APHIS will develop co-operative agreements with state departments of natural resources, state departments of agriculture, tribal agencies and other appropriate agencies for surveillance and compliance efforts. Surveillance data collected in the coming months will give APHIS more information on VHS to better target future surveillance and regulatory actions.

On October 24, 2006, APHIS issued a federal order to immediately prohibit the importation of 37 susceptible species of live fish into the United States from Ontario and Quebec, Canada, the two provinces that have reported VHS outbreaks. The order also prohibited the interstate movement of the same fish species from Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania and Wisconsin, which have reported incidences of VHS in wild fish or that are at immediate risk of acquiring the disease because they share watershed areas with states in which the disease has been detected.

APHIS amended the order on November 14, 2006 to allow for the movement and importation of susceptible fish species under conditions that mitigate the risk of spreading VHS, and on May 4, 2007, to allow for catch-and-release fishing activities. Restrictions under the federal order will continue until APHIS publishes an interim rule establishing appropriate VHS-related fish importation and interstate movement criteria.

For additional information, please contact P Gary Egrie at (301) 734 0695 or email Paul.G.Egrie@aphis.usda.gov

CANADA

Approved therapeutants for aquaculture use amended Source: AquaVetMed Distribution List

Amendment No. 11 of the Fish Products Standards and Methods Manual has been issued. The amendment modifies Appendix 1, Approved Therapeutants for Aquaculture Use. Information on emamectin benzoate has been updated. The updated Appendix 1 is available at:

http://www.inspection.gc.ca/english/anima/fispoi/ manman/samnem/app1e.shtml

Replaced material may be found in the archive at: http://www.inspection.gc.ca/english/anima/fispoi/ manman/samnem/archive/amemod11e.shtml

A pdf version of the updated policy is available at: http://www.inspection.gc.ca/english/anima/fispoi/ manman/samnem/app1e.pdf

The "Approved Therapeutants for Aquaculture Use appendix provides information on the authorised use of drugs and pesticides in the aquaculture of fish and crustaceans.

A drug used in aquaculture must be:

- approved by Health Canada specifically for use in fish or crustaceans, or
- authorised as an emergency drug release, or EDR, by Health Canada when the drug has not been approved in Canada (ie, the drug has not been assigned a drug identification number by Health Canada)
- authorised for testing purposes under an experimental studies certificate issued by Health Canada
- approved as an investigational new drug submission by Health Canada for clinical trials, or
- prescribed by a licensed veterinarian for "off-label" use (only applies to products with an assigned drug identification number).

Health Canada's Veterinary Drugs Programme is responsible for establishing the maximum residue limits (MRLs), administrative maximum residue limits (AMRLs) or residue limits for these drugs. MRLs are published in Table III of Division 15 of the Food and Drugs Regulations. Administrative MRLs or residue limits are established as policy by the Veterinary Drugs Programme of Health Canada. If levels of drug residues in excess of these limits are found in fish intended for human consumption, the fish will be considered "unwholesome", according to Section 6.(1)(a) of the Fish Inspection Regulations.

Dosages and withdrawal times for veterinary drugs must be followed as indicated in the veterinary prescription or, in those cases where a prescription is not required, in the Compendium of Medicating Ingredient brochures published and maintained by the CFIA.

When an antiparasitic product is orally administered to fish (via feed or another mechanism), it is deemed to be a drug and is regulated by the Food and Drugs Act and Regulations.

When the same antiparasitic is applied externally to fish (not ingested), it is deemed to be a pesticide and is regulated by the Pest Control Products Act. The Pest Management Regulatory Agency within Health Canada approves or grants emergency release permits for pesticides under this act.

The Veterinary Drugs Programme of Health Canada has approved, or temporarily authorised as an EDR, the use in aquaculture of the following veterinary drug products. (See updated policy for additional details):

- Terramycin-Aqua
- Romet
- Tribrissen
- Aqua Life TMS
- Aquaflor
- Formalin-R
- Parasite-S
- Perox-Aid
- Calicide

NOTE: Emamectin benzoate (SLICE[®]) is only authorised by Health Canada on a case-by-case basis under the EDR programme. When used under Canada's EDR programme or a process providing equivalent controls, the residue limit for emamectin benzoate is $0.042 \ \mu$ g/g. In all other instances, as part of an interim risk management strategy, the CFIA will review the presence of emamectin residues in fish on a case-by-case basis.

Provincial government moves on aquaculture infrastructure

New aquaculture infrastructure on Newfoundland and Labrador's southern coast will ensure increased expansion of the aquaculture industry and enable it to become more competitive in attracting international investment, says the Minister of Fisheries and Aquaculture, the Hon. Tom Rideout.

"A new aquatic veterinary diagnostic facility to be located in St Alban's, and new wastewater treatment infrastructure for processing plants in the area are milestones that will strengthen the industry's capacity to realise its vast potential, as well as generate new economic and employment growth in rural areas," said Rideout.

Funding for both projects was provided in the 2007 Budget of C\$1.2 million toward the estimated \$4.3 million cost of the aquatic veterinary diagnostic facility, and \$1.9 million for wastewater treatment equipment for processing facilities in the southern coast region. This increased the department's funding for aquaculture to over \$14 million.

"Through our strategic investments, the salmonid industry on the southern coast is seeing additional and substantial investment from companies both within and outside the province," said Rideout. "The new investments for infrastructure will make the industry even more competitive with other aquaculture-producing regions and help ensure the industry's long-term sustainability, as well as that of rural areas where aquaculture can grow and thrive."

Following the announcement of the funding for the diagnostic facility the provincial government had engaged a consultant to assist with site selection, planning and design work. Construction is expected to be completed in 2008.

"This new facility will enable the government ensure that farmed seafood products in this province are kept in the healthiest state possible," said Rideout. "This is a critical part of protecting the environment and ensuring the best margins for companies that choose to come here and set up aquaculture operations."

The diagnostic facility will be the regional office for the provincial government's aquaculture staff, and will house approximately 10 staff including development, aquaculture health and inspection personnel. The Department of Fisheries and Aquaculture is increasing its fish health capability and is hiring staff to work at the new facility.

Some diagnostic samples are currently sent out of the province for tests, but the new facility will include laboratories to enable rapid diagnostic testing of growing fish, and enable its staff to develop and use innovative diagnostic techniques that were currently not an option, he said.

It will also help the government to protect the aquaculture resource through effective disease management, while enabling producers to operate in an environmentally sustainable manner.

"The new facility will meet federal and ISO international standards," said the minister. "It will be a model for aquatic diagnostics, effluent control and energy conservation. The result of maintaining such high laboratory standards is that validated, accurate diagnostic results will be achieved."

There will also be opportunities for visiting researchers and students to use the facility to complete work that will maintain the province's lead in aquaculture-related research and development.



Rideout said equipment for the new wastewater treatment plant would be installed over the next six months. Funding for wastewater treatment systems would be provided to processing plants at Harbour Breton, Gaultois and Hermitage, all of which are located in the salmonid culture zone. These systems will allow all wastewater from fish processing activities to be thoroughly disinfected before being discharged to ensure that it is free of any substance that can harm aquaculture species.

"This new infrastructure will give our province the opportunity to position itself among aquaculture-producing regions that have the highest level of bio-security, fish health monitoring and surveillance, and will ensure the long-term sustainability of our industry," said Rideout. "Our investments are putting the Newfoundland and Labrador aquaculture industry in a more competitive position relative to other salmon growing areas in working to attract investment from across the globe."

The value of the aquaculture industry increased 60 percent from C\$33.5 million in 2005 to \$53.5 million in 2006. At present there are 370 full-time equivalent positions in the province's aquaculture industry.

GERMANY

Malachite green identified as an environmental contaminant

Source: Federal Institute for Risk Assessment, Berlin

A BfR study is said to be the first in the world to identify signs of the background contamination of wastewater and municipal water bodies.

Malachite green is frequently used as a veterinary medicinal product for ornamental fish. It may not be applied to fish intended for human consumption and a zero tolerance principle applies.



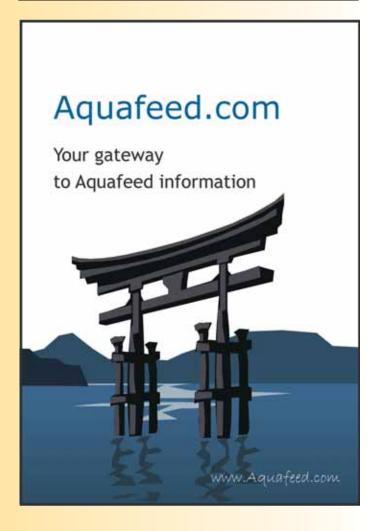
Detection of the substance is seen as an indication of illegal use, and the fish may not be placed on the market.

In a pilot project, scientists at the Federal Institute for Risk Assessment (BfR) have now proven for the first time that untreated, free-living fish may be contaminated with malachite green. They examined eels captured in the wild from Berlin water bodies for residues of malachite green and found them in 20 out of 40 samples. All the samples that were tested positive came from water bodies into which cleaned wastewater from wastewater treatment plants had been discharged.

For BfR this is a clear indication that malachite green must now be seen as an environmental contaminant, and that widespread use leads to the background contamination of wastewater and municipal water bodies.

This situation has prompted reflections on whether the zero tolerance principle can be sensibly applied to free-living food fish from inland waterways. "Irrespective of this, exposure to malachite green should be reduced", says the president of BfR, Professor Andreas Hensel. "In the case of fish from aquaculture we advocate the continued application of the zero tolerance principle. As the fish are kept under controlled conditions, the detection of malachite green in samples of this kind must always be interpreted as an indication of the possible illegal use of the veterinary medicinal product," said Dr Hensel.

Malachite green is a triphenylmethane dye. It is used as a dye to colour synthetic fibres, silk, leather and paper products, and in forensic medicine to detect traces of blood. It is also used as a veterinary medicinal product for treating ornamental fish and fish roe to combat parasites, fungal attack and bacterial infections. There are grounds for suspecting that this substance damages the genotype and triggers cancer. A toler-



able daily intake cannot be established for substances of this kind on the basis of the current level of scientific knowledge.

In contrast to ornamental fish breeding and farming, fish and fish products intended for human consumption may not contain malachite green.

The situation is different in the case of aquaculture products imported into the EU. They may be sold within the EU when the residue levels of malachite green are less than two micrograms per kilogram. This intervention value was defined as a minimum required performance limit, or MRPL, for analytical methods used internationally, and it seeks to facilitate international trade.

