

Newsletter

June 2001

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AQUATIC ANIMAL HEALTH IN INTERVET INC.

After over 15 years in the poultry, swine, cattle, dog and cat health industries, a decision was made to enter the Aquatic Animal Health market in the USA following the lead of Intervet International. The first product licensed was CHORULON® (hCG) which received an FDA license for use as a spawning aid in all finfish in August of 1999. The second product licensed was a live attenuated vaccine for the prevention of Enteric Septicemia of Catfish (ESC) caused by *Edwardsiella ictaluri*. This USDA license was granted in January of 2000. It was at this time that an Aquaculture Group was established in Intervet Inc. with two full time personnel, Kurt Schuster as Business Manager, located in Millsboro, Delaware at the headquarters site, and Clint Dees, Sales Representative located in Indianola, Mississippi, in the heart of the catfish industry.



In addition, the group enjoys support from Ms. Kim Powell in the R&D Dept. of Intervet Inc., Intervet International's Chief Technical Officer Aquatic Animal Health, Dr. Bob Busch (based in Seattle), Mr. Ruud Hein, Director of Technical Services for Poultry and Aquaculture, and the Aquatic Animal Group, International Marketing Dept. and the R&D Division of Intervet International in The Netherlands.

Because of the large market size and potential, the focus to date has been on providing help to the catfish industry in the form of the ESC vaccine. However, evaluation of the needs for other products for catfish and other fish species is on-going.

PRODUCT NEWS

Norvax® Vibriose Mediterranean

Mediterranean marine fish farming is hit with major losses the whole year round due to vibriosis caused by *Vibrio anguillarum*. Norvax® Vibriose was developed specifically for European sea bass and provides adequate

protection against the *Vibrio anguillarum* serotypes found in the Mediterranean area.

The vaccination strategy was developed in such a way as to comply with routine farm practices. It includes a first immersion vaccination at the hatchery on 1-1.5 gram fry. A second (booster) vaccination is absolutely necessary in order to reach long-lasting high levels of protection. The ideal moment to perform this booster vaccination is when the fry has reached 5 to (maximally) 10 grams. Depending on the length of the rearing period until harvest, the booster immersion vaccination should be followed by a second injection booster vaccination in order to provide life-long protection. As it is a water-based vaccine, injection vaccination is free of local reactions.

Cost:benefit analysis example for vaccination of European sea bass with Norvax® Vibriose.

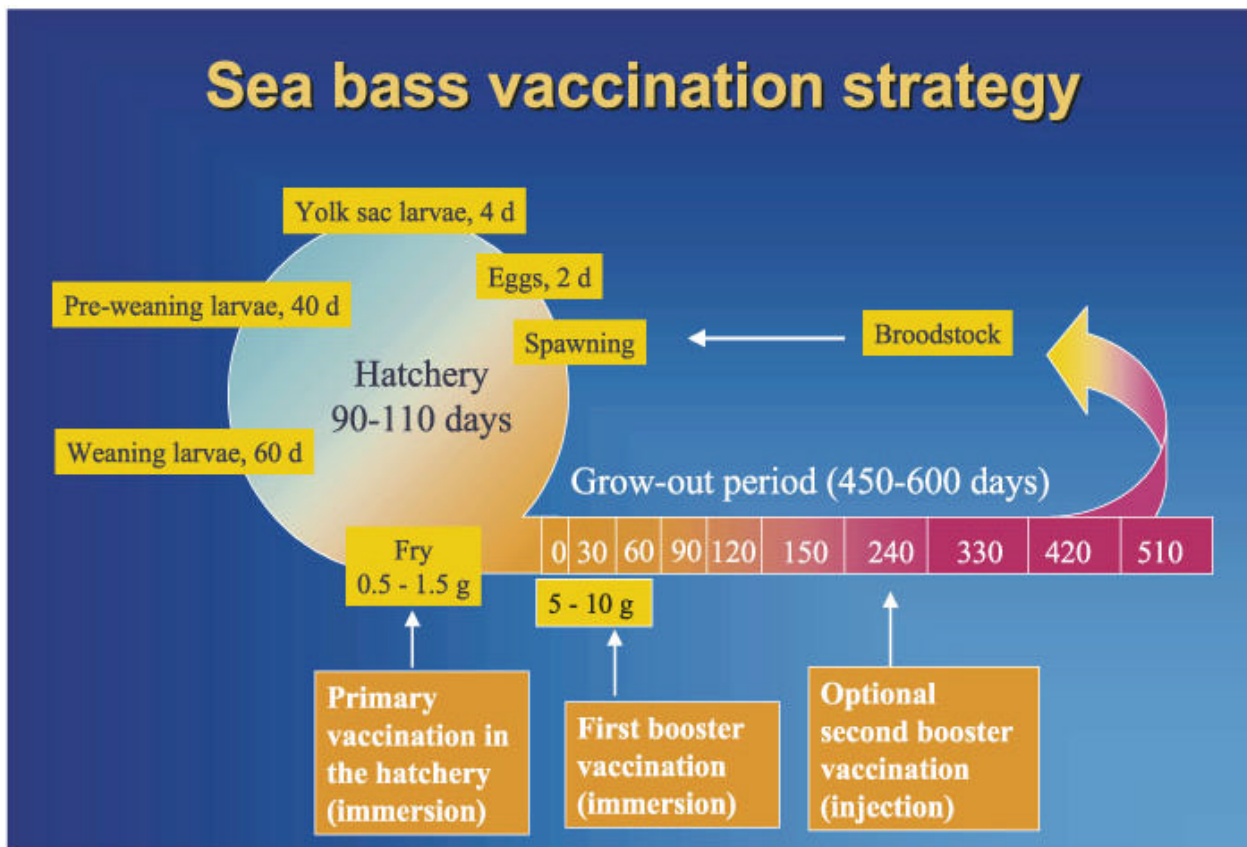
Assumptions:

