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GLOBAL AQUACULTURE AND AQUATIC ANIMAL HEALTH

Aquaculture is the world's fastest growing (10-20% per year) supplier of animal protein for humans and it should overtake beef output by 2015. Intervet was the first multinational animal health company to realise and act on this potential by purchasing (in 1993) the Norwegian R&D-based biotechnology company, Norbio, which was producing fish vaccines since 1985.

Whenever incomes rise, people want more animal protein. Thus, between 1950 and 1997, world meat consumption went from 17 to 36 kg per capita. The per capita wild fish catch (only 60% for direct human consumption) went from 8 kg in 1950 to 17 kg in 1988, but has since levelled off, its growth falling below that of population growth. Therefore, future growth in animal protein supply will have to come from "fed" sources such as feedlot-produced beef, pork, poultry or farmed aquatic animals. Because intensively fed animals rely on grain, feed conversion efficiency (kg of grain per kg of body weight) will become more important; for beef, pork and poultry it is about 7, 3.5 and 2.2,

respectively, while it is only about 1.5 for most farmed fish. At the World Aquaculture '91 conference, the FAO predicted that the significant gap between demand and supply of fish would have to be bridged by aquaculture production (Table 1).

The global aquaculture industry is under-going a rapid development and intensification. Most recently, indiscriminate expansion of production areas, a largely uncontrolled concentration of production sites and rapid intensification, have brought about increasing pollution and disease problems. Thus, while the average growth rate of fish and shellfish production throughout the 90's is still well above 15% annually, further expansion of aquaculture is likely to occur through major improvements in sustainability and efficiency, including the adoption of effective, preventive disease control methods, instead of curative control methods like antibiotics.

Table 1. World population and aquaculture production needed to bridge the shortfall from the wild fish catch.

Year	Population (millions)	Aquaculture prod. (million tons)
1989	5,200	12.4
1996	5,836	19.5
2000	6,260	37.7
2010	7,200	52.3
2020	8,060	65.7

All this will require major advances in production systems, including improved understanding of fish physiology, nutrition, breeding/selection and diseases, and management systems, and especially development and implementation of good aquatic animal health programmes. Despite intensification and improved technologies, aquaculture in most parts of the world is still in its

infancy, often characterised by traditional traits, a relatively low level of education and know-how, and continuing changes in production systems and conditions. But the overall prospects for the global aquaculture industry are still very positive. With a 1997 first-sale value in excess of US\$ 50.3 billion and attractive annual growth rates, aquaculture is an increasingly significant market for life-science products. Indeed, the aquaculture sector is expected to double or triple in size until 2010 but, for this to be achieved, a much greater range of authorised, ecologically sound, effective and preventive aquatic animal health products will be essential. In conjunction with improved management methods, vaccines will become the most dominant disease control strategy in industrial fish farming, along with diagnostics, disinfectants and anti-parasitics (and, indirectly, endocrines and anaesthetics), while the use of other therapeutics (i.e., antibiotics) is likely to continue to decline.

Intervet is a dominant player in the world aquaculture vaccine market with Aquatic Animal Health R&D programmes present in Bergen, Singapore, Boxmeer and Millsboro, designed to cover the whole range of commercially important species of fish and shellfish. And with our recently vastly increased product/ molecule pharmaceutical portfolio due to the acquisition of Hoechst Roussel Vet in 1999, the future looks bright.

INTERVET NORBIO SINGAPORE

Intervet Norbio Singapore Pte. Ltd., built from scratch, started to operate in January 2000. The centre's main goal is to perform research, development and promotion of state-of-the-art aquatic animal health products and application strategies for the Asian Pacific region. It is located at the Lim Chu Kang Agri-Bio Park outside Singapore.



