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EPITHELIOCYSTIS - A VILLAIN OR AN INNOCENT BYSTANDER?

PROMOTING SUSTAINABLE AQUACULTURE AND FISHERIES

CENTEX SHRIMP – A MAJOR SERVICE PROVIDER

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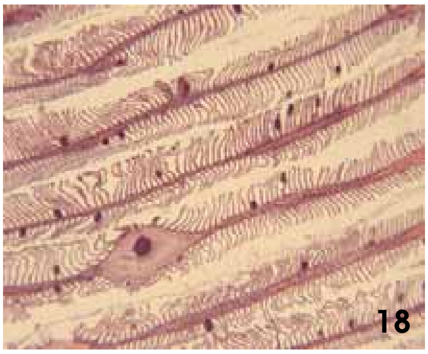

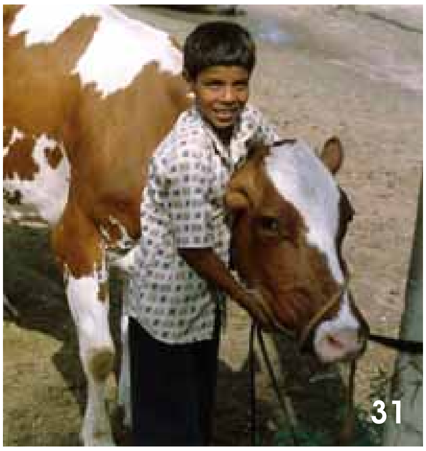

ON THE COVER:

An impressive example of a brooder shrimp

PHOTO COURTESY OF PROFESSOR TIM FLEGEL, CENTEX SHRIMP, THAILAND

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FISH WELFARE – A COMPLEX QUESTION

DR SCOTT PEDDIE, EDITORIAL DIRECTOR

Some animal welfare activists create the impression that farmed fish welfare is a straightforward issue. But is it? The science of fish welfare is complicated and fraught with interpretative difficulties that make it difficult to formulate hard and fast rules.

The issue of aquatic animal welfare is gaining increasing currency in the scientific community across the globe. A burgeoning aquaculture industry has lead decision-makers to divert increasing resources into projects aimed at devising measurable, practical and meaningful welfare indicators. But this in itself raises many questions.

In general terms, it is widely accepted that studying welfare in fish is more difficult than studying the welfare of terrestrial animals. Not only that, the underlying physiology and neurobiology of cows, pigs and sheep is more widely documented and understood than it is for sea bream or Atlantic salmon. A question like 'do fish feel pain'? is therefore more difficult to answer than the corresponding question directed towards farmed terrestrial animals.

The question of elucidating the extent and scope of sensory perception in fish has received increasing attention in recent years, and rightly so. But there are still large knowledge gaps in this respect. Nevertheless, it seems clear that in order to understand the world in which the fish inhabits, it is essential that we have a detailed understanding of piscine neurobiology. Interestingly, comparative studies have shown that fish brain structure is in some ways very different to that of mammals. But even more interesting is the fact that notwithstanding these structural differences, functional studies show that fish demonstrate behaviours consistent with sentience. So some joined up thinking is required in order to come to meaningful conclusions and to formulate practical guidelines of use to the 'end user' – the fish farmer. In this respect I am grateful to Bernice Brewster for penning an article in this issue of Aquaculture Health International that tackles this issue from her own personal perspective.

That the fish welfare debate is a complicated one goes without saying for those of us who are involved in the aquaculture industry in a professional capacity. The debate has inputs from a wide range of vantage points; it encompasses basic and applied biology at one end of the spectrum, and economics, ethics and metaphysics at the other. So the next time you hear a glib statement about fish welfare from someone critical of the aquaculture industry, perhaps a gentle reminder that like many facets of life, it's not all that simple! ■

WHO HEARS THE FISHES WHEN THEY CRY? – SENSORY PERCEPTION IN FISH

BY BERNICE BREWSTER (AQUATIC CONSULTANCY SERVICES, UNITED KINGDOM)

It is hard not to apply anthropomorphic values to the subject of pain in any animal. In many instances the term "animal" applies only to mammals, and on the whole it does not include fish or invertebrates.

It is not possible to quantify pain, and we know pain tolerance varies between individual people. Fish are generally regarded as being silent, unfeeling and possessing only a limited, five-second memory. While we cannot categorically state that animals feel pain, there is probably an overall consensus that other mammals (i.e. other than humans) can feel pain, but there is strongly divided opinion for fish and complete indifference to invertebrates, possibly with the exception of the giant squid, *Architeuthis princeps*. What cannot be denied is that the subject of whether fish feel pain is very emotive.

HEAT SENSITIVITY

Lynne Sneddon and co-workers (2003) produced evidence that rainbow trout are sensitive to heat. Among koi keepers, it is very popular to heat their pond water through the winter months. From personal observation, once the water begins to warm, koi, ornamental varieties of the common carp, *Cyrpinus carpio*, turn to face the incoming water, and over a period of a few hours gradually migrate to the pond inlet supplying the heated water.

While working within the koi industry, I was responsible for a particularly large koi pond of some 136,400 litres. In the winter months, heated water initially passed through a small pond of 13,600 litres. This smaller pond had a benched base, and water entered the large pond by transfer pipes connecting the two.

On one occasion the boiler system developed a fault, and heated the water in the small pond to a very comfortable 25° Celsius in January. Although the warmer water passed through to the larger pond, the sheer volume of water prevented it from becoming as hot.

On my arrival at the site, the majority of the koi in the pond had already jumped into this small pond, and others were still attempting to do so. It was quite a spectacle, with koi of all sizes from 15cm to 30cm all jockeying for space within this small pond.

More recently, common carp living in a lake of approximately one hectare that received thermal effluent from a factory were found in the immediate area around this pipe when netted in my presence. While I have only recently read Sneddon *et al* (2003), on the basis of the behaviour primarily of carp, I have confidently assumed that this species, and probably others, is sensitive to heat.

THE AUTHOR, BERNICE BREWSTER, AT WORK

SENSITIVITY

What about other perceptions, such as touch and of course pain? Sneddon *et al* (2003) also performed some experiments using chemical irritants, acetic acid and bee venom, and observed a change in behaviour following injection of these noxious substances into the "frontal lips" of rainbow trout (*Onchorhyncus mykiss*). A third group of trout received an injection of sterile saline, and a fourth group was handled but not injected.

While this provided some interesting behavioural changes, the truth is that for most scientists in the field, the only acceptable definition of a response is one that is quantifiable. I should not wish to dismiss observable changes in behaviour out of hand.

Fish affected by a heavy ectoparasite load show differing behaviour from uninfected fish. The fins of ectoparasite-infected fish can be seen to twitch; the fish repeatedly rub themselves against a solid surface or keep jumping out of the water and even skim along the water surface using their tail to propel them.

Taking this one stage further, tropical marine fish will patiently queue for a cleaner fish or shrimp, and observe a behavioural code to elicit parasite removal. The fish being cleaned allows the cleaning animal access to the delicate gill tissue plus other areas such as the mouth, and renders itself extremely vulnerable during this symbiotic process. If fish are insensitive, then one has to wonder, why do they behave in this manner, and how do they know they are suffering from a heavy parasite load?

Why should reef fish seek the attention of a cleaner animal, and what mechanism could cause this behaviour to evolve if they are insensitive to the parasites? Fish inadvertently presented with a noxious chemical, such as chlorine in tap water, begin to leap and dart, and display an apparent avoidance behaviour. Such a rapid onset of avoidance would indicate a response to the chemical, rather than secondary life-threatening effects such as hypoxia caused by damage to the gills.

Most of us have seen that sick fish behave differently, usually becoming very lethargic, with the fins clamped against the body and a lack of appetite. It could be construed that the fish are in discomfort or pain, but it has been pointed out to me that with the onset of disease and its effect on enzymes and other proteins it could simply be the resulting biochemical effects on physiology.

PAIN AND CONSCIOUSNESS

Pain is regarded as an unpleasant sensory and emotional event associated with injury or tissue damage, and this requires a level of consciousness. The problem is that it leads into philosophical arguments in defining consciousness, and more importantly whether fish have a level of consciousness that enables them to experience pain.

Sneddon *et al* (2003) carried out experimental work on rainbow trout that indicates the presence of nociceptors - free nerve endings that respond to noxious stimuli or injury in rainbow trout (*O. mykiss*).

In a slightly earlier paper, Rose (2002) discusses the issue of nociception in fish, and admits that this function has been more thoroughly studied in mammals. While there are similarities in teleost and mammal peripheral nerve, spinal and brain stem components, Rose concludes that the reduced size of the teleost neocortex precludes the perception of pain. There is a consensus among other scientists within the field that Rose's opinion is flawed, as we cannot dismiss that other parts of a fish's brain perceive pain, other than the neocortex.

There are many examples where an organ or tissue differs in function to that of another animal, and indeed a classic example is found in fish, where the anterior kidney is responsible for white blood cell formation.

As a scientist, I must accept that there is no irrefutable evidence that fish do or do not feel pain. Many potentially painful events are also stressors, and fish experience stress. It elicits a hormonal response similar to that seen in humans. The effects of stress in fish are well documented and are known to be deleterious, affecting growth, the immune system and in aquaculture the quality of gametes and thus fertility (Iwama *et al* 1997, Wedermeyer 1996). Irrespective of our involvement with fish, as fish farmers, coarse fishery workers or hobby fish keepers, it is our responsibility to avoid subjecting fish to potentially painful or stressful experiences.

The title of this article is taken from A Week on the Concord and Merrimack Rivers: The Writings of Henry David Thoreau (Boston: Houghton Mifflin, 1906)

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STRATEGIES FOR DISEASE CONTROL IN MARINE INTEGRATED SYSTEMS

BY DR ANGELO COLORNI, DR ARIEL DIAMANT (BOTH OF THE ISRAEL OCEANOGRAPHIC AND LIMNOLOGICAL RESEARCH LTD, ISRAEL) AND PROFESSOR ED NOGA (NORTH CAROLINA STATE UNIVERSITY, USA)

The economic success of intensive mariculture is largely because the cost of effluent treatment has never been taken into account. However, this lack of concern for the environment is coming to an end, as mariculture systems that do not address ecological management are proving to be unsustainable in the long run.

With heightened awareness of the potentially detrimental impact that fish farms have on the environment, stricter policies are being implemented to regulate effluent discharge. The pressing need for developing environmentally friendly but still economically viable culture technologies has led to a renewed interest in integrated mariculture. Such systems incorporate multiple trophic levels (fish, invertebrates and algae) in a cascade-like manner in which energy and matter flow between the constituent elements (Figure 1).

INTEGRATED SYSTEMS AND PATHOGENS

Major resources in several countries are being invested in developing more cost-effective models of integrated systems, because capital and operating costs are generally much higher than in open (flow-through) systems. Integrated systems are often semi-recirculating, so environmental pollution can be drastically reduced.

However, these systems must typically be highly intensive to be economically viable, and under such stressful conditions for the organisms raised, infectious diseases are a major constraint, as new health management problems related to the nature of these systems have arisen. Physico-chemical factors such as salinity, organic matter, nitrogenous wastes, suspended solids, pH, carbon dioxide levels, temperature, solar radiation and the use of drugs each play a different epidemiological role according to the nature of a particular pathogenic agent.

Outside their natural hosts, certain aquatic bacteria (e.g. certain *Vibrio* spp.) fail to grow on standard laboratory media. This viable but non-culturable (VBNC) state frustrates efforts to detect and monitor such pathogens.

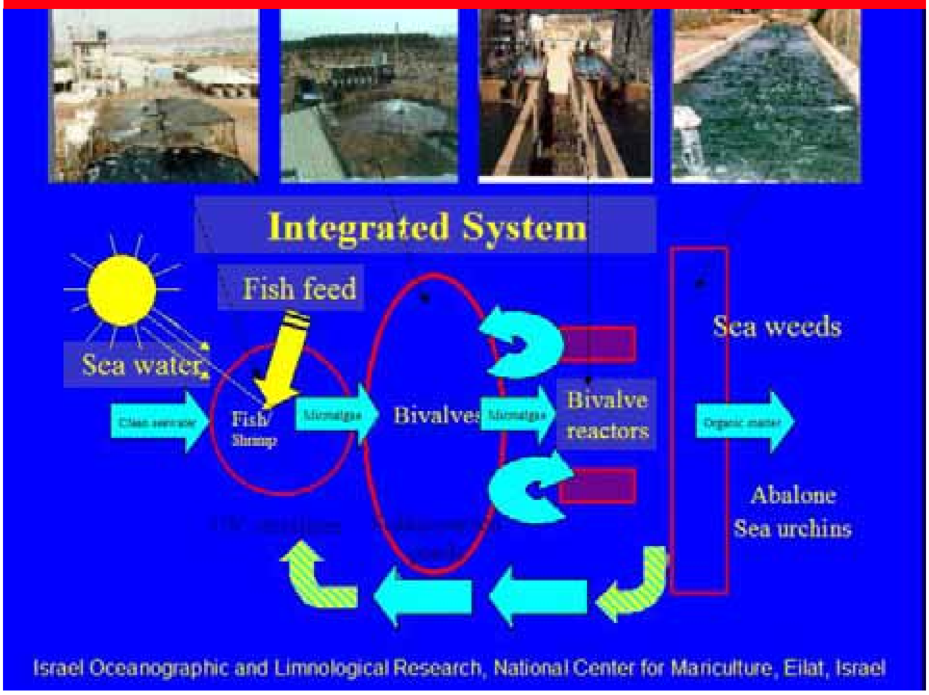
Certain parasites (e.g. *Cryptocaryon irritans*) have asynchronous excystment, with same-age tomonts (reproductive stage) developing and releasing hundreds of theronts (free-swimming infective stage) weeks apart, a survival strategy that thwarts efforts to control the infection in a confined environment. Interaction or competition of pathogens with the microflora and microfauna residing in the sediments and bio-filters determines the pathogens' ability to survive, remain infective, or propagate. Also, the long-term effects of various drugs on each cultured species, as well as their degradation rates in these man-made ecosystems, are largely unknown.

The stressful conditions of intensive mariculture can also facilitate the breaching of natural barriers to pathogen transmission (Colorni 1994, Colorni and Diamant 2005). Non-fastidious pathogens can easily jump species to include an even broader host range. For example, the same strain of *Mycobacterium marinum* was found to infect not only several genera of captive Red Sea fish, but also the hawksbill sea turtle *Eretmochelys imbricata* (Ucko *et al* 2002). Penaeid shrimp are also affected by mycobacteriosis (Brock and Main 1994, Lightner 1996). When such insidious pathogens gain entry into a system, destruction of the entire stock, disinfecting the facility and restarting the culture with clean stock may be the only reasonable choice available to the farmer, as fish that have survived an epizootic and haverecovered may be latent carriers, posing a significant risk to the entire population.

DISEASE MANAGEMENT

Concerns about bioaccumulation of drugs (eg antibiotics and heavy metals) in both the culture system and in non-target organisms have underscored the need for innovative therapies that avoid the use of drugs. The most serious parasites threatening fish in warm water marine intensive systems are two protozoan ectoparasites, the dinoflagellate *Amyloodinium ocellatum* (Figure 2) and the ciliate *Cryptocaryon irritans* (Figure 3).

FIGURE 1: INTEGRATED MARICULTURE SYSTEMS INCORPORATE MULTIPLE TROPHIC LEVELS (FISH, INVERTEBRATES AND ALGAE) IN A CASCADE-LIKE MANNER IN WHICH ENERGY AND MATTER FLOW BETWEEN THE CONSTITUENT ELEMENTS



When fish are chronically stressed, AMPP levels can decrease significantly before the fish show any signs of disease

Controlling these and other pathogens using traditional chemotherapeutics can have serious side effects on both the host fish and non-target organisms (Diamant and Colorni 2003).

Increasing water-flow velocity in tanks holding *I. multifiliis*-infected fish can sweep theronts away, and was suggested as an effective treatment (Bodensteiner *et al* 2000). Due to the extraordinary similarity of *Ichthyophthirius multifiliis* to *Cryptocaryon irritans* (the two ciliates have often been referred to as each other's freshwater and marine counterpart), this method would most likely be successful in hindering host interception by *C. irritans* theronts as well (Diamant and Colorni 2003).

Theronts' life span can extend from 24 to 48 hours post-excystment, but their infectivity sharply declines after the first six to eight hours. Furthermore, taking advantage of *C. irritans*' regular circadian rhythms, the treatment can be administered for a few hours late at night, when mature parasites exit the fish epithelia and mature theronts exit the mother cysts (Colorni and Burgess 1997).