1. 100,000 fish vaccinated at both 1.5 and 6 g, and compared with 100,000 contemporary non-vaccinated fish.
2. Mortality due to vibriosis is 20% in the non-vaccinated fish and 2% in the vaccinated fish (relative percent survival of 90% due to vaccination).
3. Current market fish (350 g harvest weight) and vaccine prices.

Results:

1. No. harvestable fish: 98,000 vaccinated vs 80,000 non-vaccinated.
2. Total harvest biomass: 34,300 vaccinated vs. 28,000 kg non-vaccinated.
3. Vaccine cost as a percent of fish revenue: 0.68

Cost:benefit ratio of 1:26 by using vaccination with Norvax® Vibriose.



Intervet's new ESC vaccine for catfish

Intervet's new ESC vaccine for catfish is unique in many ways, including the fact that it is the first live attenuated vaccine ever licensed for fish anywhere in the world. This breakthrough product is based on a patented mutant strain of the organism designated as RE-33, which is a natural Rifampin escape mutant of *Edwardsiella ictaluri*, the Gram negative enteric bacteria which causes ESC disease. Due to the unique pathogenesis of *E. ictaluri* in channel catfish, which includes a sequestered infection of macrophages, vaccine antigens based on killed bacterins (which induce primarily a humoral immune response) are not particularly effective in preventing the disease. However, Intervet's live attenuated vaccine stimulates both a humoral as well as a strong cellular response that provides improved long-term protection.

The new ESC vaccine was extensively field tested in the USA in 1998 and 1999, and first introduced for sale in Spring 2000. Based on the performance of the vaccine and the increased survival, growth and productivity experienced by the farmers, use of the product increased significantly this past season (2001) and shows a promising future.

REVIEW ARTICLE

Farm-raised catfish in the U.S.A.

Farm-raised catfish is a highly prized food fish in the United States of America as evidenced by the fact that per capita consumption has doubled since 1985, reaching an all time high of 0.45 kg in 1997. It is now the fifth most popular fish in the USA behind tuna, pollock, salmon and cod. The popularity of farm-raised catfish is due to its consistent quality, delicate flavour, firm texture, versatility, year-around availability and nutritional value. Production of farm-raised catfish was approximately 272 million kg in 1999 and accounted for two-thirds of the annual aquacultural production in the USA.

The farmed-raised catfish industry is centred in the Southeastern USA, primarily on the lower Mississippi River flood plain, in a region locally referred to as "the Delta". A unique combination of physical and socio-economic factors was favourable for development of the industry. Four states (Alabama, Arkansas, Louisiana and Mississippi) account for 95% of catfish production with Mississippi producing 70% of the total. The industry employs over 13,000

people in production, processing, feed manufacturing and related support industries. Sales of farm-raised catfish total about US\$600 million annually, but the total impact on the economies of the four major catfish-producing states exceeds US\$4 billion annually.

Catfish are raised in earthen ponds that are generally 4 to 8 hectares in size and 1 to 2 m deep. Most of the ponds in the Delta are built on flat land with dirt removed to build levees around the perimeter of the pond, and they use ground water supplied by wells as a water source. Some ponds outside of the Delta are watershed ponds that collect rainfall as a water source. There are about 71,000 hectares of catfish ponds currently in production. The production cycle for catfish includes egg and fry production, fingerling production and food fish production.

Spawning of catfish is usually performed by using the open pond method. Male and female brood fish are allowed to spawn in earthen ponds in which spawning containers (plastic buckets, recycled ammunition cans, etc.) have been placed to serve as nesting sites. When the water temperature reaches about 20° C, generally around May in the Southeastern USA, the fish mate. Female brood fish lay eggs (an adhesive mass) in the container and the male fertilises the eggs. The fertilised eggs are then removed from the container and taken into the hatchery. The eggs are hatched in a controlled environment and generally remain in the hatchery for 5 to 10 days. Immediately after the hatching, the fish are referred to as "sac fry" because they have a yolk sac attached which serves as a nutrient source. After a few days, the yolk sac is depleted and the fry swim up to the surface of the water in the hatching tank seeking food. At this point, depending on the preference of the producer, the fry may be left in the hatchery for a few days where they are fed a 50% protein diet made up primarily of marine protein sources or placed into nursery ponds typically at a rate of 250,000 fry per hectare. Once the fry are placed in the nursery ponds, they remain in schools and feed primarily on natural foods found in the pond. The fish are fed a high-protein, powdered feed and, although the fry consume some of the feed, the feed apparently serves more as a fertiliser for pond organisms than as a direct source of food for the fry. Once the fry swim up to the water surface (2 to 3 weeks after stocking) they are fed a pelleted, floating diet containing 35 to 40% protein. Fry stocked in the summer are of suitable size (13 to 15 cm or 16 to 27 kg per 1000 fish) for stocking into grow out ponds by late fall or early winter. Once the

fish are 5 to 8 cm in size, they are commonly referred to as fingerlings.