The MRPL value has not been established toxicologically and does not apply to products that are only traded within the EU. Although the zero tolerance applies to them in the EU, residues of malachite green and its degradation product, leucomalachite green, are regularly detected in Germany in edible fish as well as in fish roe (trout caviar). For instance, in 2005 it was found in almost 10 percent of edible fish samples examined by the Institute for Ichthyology in Cuxhaven during routine controls prescribed under the National Residue Control Plan.

Up to now, positive results were always taken as an indication of the illegal use of malachite green as a veterinary medicinal product in the production of edible fish. This situation has changed as a consequence of the results of the BfR pilot study. In a study on wild eels from Berlin inland waterways, scientists at the institute proved that malachite green residues can also be environmental contamination.

Almost half of the samples collected tested positive for malachite green or leucomalachite green. However, the levels were very low. Depending on the fishing area they were between 0.04 and 0.8 μ g/kg eel fillet. The residues could only be detected in samples of fish from water bodies into which wastewater had been discharged from wastewater treatment plants.

The institute said this was proof of background contamination of these water bodies with malachite green. Fish can then take up this substance as an environmental contaminant. The assumption is that the substance comes from various sources such as ornamental fish aquaria, textiles or laboratories, and reaches the water bodies from cleaned wastewater from municipal wastewater treatment plants.

The health risk for consumers who eat contaminated eels only once or occasionally is very low, the institute said. Nevertheless, the BfR said the wide distribution of malachite green in the environment was worrying, given its toxicological properties.

It called on the users of malachite green to minimise the input into the environment. The competent authorities must discuss and then decide whether the zero tolerance principle should be applied to and upheld for a substance which may not be used as a veterinary medicinal product, but which is widespread as an environmental contaminant in water bodies in the case of free-living edible fish until minimisation measures take effect.

The BfR said that in its opinion the levels should not exceed the MRPL value of 2 μ g/kg.

See http://www.bfr.bund.de/cd/10136

ISRAEL Whitening agents found to be an effective disinfectant

A technology transfer company in Israel said in September that it was engaged in an advanced study of specific types of fluorescent whitening agents as a treatment for *Saprolegnia parasitica*, one of the most prevalent fish pathogens. The agents are being studied to both prevent and treat *Saprolegnia* infections that occur in aquatic organisms, including fish and fish eggs. The study, funded by the chief scientist of the Ministry of Agriculture of Israel, is being conducted at the Hadassah-Hebrew University Medical Centre and the Laboratory of Fish Health in Nir David, Israel. It is anticipated to last for two years. The data will be presented to the Veterinary Chemical Products Registration Committee, Israel's regulatory body for chemical permits.

The immune systems of fish decrease when in stress conditions, such as a cold environment. Deadly zoospore, the infective unit of the water mould, which thrives in cold water, capitalises on the fragile condition of the fish by latching on and spreading its hyphae on the fish skin, said Professor Itzhack Polacheck, who is the head of the Medical Mycology Laboratory in the Department of Clinical Microbiology and Infectious Diseases at the Hadassah-Hebrew University Medical Centre in Jerusalem.

This resulted in white patches of filamentous mycelium on the body and fins of the fish. Malachite green had been used to treat this situation, but this agent was prohibited due to carcinogenicity. "We have identified an alternative treatment that is non-carcinogenic, non-toxic and very economical," Professor Polacheck said.

The economic loss from *Saprolegnia parasitica* infection is in the tens of millions of dollars for many countries worldwide. In the United States alone, "winter kill" in catfish caused by *Saprolegnia* costs the industry an annual US\$40 million. In Japan, there is an annual mortality rate of 50 percent in coho Salmon and elver due to this water mould. Aquaculture businesses in Scotland, Scandinavia, Chile, Canada and Israel suffer similar "winter kills" on their respective local fish populations.

Diaminostilbene derivatives, used as fluorescent whitening agents, were highly water-soluble, safe to living organisms and the environment, therapeutically effective, and economical, he said. "In fact, they are part of a large group of substances that are already widely used in commercial products in the textile industry. Our research group has discovered a new application of these compounds. Though a large body of information on this group of compounds does currently exist, our study is mandated to further investigate the toxicity and the residuals during and after treatment of fish."

Dr Rama Falk, of Hadassah, said the study would also focus on the analytical parts of the research and the mode of mechanism of antifungal activity of this compound. The study team also includes fish veterinarian Dr Simon Tinman and Nir Froyman, the head of the Laboratory of Fish Health, both from the Central Fish Health Laboratory of the Ministry of Agriculture of Israel.

Hadasit said it was is actively pursuing potential partners interested in supporting the project.

NOTE: Hadasit (www.hadasit.co.il) is the technology transfer company of the Hadassah Medical Organisation in Jerusalem. For further information on this project, see Rama Falk's article in this issue of *Aquaculture Health International*.

Vaccination benefits highlighted as Schering-Plough reinforces commitment to asian aquaculture

A series of key aquaculture events and presentations has confirmed Schering-Plough's growing presence and support in South East Asia. Aquaculture is continuing to develop at a pace in the region where the demand for tilapia is especially strong.

As producers look to establish long-term and sustainable operations, the focus on health management becomes crucial. While the returns are attractive, endemic disease is a factor in Asia as much as anywhere else. Schering-Plough's strategy is to work across all the major stakeholder groups with education and technical awareness programmes.

The company has a longstanding relationship with The World Aquaculture Society (WAS), which hosted this autumn's key event, the WAS Asia-Pacific conference in Hanoi, Vietnam. Among the key speakers was Schering-Plough's leading immunology and fish vaccine expert, Professor Patrick Smith, who presented an informative paper that summarised the global successes of fish vaccine programmes.

Professor Smith highlighted how vaccine programmes are now impacting on five key areas of world aquaculture. Sighting firstly pathology, he explained how the introduction of vaccine programmes had greatly reduced the threat and burden of disease across a wide spectrum of species.

Welfare, an issue of increasing global focus, correspondingly benefits from improved tools for disease prevention. Moving to the environment, he confirmed the clear advantages of introducing vaccines to replace the number of chemicals and antimicrobials that have historically been required in disease control.

The link between vaccination and nutrition, another key area in modern aquaculture, was highlighted, with Professor Smith explaining that through improved health and well formulated diets, farmed fish now have the ability to grow and convert to a level nearer their genetic potential.

The fifth of these critical factors was that the benefits from the other key areas provided a range of significant commercial benefits ranging from the improved cost of production through to the ability to build and sustain fish farming operations with control and confidence.

In addition to its exhibition stand, Schering-Plough hosted a number of private customer consultation sessions.

"We have had very good feedback on these sessions at previous events," said the company's director of marketing and technical services, Robin Wardle, "The opportunity to understand the issues that are currently facing operations in the area allows us to develop a dialogue on suitable vaccination strategies.

"WAS Hanoi was particularly successful in this respect, and we have made firm commitments to follow up with specific visits," Wardle said.

Vaccination for Streptococcosis in tilapia is one specific area where preventive programmes are delivering significant protection around the world. Schering-Plough's regional technical manager, Aries Madethen, presented a technical paper reporting on recent trials conducted in Latin America and Asia using its AquaVac Garvetil* and AquaVac Garvetil* Oral vaccines as part of the company's Total Protection Strategy, a tailored programme to prevent disease through appropriate vaccination.

The trials demonstrate that AquaVac Garvetil, and AquaVac Garvetil Oral vaccines are safe and highly effective for use in tilapia. They also demonstrated significant improvements in survival when the fish are exposed to a natural challenge. Added benefits in increased feed efficiency and the quality of production were also identified.

This theme continued at Tilapia 2007 in Kuala Lumpur, Malaysia, later in August. Producers again registered an increasing interest in developing vaccination programmes. Wardle said he saw Schering-Plough's involvement increasing. "Our commitment is to continue to develop relationships with producers in the region, and in addition to supplying vaccines we look to build and support long-term health programmes to help take their businesses forward."

Contact Schering-Plough Aquaculture on +44 1799 528 167, see http://www.spaquaculture.com/ or email spaquaculture@ spcorp.com

NEWS ROUNDUP

BY SUZI FRASER DOMINY

This news roundup will be a regular feature in *Aquaculture Health International.* It appears courtesy of the Aquafeed website. See www.aquafeed.com

COD GENES ON DATA CHIPS

A senior scientist with Fiskeriforskning, Madjid Delghandi, has developed an award-winning idea for a data chip that shows the variations in a fish's genetic material.

Small variations in cod genes determine differences in how fast it grows and how resistant it is to diseases and deformities. Traditional analyses of these variations are both time-consuming and expensive.

The data chip, which won Delghandi first prize for the best research-based commercial idea from the Technology Transfer Office during Norway's National Science Week, will enable several thousand variations to be analysed simultaneously. Analyses which today take up to six months to complete will be possible in just one week.

The developers now want to commercialise the idea and are currently in exploratory talks with a view to establishing an enterprise that will offer a large-scale analysis concept for the genetic identification of cod.

For further information, please contact madjid.delghandi@ fiskeriforskning.no or Magnus Seppola at TTO Nord at magnus. seppola@ttonord.no

US APPROVES AQUAFLOR FOR CONTROLLING MORTALITY DUE TO FURUNCULOSIS IN FRESHWATER-REARED SALMONIDS

The United States Food and Drug Administration has approved Aquaflor (florfenicol), the fast-acting in-feed antibiotic developed by the Schering-Plough Animal Health Corporation, for controlling mortality in freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida*.

"Aquaflor has been shown in clinical field trials to be highly effective for the treatment of furunculosis, which is a serious disease concern in salmonid species. Furunculosis outbreaks often result in substantial losses," says Dr David Erdahl, fisheries biologist and branch chief of the US Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership Programme in Bozeman, Montana. "This is the first new oral antibiotic available for the treatment of furunculosis in 20 years, and represents an important new management tool for fisheries programmes throughout the country," says Erdahl.

The Fish and Wildlife Service conducted the efficacy studies demonstrating the effectiveness of Aquaflor to control mortality due to furunculosis. Aquaflor administered in feed to either fingerling coho salmon or fingerling chinook salmon with naturally occurring furunculosis significantly reduced mortality compared with untreated controls. For instance, in a study with fall chinook salmon fingerlings, mortality in fish treated with Aquaflor was reduced by over 80 percent. No adverse reactions to treatment were reported in any of the studies.

Outbreaks of furunculosis associated with *Aeromonas salmonicida*, a Gram-negative bacterium, can cause the rapid onset of high mortality. Sub-acute or chronic forms of the disease lead to lower mortality and the formation of external "boil-like lesions" or furuncles.