Filtering activity by some invertebrates can experimentally reduce the concentration of *Amyloodinium* dinospores in seawater (Oestmann *et al* 1995). Biofiltration by sponges (*Negombata magnifica*, syn. *Sigmosceptrella laevis*) (Hadas 2006) and hard corals (*Acropora* sp. and *Stylophora pistillata*) (Shpiegel, unpubl. data) was tested at IOLR (Figure 4, page 7), as sponges sieve and digest particles of various sizes, from viruses to bacteria, while coral tentacles can easily trap particles the size of *Amyloodinium ocellatum* dinospores (12-15µm) and *Cryptocaryon irritans* theronts (25 x 57µm).

In addition to their high filtering or entrapment rates, some species of sponges and corals appear to produce powerful antibiotics (Baslow 1977). Despite encouraging preliminary results, culturing these organisms requires expertise and facilities that as of now are only within the realm of research institutes.

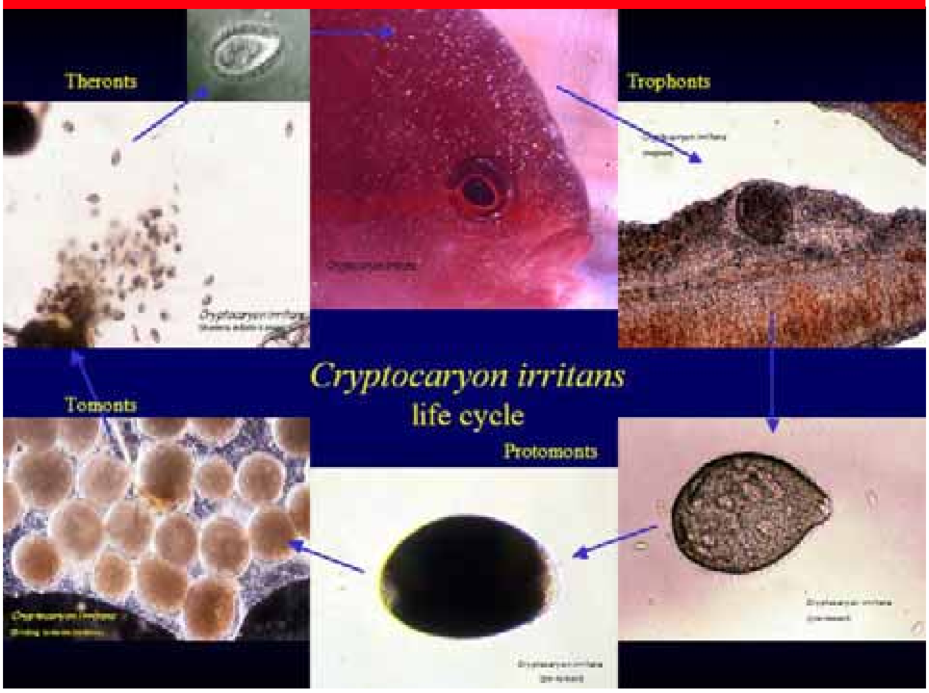
The desire to reduce drug use to a minimum has provided the incentive to develop means of enhancing immunity to various infectious diseases. A number of vaccines (particularly against certain bacterial diseases) are commercially successful and widely used. Ideally, a vaccine should not only be effective but also safe, easy to administer and inexpensive to produce. Unfortunately these features are often mutually exclusive.

Most recently, attempts to improve the efficacy of vaccines have focused on recombinant or DNA vaccines based on a narrow suite of selected antigens. However, these preparations typically appear to be less protective than traditional vaccines prepared from whole microbes (Ellis 1999, Bruno and Woo 2000). Another problem facing vaccine use is that the immune system of marine fish is generally not well developed before the first two to four months of life, during which time competence in responding to specific antigens is virtually non-existent (Manetti *et al* 1999, Toranzo et al 2003).

Even after the lymphoid organs have become functionally mature, the specific immune response can be slow to respond, highly suppressed by low temperature and have a short memory. Only IgM-like antibodies are produced, which have intrinsically low avidity and lack ▶

FIGURE 2: THE DINOFLAGELLATE *AMYLOODINIUM OCELLATUM* IS A SERIOUS THREAT TO WARM-WATER MARINE INTENSIVE SYSTEMS

FIGURE 3: YET ANOTHER SERIOUS THREAT IN WARM-WATER SYSTEMS - THE CILIATE *CRYPTOCARYON IRRITANS*



STRATEGIES FOR DISEASE CONTROL IN MARINE INTEGRATED SYSTEMS

FIGURE 4: BIOFILTRATION BY SPONGES AND CORALS HAS BEEN TESTED AT IOLR

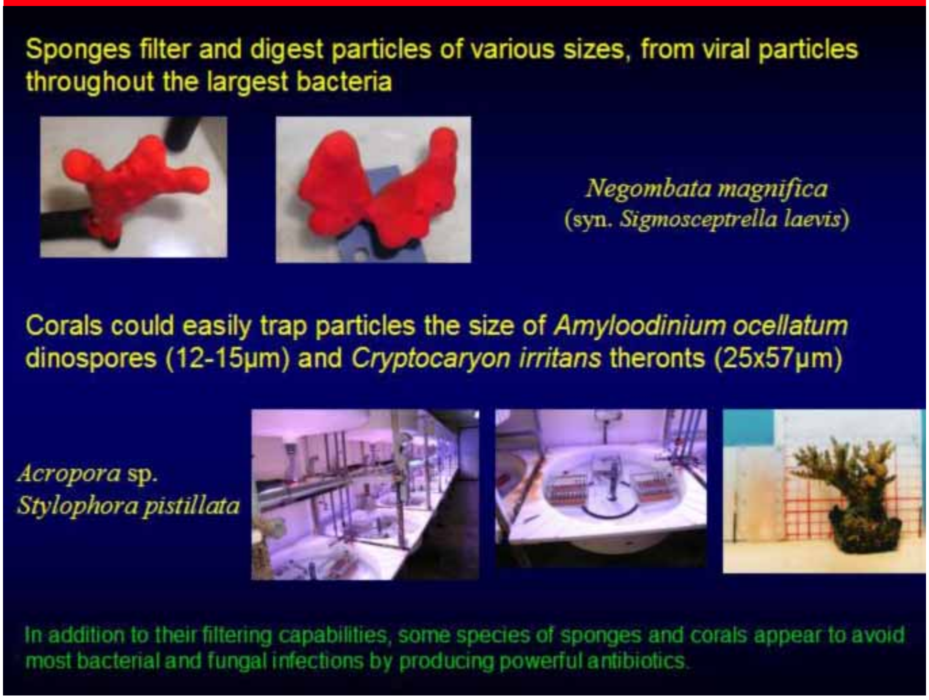


FIGURE 5: AMPPS HAVE BEEN ISOLATED OR OTHERWISE IDENTIFIED FROM AN INCREASINGLY LARGE NUMBER OF FISH

Examples of Antimicrobial Polypeptide (AMPP) Defenses in Fish

Pardaxin from sole *Pardachirus pavoninus* (Thompson et al. 1986)

Pleurocidin from winter flounder *Pleuronectes americanus* (Cole et al. 1997, 2000)

Misgurin from loach *Misgurnus anguillicaudatus* (Park et al. 1997)

Parasin I from catfish *Parasilurus asotus* (Park et al. 1998)

Histone-like proteins (HLPs) from channel catfish *Ictalurus punctatus*, rainbow trout *Oncorhynchus mykiss*, and hybrid striped bass *Morone saxatilis* ♂ x *M. chrysops* ♀ (Robinette et al. 1998, Noga et al. 2002)

AMPP from hagfish *Epatretus burgeri* (Seo et al. 2000)

Histone H1 from Atlantic salmon *Salmo salar* (Richards et al. 2001)

Piscidins from hybrid striped bass *M. saxatilis* ♂ x *M. chrysops* ♀, spot *Leiostomus xanthurus*, and croaker *Micropogonias undulatus* (Silphaduang and Noga 2001)

Moronecidins from white bass *M. chrysops* and striped bass *M. saxatilis* (Lauth et al. 2002)

affinity maturation, so that even high titers of circulating antibodies do not always correlate well with protection (see Kaattari and Piganelli 1996). These shortcomings have been responsible for the relatively minor progress made in the development of effective vaccines for many commercially important fish diseases.

The fact that healthy, unstressed fish are normally capable of effectively controlling their resident flora and limiting infection suggests that these lower vertebrates depend heavily on innate, non-specific immunity. Antimicrobial polypeptides (AMPPs), host-produced peptides and small proteins with powerful antimicrobial properties are increasingly recognised as playing a critical role in defence against pathogen invasion (Finlay and Hancock 2004).

They have been isolated from all major classes of vertebrates as well as many invertebrates (shrimp, crabs, oysters, etc) and are probably present in virtually all animals. AMPPs have been isolated or otherwise identified from an increasingly large number of fish, including many commercially important species (Figure 5).

In some cases, a single AMPP can kill pathogenic viruses, bacteria, fungi and parasites. AMPPs are typically expressed at major portals of pathogen entry, such as the epithelial surfaces of skin, gills and intestinal tract (Noga and Silphaduang 2003).

AMPPs such as piscidins and HLPs appear to be present in a wide range of fish species. HLPs are lethal to a number of important pathogens, including water moulds and *Amyloodinium*, at concentrations that are well within the levels that appear to be present in healthy tissues (Robinette *et al* 1998, Noga *et al* 2002).

When fish are chronically stressed, AMPP levels can decrease significantly before the fish show any signs of disease (Robinette and Noga 2001). Thus, depressed AMPP levels might be a significant risk factor for increasing the susceptibility of fish to any of a number of infections. This suggests that monitoring AMPP levels in fish might be a highly effective means of providing an early warning of when a fish population might be at risk of disease, and thus allow the farmer to implement management changes before an epidemic ensues.

While AMPPs in fish have been poorly investigated, a number of studies in other animals have shown that these polypeptides can be rapidly up-regulated after exposure to certain stimuli, such as bacteria, lipopolysaccharide or beta-glucan. This is reminiscent of compounds tested as immunostimulants, probiotics or “anti-stress” factors.

While such formulations have been studied for many years, results have often been too modest for these preparations to step beyond the laboratory threshold and into widespread commercialisation. Even for those products that are currently commercialised, the mechanisms responsible for the putative protection are poorly understood and often difficult to reproduce. It is likely that AMPPs play some role in the efficacy of these immune enhancers. Determining the exact molecular mechanisms by which AMPPs are up and down-regulated in fish, including the specific microbial, physiological and environmental factors affecting their expression and function, is a critical area of future research, because the ability to up-regulate or at least maintain optimal levels of AMPPs will reduce the need for and dependence on drugs, and antibiotics in particular.

Just as scientific research of some 70 years ago produced antibiotics and pesticides, with immediate advantages and a long-term downside, so the discovery of the fish endogenous antimicrobial polypeptides may ultimately lead to an organic, healthier and in the long-term environment-friendly mariculture.

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news

CANADA: NEW HEAD FOR HEALTH SCIENCES

Ms Linda Sams is the new chief executive officer of the British Columbia Centre for Aquatic Health Sciences.

Prior to her new position, which took effect on June 1, Ms Sams was a member of Marine Harvest Canada's executive management team, serving as senior biologist and subsequently manager of environment and community relations.

In recognition of her success in these positions, Ms Sams won five Aquaculture Achievement Awards between 1999 and 2006; in 2001 she also shared the Herb Dhaliwhal Environmentally Sustainable Aquaculture Award.

"We're delighted that Linda has agreed to accept this new position," said the former chief executive of the centre, Dr Jim Brackett. "The CAHS is dedicated to facilitating the economic, social and environmental sustainability of BC's aquatic-based resource industries. Linda's education and experience in environmental management, together with her expertise in business and strategic planning, made her the ideal choice," he said.

Dr Brackett will continue to serve on the board of directors. "Dr Brackett's strong leadership in establishing the concept of a collaborative aquatic research facility will make my job a lot easier," said Ms Sams. "As a result of his and the board's foresight, the CAHS has established a solid foundation for an integrated approach to aquatic health sciences in British Columbia."

Ms Sams says she is looking forward to further strengthening the centre's existing relationships - and establishing new relationships - with the government, First Nations representatives, researchers, industry and the public. In addition to conducting cutting-edge research, she envisions CAHS functioning as a community-based facilitator capable of drawing together diverse expertise to address specific aquatic health issues.

"We all have a responsibility to ensure the health and sustainability of our marine-based industries," said Ms Sams. To fully address issues of aquatic animal health and welfare, all parties had to work together in a spirit of cooperation and collaboration.

SINGAPORE: NEW MANAGER AT INTERVET NORBIO

Dr Brian Sheehan has taken up the position of R&D and Site Manager at Intervet Norbio Singapore. Prior to this, Brian worked as a project manager in the R&D department of Intervet UK. A native of Ireland, Brian obtained a PhD in Microbiology and Infectious Diseases in 1992 from Trinity College Dublin.

After graduating, he conducted research on the pathogenesis of a diverse range of bacteria of medical and veterinary importance, holding positions in the Institute Pasteur, Paris and Imperial College School of Medicine, London, before joining Intervet in 2001.

BRIAN SHEEHAN AND HIS DAUGHTER ENJOY THE WILDLIFE IN SINGAPORE

AUSTRALIA: POLICY MEMORANDUM ON PRAWN IRA

Biosecurity Australia has recently released a Policy Memorandum, or BAPM, informing stakeholders of a change to the prawns and prawn products IRA team, and of the outcome of initial research into the susceptibility of Australian prawns to the taura syndrome virus.

The prawn IRA began in 1996 with an issues paper entitled Animal Quarantine Policy Memoranda 1998/96 released in 1998, and a draft IRA report, the Animal Biosecurity Policy Memorandum (ABPM) 2000/41, released in August 2000.

Interim measures for uncooked prawns were introduced in 2000 and 2001 to address risks associated with the white spot syndrome virus, WSSV, and the yellowhead virus (ABPM 2000/57, 2001/06 and 2001/11).

These include size limitations, health certification from the relevant government authority in the exporting country, post-arrival inspection in Australia by the Australian Quarantine and Inspection Service and testing for WSSV. In May 2002, it was decided to release a revised draft IRA report that is now at an advanced stage of preparation.

The IRA team has continued to meet regularly to prepare the revised draft report. Additional resources within Biosecurity Australia have been allocated to the IRA, and the principal scientist of Biosecurity Australia, Dr Mike Nunn, has taken over as chair of the IRA team. He comes with extensive experience in scientific analysis and providing advice in animal health policy.

Dr Robyn Martin, the general manager, animal biosecurity of Biosecurity Australia, will remain a member of the IRA team. The overall membership of the prawn IRA team is therefore:

- Dr Mike Nunn, principal scientist, animal biosecurity, Biosecurity Australia (chair)
- Dr Leigh Owens (James Cook University) aquatic animal health expert
- Dr Brian Jones (Department of Fisheries, Western Australia) aquatic animal health expert
- Glen Hurry (DAFF) fisheries and aquaculture management, and
- Dr Robyn Martin, general manager, animal biosecurity, Biosecurity Australia.

Biosecurity Australia and the IRA team have been monitoring developments regarding the emergence of TSV, including the spread of TSV into parts of Asia, the increased volume of imports, the lower cost of vannamei imports from Asia, and scientific information on the virus. TSV is exotic to Australia and is not a human health concern.

Taura syndrome is an internationally reportable viral disease affecting mainly vannamei prawns (*Litopenaeus vannamei*). Originally reported from Ecuador in cultured *L. vannamei*, the disease has since been associated with production losses in the Americas, and more recently in Asia. Its spread is usually associated with movements of live prawns. It is not known to manifest as a significant disease in the prawn species produced in Australia, and there is no commercial vannamei prawn fishing or farming in Australia.

Biosecurity Australia has commissioned the Aquaculture Pathology Laboratory, University of Arizona in the United States to research the susceptibility of five Australian crustacean species to infection with the Thai and Belize isolates of TSV by ingestion of infected prawn meat or by injection. The species are:

- Banana prawn, *Fenneropenaeus merguiensis* (challenged only with Thai TSV isolate),
- Black tiger prawn, *Penaeus monodon*
- Redclaw, *Cherax quadricarinatus*
- Marron, *Cherax tenuimanus*, and

- the giant freshwater prawn *Macrobrachium rosenbergii*.

Significant clinical disease due to TSV was not observed in any of the Australian species challenged. Nor did challenge with TSV result in cumulative mass mortalities that are typical of TSV outbreaks among susceptible species.

TSV nucleic acid was detected in all the species, although an active (replicative) infection was only detected in *Fenneropenaeus merguiensis* and *Penaeus monodon*, following injection challenge. The positive control prawns, *L. vannamei*, which were treated in the same way as the Australian animals, developed the disease and died. Hard copies of the report are available from Biosecurity Australia, or see www.biosecurityaustralia.gov.au

Due to the low number of some experimental animals, Biosecurity Australia has commissioned the University of Arizona to repeat the study for banana and black tiger prawns.