The U-shaped building is composed of 207 M² office space, 289 M² dry laboratory and 289 M² fish facilities. The fish facilities consist of a quarantine room and five experimental rooms. Each experimental room can independently be controlled for temperature and the salinity of the water used can be adjusted from fresh water to full strength seawater. A total of 66 fish tanks with volumes ranging from 50 to 1300 L are installed. The centre is being managed by a Belgian couple, Dr. Luc Grisez and Dr. Ann Grisez-Tips, whose backgrounds are in aquaculture, bacteriology and physiology. Both previously worked for Intervet at it's headquarters in Boxmeer.

INTERVET HELLAS: NEW AQUATIC ANIMAL HEALTH SPECIALIST

Dr. Elias Tiligadas has joined Intervet Hellas from September 1, 2000. He will be responsible for Intervet's Aquatic Animal Health technical services in Greece. After his graduation in Aquaculture and Fisheries from the Mesolongi Institute in Greece, Elias Tiligadas obtained his Ph.D. at the University of Bangor in the UK on the Biochemistry, Nutrition, Physiology and Reproduction of *Pecten maximus*. He then worked in Greek fish farms and research laboratories.

As the fish specialist, he will be the key person to whom Greek fish farmers can address their questions on aquatic animal health issues.

PRODUCT NEWS

Norvax[®] Compact 6

Intervet Norbio has developed a new vaccine, Norvax[®] Compact 6, that offers long-lasting protection against the major diseases in salmon, specifically against the relevant pathogenic serotypes of *Vibrio anguillarum*, *Vibrio salmonicida*, *Aeromonas salmonicida*, *Vibrio viscosus* and Infectious Pancreatic Necrosis Virus.

This vaccine is part of a new innovative range of vaccines called the Compact range which feature unique characteristics compared with traditional vaccines. The injection volume has been halved from the standard 0.2 ml to 0.1 ml while the amount of antigens has not been changed - this innovative feature allows for reduced vaccine



leakage and reduced side effects. These reductions have been achieved by reducing the amount of oil adjuvant and by the standardisation and refinement of the antigens.

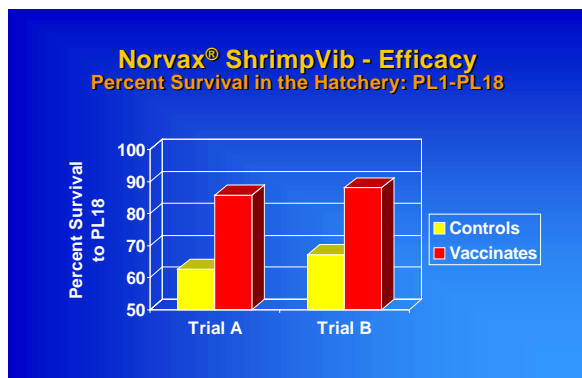
Extensive field trials have demonstrated the excellent efficacy of Norvax[®] Compact 6. The viral VP2 antigen is produced by recombinant DNA technology and is the only IPNV component proven to give effective protection. The bacterial antigens have been thoroughly selected from field strains that showed excellent expression of protective antigens. Norvax[®] Compact 6 helps salmon farmers to achieve profitable harvests in a sustainable way.

Norvax[®] ShrimpVib

A major breakthrough in sustainable shrimp farming and disease prevention was achieved by Intervet with the development and recent launch of the world's first commercial multivalent vaccine against vibriosis in shrimp.

Norvax[®] ShrimpVib is an inactivated, multivalent and uniquely adjuvanted vaccine for the enhanced resistance and protection of penaeid shrimp against the major pathogenic *Vibrio* species infecting shrimp, including the so-called luminous bacterial disease.

Norvax[®] ShrimpVib is easily incorporated into normal hatchery practices as enrichment of *Artemia* nauplii (stage II) with the vaccine is all that is required. The protection induced in the hatchery is carried over into the critical stage in the grow-out phase (days 30 to 80 after transfer).



Field trials performed in Asia and South America have proven the excellent efficacy of the vaccine as the survival, quality and growth of the vaccinated shrimp were significantly improved compared with non-vaccinated shrimp. In addition, the need for the use of costly and environmentally-damaging antibiotics is reduced.