Fingerling catfish are stocked into ponds at a rate of 12,500 to more than 25,000 fish per hectare for grow out to food fish. They are fed a 28 to 32% protein feed once daily for 150 to 180 days prior to harvest. Catfish may feed at temperatures as low as 16° C, but 30° C is considered optimum. Feed consumption is more consistent at temperatures above about 24° C. Thus, the prime feeding period in the Southeastern USAs is from May to October. Feeding rates typically range from 100 to 120 or more kg per hectare per day. When the fish reaches 0.45 – 0.7 kg in weight, it can be harvested for processing.

Once a crop of fish is of proper size for harvesting, most producers selectively harvest ponds 2 or 3 times each year using seines (weighted nets) with a mesh sized to capture fish of the size suitable for processing while letting smaller fish grade back into the pond. This is commonly referred to as "topping." Under this scenario, once the ponds are "topped", new fingerlings are placed into the pond to replace those that were removed and to replace any losses that may have occurred. This is referred to as "understocking" and is repeated for several years without draining the ponds. Thus, several different year-classes of fish are in the same pond at any given time. Another type of cropping system, referred to as "clean harvesting", in which all fish are removed from the pond and the pond is drained and refilled for restocking is used less extensively than topping. Once the fish are harvested they are placed into aerated tanks on trucks and taken to the processing plant alive. They remain alive until they are processed, which usually occurs within 30 minutes after arriving at the processing plant. The resulting fillets, steaks, nuggets or whole-gutted fish are either individually quick frozen (IQF) or placed on ice for shipment to the various markets.

The farm-raised catfish industry has enjoyed phenomenal growth over the past two decades and, although the growth rate has slowed, the industry is still growing at a rate of 5 to 10% annually. This phenomenal growth of the industry can be attributed in large part to the marketing efforts of the industry through The Catfish Institute (TCI), which was formed in 1986 to educate consumers on the positive qualities of farm-raised catfish. Considering that TCI is continually expanding existing and opening new markets and that abundant natural resources are readily available, the potential for continued expansion of the industry is good.

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Fact Sheet 005 (6/2000) of Mississippi State University

DISEASE PROFILES

Pasteurellosis

Since the 1960's, a variety of marine fish in several parts of the world have been frequently infected with a highly virulent bacterium originally called *Pasteurella piscicida* (thus the name Pasteurellosis). It was renamed in 1995 and is now officially called *Photobacterium damsela* subspecies *piscicida*. This disease is particularly common in Japan (yellowtail), the Mediterranean countries (sea bass and seabream) and the USA (*Morone* spp.). It is a typical summer disease, occurring when the water temperature is high. The mortality can be substantial, especially when occurring in the hatchery.

Etiology

Photobacterium damsela subsp. *piscicida* is a Gram-negative, pleomorphic, non-motile bacterium that grows slowly on NaCl-supplemented media. Serologically, the strains found all over the world are highly homogeneous and there seems to be only one serotype. However, ribotyping revealed genetic variation of different geographical isolates.

External symptoms

The external symptoms vary according to the fish species but are generally not very conspicuous. The fish swim lethargically at the surface, usually gasping for air. The pigmentation of the skin is usually altered (becomes darker or lighter). Slight bleeding can be observed in the fin and head areas, while the gills become paler.

Internal symptoms

Internal organs such as the spleen and kidney are often enlarged and paler. Haemorrhages are sometimes observed on the liver. In the chronic stage of the disease, white nodules typically appear on an enlarged spleen.

Diagnosis

Isolation on NaCl-supplemented media shows the growth of small, shiny, translucent colonies after 48 to 72 hours when incubated at 26° C. It does not grow on TCBS. When using the API-20 E identification kit (BioMérieux Vitek Inc.), a specific code (2005004) is obtained. It is sensitive to vibriostatic compound O/129. Gram staining reveals bipolar staining. Commercial test kits, e.g., those based on agglutination with specific antibodies, are available and easy to use (not requiring a specialised lab), using either infected fish tissue or purified colonies. Specific antibodies can also be used in immunohistochemistry, ELISA, immunofluorescence, etc., but these techniques require specialised lab equipment.

Histopathological examination shows an inflammatory, necrotic reaction caused by bacterial septicaemia, eventually leading to granuloma formation. The bacteria are easily observed in macrophages.