According to the FDA, losses due to furunculosis associated

with Aeromonas salmonicida are significant in hatchery-reared salmonids, including those at state and federal hatcheries producing fish for native species restoration programmes.

The agency said it reviewed "extensive data" on the antibiotic's effectiveness against furunculosis and its safety for treated fish and the environment. The FDA also said it found that freshwater-reared salmonids fed Aquaflor are safe for human consumption when the product is administered according to the label directions.

Aquaflor has been shown to be effective for several other diseases in aquaculture, such as coldwater disease (associated with *Flavobacterium psychrophilum*) in freshwater-reared salmonids, and enteric septicemia in catfish associated with Edwardsiella ictaluri. Earlier in 2007, its sister product, Aquaflor-CA1 (florfenicol), received conditional approval for the control of mortality in catfish due to columnaris disease associated with *Flavobacterium columnare*.

Studies show that Aquaflor does not lead to reductions in feed consumption or growth. Aquaflor also has a short, 15-day withdrawal period in freshwater-reared salmonids.

Aquaflor is the first in-feed antibiotic in aquaculture and the second for all food-animal species to be classified by the FDA as a Veterinary Feed Directive drug. VFD is a category established in 1996 to help the agency more closely control new therapeutic products, primarily antimicrobials and their use in food animals.

The VFD classification applies only to new in-feed therapeutics approved by the FDA after 2000. Aquaculturists may obtain VFD drugs through normal feed distribution channels, but they will be required to obtain a signed Veterinary Feed Directive from a licensed veterinarian.

For more information on Aquaflor, producers should contact their extension specialist, veterinarian, diagnostician or feed company representative.

See www.Aquaflor-USA.com

TASMANIA RAISES GUARD AGAINST ABALONE VIRUS

Tasmania's fisheries minister has been given increased powers to introduce stringent biosecurity measures in an effort to prevent the spread of the deadly ganglioneuritis virus that has already devastated abalone in Victorian waters. Measures will encompass all aspects of the production chain.

The previously unknown herpes-like virus, which affects the abalone's nervous system, was first detected on Victoria's commercial farms in 2005, and has since spread to wild populations across the state's southwestern coast, posing a threat to South Australia and Tasmania.

Abalone forms the basis of a developing aquaculture industry in Victoria and is the state's most valuable commercial fishery, with a landed value in excess of A\$60 million in 2005/06.

In a potential breakthrough in control of the disease, which causes inflammation of the nervous tissue, resulting in curling of the foot and swelling of the mouth, scientists from Australia's Fisheries Victoria have announced they have succeeded in sequencing its DNA. The DNA profile will help authorities develop quicker and more accurate tests for the disease.



FORTHCOMING FISH AND SHELLFISH EVENTS

FAO CONFERENCES

FAO TCP/BiH/3101 training/workshop
on Diagnostics and Surveillance
First week of March 2008. Sarajevo, Bosnia and Herzegovina
FAO TCP/BiH/3101 Balkan States Regional Conference on
Aquatic Animal Health/Project Terminal Workshop
First week of May 2008. Sarajevo, Bosnia and Herzegovina

FEATURED EVENTS

INTERNATIONAL WORKSHOP ON CYHV-3 (KHV)

Dan-Caesarea Hotel, Caesarea, Israel February 17-18, 2008 Cyprinid Herpes Viruses: basic and applied aspects Tentative programme Saturday, February 16 8pm. Welcome get together and dinner Sunday, February 17

9am. Opening session. Professor H Bercovier, vice-president of the Hebrew University.

9.30am-12 noon. Characterisation of CyHV-3 (biological, chemical, molecular biological) in relation to other herpes viruses isolated from aquatic environment and from other animals.

Taxonomic grouping of the aquatic herpes-like viruses and related viruses.

Virus replication in cultured cells and in vivo.

2pm-5pm. Pathogenesis of the CyHV-3; route of infection, distribution of the virus in the fish body, CyHV-3 disease, virus load in fish organs. Immunogenesis of CyHV-3 infection (antibody kinetics). Immunoprophylaxis/Metaphylaxix/Prevention/ Disinfection.

Monday, February 18

9am-12 noon. Latency/Persistency/Carrier. Cross-breeding experiments. Methods of diagnosis and confirmation.

12 noon-5pm. Tour.

There will be a round-table discussion at the end of each session. For further information, please contact Prof Moshe Kotler, email mkotler@cc.huji.ac.il

VII CURSO INTERNACIONAL DE ACUACULTURA CON ESPECIES PROMISORAS AMAZÓNICAS

Faculty of Veterinary Sciences, University of Santa Cruz, SANTA CRUZ, Bolivia April 21-25, 2008

The VII International Course on the Aquaculture of Promising Amazonian Species is intended to provide basic theoretical and practical training in the production of species native to the Amazon Basin which have an aquaculture potential, to members of rural communities, including small farmers and producers.

In addition, it will provide more advanced training in specific aspects of Amazonian fresh water aquaculture (eg nutrition, genetics, pathology, marketing, processing and health inspection) to biologists, veterinarians and others with professional and/or technical backgrounds.

The course and its related activities will count on the attendance of interested parties from Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, Surinam and Venezuela, as well as of representatives from national, regional and international institutions, NGOs, technical assistance agencies and others involved in aquaculture programmes in the Amazon region.

The theoretical training will be given in the Faculty of Veterinary Sciences, and the practical sessions will be undertaken in the university's El Prado Fish Culture Station located near the city of Santa Cruz.

The various courses will be given by regional and international experts in their corresponding areas. It is anticipated that the potential attendance will be about 150 people.

Contact Dr Soledad C González (coordinator, Centro de Estudios Amazónicas, CEAM -Bolivia) at ceam@ceam-ong-org, or Dr Jordi Pascual Sala (director, Hoyam-Mojos) at hoyam.mojos@gmail.com

FEED INGREDIENTS AND ADDITIVES ASIA PACIFIC CONFERENCE

Queen Sirikit National Convention Centre, Bangkok, Thailand March 5-7, 2008

An exciting and well-rounded line-up of presentations is in store for delegates to the FIAAP conference. Topics will focus strongly on innovative ingredients, natural solutions and quality issues in feed ingredients.



Register now at <u>www.aquafeed.info</u> for Aquafeed Horizons Asia '08

The technical conference for professionals

March 6, 2008, Bangkok, Thailand An Aquafeed.com Symposium in association with Victam Asia 2008. 2007 was a year of skyrocketing commodity prices and concerns about the availability of feed grains. Dr Robert A Swick, technical director, ASA International Marketing, Singapore, will address this pressing issue with an analysis of raw material supply and demand trends for feed manufacturers in the Asia Pacific region.

Questions always surround those other essential feed ingredients, protein and lipid sources. Quality issues will be discussed in two presentations. Degussa Feed Additives has analysed a large number of fish meal samples from India and South America to give a better insight into the levels of the first limiting amino acids contained. They have found huge differences in crude protein content as well as in the amino acid profile. Results and implications of this work will be shared with delegates. A comparison of the digestible protein and AA content from terrestrial protein meals to meet the requirements of high-value carnivorous fish and shrimp will be the topics of another presentation, with cost-effectiveness measured from growth performance and feed utilisation.

Feed hygiene and quality assurance will be addressed in two presentations. The importance of controlling moulds and mycotoxins in animal feed is widely known. Mycotoxins can seriously impact animal health, causing liver damage, reproduction problems, kidney failure and lung damage. Recent research indicates that the bio-transformation of mycotoxins using enzymatic preparations gives promising results. They offer feed manufacturers new opportunities, since enzymes can have a specific action and their reaction, compared to binding, is

Naturally derived ingredients that deliver optimal nutritional performance and health benefits are no less in demand for aquafeed production

not reversible. The importance of bacterial contamination and control is less well understood and is frequently overlooked.

The source, level and type of bacterial contamination of different feed ingredient types will be examined and the need for measures to control bacteria at all steps of feed processing highlighted in the second presentation. The potential for recontamination during this process will also be discussed, and the relative merits of the various chemical and physical control measures available for bacterial control reviewed, both from the technological and economic perspectives.

Delegates will learn how the selection and encapsulation of specific molecules, such as organic acids, for slow release of the active ingredients throughout the hind gut during transport, is offering a new approach to gut health and animal performance for monogastrics.

Results of feeding trials that showed the benefits and economic advantages of a defined preparation of phytogenics in commercial layers in the early stages of the egg production cycle also will be presented.

Naturally derived ingredients that deliver optimal nutritional performance and health benefits are no less in demand for aquafeed production, spurring the development of a plethora of novel ingredients. Some of the solutions offered, the claims made for them and at the processing issues that may result from their inclusion in diets will be examined.

The FIAAP Conference will be held alongside the new FIAAP trade show and Victam Asia at the Queen Sirikit National

Convention Centre. Delegates to the FIAAP can now combine their registration with Aquafeed Horizons Asia '08, to run at the same venue on the following day, March 6, 2008.

For full details see the conference website, www.feedconferences. com, or email conferences@aquafeed.com

The FIAAP Conference is presented by Aquafeed.com Conferences in association with Linx Publishing LLC, publishers of Feed Technology Update and FeedLink.com, and with FIAAP 2008 and Victam Asia 2008. It is supported by the Thai Department of Livestock Development, Thai Department of Fisheries, Thai Feed Mill Association, Thai Tapioca Trade Association, Thai Chamber of Commerce and the Thai Ministry of Agriculture and Co-operative.

The programme chairman is Patrick Keereman, chief executive officer and managing director, Vitamex group of companies, the Netherlands. Other sessions include:

- Raw material supply and demand are they in balance? Dr Robert Swick, ASA-IM, Singapore
- Amino acid content in fish meal shows high variation. Dr Torben Madsen, Degussa, Singapore
- A comparison of digestible protein and amino acids content and value of terrestrial animal protein meals for aquafeeds. Dr Yu Yu, National Renderers Association, Hong Kong
- Mycotoxins in animal nutrition-problems and solutions. Dr Mathieu Cortyl, Impextraco, Singapore
- Bacterial contamination of feed and feed ingredients the importance of control for food safety and animal performance. Dr Adam Smith, Anitox, UK
- A healthy gut for optimal performance through target release concepts in animal diets. Dr Koen Schwarzer, Nutri-Ad International, Belgium
- Efficacy of phytogenics in commercial layers. Dr Robert Nichol, Biomin, Singapore
- Turning bad news into good-feeding opportunities for the Asia Pacific animal industry to maximise profitability. Dr Andeas Kocher, Alltech Biotechnology, Australia
- Natural ingredients product opportunities and process issues. Colin Mair, Cormal Technology, UK

SEVENTH SYMPOSIUM ON DISEASES IN ASIAN AQUACULTURE

Taipei, Taiwan

June 22-26

The Fish Health Section of the Asian Fisheries Society has recently announced that it is holding DAA VII with the theme of Communication, Cooperation and Coordination: key issues on aquatic animal health management. The eighth triennial general meeting of the FHS will also be held in conjunction with DAA VII.