In the next issue of the Newsletter, more information will be provided on the use, safety and efficacy of Norvax[®] ShrimpVib.

DISEASE PROFILES

Vibriosis and Luminal Bacterial Disease in Shrimp

Commercial shrimp culture in Asia and Latin America has suffered huge mortalities due to bacterial pathogens belonging to the genus *Vibrio*. The most frequently encountered *Vibrio* spp. in hatchery and grow-out facilities are *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus*. These are Gram-negative, motile, rod-shaped bacteria that require NaCl for growth. Vibriosis affects all stages of shrimp development but juveniles during the first 30 to 80 days after transfer to the grow-out ponds are especially susceptible.

Some *Vibrio* strains are luminescent and infected shrimp often appear luminescent at night, hence the name luminescent shrimp disease or fire-fly disease. Infections of the exoskeleton often develop into a digestive tract infection, including the hepatopancreas, and eventually systemic septicaemia occurs.

External symptoms

- ◆ Affected shrimp gather in shallow water and at the edges of the tank/pond, are inactive and anorexic and will eventually die, floating to the surface.
- ◆ Melanised shell lesions and/or tail rot.
- ◆ Darkening or reddening of the body.
- ◆ Discoloration of the gills are also seen.

Internal symptoms

- ◆ The hepatopancreas is pale but dark areas (melanised tubules) can be observed.
- ◆ Melanised areas on the gills.
- ◆ Cuticular lesions infiltrating into the muscle showing necrosis (opaque areas).

Diagnosis

Bacteriology: Inoculation of infected tissue or hemolymph on NaCl-enriched media such as TCBS, TSA and BHIA. Further identification of the obtained and purified bacterial strains needs to be carried out using, for example, a biochemical reaction profile (like API), gel electrophoresis of purified LPS or DNA extracts, or slide agglutination tests using specific antibodies.



Histopathology: Cuticular areas (exoskeleton and/or the lining of the oesophagus and stomach) that are colonised by the bacteria show necrosis followed by haemocytic infiltration and melanisation. Infection of the hepatopancreas shows a severe haemocytic encapsulation of the bacterial colonies filling the tubular lumen.

Therapy

Antibiotics have been used excessively with limited and questionable effect, leading to resistance in the bacteria and environmental concerns.

Prevention

Hatchery: Disinfection of eggs, wild nauplii and incoming water. Immunostimulation using Norvax® ShrimpVib vaccine.

Grow-out: Good water quality control. Appropriate selection of good quality fry. Good pond management practices, including fallowing and disinfection. Adequate nutrition.

Winter Disease Syndrome

Over the last few years a disease referred to as Winter Disease Syndrome (WDS) has been affecting (mainly) sea bream in the Mediterranean area. The diseased fish show typical behaviour for this disease, i.e., they swim belly-up and, as the name indicates, they are affected mainly during winter when water temperatures are low.

WDS was first seen in the Mediterranean area in cultured gilthead seabream in 1991; sea bream are apparently affected most but infections in turbot and sea bass have also been reported.

Outbreaks can occur in hatcheries but are mostly found in fish at the grow-out stage. The disease typically starts in fish of medium body weight (100-200 g) and later spreads to the lower and higher weight classes. Mortalities can be over 5% of the total population. Usually, outbreaks occur when the water temperature drops below 14 °C (except in hatcheries where it can occur at higher temperatures) and persist until the temperature has risen to 18 °C.

Etiology

The precise etiology is still a point of discussion amongst researchers. Some consider the disease to be related to a dysfunctional immune system at low water temperatures while others think that a nutritional aspect can not be excluded. We have found a single bacterial species, *Pseudomonas anguilliseptica*, in all the cases that were analysed in our laboratory. This

observation is in accordance with the findings of most other research groups.