Biosecurity Australia and the IRA team will continue to monitor and review the situation in light of the latest scientific information on TSV, and will continue to assess the suitability of the current controls to meet any new or changed risks.

See www.biosecurityaustralia.gov.au. For further information on the IRA team members, contact Warren Vant +61 (02) 6272 4436 or email warren.vant@daff.gov.au

ORNAMENTAL FISH DANGERS

Imported ornamental fish pose a serious threat to Australia's aquaculture industries and the environment, says the chair of Farm Animal Health at The University of Sydney, Professor Richard Whittington. He warned delegates at the Australian Veterinary Association's annual conference it was unlikely that current controls over the importation of exotic fish would prevent the establishment of new pathogens and parasites with the potential to cause serious animal health problems in Australia.

"Despite stringent quarantine protocols, the evidence is that diseases from ornamental fish are spreading across Australia," Professor Whittington said. "In 2006 there were 22 species of alien ornamental fish with established breeding populations in the waterways of Australia. These have the potential to become invasive and alter the environment, much like carp, or become agents for the spread of disease."

Professor Whittington presented case studies of a number of infectious agents and parasites that have already been introduced to Australia, including:

- a virus, Gourami iridovirus, introduced by the gourami goldfish that killed 90 percent of murray cod in an aquaculture facility in 2003
- a bacterial pathogen, *Aeromonas salmonicida*, associated with goldfish imported from Japan, and now identified in native silver perch, and
- a bacterial pathogen, *Edwardsiella tarda*, isolated from fighting fish which was identified as a cause of mortalities in farmed rainbow trout.

Professor Whittington said that the high number of imported species, a high prevalence of pathogens and parasites in imported fish, the lack of post-border quarantine and the potential transmission of disease by asymptomatic carriers were all factors that led him to believe imported fish pose a continuing threat.

"Either the international community must adopt policies that reduce the potential risk of spreading pathogens, or Australia should consider dramatically reducing the number of imported ornamental fish," he said.

CHILE: SALMON VACCINE GAINS APPROVAL

AquaVac™ Oral IPN vaccine for farm-raised salmon has been approved for use in Chile. According to the Schering-Plough Animal Health Corporation, which markets the vaccine, it provides a highly effective and easy way for fish farmers to prevent infectious pancreatic necrosis, or IPN, and cut losses from the viral disease. The vaccine is said to be the only oral vaccine available for IPN.

"AquaVac™ Oral IPN is an excellent tool for the control and prevention of IPN in fish that are too small to inject," says Dr Jorge Martinez, a veterinarian and regional technical manager for Schering-Plough Animal Health. "The vaccine is simply mixed into feed and is totally stress-free, because the fish do not need to be handled. Salmon producers can now produce the required quantity and quality of smolts without fear of deadly IPN outbreaks."

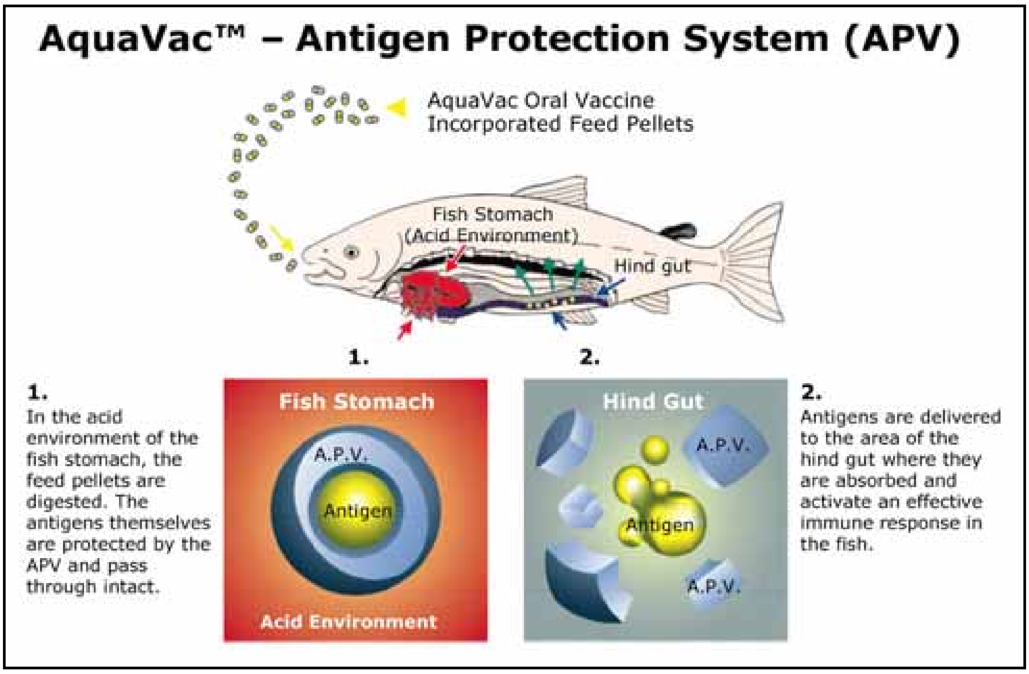
He says an important key to the efficacy of the vaccine was the antigen protection vehicle developed by the corporation. The antigens in the vaccine pellet are encapsulated and protected from destructive gastric acids until they reach the hind-gut, where they initiate immunity.

IPN causes significant mortality, particularly in fry. Those that survived IPN showed reduced smolt quality, higher variation in fish size and reduced feed conversion efficiency, leading to increased costs for fish farmers, said Dr Martinez. Survivors are presumed to be carriers of the disease.

IPN is a serious problem for salmon producers not only in Chile, but also in Scotland, Ireland and Norway. Average losses in Chile in fry from IPN are about 15 percent, but in many cases mortality reached 70 percent, he says.

In a tank challenge study, the vaccine significantly reduced mortality. Relative percent survival was improved by up to 87 percent. In addition, three separate field trials in Chile showed that survival after administration of the vaccine at the hatchery exceeded 60 percent. The vaccine reduced size variation by 36 percent and improved the specific growth rate by 13 percent after 10 weeks, he said.

An economic analysis revealed that salmon farmers who ▶



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correctly implemented the vaccine would gain a financial advantage.

"Ultimately, the rigorous studies demonstrating the value of the vaccine led to the granting of approval from the Chilean authorities," Dr Martinez said. "We expect salmon farmers in Chile to see a significant reduction in IPN losses."

Contact Chris Haacke at +44 (0) 1799 528 167 or email chris.haacke@spcorp.com. See www.spaquaculture.com

UNITED KINGDOM: BONAMIA DETECTED IN WALES

According to the OIE website, www.oie.int, *Bonamia ostreae* has been detected in a harvested natural bed of flat oysters, *Ostrea edulis,* in the River Cleddau in southwestern Wales.

Bonamia ostreae was suspected in one oyster following histological examination of a routine sample of 30 *Ostrea edulis* collected in late March from Burton Beach (Site A). Two further samples of 150 oysters (from site A), and 60 oysters from Hazelbeach (Site B) were collected and processed in late April. Two oysters from site A were found to be positive for *Bonamia ostreae* by heart smears and histology.

Sections were also examined by electron microscope and samples of tissue analysed by molecular biology techniques. Confirmation of identification of the *Bonamia ostreae* parasite was made by PCR. The source of infection is unknown and is under investigation.

See www.oie.int/Messages/060522GBR.htm

OUTBREAK OF KOI HERPES VIRUS

The Environment Agency and the Centre for Environment, Fisheries and Aquaculture Science are investigating outbreaks of koi herpes virus, KHV, disease at three stillwater fisheries in the south and east of England. These are the first KHV mortalities recorded this year in fisheries, and the most serious losses since the disease was first recorded in the United Kingdom.

To help prevent further spread of the disease, the Environment Agency has announced that it will not permit any live fish movements off the affected sites until further notice. The agency says it is working closely with CEFAS to find the source of the outbreak and identify any further sites that may be infected.

KHV is not currently one of the diseases subject to specific control measures under EU legislation. However, the disease has been included in the list of non-exotic diseases that will be subject to control on a European Community-wide basis in the proposal for a new council directive on animal health requirements for aquaculture animals currently being negotiated in Brussels.

Contact fish.health.inspectorate@cefas.co.uk, or the Environment Agency, National Fisheries Laboratory, Brampton on +44 (0) 1480 483849, email nflfh@environmentagency.gov.uk

SECOND EDITION OF MAJOR FISH PATHOLOGY BOOK

The second edition of Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and their Response in Disease, edited by Professor Hugh Ferguson of the University of Stirling, has been published. (Scotian Press, from £120, including delivery)

This edition has been expanded to 368 pages and almost 700 figures, including light, scanning and transmission electron micrographs. It retains the hardback special-bound format of the first edition, but is now in full colour on high quality non-reflective paper, with integrated text and figures. The page size has been increased to make best use of the illustrations, some of which are half or full page. Numerous high-quality images of fish have been used to separate and introduce the chapters to enhance readability and overall appearance.

As before, the book is not intended to be simply a list of aetiologies found in various species under differing environments. Instead, the systemic approach to understanding disease has been maintained, with chapters on all organ systems, and descriptions on how involved tissues respond to a variety of insults. With the addition of an extra chapter on pathophysiology, there are now 14 chapters overall, each with selected references, plus an index. There has been no compromise on quality in the production of this book, and a serious attempt has been made to blend aesthetics with scientific rigour and ease of use.

While a working knowledge of the language appropriate to the subject will be useful to readers, the book is intended for research workers, students and diagnosticians who are experienced with fish, but who are not necessarily familiar with the discipline of pathology. The book is also aimed at experienced veterinary pathologists who see only the occasional fish case.

Accordingly, each chapter starts with information on normal structure and function, and tries to place this within a vertebrate context. Examples chosen to illustrate tissue responses are selected from intensively reared food fish, as well as species in the aquarium and the wild.

The list of contributing authors is extensive, and includes Ellen Bjerkås, Øystein Evensen, Hugh W Ferguson, Salvatore Frasca Jr, Erling Olaf Koppang, John F Leatherland, John S Lumsden, Daniel Martineau, Edward J Noga, Trygve T Poppe, Mark Powell, Renate Reimschuessel, David J Speare and Jimmy Turnbull.

The editor, Professor Ferguson, has spent his entire professional life of almost 35 years as a diagnostic veterinary pathologist, research scientist and teacher, focused mainly on fish and the aquatic environment. He wrote or co-wrote roughly half of the book and supplied most of the images.

Scotian Press is a new publishing company dedicated to producing textbooks for the veterinary and scientific community. A full review of the book will appear in the next issue of *Aquaculture Health International.*

See www.scotianpress.com

BKD OUTBREAK IN DORSET

The presence of bacterial kidney disease has been confirmed in a trout farm in Dorset. The Department of Environment, Food and Rural Affairs issued an order under the Diseases of Fish Act 1937 prohibiting all movement of fish to and from the infected fish farm and fishery.

The disease was found in a trout sample during a routine fish health-monitoring visit to the farm. Fish health inspectors examined the source of the outbreak and investigations were continuing. While the disease is considered serious and notifiable under European Community law, it is not widespread in Great Britain and occurs only sporadically.

Fish infected with BKD may display a number of characteristics, including protruding eyes, a swollen abdomen, pale, anaemic gills and haemorrhaging at the base of the gills. BKD can cause large numbers of mortalities in both farmed and wild salmon and trout. It was first recognised in Atlantic salmon on the River Dee, Scotland in the 1930s, and in 1976 there was the first notable case of BKD in farmed rainbow trout.

UNITED STATES: KOI HERPES VIRUS FOUND IN TEXAS

Koi herpes virus was detected in a common carp (*Cyprinus carpio*) collected from a fish kill during May 2006 at the Twin Buttes Reservoir near San Angelo, Texas.

Also known as carp nephritis and gill necrosis virus, or CNGV, KHV is highly contagious, infects both common carp and ornamental koi carp, and can result in high mortality rates.

Biologists from the Texas Parks and Wildlife Department investigated the kill and estimated 500 dead common carp along a 1km segment of shoreline, which converts to thousands of dead fish in the reservoir. The surface water temperature was 22° Celsius, and dissolved oxygen measured 9.2 mg/l.

One moribund fish was collected and shipped to the Southeastern Cooperative Fish Disease Project at Auburn University, where KHV DNA was detected using the polymerase chain reaction. A concomitant *Flavobacterium columnare* infection was also observed, and it is unclear what role the virus played in the fish kill.

This is the first known report of KHV in the state, and the virus has the potential to affect wild populations as well as TPWD hatcheries, which use koi carp as a forage for some species of captive broodstock.

Contact Greg Southard, Fish Health Inspector, Texas Parks and Wildlife Dept, AE Wood Laboratory, San Marcos, Texas 78666 USA, or email greg.southard@tpwd.state.tx.us

VHS FOUND IN YORKSHIRE

Scientists at the Centre for Environment Fisheries and Aquaculture Science are investigating the origins of an outbreak of a notifiable fish disease at a trout farm in England. Movement restriction orders on fish farms along the entire River Ouse catchment area in Yorkshire were put in place following confirmation of viral haemorrhagic septicaemia.

CEFAS later confirmed that the VHSV virus had been detected in a sample of grayling taken from the River Nidd below the outlet of the farm infected with the disease. This is the first outbreak of VHS recorded in mainland Great Britain, although there was an outbreak of the marine form of the disease in farmed turbot in the Isle of Gigha in 1994.

See www.efishbusiness.co.uk/news/default.asp

GENEROUS GRANTS FOR WHIRLING DISEASE STUDY

Whirling disease remains a high-profile issue a dozen years after it was discovered in wild trout and streams of the Intermountain West, say officials at the Montana Water Centre of the Montana State University in Bozeman, Montana.

Six research teams in the west received more than US$650,000 in the latest round of grants from the Whirling Disease Initiative to advance management solutions. Research teams based in seven western states will tackle investigations ranging from regional risk assessments to intensive laboratory studies.

The initiative is a national whirling disease research programme administered by the Montana Water Centre. The research projects will be conducted between May 15, 2006 and the end of 2008.

Billie Kerans of the university is leading two of the studies. Kerans and Thomas McMahon, also of MSU, received almost $246,000 to carry out a state-wide study in Montana of patterns in whirling disease risk and salmonid population response.

In a separate study, Kerans and Todd Koel from the National Park Service received $68,283 to study whirling disease as it relates to Yellowstone cutthroat trout in Yellowstone National Park, and variations in the aquatic worm *Tubifex tubifex*.

A UNIVERSITY OF MONTANA STUDENT INSPECTS *TUBIFEX TUBIFEX* WORMS FOUND IN THE ROCK CREEK DRAINAGE. THIS INFORMATION WILL BE USED IN A NEW STATE-WIDE WHIRLING DISEASE STUDY TO BE CONDUCTED BY BILLIE KERANS AND THOMAS MCMAHON OF MONTANA STATE UNIVERSITY (PHOTO COURTESY OF MONTANA WATER CENTRE).

Whirling disease was discovered in the United States in a Pennsylvania fish hatchery in 1956. However, it wasn't until 1994 that scientists determined that the disease caused population losses in wild trout. The disease is caused by the *Myxobolus cerebralis* parasite, which uses salmonid fish and *Tubifex tubifex* worms as hosts, and has been a major contributor to the loss of wild trout in the Intermountain West.

Other research projects that received funding this year are based in Colorado, Oregon, California, New Mexico, Arizona and Utah. Those grants received funds ranging from more than $20,000 to more than $196,000. These studies deal with:

- resolving uncertainties in the introduction of whirling disease and establishment risks
- evaluating the effect of substratum on the development and release of a certain stage of the whirling disease parasite in resistant strains of *Tubifex tubifex*
- developing a regional risk assessment for native salmonids in arid and semi-arid lands of the Southwest, and
- testing competition among *Tubifex tubifex* lineages and the potential for biological control of whirling disease in natural streams.

The Whirling Disease Initiative supports research that looks for practical ways to maintain viable, self-sustaining wild trout populations. See http://whirlingdisease.montana.edu, or contact the outreach coordinator and programme biologist, Kajsa Stromberg, on +1 406 994 2550, email kstromberg@montana.edu

VIRUS ISOLATED FROM WILD FISH

According to the OIE website, www.oie.int, strain IVb of the viral haemorrhagic septicaemia virus has been confirmed in a number of species of wild fish in fresh water. Many of these species are currently not listed in the OIE's Aquatic Animal Health Code as being susceptible to VHS. The species affected are:

- round goby (*Neogobius melanostomus*) and muskellung (*Esox masquinongy*) in New York State
- gizzard shad (*Dorosoma cepedianum*), yellow perch (*Perca flavescens*), northern pike (*Esox lucius*), silver redhorse (*Moxostoma anisurum*) and shorthead redhorse (*Moxostoma macrolepidotum*) in Michigan State
- freshwater drum (*Aplodinotus grunniens*), yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieui*), walleye (*Stizostedion vitreum*) and white bass (*Morone chrysops*) in Ohio State.