Following DAA VII, there will be a two or three-day special seminar on recent advances in the immunology of fish and shellfish. As part of the continuing professional educational programme of the FHS, there will also be a three-day training workshop on pathogen risk analysis for aquatic animal movement.

DAA VII participants will also be offered a welcome/icebreaker cocktail, a student reception, an FHS member reception, a farewell dinner and a wide choice of technical and cultural tours.

See the symposium website http://homepage.ntu.edu. tw/~daaseven/index1.htm



FORTHCOMING PUBLICATION: IMPORTANT INFECTIOUS AND PARASITIC DISEASES OF FARMED TILAPIAS

BY GINA CONROY AND DAVID A CONROY. OTHER CONTRIBUTORS: PHIL KLESIUS, JOYCE EVANS AND CRAIG SHOEMAKER EDITED BY SCOTT PEDDIE, FOREWORD BY KEVIN FITZSIMMONS

Format: Bilingual (Spanish and English) CD-ROM **Publisher:** Schering-Plough Animal Health, UK

FROM THE EDITOR'S PREFACE:

Concomitant with the rapid expansion of tilapia farming activities has been an increasingly focussed research effort in the arenas of basic and applied tilapia biology. Such research has been necessary to enable aquaculturists to better understand the underlying physiological processes that drive the growth and optimise the health of the animals under their care. It is not surprising therefore that fish health research, teaching and extension are now core activities in many universities across the globe.

It is against such a backdrop that *Important Infectious and Parasitic Diseases of Farmed Tilapia* has been written. By drawing on a wealth of international practical experience gained over many years in active

tilapia health research and consultancy, David and Gina Conroy have written a wide-ranging, accessible and authoritative guide to tilapia diseases.

This CD-ROM document contains 17 chapters that deal with various aspects of tilapia pathology associated with viruses, bacteria, fungi, protozoans, monogenetic and digenetic trematodes, cestodes, nematodes, acanthocephalans, annelids and crustaceans. Haematological disorders related to environmental factors, nutritional deficiencies and infectious and parasitic agents are also considered.

The recognition, diagnosis and aetiology of the disease processes mentioned are outlined, as also is the economic impact of these conditions on tilapia farming operations in fresh water, brackish water and coastal marine environments. The prevention and control of infectious and parasitic diseases in tilapias is given due consideration.

The particular emphasis laid on tilapia diseases which have been detected in Latin America is a reflection of the authors' own wide experience in that region. The chapter on the immune system of tilapias has been specially written for this CD-ROM by Professor PH Klesius and Drs CA Shoemaker and JJ Evans.

This document contains numerous informative tables and figures, plus almost 100 colour plates. It also includes three annexes which provide flow charts for the systematic recording of appropriate basic data from the farm or production centre, during autopsy procedures and for the tentative laboratory identification of potentially pathogenic bacterial isolates obtained from tilapias. A fully comprehensive bibliography is provided.

By keeping the text to a minimum, while still facilitating maximum information transfer, the authors have focussed on providing the reader with a practical manual that can be used in the field, on the



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farm or in the laboratory. Given its format and style, it is intended to be useful to both inexperienced and experienced investigators. The end product is a portable and highly visual CD-ROM book that will be an invaluable addition to the library and the

Important Infectious and Parasitic Diseases of Farmed Tilapias

laboratory bench of fish health professionals and aquaculturists with a particular interest in tilapia.

REVIEW BY MARIO AGUIRRE, SCHERING-PLOUGH CA

As a student of marine biology in 1979, I had the opportunity to meet Dr David Conroy, one of the authors of this CD-ROM on tilapia diseases, who afforded me the opportunity to undertake my thesis work as my tutor. A few years later I met Mrs Gina Conroy. They both guided me with respect to acquiring a basic knowledge of aquatic pathobiology, and this basis has enabled me to appreciate the present text.

The authors describe in detail and with illustrations the different diseases that affect tilapias, and provide fish farmers and others interested in tilapia farming with a text that is an obligatory source of reference. The text emphasises the need for an opportune diagnosis and the importance of this when adequate therapeutic measures are to be applied.

An example is the clear difference that needs to be made with respect to the diagnosis of the principal diseases of bacterial origin. A special mention is warranted with reference to the diseases of intra-cellular origin and streptocococcosis, which have been reported from the Latin American countries in which tilapias are farmed, and which have a very heavy impact on the economic results of the activity.

The different aetiological agents of the various pathological conditions make their appearance in the correct evolutionary order, and this confirms to me that diseases never appear out of the blue. In the same way, the several challenges which the fish have to confront in the different types of culture conditions to which they are submitted are impressive.

An innovative chapter in this type of pathology textbook is the 🕨

review of the immune system and the immune response in tilapias, which is presented in a clear and simple way so that the reader can understand how these fish defend themselves. In this respect, the immunological bases which facilitate the use of vaccines as tools in the early prevention of tilapia diseases are explained.

After so many years, I must admit that the differences between the two principal families of monogenetic trematodes which affect farmed tilapias, the Dactylogyridae and the Gyrodactylidae, have now become quite clear to me.

Lic. Mario Aguirre,

Aquaculture Manager for Venezuela, Central America and the Caribbean

Schering-Plough CA, Caracas, Venezuela

REVIEW BY PABLO GONZÁLEZ-ALANIS, UNIVERSITY OF ARIZONA

This bilingual (Spanish and English) CD-ROM covers the most important topics in tilapia pathology, with a graphic content that makes its interpretation much easier. Although its content is destined for tilapia farmers and producers, it does not minimise the need to count on the support of pathology specialists, for which reason the authors develop its content in a way in which the text and the figures serve as a guide to adopt the appropriate steps to identify and study the diseases, and in that way resolve the disease problems as quickly as possible.

In the introduction there is a short list of books on aquatic pathology that are commonly used in aquaculture to identify diseases in aquatic organisms, including taxonomic aspects, comparative normal histology, histopathology, diseases of native species in Central and South America, techniques for diagnosing and isolating pathogens, including bacteria and parasites, and basic concepts of pharmacology.

In my opinion, there are two big challenges on writing about pathogens in aquaculture. The first is the presentation and explanation of the pathological problems in a clear and precise manner, and the second is the implementation of the use of the text as a tool in an accessible and practical form.

This CD-ROM considers the most important diseases in tilapia farming, with emphasis on the environment, management and control of the physico-chemical parameters on site, the importance of these factors in the production of stress in aquatic organisms, and the success which is achieved in order to keep the culture practices economically viable. Conroy and Conroy compare detailed descriptions of viruses, bacteria, fungi and protozoans present in different species of tilapias with information from various peerreviewed publications. The audience to which this CD-ROM is addressed should possess a basic knowledge of biology and histopathology in order to obtain an objective understanding of the text.

Chapter four cites the opinions of Evans *et al* 2006 and Roberts and Sommerville 1982 in the constant argument about the lack of pathologists specialised in the field of aquatic organisms and the lack of suitably equipped laboratories, principally in those countries where tilapia farming has been operating for a longer time. They mention isolated cases where epidemiological outbreaks have resulted in the hospitalisation of people who have consumed contaminated fish, and the negative impact which those cases had on public opinion, underlining once again the lack of knowledge with regard to the relationship between aquatic organisms and their pathogens which affect humans.

They also detail the species of organisms present in culture conditions and how these can be used to monitor the health status of the stock: "The trichodinids are excellent examples of how their presence can be used to monitor the health status of tilapia populations". Observing the number of these "indicator organisms" on a routine basis can allow a disease problem to be anticipated with sufficient time to correct or to control the situation.

This type of advice comes directly from the personal experience of the authors themselves, and offers practical advice to the fish farmer when compared with much of what is written in more specialised textbooks.

The authors conclude their work with a justification for tilapia farming, and with evidence of it being a profitable activity with a positive social and economic impact, occupying third place at world level with regard to aquaculture species, in addition to its importance in the creation of jobs. On the other hand, the authors stress the negative impact of diseases and parasites which affect tilapias, and the need to improve the techniques of diagnosis, prevention and control in the production centres, mentioning the Revised Code for the Reduction of the Risks of Adverse Effects due to the Introduction of Exotic Marine Species as given by Sindermann 1988, and the article published by Welcomme in 1998, which discusses the introduction of different tilapia hybrids in Latin America without a fish health certificate issued by the authorities of the countries into which these fish were introduced.

From my own point of view, this CD-ROM prepared by Conroy and Conroy provides a well-founded and detailed appreciation of the importance of diseases and parasites as much in tilapia farming itself, as to public health aspects of these, and the preventive measures which must be implemented in each situation. In this document, the lack of tilapia health specialists and infrastructure in the countries where diseases problems in these species are most frequent is recognised, and the authors also recommend the implementation of theoretical-practical courses or workshops involving private companies with interests in animal health and the development of novel products, in addition to making available the services of a pathologist to confirm the diagnoses.

Finally, they suggest as a very important point the maintenance of a close relationship between the productive sector, the academic/ scientific community and governmental authorities, and the need to attend congresses and other such meetings to be kept fully informed on these matters. With respect to the increasing growth of aquaculture, and specifically tilapia farming, there is no doubt that this CD-ROM will be a great utility for producers and research workers, and it will improve the fish health situation in those places which still lack disease specialists and infrastructure.