However, the disease condition is most probably multifactorial in etiology. This viewpoint is further highlighted by the difficulties different researchers have when trying to establish experimentally-induced infections. Most reports on artificial challenge show that the disease can be reproduced only in conjunction with a severe external stressor. Thus, the disease probably arises from the combined action of low temperature, nutritional imbalance, immunosuppression and the presence of *P. anguilliseptica*.

Incidentally, *P. anguilliseptica* was first described as the causative organism for 'red-spot disease' in eels in 1972 in Japan. Since the original papers, infections in European eel, turbot, Atlantic salmon, rainbow trout, sea bass, herring and others have been described, so freshwater fish as well as marine fish can be affected.

External symptoms

- ◆ Swimming belly-up or on the side
- ◆ Distended abdomen
- ◆ Slightly protruded red vent
- ◆ Occasionally, small haemorrhages around the base of the fins

Internal symptoms

- ◆ Distended intestine filled with clear fluid
- ◆ Ascitic fluid in abdominal cavity
- ◆ Distended gall bladder
- ◆ Pale liver with occasional blood-shot areas
- ◆ Enlarged spleen

Diagnosis

Bacteriology. Samples from brain, kidney, liver and spleen tissues plated on standard, non-selective TSA or BHIA media supplemented with NaCl or MA, incubated at 15 to 20 °C for 3 - 5 days might show small, translucent colonies. These colonies, which are 'sticky', can further be identified using classical identification tests. *P. anguilliseptica* is a Gram-negative long slender rod which is oxidase and catalase positive, non-fermentative and doesn't require NaCl for growth. The motility is temperature and magnesium dependent. The organism is fairly non-reactive and will score negative on most phenotypic tests.

Histopathology. Except for a non-specific inflammatory reaction in the internal organs, no particular features are seen in routine haematoxylin-eosin stain. Modified Gram's stain shows the presence of rod-shaped bacteria in the infected organs.



Therapy

Antibiotic therapy. Oxolinic acid (30 mg/kg of fish) has proven to be effective if administered daily in the feed for a minimum of 3 weeks.

Water temperature. Increase the temperature above 18 °C.

Prevention

As it is considered to be a multifactorial disease, improve the immunological status and give the optimal feeding. Adapt the feeding (reduced fat) to the digestive physiology of fish in winter time.

METHODOLOGY

Fish and Shellfish Vaccination I. Immersion Vaccination

As we often need to vaccinate extremely large numbers of fish at the same time, a quick and efficacious method of administration (i.e., mass application) is beneficial. Such a method is immersion vaccination where fish are immersed in water containing the vaccine. This is an excellent method for vaccinating very small fish, those that are too small to inject. It is also quick, simple and effective, requiring only a minimum of equipment and minimal handling of the fish.

Advantages	Disadvantages
Suitable for small fish	Some handling of fish necessary
Low cost	Suitable only for small fish economically
Not labour intensive	
Safe for vaccinator	
Quick	

Immersion vaccination is ideally performed by immersing the fish in a small quantity of water containing a concentrated vaccine dilution for a very short time (30 seconds to 1 minute), the so-called **dip** method. [Another immersion method, the bath method, where the fish are allowed to swim in larger water volumes containing a more diluted vaccine dilution for 0.5 to 1 hour, is not recommended.] The **dip** method induces good protection in the fish as the most important factor contributing to the success of immersion vaccination is the concentration of antigen.

With immersion vaccination, the vaccine is taken up through the gills, skin (including the lateral line), nares and mouth, and is processed by the local mucosal immune system. After immersion vaccination, antigen-specific antibodies can be found in epithelial cells and phagocytes in the

skin mucous and gills. Once the vaccinated fish come in contact with the live virulent pathogen, the mucosal immune system will immediately react as fish possess an immunological memory.

Unfortunately, not every type of antigen can be applied successfully or induce satisfactory protection by using the immersion method. For those pathogens (e.g., *Aeromonas salmonicida* - Furunculosis), injection vaccination is still required for effective protection.