See www.oie.int/eng/info/hebdo/AIS_10.HTM#Sec7

INTERNATIONAL: MEMBERSHIP STATISTICS ANALYSED

The Permanent Advisory Network for Diseases of Aquaculture, known as Panda, has released a detailed analysis of its membership, currently running at 293 individuals from 50 different countries.

See www.europanda.net/docs/PANDA_network_ membership.pdf

VACCINE DATABASE OPENS IN EUROPE

A searchable database of fish vaccines is now available in Europe.

The Knowledge Database is the Aquaculture Innovation Network's searchable repository for sharing information useful for innovation and technology transfer in European aquaculture. A relatively recent addition to the network's website is a searchable database of fish vaccines and suppliers in Europe.

To date, the database contains information on a range of diseases, including Coldwater vibriosis, Columnaris disease, Edwardsiellosis, ERM, Furunculosis, IPN, Pasteurellosis, Vibriosis and Winter ulcer, and species, including Channel catfish, marine species and salmonids.

See www.aquainnovation.net/aquainnovation/knowledgebase/vaccines_en.asp ■

CONFERENCES AND MEETINGS

EUROPE

Advances in Fish Disease, Diagnosis and Treatment

Birkbeck College, London, UK. October 6

European Scientific Conferences (EuroSciCon) is organising a meeting that will highlight advances in a range of fields, including molecular diagnostics, virology, serology, immunodiagnostics and parasitology, and show how these can be applied.

Talk titles include:

- Detection of viraemia and virus-neutralising antibodies - complementary tools for the diagnosis of salmonid alphavirus infections (Dr David Graham, Fish Diseases Unit, Belfast, UK)
- Does the rosette agent have relatives in Europe? (Dr Rodolphe Gozlan, CEH Dorset, Natural Environment Research Council, Winfrith Technology Centre, Dorchester, UK)
- Development and use of probiotics in aquaculture (Prof Brian Austin, Heriot-Watt University, Edinburgh, UK)
- Serum pentraxins: a diagnostic technique for monitoring general health status in fish (Dr Dave Hoole, University of Keele, UK)
- Progress in Fish Immunodiagnostics (Professor Sandra Adams, University of Stirling, UK)
- Immunostimulants: their use in fish health management and disease control (Dr Ian Bricknell, Marine Laboratory, Aberdeen, UK)

See www.regonline.co.uk/eventinfo.asp?EventId=25344

Fish Breeders' Round Table

Ålesund, Norway. June 11-13, 2007

The Fish Breeders' Round Table is an international forum where knowledge and experience is exchanged between fish breeding researchers and those involved in applied genetic improvement work on a commercial basis. The planned 2007 event follows on from the highly successful meeting held in 2004.

In 2007 the main areas of discussion will include:

- the use of molecular genetics in selective breeding programmes for aquaculture species: QTL detection, marker-assisted selection and genomic selection, genetic markers for parentage testing, and so on, and
- selective breeding and animal welfare: definition of animal welfare traits, analysis of challenge test data, sustainable breeding.

There will also be sessions with open discussion. In order to stimulate active discussion, the organisers have stipulated that each company in attendance should give at least one presentation.

This prerequisite was appreciated at the last meeting, as it facilitated greater participation in the discussion. No proceedings will be published, and presentations will not be put onto CDs.

See www.akvaforsk.no/fbrt/index.html

Seventh Nordic Symposium on Fish Immunology

University of Stirling, Stirling, Scotland. June 17-22, 2007

The seventh international symposium on fish immunology, organised by the Nordic Society for Fish Immunology (NOFFI), will be held in June 2007 in Scotland.

This event is held every three years, and as with previous meetings, scientists from around the world are invited to attend to discuss recent advances in fish immunology. The conference will last for three-and-a-half days, and will include plenary and keynote lectures, and oral and poster presentations covering basic and applied fish immunology. There will also be a workshop on the day before the conference aimed at PhD students and young researchers which will focus on new, cutting-edge technologies in fish immunology.

See www.noffi.org

13th International EAFP Conference on Diseases of Fish and Shellfish

Conference Centre, Grado, Italy. September 17-21, 2007

The 13th EAFP International Conference on Fish and Shellfish Pathology will be held at Grado, Italy. Scientific and technical sessions consisting of poster presentations, invited talks, keynotes, oral presentations, workshops and an EAFP general assembly will take place during the conference. Planned social events include a welcome cocktail party, a civic reception and the traditional conference banquet.

The conference is being organised by the council of the EAFP and the local organising committee. The committee members are the chairman, Marco Galeotti, plus Giuseppe Bovo, Letizia Fioravanti, Claudio Ghittino, Donatella Volpatti and Paola Beraldo.

In order to ensure that appropriate topics are covered, including emerging areas, the EAFP would be interested in hearing from individuals with suggestions for topics for a session or a workshop at the conference. EAFP members and non-members are encouraged to submit ideas.

A form can be downloaded from the EAFP website (www.eafp.org) and mailed, emailed or faxed. Alternatively, any views, thoughts or proposals can be emailed to the EAFP meetings secretary, stephen.feist@cefas.co.uk.

See www.eafp.org

NEW MEDICATIONS AND DISEASES IN THE US ORNAMENTALS INDUSTRY

BY DR ERIK JOHNSON (JOHNSON VET SERVICES, USA)

In this article I want to talk about the emergence of new medications and new diseases of importance to the ornamental fish farmers and hobbyists in the United States.

Over the years we've homed in on what parasites and pathogens mean the most to koi and goldfish. When we started out in 1990, not as much was known about how to combat various protozoan and trematodes. A lot of what we did for parasites was derived from utilitarian lake and pond fish medicines. We used to use a lot of salt for protozoans and we used organophosphates for flukes. We used high levels of organophosphates for crustacean parasites and sometimes even Formalin and potassium permanganate.

Over time, we arrived at low-level salting (0.3 percent) for Ich, Chilodonella, Epistylis, most Trichodina and some Costia. The Costia and Trichodina that was resistant to 0.3 percent would sometimes respond (via clearance) to double-dose salt at 0.6 percent. Trematodes used to require caustics like Formalin, chloramine-T and potassium permanganate.

However, in the last three years, the ornamental side has been introduced to Praziquantel and Supaverm, among others, for speedy, painless clearance on non-food fish. This was a great breakthrough. Crustacean parasites which were once treated with organophosphates are now treated with the chitin synthesis inhibitors such as Dimilin (API), Anchors Away (Jungle) and Anchor Control (Nursery Pro EcoRx). Again, branchiurians like fish lice and anchor worm that were once pretty devastating to ornamental fish farmers are now easily and quickly controlled.

Still, the lingering parasite that we have been trying to eradicate with new designer medicants is Costia, known to many as *Ichthyobodo necatrix*. It's the smallest of the parasites, but when it occurs in large numbers on the gills in the cold water of spring it can be cataclysmic to the fish.

A simple method has been developed which excludes the Costia from retailers' quarantine facilities. It includes two "receiving tanks" labelled Tank A and Tank B, and Formalin for short-term exposure.

Here is how it works: Fish that have just come off an airplane are consistently stressed and often bag-burned by ammonia and other toxins that accumulate in the shipping bags. It is therefore unwise to treat these fish with anything like Formalin during the first day they arrive.

The recommendation is to place these fish in spacious holding facilities (receiving Tank A) with absurd amounts of aeration and filtration. Let them acclimatise and rest for one day.

The following day, a single treatment with 50ppm 37 percent Formalin is instituted, maintaining the absurdly high aeration, as Formalin consumes prodigious amounts of dissolved oxygen. If the tanks are warm, partial water changes are recommended to cool the tank before treatment. Leave the 50 parts-per-million Formalin treatment in effect for 120 minutes.

If you have to treat for up to 125 minutes, it's okay. I've done it with some success. But not 126 minutes. You get the "idea" that that's a lot of Formalin, and the timing is crucial to avoid burning the fish up. For valuable fish, or small groups of fish that can be hand-managed, they are treated even better by placing them in clean shipping bags with water from Tank A and Formalin at 50ppm, all under pure oxygen in the bag. It does not interfere with the Formalin, but it ensures that the fish don't have any oxygen stress.

COSTIA UNDER THE MICROSCOPE

At the 120-minute mark, the fish can be moved from Tank A (or the oxygenated bags) to their actual quarantine facility, also equipped with absurd aeration, Tank B.

Tank A is broken down and disinfected. From there, a routine quarantine with medicated food, salt at 0.3 percent, heat at 23° Celsius, Praziquantel and Dimilin can be followed through. I realise this sounds like a lot of medicine but none of them are caustic, dangerous or expensive in holding facilities, they ensure a "clean" fish, and they compensate for what some wholesalers and retailers do; sell fish without performing quality control biopsies to actually know what pathogens exist in their livestock. Huh.

I'd like to take a moment to fill you in about Praziquantel and what it does. The trematode de-wormer Praziquantel has been available for some time for use by veterinarians in the form of Droncit® and to human doctors in the form of Biltricide®. I don't know what day it must have been, but some researcher put some Praziquantel in with some fish with parasites, and noted that the flukes/trematodes on the fish, and the parasites in the fish, were eliminated handily. Too bad the medicine was so expensive.

For years we knew Praziquantel was excellent for eliminating trematodes and intestinal worms, but it was prohibitively expensive until a gentleman in California checked pricing with a Chinese distribution company and imported barrels of Praziquantel, bringing the price within reach of everyone.

Now available under the labels Prazi®, PraziPro® and PraziPond®, as well as in bulk from various pharmacies, this medication is now useful in the safe, painless clearance of flukes on koi and goldfish. Dissolution is difficult; the medicine can be made soluble with a high-speed blender, Formalin or other solvents. It's working very well.

It is not yet cleared for use on food fish, but it has revolutionised the treatment of flukes on ornamental livestock. It has no effect on plants, beneficial bacteria and fish young and old, South American or elsewhere.

MAPPING COMPONENTS OF THE IMMUNE SYSTEM IN ATLANTIC COD

MARIT SEPPOLA (NORWEGIAN INSTITUTE OF FISHERIES AND AQUACULTURE RESEARCH, NORWAY)

Atlantic cod (*Gadus morhua*) is recently introduced to aquaculture industry, and currently little is known about what diseases may become a problem in intensive commercial culture systems. Also, the immune mechanisms against infectious agents are far from solved. Today, it is known that cod is susceptible to viral haemorrhagic septicaemia virus (VHSV), nodavirus and infectious pancreas necrosis virus (IPNV). In addition, several bacterial infections have been reported.

The immune system of vertebrates is divided in two parts, where the innate/non-specific system is the primary barrier to combat an infection, while the adaptive/specific system has a delayed secondary response against the pathogen. Previous reports have shown that Atlantic cod is a weak producer of specific antibodies following vaccination, and it has been suggested that the innate immune response could be more important to cod relative to other species. These responses have been characterized to a lesser extent due to a general lack of molecular tools.

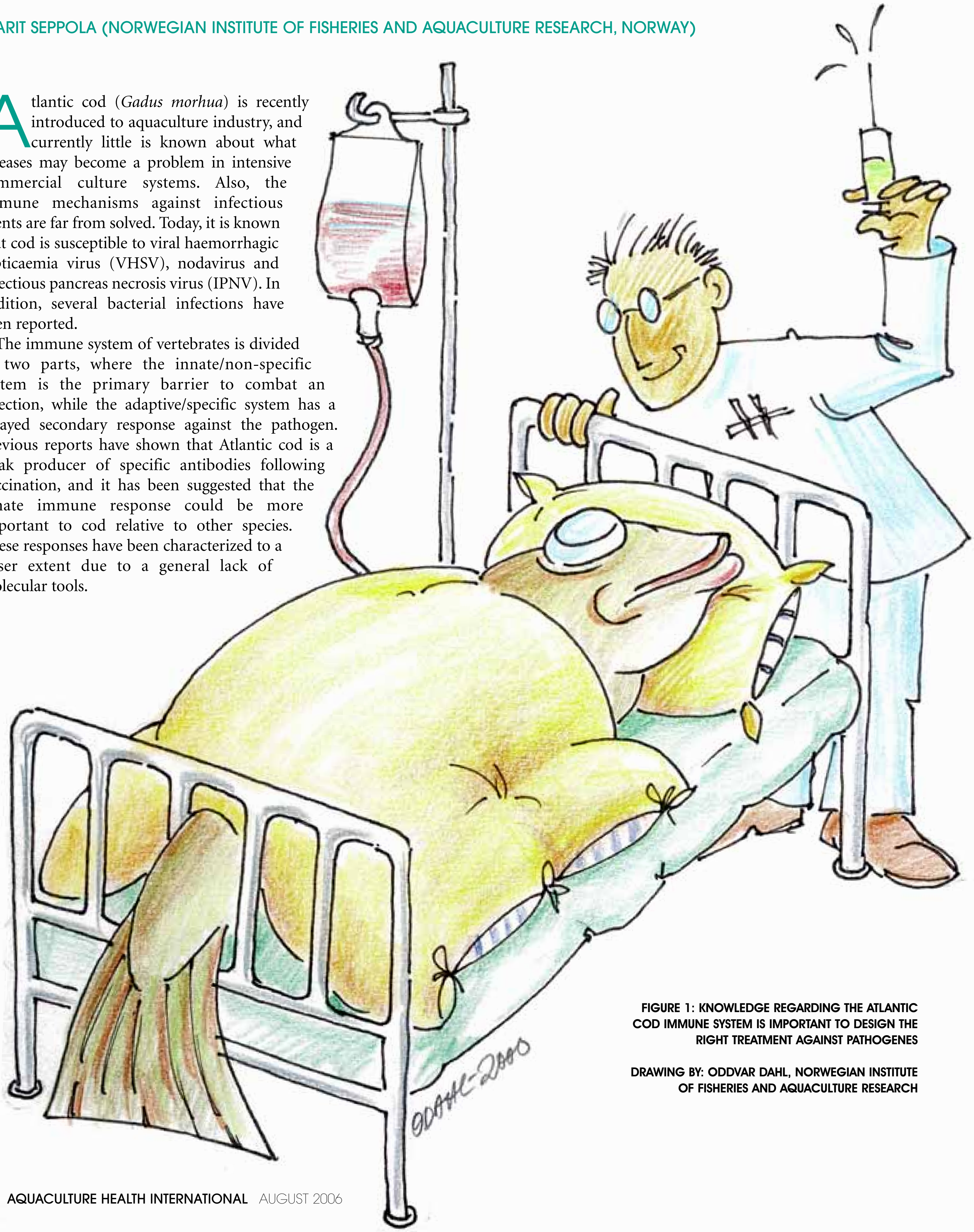
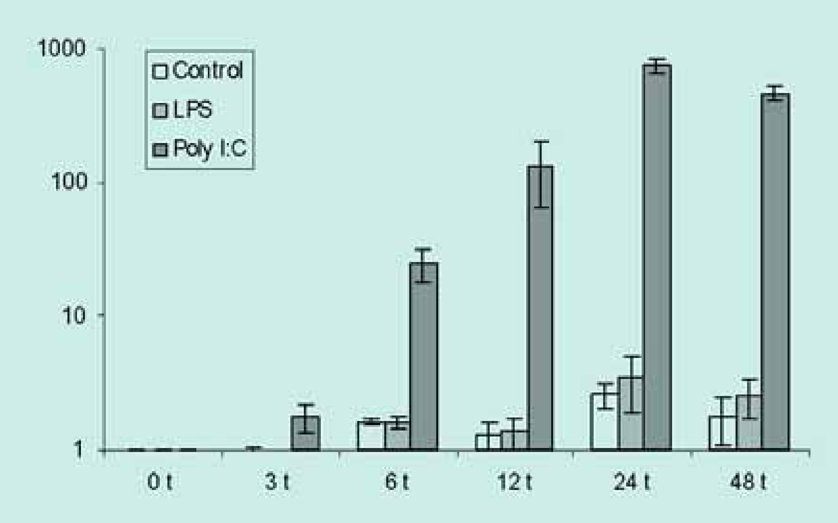


FIGURE 1: KNOWLEDGE REGARDING THE ATLANTIC COD IMMUNE SYSTEM IS IMPORTANT TO DESIGN THE RIGHT TREATMENT AGAINST PATHOGENES

DRAWING BY: ODDVAR DAHL, NORWEGIAN INSTITUTE OF FISHERIES AND AQUACULTURE RESEARCH

FIGURE 2: RELATIVE GENE EXPRESSION OF ATLANTIC COD ISG15 FOLLOWING TREATMENT WITH POLY I:C, LPS AND UNTREATED (CONTROL)



> We are currently identifying additional genes in the Atlantic cod immune system

Cytokines consist of a diverse array of secreted proteins with a crucial role in regulating the immune system. This group of proteins include interleukins, interferons, tumour necrosis factors, growth factors, and chemokines. Cytokines are strongly induced during infection with pathogens, and they are thus highly relevant candidates as molecular markers for activation and modulation of the immune system.