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Pablo González-Alanis, Graduate research associate, Soils, Water and Environmental Science Department, University of Arizona, Tucson, Arizona, USA

For further information on this publication, contact Dr Scott Peddie, email info@pattersonpeddie.com

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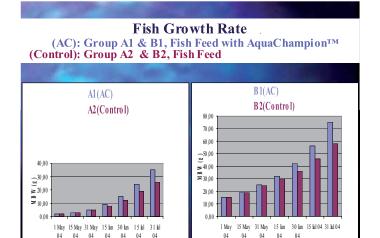
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ENDEMIC CROUPOUS ENTERITIS IN MEDITERRANEAN FARMED FISH AND ITS TREATMENT WITH THE **SPECIFIC NUTRITIONAL SUPPLEMENT** AQUACHAMPIONTM

BY GRIGORIOS KANLIS, PANAYOTIS N LOGHOTHETIS AND ELENI PAPALEXIOU LABORATORY OF FISH PHYSIOLOGY AND PATHOLOGY, DEPARTMENT OF AQUATIC BIORESOURCES, TECHNOLOGICAL EDUCATION INSTITUTION OF EPIRUS, IRINIS AND FILIAS, GREECE



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Vitamin E	25.000	mq	Manganese	Mn	12.000	mg
Vitamin K3	1.000	mg	Zinc	Zn	18.000	mg
Vitamin B1	3.000	mg	Cobalt	Co	200	mg
Vitamin B2	3.000	mg	Iodine	I	300	mg
Pantothenic acid (B3)	5.000	mg	Selenium	Se	50	mg
Nicotinic acid (B5)	12.000	mg				
Pyridoxine (B6)	2.500	mg	Antioxidant: +++			
Folic acid (B9)	1.350	mg				
Vitamin (B12)	10	mg	Flavor: +++			
Biotin (H)	100	mg				
Vitamin C	25.000	mg	Antiaggregator: +++			



The intensive raising of marine fish (mainly gilthead sea bream and European sea bass) has grown to be a significant industry in the last 20 years in Greece. This industry has developed through the gradual solution of various technical problems in the application of well-known production protocols and has now achieved maturity status.

However, profit margins have considerably decreased over recent years. Such financial constraints bear heavily on production management, thus having the potential to adversely affect product quality, and/or leading to sub-optimal fish performance, including reduced disease resistance, reduced growth rate and poor feed conversion efficiency.

CROUPOUS ENTERITIS

The origins of the above situation lie in the "classic" field of nutrition. Feed costs typically accounts for more than half of the total costs of production and is the most amenable to economic "manipulation". The use of feedstuffs of sub-optimal quality has also spread because of the lack of availability of higher quality ingredients at affordable prices.

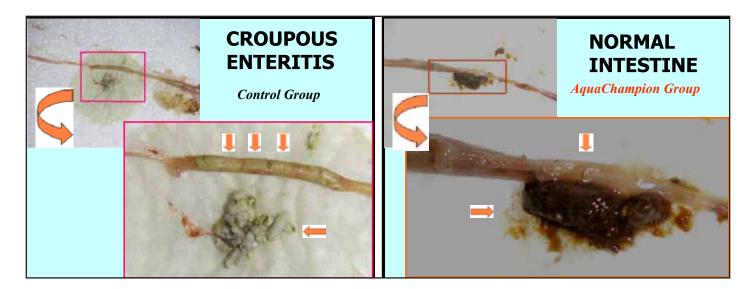
In such a context, pathological problems primarily of the digestive system can be expected to arise, as can more general conditions affecting the organism as a whole. Hence, it did not

The symptoms of the enteritis were substantially reduced, while the growth rate and the food conversion ratio improved

come as a surprise that on many an occasion in recent years, while inspecting fish farming facilities, the authors came across a clear manifestation of a possible intestine malfunctioning.

In a clinical sense, the observed condition can be described as an increased defecation rate immediately after feed administration. Moreover, it appeared that many fish produced strings (casts) of a flexible but insoluble whitish material over relatively long periods of time. On-site necropsy revealed that the intestines of most specimens contained the same material, in addition to some semi-digested feed and/or water.

This picture is clearly an indication of a chronic, mild but extensive inflammation of the enteric mucosa with the consequent production of croupous material. Also observed were the more or less "typical" liver lipidosis and some degree of degeneration in the lateral musculature (ie, this tissue was of a more fatty and watery composition and exhibited a weaker overall consistency). Many fish populations exhibited this phenomenon even at quite low body weights (eg, below 20g). The estimated rate of prevalence



among farms investigated was very high. However, no significant cases of clinical parasitic and/or bacterial infections were identified even after additional laboratory testing. Furthermore, the data on average body growth and on feed consumption (as supplied by the operating managers) showed retardation of the growth rate and worsening of the feed conversion efficiency. In addition, secondary disease outbreaks had been documented on occasion despite the preventive use of vaccines or antibiotics.

The feedstuffs used on affected sites appeared to rely heavily on sub-optimal raw materials that are cost-effective but often do not meet the needs of the fish, particularly during episodes of stress induced by poor oxygenation and regular handling of the populations, together with occasional outbreaks of endemic sub-clinical disease.

Although the energy and protein levels of such feedstuffs are at the "recommended" levels, the low quality of the individual constituents (ie, proteins and fats) may cause significant problems with regard to their utilisation. One such problem, which we believe to have resulted in the gross pathological signs mentioned above, seems to be amino-acid composition imbalance.

This, along with the high content of low quality lipids, may have had a negative impact on the metabolism, thus placing the digestive organs (ie, intestine and liver) under stress and eventually presenting enteritis accompanied by liver fatty degeneration. As a further consequence, possible malfunctioning with accompanying pathological alterations could be expected for other organs as well, notably the gills, blood and kidneys.

NUTRITIONAL INTERVENTION

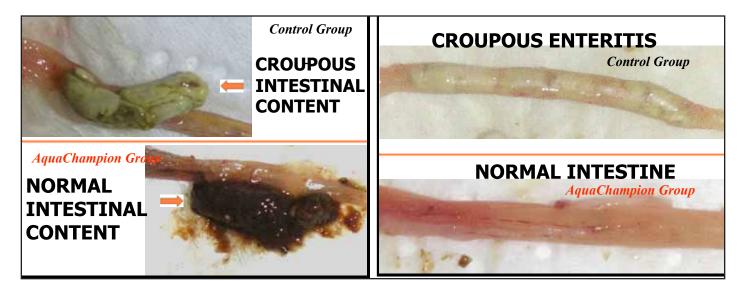
Given this situation, it was felt that some rectifying nutritional intervention was called for, aiming primarily at rectifying the essential amino-acid deficiency in basic feedstuffs, while also fortifying the novel formulation with vitamins and minerals in order to optimise availability of these important elements.

After many pilot tests under field conditions, the new product, named AquaChampion[™], was licensed and entered the commercial phase. Various administration protocols were tested (ie, beginning with high dosages for short periods and continuing with low dosages for longer periods). In nearly all the cases of its application it became evident that the symptoms of the enteritis were substantially reduced, while the growth rate and the food conversion ratio improved.

Feed supplement usage raised the costs of production, but at the same time improved the general condition and the quality of the end product (by means of the restored functioning of the digestive system and the health of the fish). Consequently, this new product can be regarded as a nutritional supplement with substantial utility in production management.

For further information contact:

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THE BLOOD CELLS OF COLOSSOMA MACROPOMUM, A NATIVE CHARACIN WITH IMPORTANT AQUACULTURE POTENTIAL IN THE AMAZON REGION

BY DAVID CONROY AND GINA CONROY, PHARMA-FISH SRL, MARACAY, VENEZUELA

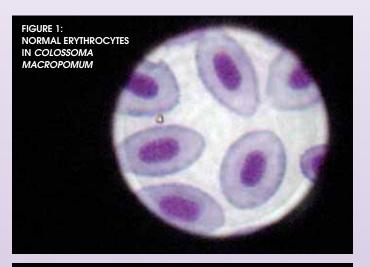


FIGURE 2: NORMAL ERYTHROCYTES IN COLOSSOMA MACROPOMUM

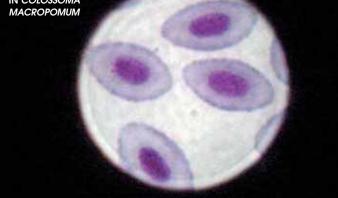


FIGURE 3: POLYCHROMATOCYTE (ARROW) IN COLOSSOMA MACROPOMUM ncreasing attention is now being given to the aquaculture potential of selected native species in the Amazon region of South America as a readily available and inexpensive source of high quality food for human consumption.

Among these native fish, the characins *Colossoma macropomum* and the closely related *Piaractus brachypomus* have been identified as being of particular interest in that respect (Hernández 1989).

As much *Colossoma macropomum* as *Piaractus brachypomus* are widely distributed in the Amazon watershed. Not surprisingly, both species are known by different "popular names" in the countries of the region, as indicated in Table 1.

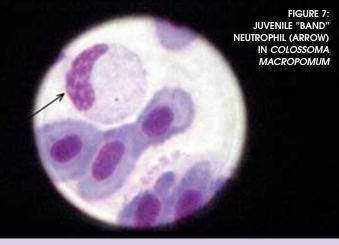
It is now becoming appreciated that the application of routine haematological techniques is of considerable importance and utility in monitoring aspects of the health, nutrition and physiology of cultured fish species. With respect to *Colossoma macropomum* and *Piaractus brachypomus*, workers in Brazil and elsewhere have made significant contributions to our knowledge of the haematology of these two types of characins and their hybrids (Martins *et al* 1995, Ranzani-Paiva *et al* 1998/1999, Tavares-Dias and Mataqueiro 2004, Tavares-Dias and Sandrim 1998, Tavares-Dias *et al* 1998, 1999a, 1999b, 1999c, 1999d, 2000, 2007 and Tocidlowski *et al* 1997).

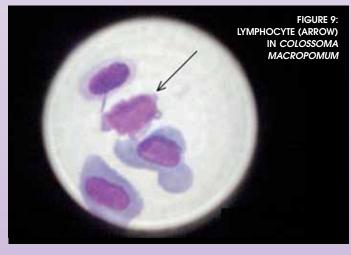
The two authors were afforded an opportunity to study the blood cells of *Colossoma macropomum* under conditions of semi-intensive culture in a warm water area of the Andean region of Táchira state, Venezuela. At the time of sampling the fish had an average weight of 250g to 400g, and had been fed on a pelleted feed available commercially on the Venezuelan market. Small, non-lethal blood samples were taken from the caudal vein, and thin blood smears were prepared from these and fixed in methanol for staining by the Giemsa technique, prior to microscopical examination.

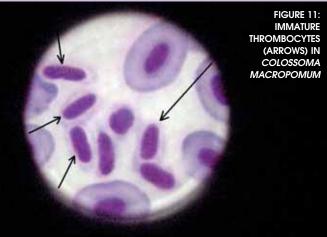
The results of these studies indicate that the blood cell picture of *Colossoma macropomum* is very similar to many other tropical and sub-tropical freshwater teleosts with respect to the nature and morphology of the red and white blood cell elements encountered. Representative blood cell types of *C macropomum* are illustrated here as a guide to others working on the haematology of this species.