Methods for Bacteriological Sampling I. Materials for Collection of Samples

A reliable and correct diagnosis of the disease affecting your fish/shrimp depends mostly on the quality of the sampling before it is handed over to the laboratory. In order to promote optimal sampling, we will focus on sampling methods for shrimp and fish in this and the next few issues of the Newsletter. Here we describe the materials required.

Agar plates are needed to seed (inoculate) the sample taken, and to provide nutrients and water for the bacteria present in the sample so that they can grow and develop colonies. It is important to take good care of the agar plates, both before and after inoculation. Agar plates should preferably be kept in the refrigerator at 2-8 °C before inoculation. In addition, even in the refrigerator, plates should be stored either wrapped in cellophane sheets, Parafilm™, Duraseal Stretch Film™ or in clean plastic bags to prevent evaporation. Plates should be discarded when the agar shows signs of dehydration. Dehydration can be noticed when a reduction in the thickness of the agar or the formation of fine wrinkles in the agar surface occurs. When agar plates are too wet, i.e., when water droplets are present on the agar surface, they should not yet be used as the presence of water droplets will interfere with the inoculation. Plates should always be stored lid-down to prevent evaporation of water from the agar.

The lid of the plate should never be removed for longer than the time needed to inoculate the plate. The growth medium in the plate is so rich in nutrients that bacteria present in the air can make contact with the medium and start to grow. Such contamination can delay the correct identification of the causative agent of the disease. Similarly, the agar (or any other) surface inside the plate should never be touched with fingers or any non-sterile object.



The most common **media** used are: Brain-Heart Infusion agar (BHIA), Tryptic Soy agar (TSA) and Thiosulphate Citrate Bile salts Sucrose agar supplemented with 0.5% NaCl (TCBS). In addition, Blood agar or other more specific media can be used in conjunction with either BHIA or TSA. The media should be supplemented with NaCl to a final concentration of 1.5% if used for the isolation of marine bacteria (e.g., *Vibrio* spp.). See below.

For the **preparation of media**, each brand has its own recommendations. Always follow the instructions on the package. However, most media are not ready to be used for isolation of marine bacteria. In order to allow these to grow, you must adapt the salinity of the media. For marine bacteria in general, a final NaCl concentration of 1.5% (= 1.5 gram NaCl per 100 ml of dissolved media) is used. Look at the product leaflet to see how much NaCl is already present and add the amount that is necessary to reach a final concentration of 1.5%.

Most media can be sterilised in the autoclave or in a high-pressure cooking pot. Use glass material that is heat resistant. Don't tighten the cap on the bottle too much but rather leave it a bit loose. TCBS can not be autoclaved. It must be brought to its boiling point and then taken off the flame. [Ideally, a **hot-plate** with a magnetic stirring element should be used - a **magnetic stir-bar** inside the glass beaker then helps prevent the agar from sticking to the bottom when it is boiled. This hot-plate and stir-bar combination also makes it possible to cool the media down slowly and evenly, thus preventing solid material from building up on the sides of the beaker.] Leave the bottle/beaker with media cool down to 50 to 60 °C (the temperature at which you can just bear to hold it without needing gloves) and pour the liquid media into the plates. Pour sufficient liquid media into each sterile plate: the bottom should be covered with a minimum of 5 mm of media in order to provide enough nutrients for the bacteria.

Inoculation loops are used to inoculate a small amount of water or tissue samples onto agar plates. Different types of inoculation loops exist. When no routine laboratory is at hand, it is most convenient to use plastic pre-sterilised disposable loops. These loops can be purchased from several suppliers.

Metal inoculation loops can be reused. However, when using these loops, a Bunsen burner or gas flame (alternatively a gas cooker) should be available, as the loop has to be sterilised between inoculations by holding it in the flame until red-hot. Sufficient time should then be

allowed for cooling of the loop before a sample or an agar plate is touched. The time needed for this is 5 to 10 seconds.