DISCOVERING GENES IN THE IMMUNE SYSTEM

The immune systems of bony fish is considered to be equivalent to the immune system of higher vertebrates, but the genes involved show high extent of sequence divergence, even between related fish species. Gene duplication followed by positive selection is typical in several gene families involved in immune responses. Divergence of these immune genes may lead to additional layers of functional redundancy or to acquisition of novel functions, both of which conceivably help the host cope more effectively against a broad range of pathogens. However, high sequence divergences makes it difficult to identify genes in a new species by a targeted approach (homology cloning), and gene discovery by screening methods is thus more efficient. The use of techniques like suppression subtractive hybridization (SSH) makes it feasible to extract expressed genes (ESTs) that are up-regulated by a particular stimulus. We have thus generated an EST library with the use of polyinosinic polycytidylic acid (poly I:C) which is known to have strong immune stimulatory properties, mimicking a viral infection. We have sequenced about 1000 ESTs and analyzed them by comparison against sequences in the GenBank. These analyses revealed that approximately seven percent of the ESTs were immune related, 41 percent represented other genes, while 52 percent had no apparent similarity to known genes from other species. Some of the immune related genes were used for further applications and development of quantitative gene expression assays.

IMMUNE GENE EXPRESSION

There has been a paucity regarding identified immune related genes in Atlantic cod, and most reports focused on the adaptive immunity. However, recent developments have revealed the presence of some cytokine and chemokine sequences including interleukin-1, interleukin-8 and interleukin-12. In addition, we have identified an interferon stimulated gene (ISG15) in Atlantic cod, which is known to function also as a cytokine in mammals. In mammals this gene was one of the strongest induced following immune stimulation, and our results suggest that ISG15 have an analogous role in Atlantic cod. Additionally, the response to external stimuli is rapid. Already three hours after exposure to an immune stimulant a detectable increase in gene expression is observed in primary cell cultures, with a slight decrease after 24 hours. These results suggest that ISG15 is most important immediately after an infection (Figure 2). Lipopolysaccharide (LPS) treatment resulted in a small, but not significant increase in ISG15 expression (Figure 2). Atlantic cod ISG15 is also induced by *Vibrio anguillarum* (Figure 3), suggesting that it could be involved in resistance to bacterial infections as well.

To monitor gene expression we use the quantitative real time PCR technique, and we have developed several assays to examine the gene expression as a response to a diverse array of stimuli. We are currently identifying additional genes in the Atlantic cod immune system and are developing real-time assays to examine their expression pattern.

Fish farming has large economic importance in Norway as well in other countries, and fish health is essential for this industry. More information regarding components of the immune system, including Atlantic cod, is thus important. Identifying gene sequences involved in response to diseases will enable us to expand the current knowledge concerning how the immune system behaves in Atlantic cod.

FURTHER READING

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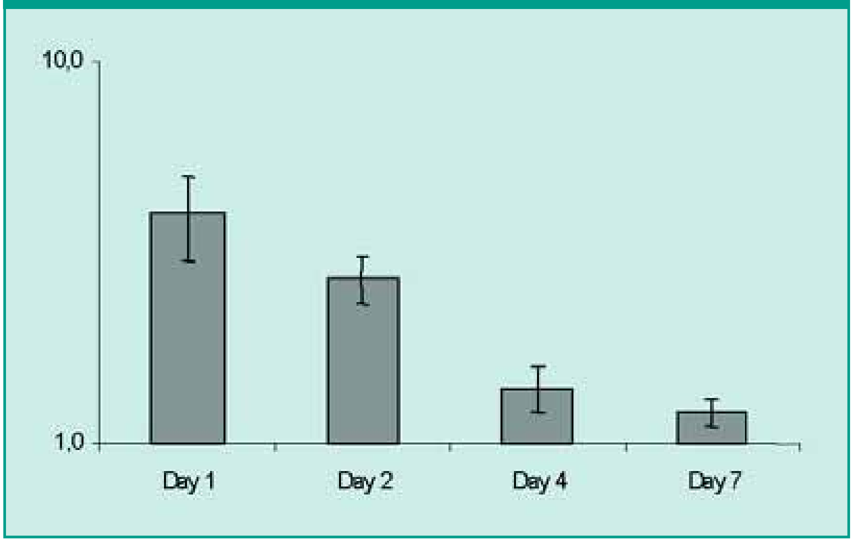
FIGURE 3: RELATIVE GENE EXPRESSION OF ATLANTIC COD ISG15 FOLLOWING INJECTION WITH *V ANGUILLARUM*



EPITHELIOCYSTIS – A VILLAIN OR AN INNOCENT BYSTANDER?

BY DR BARBARA NOWAK (UNIVERSITY OF TASMANIA, AUSTRALIA)

Epitheliocystis, a common condition in fish, affects gill and skin epithelium. It is also reported to infect chloride cells, mucous cells, pillar cells and macrophages.

Epitheliocystis has been described in more than 50 marine and freshwater fish species, and has been found not only in teleost fish but also in sharks, rays and sturgeon. Additionally, epitheliocystis has been reported in both cultured and wild fish.

Epitheliocystis is an intracellular infection with gram-negative bacteria, resulting in hypertrophy of host cells. The condition has been diagnosed mostly in histological sections. Sometimes, particularly in its proliferative form, epitheliocystis can be seen grossly or in wet preparations. However, histopathology has been used to provide definitive diagnosis.

There are no culture methods for the causative agent, which means that the disease cannot be diagnosed through a routine microbiology screening. As a result, the disease can go unreported if histology is not used.

TREATMENT

Oxytetracycline (25ppm active ingredient, twice a day for three days) was successfully used to treat epitheliocystis in farmed largemouth bass. Sterilisation of rearing water using ultra-violet light was reported to control outbreaks of epitheliocystis in marine hatcheries.

KNOWLEDGE GAPS

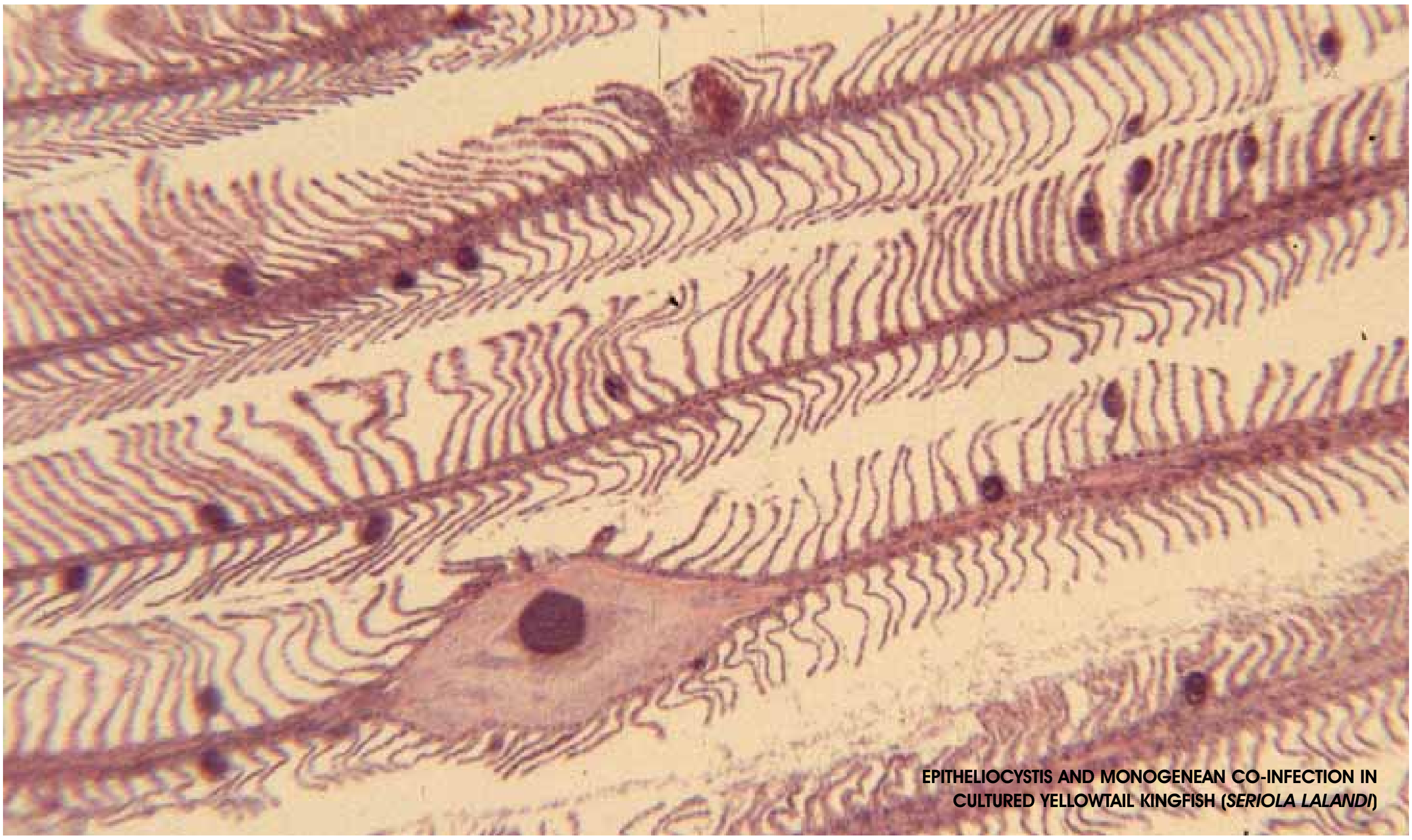
Epitheliocystis is not well understood, despite being frequently reported. It has been associated with morbidity and mortality in cultured fish species. However, the direct link between epitheliocystis and mortalities has not been established due to the lack of an experimental challenge model.

Recently progress has been made in identifying the epitheliocystis agent, at least partially, in some fish species, including Atlantic salmon. The results to date suggest that different pathogen causes epitheliocystis in different host species, and that they are not related to each other more than to other bacteria from the order Chlamydiales.

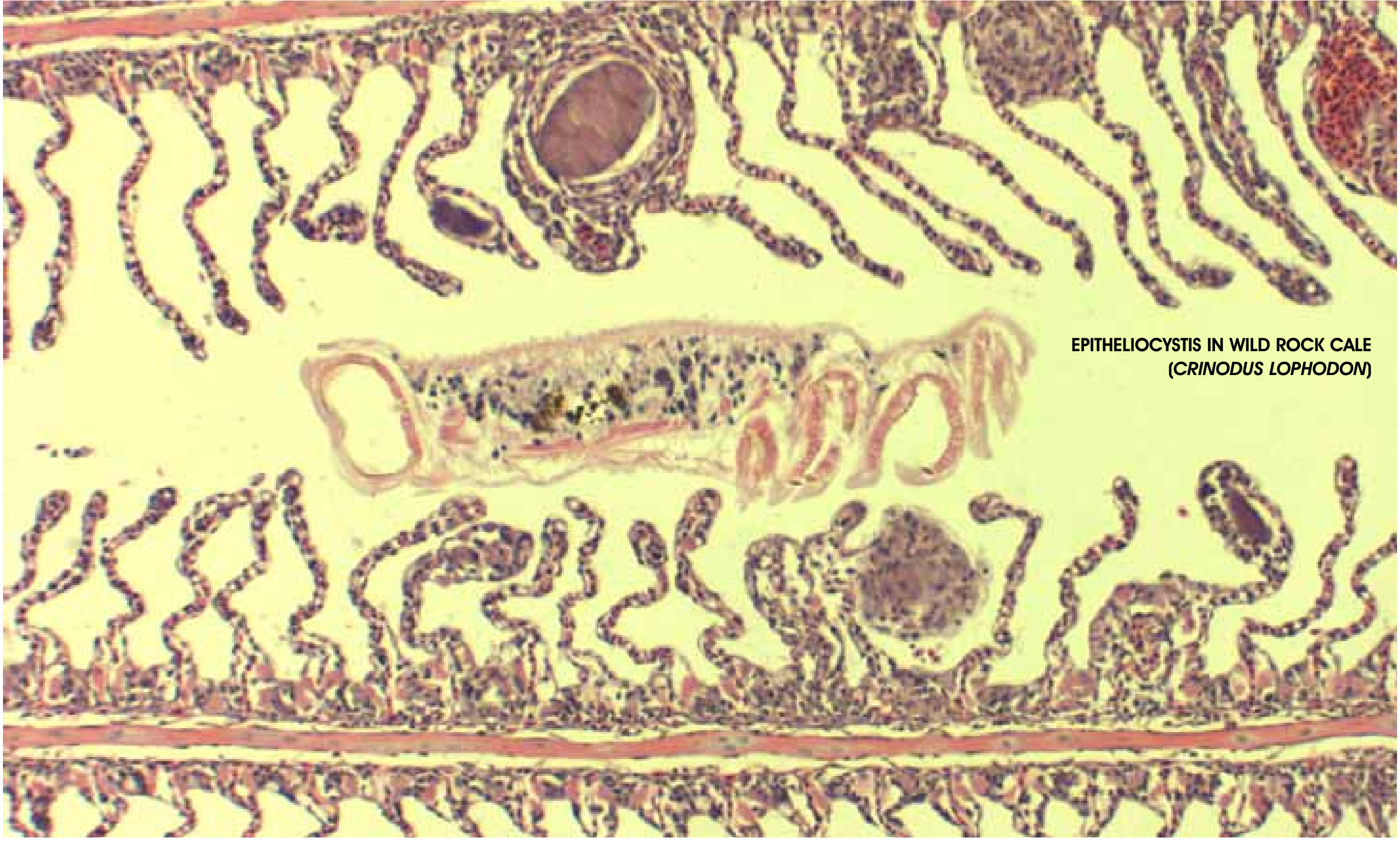
The bacteria all belong to the order Chlamydiales, in a lineage separate from Chlamydiaceae. This confirms the high diversity and host-specificity of the pathogen. However, so far it discounts the previous hypothesis that rickettsia-like organisms caused some of the outbreaks.

Further research is needed to understand the role of epitheliocystis in aquatic animal health

The typical chlamydial developmental cycle includes infective elementary body, intermediate cell/round body and reticulate body. In some fish species other stages have also been described. This suggested that the epitheliocystis agent could undergo



EPITHELIOCYSTIS AND MONOGENEAN CO-INFECTION IN CULTURED YELLOWTAIL KINGFISH (*SERIOLA LALANDI*)



EPITHELIOCYSTIS IN WILD ROCK CALE (*CRINODUS LOPHODON*)

other cycles, in addition to the typical chlamydial developmental cycle.

Developmental cycle II includes primary long cell, intermediate long cell and small cells. The factors proposed to cause cycle-switching in fish epitheliocystis were host age, stress or environmental conditions and the type of cell infected.

The developmental cycle does not appear to be specific for host species or bacteria, as cells characteristic of both developmental cycles can be found in the same fish species.

While it has been suggested that amoeba could potentially transmit epitheliocystis agent, microbiological and epidemiological studies showed that *Neoparamoeba* spp., the causative agent of amoebic gill disease, has not been significantly involved. Temperature, season, culture conditions and age of the fish were all considered risk factors for epitheliocystis.

FUTURE DEVELOPMENTS

The application of molecular techniques has greatly increased our knowledge of epitheliocystis. Further research is needed to understand the role of epitheliocystis in aquatic animal health. Development of real-time PCR for epitheliocystis will allow for epidemiological study and a better understanding of risk factors.

New sequence data is required to evaluate the relationships between bacteria from different hosts and to design effective diagnostic and research tools.

The development of in-vitro culture techniques and a challenge model will allow the assessment of the real significance of this disease in aquaculture and fisheries.

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CENTRE OF EXCELLENCE FOR SHRIMP MOLECULAR BIOLOGY AND BIOTECHNOLOGY

BY PROFESSOR TIM FLEGEL (CENTEX SHRIMP, THAILAND), DR DAVID GROMAN AND DR FRANCK BERTHE (UNIVERSITY OF PRINCE EDWARD ISLAND, CANADA)

The production of cultivated shrimp has been a major industry for Thailand since the late 1980s. The industry employs approximately 300,000 people nationwide, and exports approximately US$2 billion of shrimp per year.

Because Thailand is strategically positioned both geographically and technically, it is a world leader in the production of premium, value-added shrimp products and shrimp seed. In order to maintain this leadership, Thailand must develop and train specialists to carry out relevant research and provide continuous support to the industry.