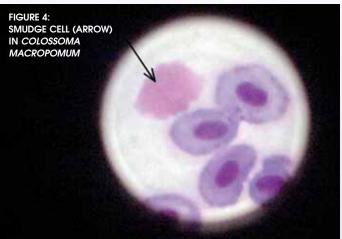
TABLE 1: COMMON VERNACULAR NAMES BY WHICH COLOSSOMA MACROPOMUM AND PIARACTUS BRACHYPOMUS ARE KNOWN IN THE COUNTRIES OF THE AMAZON REGION.					
COUNTRY	Colossoma macropomum	Piaractus brachypomus			
BOLIVIA	pacú	tambaquí			
BRAZIL	tambaqui	pacu, pirapitinga			
COLOMBIA	cachama, cachama negra	cachama, cachama blanca			
PERU	gamitana	paco			
VENEZUELA	cachama	morocoto			

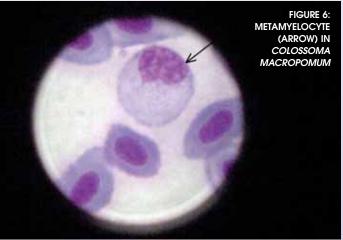




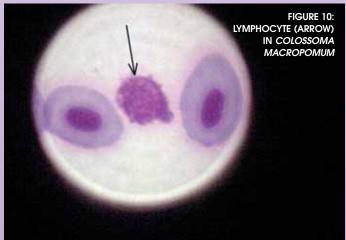




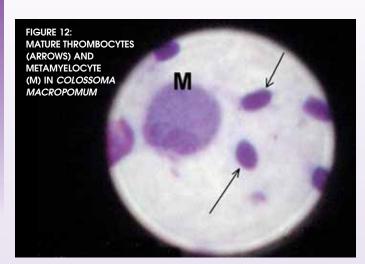








THE BLOOD CELLS OF COLOSSOMA MACROPOMUM, A NATIVE CHARACIN



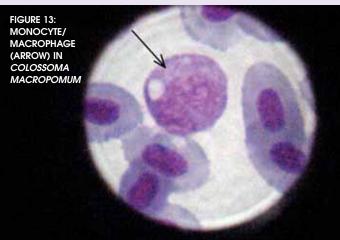


FIGURE 14: MONOCYTE/ MACROPHAGE (ARROW) IN COLOSSOMA MACROPOMUM

The red blood cells in farmed *Colossoma macropomum* are represented by normal erythrocytes (Figures 1 and 2), polychromatocytes (Figure 3), poikilocytes and smudge cells (Figure 4). The white blood cells, or leucocytes, include neutrophils (Figures 5, 6 and 7), lymphocytes (Figures 8, 9 and 10), and thrombocytes (Figures 11 and 12).

Although not illustrated here, it must be mentioned that neutrophils with two nuclear lobes appear to be the most advanced "adult form" of this type of cell in blood smears obtained from specimens of *C macropomum*. It is also of interest to note that monocytes/macrophages (Figures 13 and 14) may also be encountered occasionally.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Manuel Useche of the Universidad Nacional Experimental del Táchira – UNET, San

Cristobal, Venezuela, for making available suitable facilities for collecting blood samples from cultured specimens of *Colossoma macropomum*.

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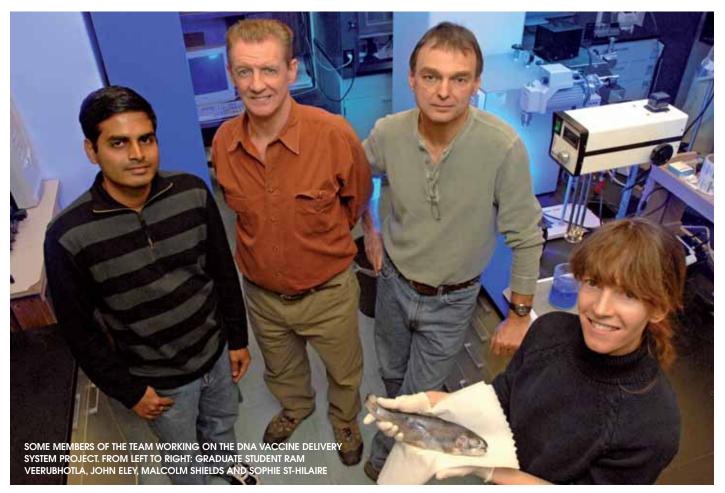
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US RESEARCHERS AWARDED A GRANT TO WORK ON VACCINE ORAL DELIVERY SYSTEMS

BY SOPHIE ST-HILAIRE (IDAHO STATE UNIVERSITY, USA)



Researchers in Idaho have been awarded a US\$550,000 grant from the Idaho State Board of Education to investigate an oral delivery system for DNA plasmid vaccines in rainbow trout. The team consists of

- Dr John Eley, a drug delivery specialist in the Department of Biomedical and Pharmaceutical Sciences at Idaho State University
- Drs Malcolm Shields and Peter Sheridan (microbial molecular biologists) and Sophie St-Hilaire (veterinary epidemiologist) in the Department of Biological Sciences at ISU
- Drs Wendy Sealey and Ronald Hardy, fish nutritionists at the University of Idaho Hagerman Fish Culture Experiment Station
- Dr Ken Cain, a fish immunologist at the University of Idaho
- Dr Gael Kurath, a virologist at USGS
- Dr Rick Barrows, a nutritionist at USDA
- Gary Fornshell, University of Idaho extension educator, and
- partners associated with the rainbow trout industry.

The researchers will assess two experimental delivery systems for a DNA vaccine against infectious hematopoietic necrosis virus (IHNV) in rainbow trout. The current method of vaccinating rainbow trout against IHNV (immersion of the fish with a killed vaccine) does not provide sufficient exposure to the pathogen for adequate absorption and life-long immunity.

New, more efficient vaccines such as DNA vaccines are available, however, they have not been seriously considered for use in the rainbow trout industry in Idaho because the delivery system for this type of vaccine currently requires intramuscular injection, which is cost-prohibitive.

To date, there is no means of delivering DNA vaccines to fish orally, although oral delivery systems for these types of vaccines have been developed for mammals. The proposed oral delivery system is based on preliminary work done on the oral delivery of killed vaccines in fish, and more recent work on plasmid delivery systems in mammals.

Specifically, Drs Eley, Shields, Sheridan, Kurath and St-Hilaire will investigate the ability of a poly (D, L-lactide co-glycolide) (PLGA) polymer and a liposome polymer complex to orally deliver an IHNV DNA plasmid vaccine to rainbow trout.

Drs Sealey, Hardy and Barrows will investigate different methods of incorporating the nanoparticles into fish feed. The goal of the project is to protect the DNA vaccine while it is in the stomach of the fish, and then release it from the polymer once it is in the intestinal tract so that it can be fully absorbed.

The benefits of this project extend beyond reducing losses from IHNV. The technology developed will be applicable to other DNA vaccines and, potentially, to other aquatic species.



WELFARE AS A DRIVER FOR TECHNOLOGICAL DEVELOPMENT IN AQUACULTURE

BY ALISTAIR LANE (EUROPEAN AQUACULTURE SOCIETY, BELGIUM), KJELL-INGE REITAN, (SINTEF, NORWAY) AND KARI ATTRAMADAL (NTNU, NORWAY)

his article is the first of a three-part series exploring the findings of the Aqua Nor Forum held at the Aqua Nor 2007 conference in Trondheim, Norway on August 15 and 16. It deals with the "my first home" section of the forum, and subsequent articles will report on the "my land home" and "my sea home" sections of the event.

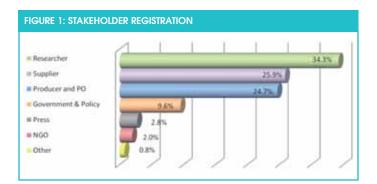
FORUM CONCEPT AND ATTENDANCE

The basis of the Aqua Nor forum concept is that too many workshops are too presentation-focused and workshop participants only have a very limited possibility to contribute. With good moderation, more discussion time allows ideas to be raised and explored in more depth, hopefully leading to consensus-based outcomes from the discussion.

The main objectives of the forum were to:

- provide a forum for science, industry, consumers and policy makers to share knowledge and ideas of the use of welfare as a driver for technological development in aquaculture
- discuss different "housing situations", and assess how our knowledge of species biology can drive the design of new and improved production systems that decrease stress and hence increase product quality, and
- provide good examples and good reasons why biologists, engineers and other stakeholders should work together today to develop the systems of tomorrow.

The three sessions (my first home, my land home and my sea home) allowed lengthy discussion around short presentations that raised issues. The principal success criterion of the organisers was active participation by the participants.





Interest in the forum was higher than anticipated and resulted in 251 registrations from an impressive 34 countries, which effectively confirmed the organisers' concept of the forum.

Figure 1 shows the stakeholder representation of registered participants. It was most encouraging to see that producers, producer organisations and industry suppliers made up the majority of participants. Having almost 10 percent of government representatives and other policy makers can also be considered a success in meeting the forum's main objective.

The forum approach and the representation of different stakeholders were underlined by the president of the Nor-Fishing Foundation, Peter Gullestad, in his welcome to participants. Being organised on the site of Aqua Nor allowed participants to come and go freely between the exhibition and the forum.

THE "MY FIRST HOME" SESSION

Session moderator: Kjell-Inge Reitan, Sintef, Norway Contact: SINTEF Fisheries and Aquaculture, Brattørkaia 17 B, NO-7465 Trondheim, Norway.

Email Kjell.i.Reitan@sintef.no

PANEL MEMBERS:

Grete Baeverfjord, Akvaforsk, Institute of Aquaculture Research, Norway. The fish larva future source of profit or potential disaster? *Eamonn O'Brien*, Skretting, Marine Feeds, Belgium. Nutritional aspects related to larval rearing success in marine hatcheries

John Sweetman, Ecomarine Ltd, Greece. The management of critical stressors in the hatchery environment and their impact on performance

Gunvor Øie, Sintef Fisheries and Aquaculture, Norway



NEW TECHNOLOGY IN LARVAL REARING

This session addressed the specific environment of the seawater hatchery and nursery, where larval fish are fragile and require special husbandry.

In his opening comments, Kjell Inge Reitan outlined the factors that influence hatchery production – notably the biological factors, the environment, the specific microbial environment and the nutritional aspects – all covered by the presentations of the panel members. Forum discussions were held after the four panel presentations.