Sterile cotton-tipped swabs are convenient for the inoculation of water samples or liquid tissue sample onto agar plates. However, sterile swabs allow only a poor 'on plate contact' of the different bacteria which might be present in the sample. Therefore, it is advisable when taking a water sample to make a first series of streaks with the sterile swab after which these first streaks are spread further with an inoculation loop. Sterile swabs can be purchased from several suppliers.

A **Bunsen burner** (or alternatively an open gas flame) is needed when taking samples of tissues or when using metal inoculation loops. The flame is normally used for all operations when taking samples, i.e., for pre-sterilising and for intermediate sterilisation of scissors, forceps, scalpel knives, etc.

A good supply of **paper tissues** is a very useful thing to have available when sampling. A **70% ethanol solution** is routinely used to disinfect materials, work area and (possibly) the skin of fish or the shell of shrimps before sectioning. For intermediate sterilisation, it is advised to keep the tips of all sectioning materials immersed in ethanol between the different operations. However, before they are used again, they should be flamed again to evaporate all the alcohol (care should be taken as 70% ethanol is flammable).

Sectioning materials include: scalpel knife with disposable sterile blades; forceps, preferably with fine tips; small scissors; large scissors.

A clean flat washable surface, preferably in a clean, dry room is needed as a **work place** for sectioning and sampling. Do not work under an air conditioner or close to a ventilator since the air flow will generate contamination on the agar plates.



SUMMARIES OF SCIENTIFIC PUBLICATIONS

Toxic factors of *Vibrio* strains pathogenic to shrimp

Diseases of Aquatic Organisms. 40:101-107, 2000.

Goarant C., Herlin J., Brizard R., Marteau A.L., Martin C. and Martin B (New Caledonia and French Polynesia).

Summary Vibriosis is a major disease problem in shrimp aquaculture. 'Syndrome 93' is a seasonal juvenile vibriosis caused by *Vibrio penaeicida* which affects *Litopenaeus stylirostris* in grow-out ponds in New Caledonia. This study assessed the toxic activities of extracellular products (ECP) from *V. penaeicida*, *V. alginolyticus* and *V. nigripulchritudo* using in vivo injections in healthy juvenile *L. stylirostris* (= *Penaeus stylirostris*) and in vitro assays on shrimp primary cell cultures and the fish cell line epithelioma papulosum cyprini (EPC). Toxic effects of ECP were demonstrated for all pathogenic *Vibrio* strains tested both in vivo and in vitro for shrimp only, but no effect was observed on the fish cell line. ECP toxicity for New Caledonian *V. penaeicida* was found only after cultivation at low temperature (20 °C) and not at higher temperature (30 °C). This points to the fact that 'Syndrome 93' episodes are triggered by temperature drops. The assays used here demonstrate the usefulness of primary shrimp cell cultures to study virulence mechanisms of shrimp pathogenic bacteria.

Surface disinfection of Atlantic halibut *Hippoglossus hippoglossus* eggs with ozonated sea-water inactivates nodavirus and increases survival of the larvae

Diseases of Aquatic Organisms. 39:89-96, 2000.

Grotmol S. and Totland G.K. (Norway).

Summary Disinfection by ozonation of sea-water may reduce the risk of transmission of nodavirus, a major fish pathogen, via Atlantic halibut (*Hippoglossus hippoglossus*) eggs. In the present study, eggs at 4 d prior to hatching were exposed to nodavirus and then to ozonated sea-water using different concentrations (0.3 to 10 mg/L) and exposure times (0.5 to 10 min). None of the larvae from virus-exposed eggs washed with ozonated sea-water developed viral encephalopathy and retinopathy (VER), which was detected in all dead larvae from eggs exposed to nodavirus but not washed with ozonated seawater. In the non-treated control group, about 20% of the dead larvae developed the disease. This suggests that the halibut eggs taken from a large-scale production facility were