The Centre of Excellence for Shrimp Molecular Biology and Biotechnology (abbreviated as Centex Shrimp) is a multidisciplinary laboratory. It was formed in October 2001 by amalgamating research laboratories from the Departments of Anatomy, Biochemistry and Biotechnology of the Faculty of Science, Mahidol University, Bangkok.

It is jointly supported by the university's Faculty of Science and the National Centre for Genetic Engineering and Biotechnology, or Biotec. Prior to amalgamation, the laboratories had been engaged in cooperative research on disease diagnosis and control in shrimp since 1990. This led to the 2000 Toray Science Foundation Award for their lead scientists in February 2001.

Centex Shrimp's mission statement is, "Premier science for premium shrimp", and its vision is that Thailand in 2007 will be the world leader in producing healthy, domesticated shrimp cultivated in biosecure ponds with no negative environmental impact.

The centre's strategy for achieving this goal is

- to become a national and regional focus for research and training in molecular biology and biotechnology for shrimp production
- to serve as a coordination centre whereby Mahidol University can cooperate with Biotec and other national and international institutions on shrimp research, and
- to operate in close consultation with the Thai shrimp industry so that Centex's work will be both academically sound and relevant to the shrimp industry's needs.

A combination of the current needs of the shrimp industry and the expertise of its staff drive Centex research. Research priorities are set by the urgency of the industry's needs in molecular biology and biotechnology, and these needs change with time. Centex Shrimp cannot serve all the industry's needs, but can coordinate with other groups to complement their work and reduce overlap.

Current priorities are disease diagnosis and control, and broodstock development. The six main areas of related research interest are:

- molecular study of shrimp pathogens, including viruses, bacteria and parasites
- development of diagnostic probes and kits for shrimp diseases
- research on shrimp humoral and cellular defence mechanisms
- shrimp genome analysis
- shrimp nutritional research related to reproduction, and
- developing and improving domesticated and specific pathogen-free (SPF) black tiger shrimp stocks.

Although hard to predict, future needs may shift to such areas as genetic improvement of domesticated stocks, improvement in shrimp production efficiency (eg reduced mortality at all stages of rearing) and support for the introduction of alternative species.

Being a "firehouse" is also an important Centex function. Centex shrimp is a storehouse of information, technology and expertise in shrimp disease diagnosis and control, and can respond quickly to assist the shrimp industry in stopping disease outbreaks.

A recent example was the introduction of exotic Taura syndrome

BROODER SHRIMPS

virus from the Americas to Thailand via the importation of infected white shrimp (*P. vannamei*) stocks. Centex Shrimp and the Shrimp Biotechnology Business Unit had prior experience with this virus and had reagents on hand for its detection. Farmers were able to receive help with diagnosis and control immediately the problem arose.

SHRIMP PATHOGENS

The rapid expansion of the shrimp farming industry has resulted in disease problems, particularly those caused by viral pathogens, and controlling them is a high priority for the industry. A major portion of Centex Shrimp's work has focused on characterisation of the causative viruses and the development of rapid diagnostic probes for them.

However, we also work on bacteria and parasites. The major viruses of concern for the Asian shrimp industry are white-spot syndrome virus (WSSV), yellow-head virus (YHV), hepatopancreatic parvovirus (HPV), monodon baculovirus (MBV) and infectious hypodermal and haematopoeitic virus (IHHNV).

Our group has developed DNA diagnostic procedures for several of these (WSSV, YHV and HPV), and they are now available together with reagents for all these viruses and others from the SBBU under the Ezee Gene brand. They are widely used in Thailand for screening broodstock and post-larvae in an attempt to stem the spread of viruses and maintain production at high levels.

The diagnostic kit for detecting YHV by nested RT-PCR has been licensed to the Farming IntelliGene Technology Corporation, Taiwan. We have also developed molecular diagnostic methods for the bacterium *Vibrio parahaemolyticus* and the microsporidian parasite *Agmasoma penaei*.

Detailed research on the total genome of YHV and the expression of its structural proteins is an example of the type of more basic study carried out by Centex on major shrimp pathogens. The complete nucleotide sequence and major structural proteins are being studied and expressed for use in the development of diagnostic kits, for "vaccine" testing and for functional studies.

GENOME ANALYSIS

Centex Shrimp research on shrimp genome analysis focuses on construction of EST libraries from normal and pathogen-challenged shrimp in collaboration with Dr Anchalee Tassanakajon of the Department of Biochemistry, Chulalongkorn University.

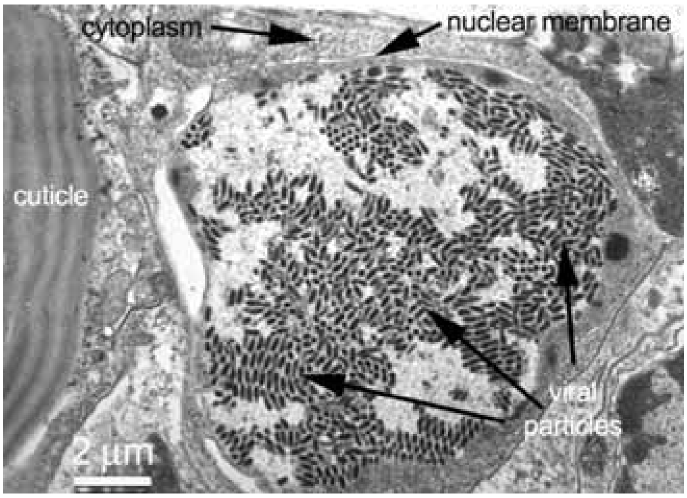
For example, EST from various tissues of normal, specific pathogen-free (SPF) *P. monodon* and those challenged with WSSV and YHV are compared in a search for various defence molecules.

Ongoing studies in functional genomics and proteomics with selected genes will help us to understand shrimp defence mechanisms and devise new methods of disease control. The EST libraries will also help in the construction of a genetic map for *P. monodon* that will be applied in programmes for genetic improvement of domesticated shrimp stocks. Genetic mapping work is being done in collaboration with research groups in Taiwan and Australia.

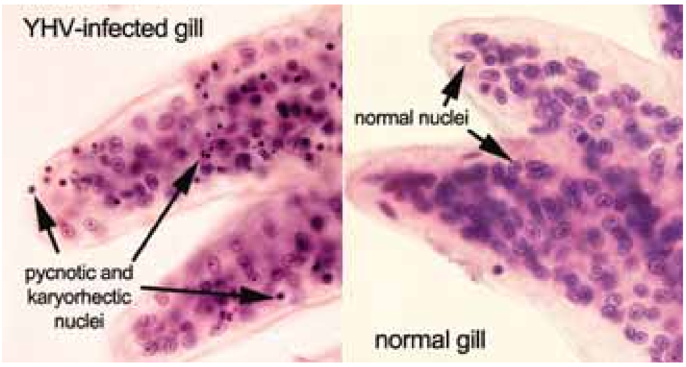
DEFENCE MECHANISMS

Shrimp viral infections are characterised by the general lack of an inflammatory response. Epizootics by newly introduced viral pathogens follow a pattern of initial, widespread and catastrophic crop losses, and then, within a year or so, sporadic crop losses, even though the pathogen is widespread in persistently infected shrimp that show little or no mortality.

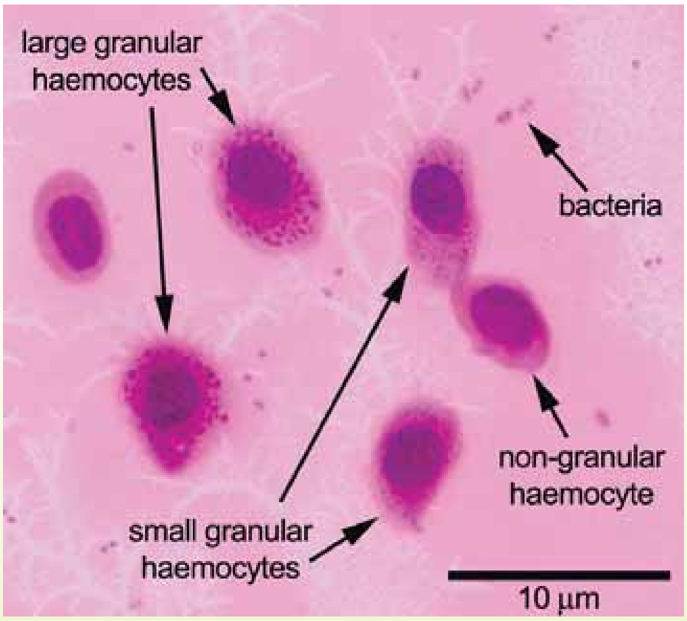
This is not due to decreased viral virulence, since viruses carried by the persistently infected shrimp remain lethal for naïve shrimp in cohabitation tests. This apparent adaptive response cannot be explained easily by current knowledge or theories regarding shrimp or crustacean defences. Understanding the phenomenon may help us to develop new methods of prevention and therapy for viral infections. Centex Shrimp scientists have proposed a radically new working concept called "active viral accommodation" to explain this



A TEM IMAGE OF A WSSV INFECTION



YHV-INDUCED CHANGES IN THE GILL



BACTERIA ARE CLEARLY EVIDENT IN THIS BLOOD SMEAR

adaptation and they are testing the concept in shrimp and insects. Recent results suggest that shrimp actively accommodate viral pathogens as persistent infections that help to reduce disease severity, possibly by inactivating triggers for viral-induced apoptosis or programmed cell death.

Specific memory for each virus is provided by the infection itself, but the basis for recently discovered cross-protective effects remains unknown, and it appears that some aspects of the host-viral interaction may be genetically determined.

We have shown in a parvovirus-mosquito model that mosquitoes are persistently infected after challenge, and that their survival increases with each challenge generation. We are studying the reasons for this improvement in survival. Since apoptosis ▶

CENTRE OF EXCELLENCE FOR SHRIMP MOLECULAR BIOLOGY AND BIOTECHNOLOGY

STAFF OF CENTEX SHRIMP

appears to play a key role in the interaction, research is ongoing towards the isolation and characterisation of apoptosis-related genes in *P monodon*.

NUTRITIONAL RESEARCH

Centex Shrimp has few activities in the area of shrimp nutrition except for some work on broodstock nutrition for maturation and reproduction. It is well known that the best results for egg and larval production in broodstock shrimp can be obtained only by using living polychaete worms. Unfortunately, these are usually captured from the wild and may thus carry pathogens that pose a risk to shrimp culturists.

Our work has shown that these marine worms do not become infected with WSSV, although they may carry it in the gut for a brief period if they eat WSSV-infected shrimp.

Our scientists have developed a method of rearing SPF polychaetes for safe feeding of broodstock, and they are trying to discover why live polychaetes are so necessary for good shrimp reproduction. We are also interested in work on probiotic microbes, immuno-stimulants and the effect of nutrition and stress on disease, but current activities in this area are minor. This is the only aspect of our research that might be considered to be in the environmental field.

REPRODUCTION

Aside from broodstock nutrition, scientists at Centex Shrimp are carrying out work to better understand the shrimp's reproductive process. The aim is to increase the efficiency of larval production and the percentage of larval survival and quality in shrimp hatcheries. For example, our recent studies have revealed that egg maturation is very fast in *P. monodon*, and that the interval available for fertilisation is very short after egg release (spawning).

We have also shown that sperm stored in the female theylicum undergoes a maturation process that is essential in order to obtain high fertilisation rates. We hope that a better understanding of these phenomena will allow us to exercise better control over the reproductive process, and perhaps allow us to eventually cryo-preserve eggs and sperm and carry out *in-vitro* fertilisation.

We are also working on the possibility of hormonally induced maturation in female shrimp in order to discontinue the practice of eyestalk ablation and allow the recycling of valuable broodstock.

PATHOGEN-FREE SHRIMP

The increasing usage of wild *P. monodon* broodstock to produce post-larvae (PL) in hatcheries in Thailand and worldwide has led to a reduction in the number and quality of captured wild broodstock available in Thailand.

Prices have risen steeply (more than five times), and this has increased the cost of hatchery operations and the price of post-larvae for farmers.

The captured wild broodstock also produce highly variable seed quality, so that some earthen ponds show slow growth and low survival rates, leading to economic loss. This is sometimes due to sub-clinical viral infections passed on from the wild broodstock to their offspring.

The best solution to these problems would be the domestication of *P. monodon*, and the Shrimp Culture Research and Development Company Limited, a joint venture between the National Science and Technology Development Agency and the private sector, began work on this in 1996. The domesticated shrimp from this programme have now reached the sixth generation (F5 starting from F0). They were screened for freedom from WSSV and YHV and have been shown to be free from them by continual monitoring since 1966 (ie they are specific pathogen-free or "SPF" for WSSV and YHV).

Centex Shrimp is closely involved in monitoring and improving the SPF pathogen list for these domesticated shrimp. It is also engaged in research on genetic characterisation and selection for improved performance of their offspring in aquaculture ponds.

TRAINING

Centex Shrimp has an active post-graduate training programme with 15 to 20 MSc and PhD students and one or more post-doctoral fellows working at any one time. It also accepts a few undergraduate students for senior projects each year.

In addition, it offers a yearly regional training course on shrimp biology and pathology and occasional courses on specialised topics according to demand. For example, it recently joined with Biotec to sponsor a specialist course on shrimp immunology for local and international participants.

In cooperation with the SBBU, it also provides consultants and training at the request of the Thai shrimp industry in disease diagnosis, disease control and broodstock development.

AWARDS

The Toray Science Foundation Award in 2000 to leading scientists Vichai Boonsaeng, Tim Flegel and Boonsirm Withyachumnarnkul in recognition of the group's work on shrimp viral disease diagnosis and control.

The Outstanding Technology Group Award in 2003 from the ▶

CENTRE OF EXCELLENCE FOR SHRIMP MOLECULAR BIOLOGY AND BIOTECHNOLOGY

AT WORK IN THE CENTEX SHRIMP LABORATORY

Science and Technology Promotion Foundation, in recognition for development of practical diagnostic reagents and tools for the black tiger shrimp industry.

COLLABORATION

Centex Shrimp attempts to work with other scientists both in Thailand and abroad who share interests in disease diagnosis and control and development of domesticated broodstock. A number of mutual benefits and joint publications have resulted. Some of the institutions involved in past and present collaborative work are listed below.

THAILAND AND ABROAD

Chulalongkorn University in the area of shrimp genetics

Srinakharinwirot University – diagnostic tool development

Rangsit University – pathogen isolation, characterisation and diagnosis

Prince of Songkhla University – disease diagnosis and control

The Thai Department of Fisheries – disease control and shrimp domestication

Mae Jo University – shrimp genes involved in apoptosis

Zoology Department, National University of Taiwan – shrimp disease and genetics

CSIRO Livestock Industries, Queensland Bioscience Precinct, Australia – shrimp disease diagnosis and control

University of Arizona, USA – pathogen characterisation and disease diagnosis and control

University of Upsala, Sweden – shrimp defence system

James Cook University, Australia – shrimp pathogen characterisation

Oceanic Institute, Hawaii – shrimp maturation and genetics

These external collaborations sometimes involve student and scientist exchanges and have led to a number of joint publications in international scientific journals.

RESEARCH STAFF

Timothy W Flegel, PhD (Fungal physiology, Simon Fraser University, Canada)

Vichai Boonsaeng, PhD (Biochemistry, Otago University, New Zealand)

Boonsirm Withyachumnarnkul, MD, PhD (Anatomy, University of Rochester, USA)

Kallaya Sritunyalucksana, PhD (Biotechnology, Mahidol University, Thailand and Comparative Physiology, Upsala University, Sweden), Nusra Sittidilokratna, PhD (Biochemistry, Mahidol University, Thailand)

Saengchan Senapin, PhD (Biochemistry, Australian National University, Australia)

Chumporn Soowannayan, MSc, Wansika Kiatpathomchai, MSc (Biochemistry, Mahidol University, Thailand)

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See www.sc.mahidol.ac.th/research/shrimp

INTERVET NORBIO SINGAPORE: AN INTEGRAL PART OF THE ASIAN AQUACULTURE INDUSTRY

BY DRS CEDRIC KOMAR, BRIAN SHEEHAN, ZILONG TAN AND WILLIAM ENRIGHT
(INTERVET NORBIO SINGAPORE PTE LTD, SINGAPORE)

During the last three decades, aquaculture has been one of the fastest expanding sectors of the agrifood industry. However, Asian aquaculture is characterised by an enormous diversity of species, with several dozen marine species being farmed.