Opening the "My first home" session, senior research scientist at Akuaforsk, Grete Baeverfjord, presented an overview of fish larval life, demonstrating that fish larvae are the most productive life stages and show great plasticity in adapting to their environment.

She focussed on our knowledge of the biological limits that we must comply with when planning their rearing conditions, adding that fish larvae are vulnerable, and the potential for damage is uncomfortably big.

Grete also presented new knowledge on fish malformations and deformities from the EU Collective Research project FineFish (See www.finefish.info), which is looking to reduce the incidence of malformations in the major fish species used in European aquaculture production and to apply this to the professional sector.

Eamonn O'Brien of Skretting then gave an overview of fish larval nutrition, addressing the different stages of the larvae from broodstock to post-weaning. His principal message was that "because we are operating at 25 percent survival as an industry, fish welfare in the marine fish hatchery today is in a state of 'perpetual improvement'. It is important for hatcheries not to stay tied to traditional ways, but to remain open to new ideas and new technology development on all fronts: nutrition, equipment."

Nutrition is just one of multiple factors (although an important one) that contribute to welfare and larval rearing success. Live feed systems have typically remained unchanged in marine fish hatcheries, and Eamonn underlined the importance of moving towards new, high quality natural diets for an optimal quality of the live feeds currently used, and the implications of intestinal flow rate at first feeding and at "later" larval stages.

No correlation has been found between the increased survival in hatcheries and increased incidence of problems

John Sweetman of Ecomarine followed up this line with a presentation that focussed on stress management, based on the direct links between stress parameters and survival and health status.

Water quality, chemistry and microbiology all effect growth and "liveability" of fish fry. Live food nutrition and microbiology are an integral essential, as they introduce heavy bacterial loads into rearing systems, which have an effect on larval pathologies. Through teamwork in the hatchery, an in-depth understanding of systems,

WELFARE AS A DRIVER FOR TECHNOLOGICAL DEVELOPMENT IN AQUACULTURE



their management and their effect on environmental, nutritional, chemical and physical stressors within the production cycle can be achieved, and this is the basis of stress reduction and welfare (and thus performance) enhancement.

Finally, Gunvor Øie of Sintef looked at new technologies to improve larval rearing. Outlining the challenges associated with automation of a process dealing with live organisms in sea water, she noted that the majority of automation currently seen in hatcheries was based on the development of tools to facilitate current processes – including automated cleaning of rotifer tanks and control of the density of rotifers in first feeding larval rearing tanks.

She did, however, provide examples of new technologies. One of these is for grading salmon eggs (this actually won the Aqua Nor 2007 Innovation Prize). The sorting machine provides exact numbers and quality assessment of eggs, greatly increasing efficiency. It is estimated that one hour of machine sorting is equivalent to 30 hours sorting by hand. The technology has been developed by Aquagen, Sintef and Maskon.

The second example is based on the utilisation of a new technology for producing copepod (*Acartia tonsa*) eggs – an EU project funded under the 5th Framework Programme (QCR-1999-72468).

FORUM DISCUSSION

The forum discussions that followed are reported here by Kari Attramadal of NTNU, Trondheim.

Is high survival the objective?

The discussion opened with the question that, in our quest for high larval survival in aquaculture, are we in a way selecting for problems? In nature, in comparison, only a very low percentage of marine fish larvae survive to the next stages.

It was, however, also commented that fish larvae are exposed to all sorts of problems and challenges in nature, so this might not be an evolutionary effect. In fact, no correlation has been found between the increased survival in hatcheries and increased incidence of problems. Furthermore, fish growth rates in nature are generally very high, which is proof of the growth potential and should be something for the intensive fish hatchery and nursery industry to work towards. To obtain maximum output from the industry, it was said, one has to strive for high growth levels.

Even if good growth in nature can be measured under less than optimal conditions, it is not directly transferable to the intensive hatchery or nursery situation, where a lot of parameters can be poor over a longer period of time, thus affecting the long-term welfare of the fish larvae. The additive effects of several parameters are also important, in addition to the fact that intensively reared larvae do not have the opportunity to move away from the poor conditions like they may have in nature.

The copepod effect?

Is the feeding of fish larvae with live or inert feed that is not natural for them good for fish welfare? Copepods, a natural prey item for many (cold water) fish larvae, have shown to increase viability and growth in fish larvae compared to rotifers. New copepod production systems allow for copepods to be used for certain limited "critical" stages of the production, but it was generally accepted that it is not practically possible to produce a stable, commercially viable output of juveniles on the exclusive use of copepods during the whole larval production period.

The discussion then focused on what the fish larvae actually get from the copepods in terms of, for example, lipid composition, protein content and digestibility, to reveal what the fish really needs to perform well. Based on this knowledge it could be possible to engineer rotifers that more closely resemble copepods.

In addition, it was also pointed out that one may have focused too much on the importance of fatty acid and protein content compared to the effect of mineral content of the feed. Little is done on mineral content in live feed and fish larvae, and copepods may well have a more sophisticated mineral composition than rotifers.

Finally, it was also pointed out that rotifers act differently from copepods, and that fish larvae are more active in their feeding on copepods due to their attractiveness as prey items. There may well be much more to the "copepod effect" than nutritional factors alone, including, for example, their digestive enzymes and hormones that we don't see and we don't know about yet.

Can we really measure welfare

There was then a call for suggestions on suitable welfare indicators for fish in their early life stages. The panel quickly agreed that the term "welfare" still remains subjective and that it needs defining.

Does it simply mean that fish larvae with good welfare grow better? Or is it a link that we create for the welfare term to be useful for the industry? It was remarked that there is an issue of productivity versus welfare that needs clarification: Is welfare something that should be defined to optimise production or for the fish in the natural environment? Some define welfare as the lack of pain and to have good growth, but is this definition good enough when hatchery managers are generally striving to keep the larvae alive? Pain, it was remarked, is very difficult to sample, and may be clear enough with immediate death, but how does one grade life-lasting pain? In the copepod versus rotifer discussion one might also have a problem of defining welfare from the potential problems of intestinal damage and pain later in life for fish grown on an insufficient diet during the larval stage.

When the survival rate is low, good growth doesn't necessarily mean good welfare

The need for good stress indicators was emphasised, and it was argued that there is a need to put actual numbers on stress to be able to monitor and find optimal welfare to raise sufficient numbers of normal healthy fish in an economically viable way. Several suggestions were made to start a potential list of "workable welfare indicators", including normal development, fin damage, general health status and parasites. It was suggested that the striking difference in growth between larvae-fed copepods versus rotifers may be a good welfare indicator, but the question remains how this is followed in other parameters.

We were then reminded that one cannot look at growth separately from survival, as when the survival rate is low, good growth doesn't necessarily mean good welfare.

It was remarked that stress indexes are not very useful, as the stress level for one fish may change continuously and the level may vary between tanks in the same nursery. These facts make it difficult to set welfare standards. The question was asked, if by trying so hard to avoid stress one is in danger of overprotecting the larvae: If the larvae are kept disease-free they may perform worse at later stages, so do we need to stress the fish a bit?

It was remarked that some stress will always be a natural part of the industrial raising of fish larvae, so it should not be a problem not to meet general stress. On the other hand, disease may be a separate issue.

If so, from when?

The question was also raised, at which point one should start to discuss "welfare" for fish larvae, since marine species have an embryonic life form following hatching, compared with the more developed salmonids. Health and normal development includes more than the welfare discussion, as everything the farmer does is to try and avoid signs of stressed larvae to secure and stabilise production.

While we should try to define welfare indicators as early as possible in the hatchery cycle, at later stages the fish become immunocompetent and the welfare indicators could compromise other important factors.

Hatchery automation - what real technical advances?

Ten years ago, automation was rated low in hatcheries, and the question was asked about which parts of the production was prioritised in this field to obtain cost-effectiveness. It was remarked

that costs in Norway made automation more lucrative, and that, for example, rotifer production and general structuring of the industry have been fields of special development, based on Mediterranean experience. It was noted that automation per se does not imply increased welfare for the fish, but it may be an important tool to increase fish welfare and make it easier for technicians to control the fish environment.

It was asked if the impressive increase in cod production in Norway in recent years is a result of technical advances or more of simple adjustments in biological measures. The reply was that it is hard to answer (for the industry as a whole) which is the more important – the success is probably partially related to better technology and a more stabilised live feed production: the combination between improved rotifer culturing techniques and automation. In a way, automation may also make biologically based improvements possible at a larger scale, so the conclusion must be that there are multiple factors contributing to the success.

Let's "smooth out" the bumps!

In the concluding remarks, it was mentioned that we certainly need to define fish welfare. It was noted that mobilisation of resources – technology, management and automation – is

the way to succeed. The objective is "smoother curves" in the growth and environmental parameters, which result in the need for more automation.

Finally, it was emphasised that welfare is an important issue to consider for the earliest stages of marine fish larvae: health, development and growth during the first couple of weeks following hatching is certainly very important for later performance and hence for overall production.

Aquatic Animal Health Services

Aquaculture Research and Development Facility of the Ocean Sciences Centre located in St. John's, NL, Canada, provides state-of-the-art facilities designed to support research, training, pre-commercial production, and smallscale commercial trials, on alternative species for marine

Services

- Aquatic ecosystem monitoring and aquaculture site
- evaluations
- Broodstock services
- Hatchery, first feeding and on-growing
- Live feed production and feed formulation

Facilities

- Seawater systems
- Hatchery and nursery operations
- Live food production facilities
- Research cage site Image analysis facility



IMMUNOSTIMULATION, VACCINE AND **PHAGE THERAPY** STRATEGIES IN AQUACULTURE

BY PHILIP H KLESIUS, JOYCE J EVANS AND CRAIG A SHOEMAKER (USDA, ARS, AQUATIC ANIMAL HEALTH RESEARCH LABORATORY, USA)

n contrast to fish, an adaptive immune system is absent in shrimp and other kinds of aquatic invertebrates that rely on only a non-adaptive immune system for defence against pathogens (Klesius and Shoemaker 1997, Table 1). Shrimp must immunologically respond to a specific pathogen each time it is encountered because of the lack of memory (Arala-Chaves and Sequeira 2000).

Shrimp pathogens are recognised by glycoprotein substances, or lectins, that react with sugar substances on the surface of pathogen to activate their non-adaptive immune responses. These responses include the antimicrobial actions of lytic peptides, free-radical oxygen substances and phagocyte-like cells (Song and Hsieh 1994). Shrimp cannot be vaccinated, but may be immunostimulated with a "shrimp vaccine". The use of the term "shrimp vaccine" is a misnomer given the known immune memory capacity of shrimp. A review of the immune system of crustaceans is provided by Stet and Arts (2005).