Therapy and prevention

Once the disease has appeared it will frequently reappear as long as the water temperature is high. Most broad spectrum antibiotics are effective as a curative therapy but resistance is often encountered, especially with repeated use. Furthermore, the bacterium has an intracellular life stage during infection, making it non-vulnerable for any kind of drug during this stage and, as such, enhancing the spreading of the infection. The disease outbreak stops when the water temperature declines. A lot of research is being conducted in order to find a highly effective vaccine.

Enteric Septicemia of Catfish

Enteric septicemia of catfish (ESC) is considered, both biologically and economically, as the most important disease of farm-raised channel catfish, particularly in the USA. ESC causes millions of dollars in losses annually to the USA catfish farming industry due to morbidity, mortality and lost production potential. The acute disease is now commonly known as ESC but chronic infections are also known as "hole-in-the-head" disease.

ESC disease may have been present since 1969 but was first recognised and differentiated from a closely related but distinctly different disease of catfish caused by *Edwardsiella tarda* in 1976 and not actually described in the literature in 1979. However, the causative agent of ESC disease, *Edwardsiella ictaluri*, was not actually described as a new species until 1981.

Etiology

ESC is caused by the Gram negative enteric bacteria *Edwardsiella ictaluri*. It is closely related to *E. tarda*, another pathogen of warmwater fish, but the two organisms cause distinctly different clinical diseases and are easily differentiated in the laboratory on the basis of biochemical and serological tests.

Host Range, Geographic Distribution

Channel catfish (*Ictalurus punctatus*) is the most susceptible host but *E. ictaluri* is also known to be pathogenic for white catfish (*I. melas*), walking catfish (*Clarias batrachus*) and brown bullhead (*I. nebulosus*), as well as ornamental fish such as the danio, rosy barb and green knife fish. Some strains of channel catfish appear to be more resistance to ESC disease than others. The European catfish (*Silurus glanis*) and many other commonly cultured warmwater fish are considered resistant. The blue catfish (*I. furcatus*) is considered to be more resistant to the disease than channel catfish and, when hybridised with channel catfish, results in progeny with an intermediate level of resistance. As a Gram negative pathogen, *E. ictaluri* appears to have a comparatively narrow host range but, under experimental laboratory conditions, it has been shown to be pathogenic for a wide variety of fish including trout, salmon, tilapia and other species of catfish and ornamental fish. However, natural outbreaks of ESC disease in these species have not yet been reported.

ESC is wide spread in the USA and considered endemic to all waters with resident populations of catfish. However, *E. ictaluri* has also been reported in Thailand and Australia. Epizootic occurrences are most common in farm-raised stocks but have also been described in wild stocks. Given the relatively slow growth and fastidious nature of the organism, *E. ictaluri* may often remain undetected by diagnostic laboratories unfamiliar with its differential diagnosis and the true host range and distribution may be wider than currently thought.

Epidemiology, Clinical Signs and Pathogenesis

ESC can occur as an acute or chronic disease depending on water temperatures as well as the species, size and prior disease history of the host. The disease tends to be most acute in fry and fingerling fish during the autumn of the first years of production when water temperatures are between 20 and 28 °C (68 to 82 °F), but more chronic infections and mortality can occur almost year around.

The clinical signs of an acute infection with *E. ictaluri* in channel catfish are typical of a Gram-negative enteric bacteremia. Diseased fish typically stop feeding, become lethargic and hang near the surface of the water and sides of the pond in a "tail-down" position. Prior to death, they will often be observed to swim in circles to exhaustion. On clinical examination, clinical signs can vary greatly but diseased fish characteristically exhibit pale gills, "pop-eyes" and a distended abdomen filled with fluid. Pin-prick haemorrhages of the skin are often apparent in the non-pigmented areas under the jaw, belly and base of the fins. Pin-prick haemorrhages are also often seen on the surface of the visceral organs. The kidney and spleen are usually enlarged. The liver is characteristically mottled and pale in colour and can show white spots of focal necrosis. The intestine is usually flaccid, void of food and filled with a bloody fluid.

In more chronic infections, often seen in larger fish or when water temperatures are outside of the optimal range for the disease, diseased fish will often exhibit numerous small (1–3 mm diameter) necrotic (white) or haemorrhagic (red) skin lesions on the sides and back of the fish. One very unique and characteristic sign is a single raised "pimple" over the brain and between the eyes. This "pimple" can progress into an open lesion directly into the brain. This condition is commonly referred to as "hole in the head" disease.

The histopathology of ESC disease is typical of a Gram-negative enteric bacteremia. It is characterised by the presence of bacteria in the blood circulation in all major organs and tissues resulting in inflammation, haemorrhage and necrosis. One unique observation is the appearance of viable organisms inside of macrophages. This is due to the fact that *E. ictaluri* is capable of surviving and even replicating within macrophages for extended periods of time.