Consequently, more resources are needed to understand the basic epidemiology of diseases in the various species. In Asia, some disease-causing agents have been described, but comparative studies between isolates from different geographical locations and fish species are generally not available. Epidemiology data is scarce, as is basic data on the immune systems of Asian fish species. This hampers development of effective strategies for disease control. Also, most farming is operated on a small scale, and technical support, including disease diagnosis and training, is often lacking at farm level.

At present, many farmers still focus more on treatment than prevention. Irresponsible use of antibiotics and chemicals in aquaculture can lead to residue problems, an increasing consumer concern, and to the development of drug resistance among the bacterial pathogens.

With the exception of Japan, few fish vaccines are yet commercially available in Asia. The major advantages of prophylactic vaccination over therapeutic treatment are that vaccines provide long-lasting protection and leave no problematic residues in the product or environment. As one of the world's leading animal vaccine suppliers, Intervet knows that vaccination is one of the key solutions when it comes to health management.

Asian aquaculture will continue to grow at a fast pace due to both area expansion and production intensification. Under these conditions, the prevalence and spread of infectious diseases will unavoidably increase as a result of higher infection pressure, deterioration of environmental conditions and movement of aquatic animals. Accordingly, the effective control of infectious diseases has become more and more important in the cultivation of aquatic animals.

Good health management is the "silver bullet" for disease control. Collectively, this includes the use of healthy fry, quarantine measures, optimised feeding, good husbandry techniques, disease monitoring (surveillance and reporting), sanitation and vaccination, and proper control and biosecurity measures when diseases do occur.

Overall, the emphasis must be on prevention rather than treatment, remembering the old adage that "a gram of prevention is better than a kilogram of cure".

MAJOR ACTIVITIES AT INTERVET NORBIO SINGAPORE

Research and development

- Disease diagnosis, including the detection and identification of new emerging pathogens
- Vaccine development for economically important fish diseases (bacterial and viral vaccines)
- Development of high quality and safe pharmaceuticals for responsible use in aquaculture

Technical support

- Regional epidemiological disease investigations
- Education of fish farmers to improve husbandry and health practices
- Technical support to farmers
- Marketing support to Intervet local companies

> Intervet has developed unique know-how and technology in vaccine development for salmon

INTERVET NORBIO'S SINGAPORE AQUATIC ANIMAL HEALTH FACILITY

INS TEAM MEMBERS WITH THE INTERVET PRESIDENT RUURD STOLP

INTERVET NORBIO SINGAPORE

With in-house expertise going back more than 20 years in fish vaccine development in Norway, Intervet has developed unique know-how and technology in vaccine development for salmon. Today, the success of the salmon industry has been achieved thanks to the widespread use of vaccines. Expanding on the expertise in salmonid vaccines and health management, Intervet set up a Research and Development Centre in Singapore in 2000 entirely dedicated to developing novel vaccines and other products for commercially farmed warm water aquatic animal species.

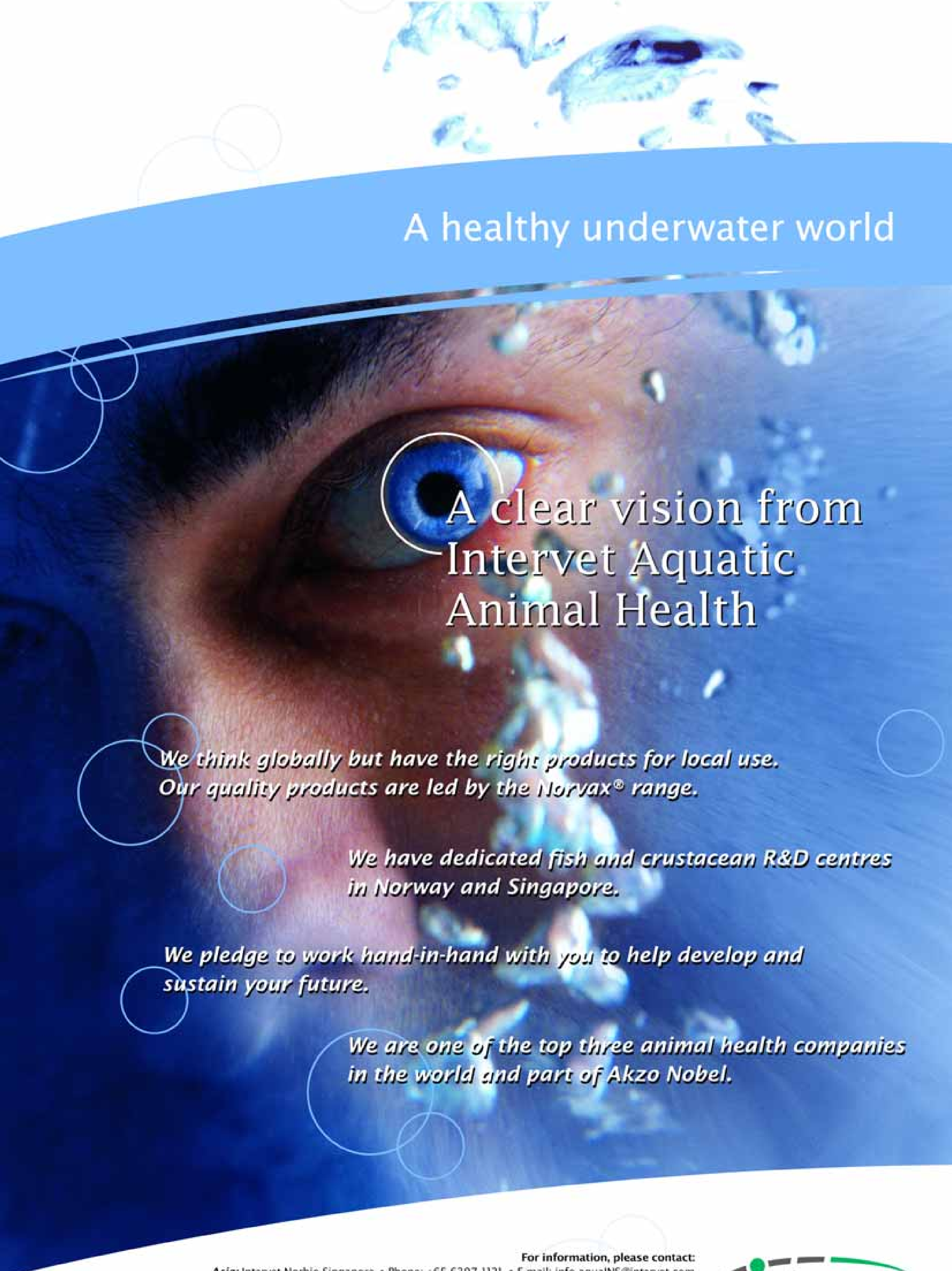
This centre, with its state-of-the-art facilities, was the first of its kind in Asia. Indeed, Intervet is using the same level of technology and innovation to develop Asian-targeted products as for its salmon-targeted products. Work is carried out in cooperation with other Intervet R&D centres and in close collaboration with leading universities and institutes. Thus, the mission of Intervet Norbio Singapore is to help develop aquaculture in the Asia-Pacific region.

The centre comprises over 200 m^2 of office space, almost 300 m^2 of laboratories and almost 300 m^2 of fish facilities. The laboratories include several functional units that perform disease diagnosis (bacterial, viral and parasitic), isolation and identification of pathogens, biochemical analysis, molecular biology, tissue culture, fermentation and vaccine formulation.

The fish facilities consist of a quarantine room and five experimental rooms. Each experimental room can be independently controlled for temperature and water salinity. There are about 100 separate fish tanks with volumes ranging from 50 to 1300 litres. ▶

SOME IMPORTANT DISEASES IN ASIAN AQUACULTURE

TEMPERATURE ZONE/SPECIES	BACTERIA	VIRUSES
Temperate seawater (yellowtail; amberjack; red seabream; Japanese flounder; turbot)	*Aeromonas salmonicida* *Edwardsiella tarda* *Lactococcus garvieae* *Listonella (Vibrio) anguillarum* *Mycobacterium* sp. *Nocardia seriolae* *Photobacterium damsela* ssp. *piscicida* *Rickettsia* sp. *Streptococcus iniae* *Tenacibaculum maritimum*	Iridovirus Lymphocystis virus Nodavirus (Nervous necrosis virus) Rhabdovirus (viral haemorrhagic septicaemia) Yellowtail ascites virus (YAV)
Tropical seawater (Asian seabass; groupers; snappers, etc.)	*Nocardia* sp. *S. agalactiae* *S. iniae* *T. maritimum* *Vibrio* sp. (big belly disease bacterium)	Iridovirus Nodavirus
Freshwater (catfish; tilapia; carp)	*A. hydrophila* *E. ictaluri* *E. tarda* *Flavobacterium columnare* *Francisella*-like organism *Nocardia* sp. *S. agalactiae* *S. iniae*	Aquareovirus (grass carp hemorrhage virus; GCHV) Iridovirus Koi herpes virus (KHV) Spring viraemia of carp virus (SVCV)
Shrimp	*Vibrio* sp.	Taura syndrome virus (TSV) White spot syndrome virus (WSSV) Yellow-head virus (YHV)


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

INTERVET NORBIO SINGAPORE

INTERVET NORBIO SINGAPORE HAS EXTENSIVE HOLDING FACILITIES FOR VACCINE DEVELOPMENT WORK

PILOT VACCINE PRODUCTION

HIGHLIGHTS OF INTERVET NORBIO SINGAPORE

2000: Intervet Norbio Singapore, first and only private aquatic animal health (AAH) R&D centre established.

2003: Intervet participated in the task force for koi herpes virus on request from NACA (Network of Aquaculture Centres in Asia-Pacific) and the Indonesian government.

2004: Intervet Norbio Singapore is recognised as a regional resource centre by NACA.

2005: Introduction of Intervet's first fish vaccine in Japan, Norvax® Vibrio mono, an immersion vaccine against *Vibrio* (*Listonella*) *anguillarum* infections of Japanese yellowtail. Introduction of Intervet's first Asian fish vaccine outside Japan, Norvax® Strep *Si*, an immersion and injection vaccine against *Streptococcus iniae* infections in warmwater fish.

2005: Development of a CD rom on Fish Vaccination and Health Management in Asia, an information/ training tool highly appreciated by the industry. To receive a copy, see www.intervet.com/aah/aahcontact.asp?cd

2005: Intervet Norbio Singapore launched the Intervet Asian Aquatic Animal News, a weekly newsletter that consists of news items on topics related to aquatic animal health or general aquaculture developments in the Asia-Pacific region. To register for the free email newsletter, see www.intervet.com/aah/aahcontact.asp?nl

2006: Intervet was one of the supporting organisations for the 2nd International Symposium on Cage Aquaculture in Asia (CAA2). Intervet and its collaborators presented five scientific papers, including one as a keynote presentation on fish diseases and health management.

Each tank can be operated either as a closed recirculation or flow-through system, allowing multiple treatment conditions to be tested simultaneously. The centre currently employs 17 scientists, technicians and support staff.

Using the centre in Singapore as a hub, Intervet is developing vaccines in Asia for the most commercially important species farmed in the region, such as Japanese yellowtail, amberjack, Asian sea bass (barramundi) and tilapia.

It normally takes five to 10 years to develop (to full commercialisation) an effective vaccine. In 2005, five years after it opened, Intervet introduced its first fish vaccine in Japan, Norvax" Vibrio mono, and the first Asian fish vaccine outside Japan, Norvax" Strep Si, an immersion and injection vaccine against *Streptococcus iniae* infections in warm water fish.

Over the last six years, Intervet Norbio Singapore has developed a unique and deep knowledge of the major diseases of finfish in the warm water aquaculture regions of the world. Intervet maintains an

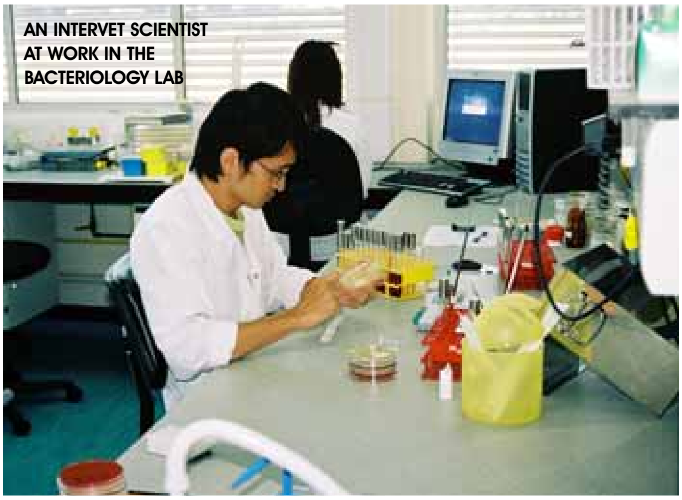
> The Asia-Pacific region holds the challenge of a large diversity of species and farming systems

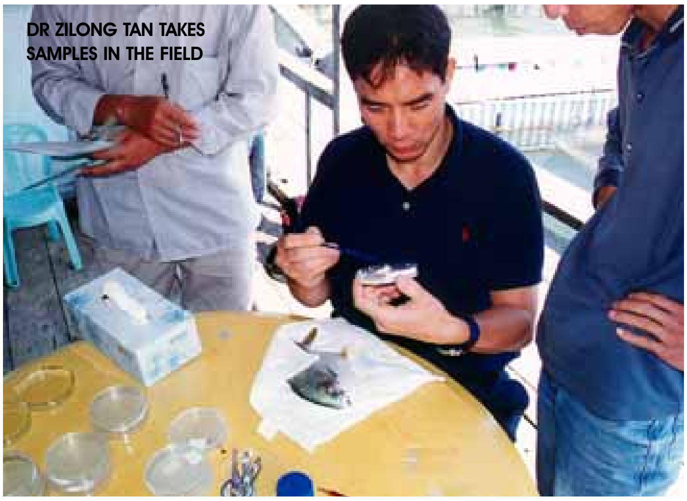
international network of centres where experts in such fields as microbiology, immunology, pharmacology, biotechnology and aquatic veterinary science carry out research and other work vital to the future of the aquaculture industry, such as epidemiology, training and technical support.

The company strongly believes in a bright future for the whole aquaculture industry, from the well developed, more species-focused areas such as Norway, the European Community, Chile and North America, to the Asia-Pacific region, which is by far the largest aquaculture region of the world (with over 90 percent of production), and which holds the challenge of a large diversity of species and farming systems.

The research and foresightedness of Intervet AAH will help the aquaculture community grow and prosper. Intervet Norbio Singapore is fully committed to developing aquaculture in the Asia-Pacific region, and will continue to support the industry with the introduction of effective vaccines for the most economically devastating fish diseases.

Contact +65 397 11 21, fax +65 397 11 31, or email info.aquaINS@intervet.com
See www.intervet.com/aah/Intervet-AAH-Asia-Pacific.asp


AN INTERVET SCIENTIST AT WORK IN THE BACTERIOLOGY LAB


DR ZILONG TAN TAKES SAMPLES IN THE FIELD

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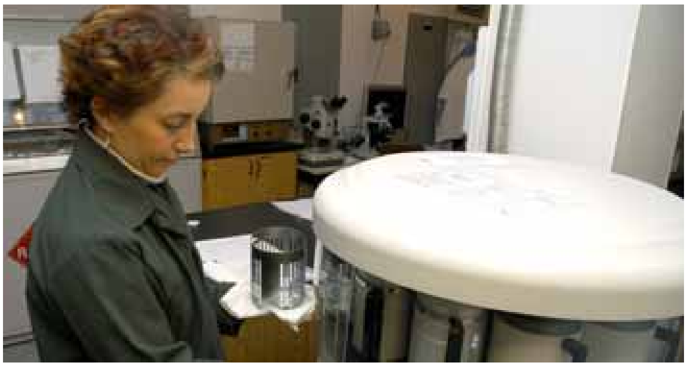
PROMOTING SUSTAINABLE AQUACULTURE AND FISHERIES

The Marine Institute (ISO-9001™ [2000 standards] registered) is Canada's most comprehensive education, training, applied research and technology transfer resource for the ocean industries. The Marine Institute's Centre for Aquaculture and Seafood Development (CASD) is internationally recognised for its applied scientific and technical expertise, facilities and commitment to clients in the seafood processing and aquaculture industries. CASD offers a complete range of fish/shellfish health services with state-of-the-art facilities for biotechnology research and product development to promote sustainable aquaculture and fisheries.

NECROPSY

CASD's fish health technicians are experienced in conducting necropsies of finfish and shellfish, and are skilled in collecting samples for parasitology, bacteriology, virology, histology and hematology.



HISTOLOGY

The histology lab is equipped with state-of-the-art equipment for processing tissue samples. Experienced fish health technicians process tissue samples using an automatic tissue processor, paraffin embedding station, cooling plates and microtome machines. This laboratory also has image analysis capability.