IMMUNOSTIMULATION

Immunostimulants encompass a variety of biological compounds capable of producing or aiding in the stimulation of nonadaptive immune responses (Werner and Jollès 1996, Robertsen 1999, Gannam and Schrock 2001). They are a heterogenous group of compounds that are derived from bacterial, plant and animal extracts.

One of the most commonly used immunostimulant is ßglucans. The glucans are extracted from cell walls of bacteria and yeasts. β - 1, 3 and 1, 6 glucans are used in fish diets to initiate non-adaptive immune responses. The chemical composition and solubility of these glucans varies considerably depending on the number and length of their sugar side chains (Robertsen *et al* 1990). These factors can reduce their ability to stimulate immunity. The results of feeding or injecting β -1,3 and 1,6 glucans into different fish species and with pathogen challenges are highly variable.

Among the other kinds of immunostimulants are ascogen (Ramadan*etal*1994), peptidoglycans, ß-hydroxyß-methylbutryate (Siwicki *et al* 2003, Whittington *et al* 2003), *oligodeoxynucleotides*

TABLE 1: The properties of fish and shrimp immune systems					
PROPERTY	FISH	SHRIMP			
Non-adaptive immunity	Yes	Yes			
Acquired (adaptive) immunity	Yes	No			
Antibody immunity	Yes	No			
Cell-mediated immunity	Yes	Yes			
Specificity for a pathogen	Yes	No			
Memory of a pathogen	Yes	No			
Duration	Months-years	1-14 days			
Immunization	Yes	No			
Immunostimulation	Yes	Yes			
Macrophage/lymphocytes	Yes	No			

(Li *et al* 2004) and LPS, DNA, chitosan A and B and thamnolan (Magnadottir *et al* 2006), lactoferrin (Welker *et al* 2007) and bacterial probiotics (Shelby *et al* 2006). The results of these studies on different immunostimulants were variable, ranging from susceptible to resistant to bacterial challenge, and from none to marginal immune function enhancement.

Activation of phagocytic and killing capacity in monocytes/ macrophages is the likely mode of action of B glucans. Increased lysozyme and complement levels may also occur following the use of B glucans.

Among the other immune and non-immune responses stimulated are increased macrophage movement (chemotaxis, chemokinesis), lymphocyte numbers, immunoglobin levels, mucous production, C-reactive protein levels and macrophage activating factor activity. However, enhanced immune and nonimmune responses are not always followed by increased pathogen protection (Whittington *et al* 2003, Whittington *et al* 2005).

A combination of ascorbic acid, a tocopherol and glucans fed to sea bass for three months at two percent of their body weight was investigated by Bagni *et al* (2000). They reported that the plasma lysozyme levels and alternative complement pathway activation was enhanced in the fish fed glucans and increased vitamin levels.

The use of immunostimulants is influenced by many variables, including the fish species, age and size, solubility, type and chemical composition of an immunostimulant, route of treatment, virulence of pathogen(s), duration and amount of immunostimulant used. Properly designed field trials and data analysis are often inadequate regarding the numbers of fish, treated replicates, untreated controls and known cause of mortality to be accurately interpreted.

The risk/economic benefit factors are often unknown. Furthermore, does an immuostimulant provide long-lasting protection in both juveniles and adult fish? More research is needed to demonstrate their efficacy and economic benefit for fish farmers to completely accept their role in promoting fish health.

VACCINATION STRATEGIES

Tilapia can be immunised with *S iniae* and *S agalactiae* bacterins that include extracellular products (ECP) by immersion, oral and injection routes to prevent streptocococcal infections (Klesius *et al* 1999, Klesius *et al* 2000, Evans *et al* 2004, Klesius *et al* 2006, Shoemaker *et al* 2006). Extensive overviews of Streptococcus in warm water fish and of streptococcal vaccinology (Klesius *et al* 2001, Evans *et al* 2006) as well as warm water fish vaccinology (Klesius *et al* 2004) have been published, and readers should refer to these reviews for information regarding vaccines against infectious pathogens.

The effective immersion immunisation scheme is based on a 20 to 30 second exposure to the diluted *S iniae* vaccine in a water bath (Klesius *et al* 2006). The *S iniae* vaccine was shown to be effective in tilapia fry as small as 0.5g wet weight. Multiple vaccine exposures were effective in protection against *S iniae* when the tilapia were immunised at 0.01, 0.3 to 0.5, five to 10g and 45-50g wet weight. Furthermore, the *S iniae* vaccine has been shown to provide therapeutic effects (Evans *et al* 2006c). The *S agalactiae* vaccine was effective in 3g and 30g tilapia with relative percent survival of 80 by injection (Evans *et al* 2004). This vaccine also provided protective immunity following immersion administration.

S agalactiae and *S iniae* ECP appear to be important to antigenicity of the *S agalactiae* vaccine (Pasnik *et al* 2005b) and to the stimulation of inflammatory macrophage responses to *S agalactiae* and *S iniae* (Klesius *et al* 2007). Specific anti-streptococcal antibody appeared to correlate with the protective immunity (Pasnik *et al* 2005a).

Passive transfer of immunity against these streptococcal pathogens was achieved using immune serum

containing antibodies against these streptococcal species (Shelby *et al* 2002, Pasnik *et al* 2006). Passive transfer of antibody also suggests an important role for humoral immunity against *F columnare* (Shelby *et al* 2007), however, complete protection was not demonstrated. Recently, a modified live *F columnare* vaccine has become available for channel catfish in the United States, and has potential for use in many species of freshwater fish (Shoemaker *et al* 2007).

A DNA vaccine is not yet available for use against tilapia pathogens. Immunisation antigen-encoded plasmid DNA may provide a strong antibody and cell-mediated immune response against fish pathogens. A study by Kumar, Parameswaran, Ahmed *et al* (2007) reported that Asian sea bass vaccinated with a single dose of DNA plasmid encoding a major membrane protein produce moderate protection against acute haemorrhagic septicemia and mortality by *Vibrio anguilarum* experimental infection. A recent review by Kurath (2005) provides additional information on the status of DNA vaccine development for fish.

Vaccination strategies should not only increase survival, but also provide additional profits to the fish farmer by increased growth of healthy fish (Klesius *et al* 2006). Vaccinology is a valid, environmentally sound approach to producing specific immune responses that can be effective in the prevention of streptococcal diseases in farmed tilapia.

BACTERIOPHAGE THERAPY

One of the first studies to use bacteriophage therapy reported that *Escherichia coli* phage was both treatment and prophylactically beneficial in farm animals and mice (Smith and Higgens 1982, Smith and Higgens 1983). Bacteriophages specific for *Lactococcus garvieae* were demonstrated to protect yellowtail against experimental *L garvieae* infection (Nakai *et al* 1999). More recently, Park *et al* (2000) suggest that phages may be used to control disease caused by *Pseudomonas plecoglossicida* in cultured ayu.

Morrison and Rainnie (2004) noted that bacteriophage therapy may be an alternative to antibiotic therapy in aquaculture. Matsuzak *et al* (2005) review the literature on the promise of phage therapy to control bacterial infectious diseases in animals. Currently, many investigators are attempting to develop effective phage therapy for infectious diseases of fish.



The use of prophylactic antibiotics in aquaculture is well recognised as a growing problem for human and animal health and for the environment (Cabello 2006). The use of safer alternative strategies such as vaccines (Sommerset *et al* 2005) and phage treatments represent progress towards solving health issues and environmental problems caused by the use of antibiotics in aquaculture.

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FEEDING STIMULANT SHOWS ENCOURAGING RESULTS

BY SCOTT PEDDIE

A fter initial laboratory tests, the first commercial trials in the use of nature-identical chemosensory fish feeding attractants have shown very encouraging results, according to Kiotech International representatives.

These findings have been outlined by Dr Andrew Moore, head of salmon and freshwater fisheries at Cefas (Centre for Environment, Fisheries and Aquaculture Science) in the United Kingdom.

According to Dr Moore, such findings could result in both significant commercial benefits to participating farmers, and also equally important environmental benefits.

The trials were carried out under the supervision of the UK government agency Cefas, in partnership with Kiotech International and in collaboration with local aquaculture and fish institutes in China and Thailand.

"These results give much encouragement and demonstrate the effectiveness of chemosensory fish feeding attractants," said Dr Moore. "This gives us the impetus to press on with the development of products tailored to other commercially important species and to start work on commercialising these current products."

TILAPIA TRIALS

The trials on Tilapia (*Oreochromis niloticus*) were conducted in Zhouhai, China, over a six-month period. According to Kiotech International, the application of the Tilapia Aquatice product produced a 17 percent increase in the average weight of the tilapia compared with the control pond.

Aquatice also increased the growth rate of the tilapia, allowing the farmer to start harvesting three weeks earlier than the control pond. In addition, it was noted that in the Aquatice-treated pond the fish appeared healthier, the water quality was better and the secondary crop of white shrimp was significantly higher with less incidence of disease. Overall, the farmer received a 50 percent higher income from the Aquatice-treated pond than the control.

SHRIMP TRIALS

The white shrimp (*Litopenaeus vannamei*) trial was conducted in Tradt, southeastern Thailand over a three-month period. The white shrimp Aquatice product was applied coated to the commercial shrimp feed. According to Kiotech International, the application of feed coated with



Aquatice produced shrimp that were 30 percent larger on average than the control shrimp and had a significantly faster growth.

In addition, less feed was required in the Aquatice pond, probably due to increased feeding by the shrimp, which was reflected in an improved food conversion rate at harvest than the control pond.

On the environmental side, Kiotech believe that the use of natureidentical chemosensory attractants will lead to a reduction in the amount of waste from uneaten feed. Moreover, in the longer term it is anticipated that this technology will be used to permit the use of more sustainable forms of proteins within feeds, which are not based on fish oils or proteins. This approach will further conserve and protect wild fish populations and provide a sustainable base for the large-scale expansion of the aquaculture sector.

Also from Kiotech International

At its annual general meeting in mid-2007, Kiotech International said it was in the final stages of agreeing to a joint venture in China, in which the company will have a majority stake.

Kiotech said it had received considerable international interest in Aquatice from the aquaculture industry and was taking steps to work with some of those parties to progress the commercial development of the product. This should help with the process of registering Aquatice (chemosensory stimulant for fish feeding) in that country.







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aquaculture. AwF has also assisted tsunami-devastated shrimp farmers in India and Indonesia.

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