Survivors of moderate to severe outbreaks of ESC disease appear to develop a high level of acquired life-long immunity to the disease and subsequent or recurrent outbreaks of the disease in these fish are rare. However, *E. ictaluri* is also known to establish a clinically apparent carrier infection in surviving fish. These carrier fish are able to reservoir and transmit the pathogen through their life, most likely through infected faeces. *E. ictaluri* has also been shown to survive in water at 25 °C for up to 15 days and to remain infective in bottom

mud for over 90 days at 25 °C. Transmission is typically due to direct fish-to-fish contact, particularly when susceptible fish, such as fingerlings, are "under-stocked" in ponds together with larger fish carrying the disease. Likewise, newly constructed earthen ponds tend to experience fewer outbreaks of ESC but the disease becomes more prevalent in later years, even though the ponds are drained, air dried and disinfected between crops.

Differential Diagnosis

ESC is typically diagnosed by culturing *E. ictaluri* from the internal organs and/or brain on an enriched media such as tryptic soy agar (TSA) supplemented with sheep's blood (5% v/v) or brain heart infusion (BHI) agar. Primary cultures should be incubated at 25 to 30 °C for five days. *E. ictaluri* is typically seen as very small (1–2 mm diameter) smooth, round, entire and non-pigmented white colonies at 48 hours or more following inoculation. If non-enriched media or lower incubation temperatures are used, *E. ictaluri* is often overgrown by other organisms and not detected. Therefore, when mixed infections are expected or the diagnostician is not familiar with the pathogen, a selective or differential media such as Shotts and Waldman's EIM media is recommended. If the API 20E biochemical identification system (BioMérieux Vitek Inc.) is used at 30 °C, *E. ictaluri* will result in the code number 4004000. *E. ictaluri* is easily differentiated from *E. tarda* on the basis that it does not produce H₂S on TSI media and is negative for indole in tryptone broth.

Confirmed identification is typically based on biochemical characteristics or serological tests. No serological cross reactions have been reported.

Prevention

Once *E. ictaluri* is endemic to an area, particularly if fish are being produced in earthen ponds, the disease is difficult to prevent through management practices alone. However, the incidence and severity of the disease can often be reduced by avoiding the practice of under-stocking susceptible or naive fingerlings together with larger fish previously exposed to or surviving the disease. Whenever possible, also make sure that all earthen ponds are well drained and thoroughly dried and disinfected before being refilled and stocked with fry or fingerlings.

The withholding of feed at critical times has also been shown to be of value in significantly reducing mortality but this practice also

significantly reduces growth rates and lengthens production cycles and the time to harvest. Unless the fish are either protected by vaccination or by acquired immunity from prior exposure to the disease, it is recommended that feeding rates and frequencies be cut in half or even temporarily suspended when water temperatures are between 20 and 28 °C (68 to 82 °F), a condition which typically occurs for several weeks in the spring and autumn, depending on local weather conditions.

Vaccines have been shown to provide significant protection against ESC disease. Their use can not only reduce the incidence and severity of the disease but also allow full feeding during the spring and autumn seasons when acute ESC disease is most prominent. Intervet now has the only fully licensed and commercially available vaccine against *E. ictaluri* and ESC disease (see later). The vaccine is the first live attenuated vaccine approved for use in fish. It is typically applied to channel catfish fry as a bath treatment in the transport tank during the process of moving fry out of hatchery buildings and stocking outdoor ponds.

Treatment

E. ictaluri is susceptible to a number of broad spectrum antibiotics. However, clinical isolates should be routinely tested for in vitro sensitivity before treatment is initiated as plasma-mediated resistance is common, depending on drug use history. As always, all drugs should be properly registered and labelled for the use intended and label directions should be strictly adhered to.

If you have questions about *Edwardsiella ictaluri* or ESC disease, require any additional information or are interested in learning more about vaccines to prevent ESC, please contact your local Intervet representative or call Kurt Schuster in the United States at +1-302-934-4250 or by e-mail at kurt.schuster@intervet.com

METHODOLOGY

Fish and shellfish vaccination III. Bioencapsulation

Bioencapsulation is a widespread technique and consists of incorporating substances into live organisms, in other words, encapsulating in biological vectors, hence bioencapsulation.

These live organisms ingest the substance during feeding. In fish and shrimp larval rearing, bioencapsulation has been since long used for enrichment of live food such as rotifers and *Artemia*. The enriched rotifers and *Artemia* will as such provide the fish or shrimp larvae with additional essential nutrients.

The major advantages of bioencapsulation is that substances can be administered to the fish or shrimp without having to handle them or causing any stress. On the contrary, since live food is part of the normal feeding regimen, no extra effort is needed. Furthermore, since rotifers and *Artemia* are filter feeders, the incorporation of small particles is very easy. The disadvantages are the rapid excretion of the ingested particles, the high variation in individual incorporated substance (dosage not uniform) and the fact that individual uptake per shrimp or fish larva is difficult to measure and to influence.

Another application of bioencapsulation is the incorporation of antibiotics or other drugs into the live food. Medication of fish larvae is as such possible when other ways of administration such as bath are not effective. Depending on the drug used, this method offers an efficacious delivery method. The bioencapsulation of several broadspectrum antibiotics have been reported to be successful to control bacterial disease outbreaks in larval fish rearing (see last abstract below). The major problem remains how to find the exact dosage.