BACTERIOLOGY

CASD's bacteriology lab is well equipped for cultivating pathogens and isolation of pure colonies, conducting motility tests, oxidative-fermentation tests, cytochrome oxidase and indole tests, antibiotic sensitivity, vibriostatic testing and biochemical testing. Media is made on-site for freshwater and seawater bacterial species.

HAEMATOLOGY

CASD's haematology services include: non-lethal blood collection; blood smear production, staining of blood smears and subsequent analysis, and blood parasite screening.

QUARANTINE FACILITY

This artesian-well supplied, flow-through containment facility with viral disinfection capabilities is ideal for studies requiring rigorous experimental design, such as disease challenges and vaccine testing (currently houses 24, 120 litre tanks). There is an alarm monitoring system with 24 hour on-call personnel.

For further information, contact Heather Manuel, director, Centre for Aquaculture and Seafood Development, Fisheries and Marine Institute, Memorial University of Newfoundland

CHARITY FOCUS: VETERINARIANS WITHOUT BORDERS IN CANADA

BY DR ENID STILES (VETERINARIANS WITHOUT BORDERS/VÉTÉRINAIRES SANS FRONTIÈRES, CANADA)

Perhaps you read about the newly formed Veterinarians without Borders/Vétérinaires sans Frontières in the fifth issue of Aquaculture Health International (May 2006). But what you haven't heard is how much we've grown and how much we've done.


SMALL-HOLD DAIRY FARMING IN INDIA

Our inaugural project in Sri Lanka in November 2005, headed by Mark Sheppard and Craig Stephens, was just the beginning, and has contributed in part to the support, initiation and development of numerous new and exciting projects and placements around the world.

Who are we? In the winter of 2005, David Waltner-Toews, an epidemiologist at the Ontario Veterinary College, approached a few interested veterinarians across Canada with a question: Is there a Vets without Borders in Canada and, if not, don't you think there should be?

And so it began, our very own VWB Canada. Similar to, but not the same as Vets without Borders Europe (although we have a strong working partnership with them), we are our own unique, charitable organisation, with an emphasis on fostering the health of animals, people and the environment that sustains us.

The net worth of the richest 358 people in the world equals that of the poorest 2.3 billion people in the world. World poverty needs to be addressed and it is our mandate to help diminish it. How are we planning to do this? Our members are currently engaged in activities around the world relating to the health of farm animals (aquaculture as well as land-based), urban domestic animals and wildlife, as well as public and ecosystem health.

We use our diverse veterinary skills to build local capacity, not only to recognise and treat diseases once they occur, but also to promote the health of these animals and thereby reduce the occurrence of disease. We want to create healthy homes for all animals, wherever these homes might be, and in so doing, foster healthy and sustainable lives for the people who live with, use the animals and share this global ecosystem.

In our short existence, we have been fortunate to receive the support of private and corporate sponsors. This has allowed us to develop and initiate a few of our own projects, as well as support other, existing projects. These pre-existing projects were selected based on criteria developed by VWB-VSF Canada. The criteria is closely linked to our guiding principles and mission and ensure that the project:

- must be in response to locally identified needs
- must be sustainable beyond our involvement (build local capacity), and
- must respect people, the environment, culture, laws, human rights, tradition and animal welfare.

These are but a few of the criteria we use to select projects and programmes that we will support, and our members are using these same criteria to develop their own projects within VWB-VSF Canada.

Encouraging and educating Canadian veterinary students has been an important aspect of our outreach programme. We are working closely with student clubs in each of Canada's four veterinary schools to promote cross-cultural sensitivity and a critical understanding of development and sustainability issues.

This year alone we have given travel support to two student projects: a Global Vets project from the Ontario Veterinary College and a spay-neuter programme from the Western College of Veterinary Medicine. The Global Vets students are assisting with an investigatory project on the impact of Cysticercosis as a public ▶


NORTHERN COMMUNITIES LEARN ABOUT ENDO-PARASITES


DR JOHN VAN LEEUWEN WITH SMALLHOLDER DAIRY FARMERS IN KENYA

CHARITY FOCUS: VETERINARIANS WITHOUT BORDERS IN CANADA


RESEARCHERS OF THE SRI LANKAN NATIONAL AQUATIC RESEARCH AGENCY

health problem in Busia, Kenya, as well as a lion conservation project in Hwange, Zimbabwe.

Meanwhile, student and faculty teams in First Nation locations of northern Saskatchewan will hold free spay/neuter clinics with the objective of reducing the semi-feral dog population. They will carry out vaccination, deworming and health examination programmes to improve the health status of the small-animal populations. In so doing, they will also contribute to enhanced health of the First Nations people while building capacity through outreach programmes.

> This year our members will return to Sri Lanka to deliver continuing education on fish health

Inadequate access to veterinary training and education, skills and services can be a major limiting factor to the health of communities, animal populations and ecosystems in many impoverished locations throughout the world. VWB-VSF Canada has entered partnerships with organisations such as the Tanzanian Veterinary Association and the Commonwealth Veterinary Association to help reduce poverty and enhance food production in a village (Ilima) by using genetically improved chickens.

We have supported participation with a Farmers helping Farmers project in Kenya that is designed to help smallholder dairy farmers living in extreme poverty by providing information on improved milk production and quality, as well as instruction leading to better health management and veterinary care.

Investigation of pathogens in Brucellosis-infected Tsaatan reindeer of Mongolia, and instruction on chemical and physical restraint of wildlife in Zimbabwe are just a few other ways in which we are helping people by helping animals. Our efforts in Sri Lanka have been very well received across Canada, as well as in Sri Lanka.

This year our members will return to Sri Lanka to deliver continuing education on fish health, and work with the Sri Lankan Department of Animal Production and Health on public health issues.

The continued enthusiasm, perseverance and motivation of our members and board has ensured the success of VWB-VSF Canada during our first year. Although our membership at this time is small, with 150 members from across Canada, it has proven to be a strong force within Canada, displaying our global citizenship and concern for the wellbeing of people all over the world.

Our members devote considerable personal time, energy, vacation days and finances to support and participate in VWB-VSF activities. With little in the way of financial resources, we are dependent on our volunteers, and hope to increase our membership significantly in the coming years.

How can you contribute to VWB-VSF Canada? Become a member, volunteer your expertise, donate equipment, host a fundraising event, or perhaps just tell your friends about us. We are struggling, as any new charitable organisation often does, but we are optimistic that we will be around for years to come. Expect to hear more about us...we are here to stay!

See www.vwb-vsf.ca. To donate aeroplane miles, see www.aeroplan.com/use_your_miles/donate_miles.d

FISH VACCINATION MACHINES IN NORWAY

BY DR SOLVEIG NYGAARD (FISKEHELSE OG MILJØ AS, NORWAY)

This first appeared as an Intervet agenda article (Norwegian) and in the Intervet AAH Newsletter No. 12 (May 2006)

THE LUMIC LV4 IN USE

PHOTO COURTESY OF ARILD KOLLEVÅG

In the early years of the salmon industry in Norway, vaccination by hand was essentially the only method used to vaccinate fish by injection. Since then, a number of vaccination machines have appeared on the market, and today more than half of Norwegian salmon and trout smolts are vaccinated by machine.

Based on the experience that the Norwegian industry has gleaned in the use of specific methods and vaccination machines, this article is an attempt to evaluate some of the current machines, as well as manual vaccination.

Because of the risk of side-effects due to injection vaccination, salmon and trout smolts should ideally weigh more than 40g at vaccination. As all of the machines considered here use fish of this size, product information related to fish size will not be discussed.

Some basic comparative information on four systems is presented in Table 1. There is most experience with manual vaccination of salmon and rainbow trout. Simple syringes that do not use compressed air are the cheapest, but they are labour-intensive and put a strain on the hands of the vaccinators.

Syringes that use compressed air cost more, at around 1300 euros each, but they place less strain on the vaccinator and allow for a higher vaccination rate.

Lumic are currently the most widely used machines for vaccinating salmon in Norway. Lumic entered the market with a two-line machine in 1992. Following further developments, it was launched as a four-line machine, the Lumic LV4, and this is currently their best selling model.

Nordic Fish Tech has been producing machines for the fishing industry for a number of years, and its first vaccination machine was introduced at AquaNor in 2003.

Rossi International developed the EasyVac 8000 (formerly the Thormed 8000), in Denmark in 1994. The technology was developed from the first vaccination machine, the SpinoVac 7000, which entered the market in 1984. The machine is sold with one, two, three, four or six vaccination units. Thirty-three machines were sold in Norway in the 1990s and it is still available.

Table 2 summarises the key operational statistics (vaccination rate, labour requirement and functionality) for each system.

EVALUATING VACCINATION SYSTEMS

In the case of manual vaccination, the vaccinator usually places the fish in a belly-up position for vaccination, although some vaccinators prefer to have the fish on its side on a table. The author is not aware of any studies where the two techniques have been compared. ▶

TABLE 1 – Basic comparative information

Name	Producer	Approx. Cost Price in 2006 (Euro)	Produced Since	No. of Machines Sold (as of June 2006)
Manual vaccination (assuming four vaccinators)	Several producers of needles and syringes	5,500 (incl. compressed air) 5,500 (incl. compressed air)	Pre-1980	
Lumic LV4	Lumic AS 5690 Lundegrend, Norway	33,000	1999	48
NFT 10	Nordic Supply System as 6260 Skodje, Norway	55,000	2003	8
EasyVac 8000	Rossi International Horsens, Denmark	25,000	1994	154

TABLE 2 – Key operational statistics

Name	No. Lines/ Injection Units	Rate (according to producer) No. Fish/Hour	Average Rate (according to user). No. Fish/Hour	No. of Vaccinators/ Operators	Sorted by Size?	Injection Point/Depth Regulated According to Fish Size
Manual vaccination	Free choice, often 4	–	10,000 2,500 per man	Free choice, often 4	No	Yes No
Lumic LV4	4	9,000	9,000	2	No	No No
NFT 10	2	7,000	6-7,000	2	Yes	Yes Yes
EasyVac 8000	4	8,000	7,000	2	No	No No

FISH VACCINATION MACHINES IN NORWAY

TABLE 3 – Manual vaccination

Prerequisites for a good result	Fresh fish
Vaccine at correct temperature	Can be kept in contact with body or in a warm room/tent
Overview of vaccination process	Good
Adjust point of injection	Depends on vaccinator's accuracy and alertness
Adjust needle angle	Possible, desirability of injection at 90 degrees
Adjust depth of injection	Determined on the basis of the needle length
Adjust amount of vaccine	Easily regulated
Replacement of needles and syringes	Easily accessible
Risk of self-injection	Yes

TABLE 4 – Lumic LV4

Prerequisites for a good result	The fish must be sorted before vaccination. The fish must be placed head first into the vaccination chute
Vaccine at correct temperature	Yes
Overview of vaccination process	Operator cannot see the vaccination process
Adjust point of injection	Adjustment is possible, but accurate adjustment is difficult with older machines. Today's machines are improved in this respect
Adjust needle angle	Not possible
Adjust depth of injection	This is done through choice of needle length
Adjust amount of vaccine	Acceptable
Replacement of needles and syringes	Acceptable as regards accessibility
Risk of self-injection	Very low

TABLE 5 – NFT 10

Prerequisites for a good result	Fresh fish
Vaccine at correct temperature	Yes. Hoses and the vaccine are kept at the correct temperature
Overview of vaccination process	The operator cannot see the vaccination process
Adjust point of injection	Regulation is possible to take account of both the length and the breadth of the fish
Adjust needle angle	Not possible
Adjust depth of injection	The depth of injection is determined by the size of the fish
Adjust amount of vaccine	This is done using chambers with different volumes
Replacement of needles and syringes	Acceptable as regards accessibility
Risk of self-injection	Very low

TABLE 6 – EasyVac 8000

Prerequisites for a good result	The fish must be sorted before vaccination. The fish must be placed correctly in the chute
Vaccine at correct temperature	Not standard. Available as extra equipment
Overview of vaccination process	Yes
Adjust point of injection	Regulation is possible to take account of both the length and breadth of the fish
Adjust needle angle	It is possible to set the angle at 15, 30, 45 and 90 degrees
Adjust depth of injection	The injection depth can be regulated, but it is the same for both small and large fish
Adjust amount of vaccine	Acceptable
Replacement of needles and syringes	Acceptable as regards accessibility
Risk of self-injection	Very low

THE EASYVAC 8000 IN OPERATION

PHOTO COURTESY OF ROSSI INTERNATIONAL

Professional vaccinators usually use compressed air syringes to reduce the strain on the vaccinators and achieve a higher vaccination rate. Experience shows that manual injection vaccination gives very good results regarding the point of injection and vaccine deposition. However, the human factor is the key to the result and, in practice, a tired or stressed manual vaccinator will produce a poorer result than that achieved with machine vaccination.

Manual vaccination is tiring work, with a far greater risk of self-injection than is the case with machine vaccination. See table 3.

In conclusion, manual vaccination can be a quick and reliable vaccination method that does not require the fish group to be well sorted. The result depends more on the vaccinator's abilities than is the case with machine vaccination. The method is not optimal in terms of the working environment, especially as there is physical strain on the hands and the risk of self-injection.

LUMIC LV4

The Lumic LV4 has four vaccination lines that are operated by two people. The anaesthetised fish are lifted out of a line and placed head first into the vaccination chute. The fish slide down a partially enclosed chute into the injection chamber, where they are stopped by a cushion. Fish that enter on their back are turned so that their belly points down.

The fish are injected intra-peritoneally from below at an angle of 45 degrees. A hatch at the start of the chute closes when the fish is being vaccinated to prevent two fish from entering at the same time. In the new models the pump for vaccine is improved and can give small vaccine doses of 0.05ml. See table 4.

In conclusion, the Lumic LV4 provides a high rate of vaccination and is reliable, provided that the fish are well sorted, the operator feeds the machine correctly and the machine is correctly adjusted.

The point of injection will vary if the fish are uneven in size. The injection depth is the same regardless of whether the fish is large or small, and the vaccination process is hidden from the operator's view, so any mistakes are not discovered unless the

FISH BEING FED THROUGH THE NFT 10 VACCINATION MACHINE

PHOTO COURTESY OF ARILD KOLLEVÅG

operator is really on the lookout for them.

Development work in the newer machines has improved the accuracy of the vaccine dose and made it possible to inject 0.05ml.

NFT 10

The NFT 10 sorts and vaccinates the fish. The fish can be sorted into four sizes, based on their length, and the machine can be adjusted so that the smallest fish are not vaccinated.

Two operators place the fish on the side of an inclined belt, and the speed of the belt is controlled. On their way to the vaccination chamber, the length of the fish is measured, and both the point and depth of injection are adjusted to take account of fish size. The two vaccination units inject the fish quickly, as the needle is inserted and withdrawn using a compressed air piston.

The speed of the operators determines how many fish are vaccinated. The machine senses the parts of the belt where there are no fish or fish in an incorrect position, and only injects where there are fish in the correct position.

If the supply of vaccine stops or if air gets into the vaccine, the machine will stop after three fish have been vaccinated. The producer considered that glass syringes were too fragile and now uses a metal pump to allocate the correct vaccine dose. The machine can inject small doses of 0.05ml. See table 5.

In conclusion, the NFT 10 is a technically sound sorting and vaccination machine which provides good precision. The machine will sort out and not inject fish in an incorrect position. It gives good results when it is correctly adjusted, and it is a great advantage that the depth of injection is adjusted to take account of the size of each fish.

EASYVAC 8000

The EasyVac 8000 can be supplied with one, two, three, four, six or more vaccine units. This machine has four vaccination units operated by two operators. The fish is placed on its side, nose first, in the vaccination chute, where it slides down until it is stopped by a cushion and held firmly in place by slight pressure from the side.

> All of the methods described above for vaccinating fish function in an acceptable way, provided that certain conditions are met

The injection takes place from the side, and it is possible to adjust the angle of the needle, the injection depth and the point of injection. The injection depth is regulated on the machine and not by changing needles. The operator is able to observe the vaccination process so that any errors can be quickly identified and corrective action taken. Rossi will shortly introduce a new machine for grading and vaccination by injection. See table 6.

In conclusion, the EasyVac 8000 provides a high rate of vaccination, provided that the fish are well sorted, the operator feeds the machine correctly and the machine is correctly adjusted. If the fish are uneven in size, the point of injection will vary according to the size of the fish.

SUMMARY

All of the methods described above for vaccinating fish function in an acceptable way, provided that certain conditions are met. The EasyVac 8000 and Lumic LV4 require the fish to be of the same size, and correct adjustment of the machine is essential to achieve good results.

The NFT 10 also requires correct adjustment of the machine to achieve good results. Of the machines described, this is the only one which both sorts and vaccinates, and where the point and depth of injection are adjusted to take account of the size of the fish. ■