already contaminated with nodavirus. The egg groups which had been treated with 4 mg O₃/L for 0.5 min or with lower total ozone exposures had a higher survival and no adverse effects on the development of the larvae after hatching were observed. Although a slight delay in hatching was found, after 2 d the cumulative hatching had normalised. In the egg groups with high total exposure (4 mg O₃/L for 1 min or higher total ozone exposures), a pronounced negative effect on hatching was observed. Our results indicate that the egg surface may be important in the transfer of nodavirus and that nodavirus associated with the surface of the egg may be inactivated by ozonated sea-water.

Streptococcus iniae, a bacterial infection in barramundi *Lates calcarifer*

Diseases of Aquatic Organisms. 36:177-181, 1999.

Bromage E.S., Thomas A. and Owens L. (Australia).

Summary The cause of ongoing mortality in barramundi *Lates calcarifer* (Bloch) in seawater culture was identified as *Streptococcus iniae* by biochemical and physiological tests. This is the first published record of this bacterial species in Australia and the first confirmed report of *S. iniae* causing mortality in barramundi. The bacterium was highly pathogenic for barramundi when challenged by bath exposure. The pathogen was found to have a LD₅₀ of 2.5 x 10⁵ and 3.2 x 10⁴ colony-forming units at 48 h and 10 d, respectively. Experimental challenge of barramundi resulted in high levels of mortality (>40%) within a 48-h period. Ten days after the challenge, *S. iniae* could not be isolated from kidney, spleen, liver or eye of surviving fish. However, the organism was easily isolated from the brain of both moribund and healthy fish, indicating that barramundi can carry the bacterium asymptotically.

Review of grouper disease and health management strategies for grouper and other marine finfish diseases.

Web site reference:

<http://naca.fisheries.go.th/grouper/Medan/006.htm>

Bondad-Reantaso M.G., Kanchanakhan S. and Chinabut S. (Thailand and Philippines).

Summary Grouper has recently become one of the most important aquaculture and trade commodities in the Asia-Pacific region. This review paper lists and gives some information on all the major diseases and parasites affecting grouper, and outlines current disease control practices.



A new bacterial white spot syndrome (BWSS) in cultured tiger shrimp *Penaeus monodon* and its comparison with white spot syndrome virus (WSSV) caused by virus.

Diseases of Aquatic Organisms. 41:9-18, 2000.
Wang Y.G., Lee K.L., Najiah M., Shariff M. and Hassan M.D. (Malaysia)

Summary This paper describes a new bacterial white spot syndrome (BWSS) in cultured tiger shrimp. The affected shrimp showed white spots similar to those caused by white spot syndrome virus (WSSV) but the shrimp remained active and grew normally without significant mortalities. The study revealed no evidence of WSSV. Grossly the white spots in BWSS and WSS look similar but under wet mount microscopy they appear different. The bacterial white spots are lichen-like, having perforated centres unlike the melanised dots in WSSV-induced white spots. Bacteriological examination in the study showed that the dominant isolate in the lesions was *Bacillus subtilis*. The occurrence of BWSS may be associated with the regular use in shrimp ponds of probiotics containing *B. subtilis*. The externally induced white spot lesions were localised in the integumental tissues, i.e., cuticle and epidermis, and connective tissues. The BWS lesions are non-fatal in the absence of other complications and are usually shed through moulting.

Evaluation of a rapid coagglutination (COA) test for the detection of infectious pancreatic necrosis virus (IPNV) in tissue samples of Atlantic salmon, *Salmo salar* L.