The latest application of bioencapsulation is using *Artemia* as a vector of vaccine antigens, the so-called "oral vaccination via bioencapsulation by *Artemia* enrichment". A good example of this in practice is with the recommended administration of Norvax® ShrimpVib. Specifically, disinfected *Artemia* cysts are incubated to the nauplii instar II stage. The required amount of *Artemia* instar II for 1 feeding are then concentrated to a density of 1 million nauplii per litre. Ten ml of vaccine is then added per litre of concentrated *Artemia* suspension and the mixture incubated for 1 hour with adequate aeration. Thereafter, the vaccine-enriched *Artemia* are immediately fed to the shrimp postlarvae (PL). Using this method (with the appropriate number and timing of feedings), each PL will receive the antigen in sufficient amounts to elicit a good immune response.

Parasitological examination at the farm

Parasites often account for poor growth, morbidity and mortality in fish farming, both acute and chronic in nature. External parasites and some internal parasites have the major advantage that a diagnosis can be made with minimal equipment, i.e., a pair of scissors and forceps, glass slides and cover slips, and a simple microscope. In contrast to ornamental fish kept by hobbyists, farmed fish offer the advantage that a few fish can easily be sacrificed, thus facilitating a parasitological examination. However, this is not the case for valuable broodstock fish. The examination should always be done in freshly killed fish as parasites tend to leave their host as soon as it is dead. Most parasites, including protozoa, are readily observable at low magnification (10x).

Methods

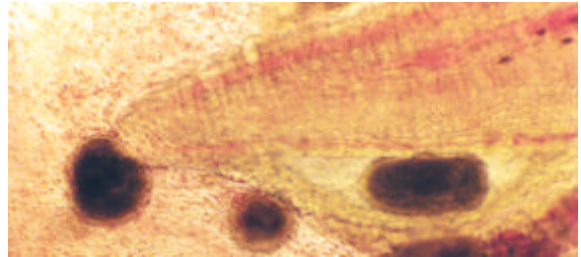
Gills: The easiest way is to perform it on dead fish as the ablation of gill filaments cause bleeding which can lead to infection. Gill filaments should be removed close to their insertion base on the gill arch, placed on a glass microscope slide with a drop of water (to allow spreading of the filaments, allowing detailed examination of each individual filament, as well as to prevent parasites dehydrating) and gently covered by a cover slip, taking care not to crush the filaments and parasites.

Skin: if performed gently, it can be performed with live fish. Slightly scraping the skin directing towards the tail, a mucus sample containing the superficial layer of the skin, including parasites, can be obtained. This mucus is placed on to a glass slide with a drop of water and gently covered by a cover slip, taking care not to crush the parasites.

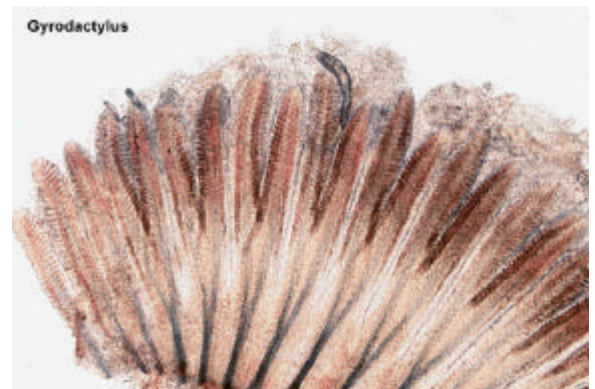
Gall bladder: Myxosporea are often found in the bile and are easily observable under low magnification. The bile is dropped on to a glass slide and covered by a cover slip.

Intestinal tract: large parasites such as roundworms and cestodes are observable with the naked eye. However, the intestinal tract is often infected with protozoan parasites that can only be observed under the microscope. The intestine is cut open longitudinally and a scraping of the inner wall is made. The obtained material is placed onto a glass slide with a drop of water and covered by a cover slip.

Fresh mount of fish gill showing three *Cryptocaryon* marine protozoan parasites, one inside the gill filament.



Fresh mount of fish gills showing the presence of three (one large one at top and two small ones towards the left) *Gyrodactylus* parasites (a freshwater trematode).



SUMMARIES OF SCIENTIFIC PUBLICATIONS

Effect of marine *Eubothrium* sp (Cestoda : *Pseudophyllidea*) on the growth of Atlantic salmon, *Salmo salar* L.

Journal of Fish Diseases. 24:111-119, 2001.

Saksvik, M., Nilsen, F., Nylund, A. and Berland, B. (Norway).

Atlantic Salmon, *Salmo salar* L., experimentally infected with marine *Eubothrium* sp. were kept together with uninfected salmon in the laboratory for 11 months in two tanks, 80 infected and 80 uninfected in each tank. The infected fish had a reduced growth rate compared with the uninfected fish. Significant differences in growth between infected and uninfected fish were not observed until several months post-infection. There was no correlation between the number of *Eubothrium* sp. and fish weight, indicating that even low intensity alters the growth rate of the salmon. The cestode had the same effect on both sexes

of salmon. Haematocrit level was found to be significantly lower in infected compared with uninfected salmon in one of the samples during the experiment.

Effect of temperature on the development of pasteurellosis in carrier gilthead seabream (*Sparus aurata*)

Aquaculture. 195:17-21, 2001.

Magarinos, B., Couso, N., Noya, M., Merino, P., Toranzo, A.E. and Lamas, J. (Spain).

In the present paper we study the effect of water temperature on the development of pasteurellosis in 60-day-old gilthead seabream larvae obtained from asymptomatic carrier broodstock. Fish were exposed to different temperatures and mortalities were recorded over a period of 5 weeks. Mortalities were very low when larvae were kept, at 15 degrees C, increased considerably after the temperature was raised to 18 degrees C or 20 degrees C, and decreased again when the temperature was lowered to 15 degrees C. These results suggest that larvae obtained from asymptomatic carrier broodstock are also carriers and can develop pasteurellosis by increasing the water temperature. However, it is possible to control the disease by maintaining larvae at low temperatures.

Antibiotic resistance of bacteria from shrimp ponds

Aquaculture. 195:193-204, 2001.

Tendencia, E.A. and de la Pena, L.D. (Philippines).

The incidence of antibiotic resistance was compared in bacteria isolated from pond water, pond sediment, water and sediment from the receiving environment (area where water from pond drains, which is 0 and 50 m away from the exit gate, in this study) and cultured shrimp from ponds that have not used any antimicrobials, ponds that have previously used antimicrobials and ponds that are currently using oxolinic acid. Most of the bacteria isolated from all sample and pond type were *Vibrios*. Among the *Vibrios*, *V. harveyi* were most commonly isolated. Multiple antibiotic resistance (MAR) to at least two antimicrobials was highest in ponds currently using oxolinic acid (24% of bacteria isolated from such ponds), followed by those that have previously used antimicrobials (19%) and the least was those from ponds that have not used any antimicrobials (17%). The lowest incidence of antibiotic resistance was observed in ponds that have not used any antimicrobials (41% of the isolates from such ponds). Among the individual antibiotics, incidence of resistance to oxytetracycline was highest (4.3% of the total

number of isolates) followed by furazolidone (1.6%), oxolinic acid (1%) and chloramphenicol (0.66%). Resistance to individual chemotherapeutants did not reflect the pattern of antimicrobial use with ponds that have previously used antimicrobials showing the highest incidence of resistance to one antimicrobial (12% of total isolates from such ponds). Resistance to both oxolinic acid and furazolidone (15% of total number of isolates) was highest compared to other antimicrobial resistance profiles (1-12%). Multiple antimicrobial resistance and intermediate reaction to at least one antimicrobial are associated with antimicrobial use.

Efficacy of hydrogen peroxide to control parasitic infestations on hatchery-reared fish

Journal of Aquatic Animal Health. 12:267-273, 2000.

Rach, J.J., Gaikowski, M.P. and Ramsay, R.T. (USA).

The efficacy of hydrogen peroxide to control external parasitic infestations on juvenile (10-33-g) rainbow trout *Oncorhynchus mykiss* was evaluated in three clinical field trials. Fish were exposed to hydrogen peroxide concentrations ranging from 0 to 560 mg/L for 30 min once every other day for a total of three treatments. Pre- and posttreatment skin scrapes and gill wet mounts of test fish were microscopically examined to identify and enumerate external parasites. Infestation severity was classified as nonexistent (0 organisms), low (1-10 organisms), moderate (11-20 organisms), or high (greater than or equal to 21 organisms). In trial 1, pretreatment skin examinations revealed a severe infestation of the protozoan *Ambiphrya* on all fish examined. Posttreatment skin examinations conducted within 24 h of the last treatment indicated that all hydrogen peroxide treatments eliminated *Ambiphrya*, whereas control fish remained severely infested with the protozoan. In trial 2, pretreatment examinations of skin and gill samples indicated a high infestation of the trematode *Gyrodactylus* (skin) and the protozoan *Trichodina* (gills) on all fish. Posttreatment examinations conducted within 24 h of the last treatment indicated that *Gyrodactylus* was eliminated from the skin of all treated fish; however, the high infestation of *Trichodina* remained on the gills of the test fish. All control fish had high infestation levels of bath parasites. A high infestation of *Ambiphrya* was found on the skin of test fish before treatment (trial 3). Posttreatment examinations conducted 14 d after the last treatment revealed that 56% of the fish were parasite free, whereas

the remaining test fish had low infestation levels. Control fish remained severely infested with the parasite. Based on the efficacy data, all hydrogen peroxide treatment regimens were efficacious in the control of *Ambiphrya* and *Gyrodactylus*.

Effects of low levels of salinity on production characteristics of fingerling channel catfish reared from fry

North American Journal of Aquaculture. 63:156-160, 2001.

O'Neal, C.C. and Weirich, C.R. (USA).