Journal of Fish Diseases. 22:117-124, 1999.
Taksdal T. and Thorud K. (Norway)

Summary The field use of a staphylococcal coagglutination (COA) test for the detection of infectious pancreatic necrosis virus (IPNV) in tissue samples from Atlantic salmon was evaluated. The COA test was compared with an immunohistochemical (IHC) method for the detection of clinical outbreaks of IPN. The paper describes the evaluation of 320 COA test results performed at local fish health laboratories in Norway from 1994 to 1996, and COA test results from two infection trials with IPNV. The agreement between the COA and IHC tests was very good and the COA test was suited for the detection of outbreaks of IPN. Covert infections with IPNV remained undetected by the COA test. The minimum IPNV titre needed to obtain a positive COA test was $\approx 10^5$ TCID₅₀/ml.

Recent advances in tilapia broodstock management

Proceedings de Acuicultura 1999, Nov 17-20, Puerta La Cruz (Venezuela)

Arul Victor Suresh (USA)

Summary Maximizing seed productivity in hatcheries is the ultimate aim of broodstock management. Traditional tilapia seed production systems suffer from productivity problems that are associated with tilapia's unique reproductive traits such as early maturity, high frequency spawning, low fecundity and high investment in parental care. Research conducted in the past 10 years has demonstrated that many of these productivity problems can be resolved through proper broodstock management. Studies have shown that younger broodstock (1-2 years) and female:male ratios of 2:1 and 3:1 are more productive than older ones and higher ratios, respectively. It has become clear that while nutrition plays an important role in tilapia broodstock performance, 28-32% crude protein feeds formulated for tilapia grow-out can be reliably used for feeding tilapia broodstock. It is also firmly established that clutch-removal (removal of eggs and sac-fry from mouth-brooding females) results in a tremendous improvement in seed productivity. Other broodstock management strategies such as rotation of broodstock and resting and reconditioning of broodstock have also been examined and proven to be effective in improving tilapia seed productivity. In conclusion, a number of technological advances in tilapia broodstock have been made in the past 10 years. Whether those advances can be applied in practical aquaculture would depend on the economic viability of the technological solutions derived from the advances. Studies comparing the cost/benefit ratios of the various solutions are lacking in the public domain at present and are required.

Dominant intestinal microflora of sea bream and sea bass larvae, from two hatcheries, during larval development.

Aquaculture. 155:387-399, 1997.

Grisez L., Reyniers L., Verdonck L., Swings J. and Ollevier F. (Belgium).

Summary The intestinal microflora of larval sea bream (*Dicentrarchus labrax*) and sea bass (*Sparus aurata*) from two marine hatcheries (Greece and Spain) was studied. Samples for bacteriological analysis were taken during feeding regimes of the larvae with rotifers and *Artemia*. A total of five production cycles were examined, namely three of sea bream and two of sea bass. When the larvae were fed rotifers, the incidence of *Vibrio anguillarum*, *V. tubiashii* and



non-vibrio groups was high. During feeding with *Artemia*, *V. alginolyticus*, *V. proteolyticus*, *V. harveyi* and *V. natriegens* were mainly isolated. During larval development, no dominant and persistent colonisation of the intestine by any given bacterial species was observed. Fluctuations in the composition of the dominant microflora appeared to reflect the bacterial composition of the ingested live feed. Selection towards the genus *Vibrio* was not observed until the larvae reached the end of the larval life stage. Two additional samples were taken during massive mortality outbreaks in bream larvae. In both samples, *V. anguillarum* was dominant, being associated with feeding with rotifers. The results suggest that disease outbreaks can occur when *V. anguillarum* dominates in the larval intestine.

Local company contact details:

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It is our hope and intention that all the information contained in this Newsletter is accurate; however, the Newsletter is intended solely to supply useful information to the aquaculture industry. Thus, Intervet International B.V. is not liable for any inaccuracies contained in this Newsletter.

If we receive specific interesting questions on aquatic animal health topics, we will try to answer (a selection of) them in a new Question and Answer section in future issues of the Newsletter.

Intervet Aquatic Animal Health Newsletter

Final copy editor and major contributor:
Dr. Thierry Latscha

Major contributor: Annick Bolland
DVM

Editor, co-ordination, feedback:
Dr. William Enright