Two production trials were conducted to determine the effects of low levels of salinity on production characteristics of fingerling channel catfish *Ictalurus punctatus* reared from fry under simulated nursery pond conditions. In each trial, fry were stocked into 10,000-L earthen-bottom pools at salinities of 0, 1, 2, or 4 g NaCl/L and reared for 170-d to produce fingerling catfish. At termination, selected production characteristics (weight, length, total yield, percent survival, feed conversion ratio, and feed consumption) were determined. In both trials, final weight and length of fish was greatest at a salinity of 1 g/L. Total yields of fish reared at salinities of 0, 1, or 2 g/L were not significantly different; however, yield was reduced at a salinity of 4 g/L. Survival and feed conversion ratio were not significantly affected by salinity levels tested. The total amount of feed fed to fish reared at a salinity of 4 g/L was significantly less than that fed to fish reared at salinities of 0 and 1 g/L in both trials. Results of this preliminary study suggest that production of fingerling channel catfish is unaffected at salinities of 2 g/L or less and may even be enhanced at a salinity of 1 g/L.

Comparative growth performance of diploid and triploid European sea bass over the first four spawning seasons

Journal of Fish Biology. 58:76-88, 2001.

Felip, A., Piferrer, F., Zanuy, S. and Carrillo, M. (Spain).

During their 3-4 first years of life, triploid sea bass *Dicentrarchus labrax* grew in a similar fashion to diploids in fork length but more slowly than diploids ($P < 0.05$) in body weight, even when the diploids reached full sexual maturity. However, from 48-53 months of age triploids exhibited non-significantly higher instantaneous growth rates, and thus when fish were 4 years or older, differences in weight with diploids were no longer apparent, suggesting that triploidy could be of benefit in the culture of large (>1 kg) sea bass. The condition factor was reduced in both ploidies during the spawning season which took place in winter when the temperature was

low. These observations suggest that any growth advantage in triploids, which were functionally sterile, may be offset by unfavourable environmental conditions. Thus, the potential gain of triploid fish, because they do not direct energy to gonadal growth, could not overcome the effects of low temperature on somatic growth, which coincided with the spawning season. This suggests that the low growth of this species during winter is more a consequence of low temperature than of the energetic cost associated with reproduction. On the other hand, the lower hepatosomatic index in triploid females in contrast to diploid females might be indicative of the lack of gonadal oestradiol-mediated hepatic synthesis of vitellogenin. Also, erythrocyte and haematocrit measurements showed an increased nuclear and cellular volume in triploids, but with similar cell numbers to those of diploids, respectively.

Recent advances in European sea bass and gilthead sea bream nutrition [Review]

Aquaculture International. 8:477-492, 2000.

Oliva-Teles, A.

European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) are amongst the most important finfish species cultured in the Mediterranean region. Production of these species is nowadays a well-controlled process, but knowledge of their nutritional requirements is still very limited. Nevertheless, a considerable amount of data has been accumulated in recent years, and the purpose of this paper is to review the recent advances on the nutritional requirements of sea bass and sea bream. The optimum protein to energy ratio of the diets of sea bass and sea bream seem to be higher than for salmonids, and there is some evidence that high dietary lipid levels have no beneficial effects on fish performances. Although the essential amino acid requirements were estimated by the ideal protein method, data based on the dose-response method is only available for a few amino acids. Essential fatty acid requirements were estimated for sea bream juveniles but data is lacking for sea bass. Vitamin and mineral requirements of these species are practically unknown. Although the importance of broodstock nutrition on gonadal development, spawning and egg quality is recognized, few studies were done to elucidate these aspects. The recent development of microparticulate diets for larvae will contribute to the accurate evaluation of their nutritional requirements.

Standardization of the bioencapsulation of enrofloxacin and oxytetracycline in *Artemia franciscana* Kellogg, 1906

Aquaculture 196:1-12, 2001.

Gomez-Gil B., Cabanillas-Ramos J., Paez-Brambila S. and Roque A. (Mexico).

Bioencapsulation of enrofloxacin and oxytetracycline into *Artemia franciscana* nauplii was standardized. Both antibacterials were delivered to the nauplii individually and the amounts used in the study were percentages of the lipid emulsion Rich((R)) added as nutrition enrichment for the nauplii. The determination of the amounts of drug incorporated in *A. franciscana* nauplii was obtained using a bioassay radial diffusion method, standardized in the laboratory and using *Escherichia coli* as an indicator. The minimum time for full enrichment for both enrofloxacin and oxytetracycline bioencapsulation in *A. franciscana* nauplii was 4 h after the initial exposure of the nauplii to the antibiotics and this was established sampling nauplii at 1, 2, 3, 4, 8 and 24 h after adding the nauplii to the mix. These experiments were carried out twice and at 4 h, 1.10 and 1.13 ng of enrofloxacin per nauplius and 9.32 and 9.37 ng of oxytetracycline per nauplius were obtained. The optimum percentages of enrichment were 40% of enrofloxacin in relation to Rich((R)) and 80% of oxytetracycline. The percentages tested were 10%, 20%, 30%, 40% for the enrofloxacin and 0%, 40%, 80%, and 160% for the oxytetracycline. The maximum time at which antibiotic was still detected in the *Artemia* after it had been introduced in seawater was 8 h for both antibacterial agents.

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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

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