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AQUATIC ANIMAL HEALTH IN INTERVET INTERNATIONAL

For nearly 10 years, Intervet has had aquatic animal health R&D activities at its headquarters in Boxmeer, The Netherlands. Activities were primarily related to (dry) laboratory research towards the development of vaccines against bacterial and viral diseases of fish, in support of our sister R&D site in Bergen, Norway (see issue 2 of the Newsletter) and Singapore (see issue 1). However, two years ago, wet lab facilities were also built; the state-of-the-art facility houses offices, and feed storage/preparation, handling and tank/aquaria rooms. The latter incorporate various size tanks and aquaria for quarantine, water quality control (in and out), fish acclimatisation, and vaccination, challenge/efficacy and GLP safety studies. Various model species, marine and freshwater, can be studied in relation to both pharmaceutical and biological R&D. Besides general R&D for bacterial and viral diseases in certain fish species, special attention at this site is devoted to oral, immersion and injection vaccination technologies and strategies, vaccine/adjuvant technology, pathology and pathogen identification; and providing continuous support to R&D in Bergen and

Singapore with respect to final product development.



DISEASE PROFILES

Pancreas Disease of farmed Atlantic salmon

Pancreas Disease (PD) is an important economic disease of European farmed Atlantic salmon. It can cause significant losses due to morbidity, mortality and reduced production. While the name suggests that the primary organ damaged is the pancreas, severe cardiac and skeletal myopathies are also key features of this disease. Chronic PD has also been known as 'sudden death syndrome' (SDS). PD was first recognised in Scotland in 1976 but not actually described in the literature until 1984. For many years there was some controversy over the aetiology of the disease, with various nutritional and infectious causes being suggested. The causative agent was only isolated in 1995 and

was shown to be an alphavirus, now known as Salmon Pancreas Disease Virus (SPDV).

Aetiology

Salmon pancreas disease virus, originally described as "Togavirus-like" in 1995, was the first alphavirus to be recorded in fish (in 1999) and recent studies have indicated that it is very similar to 'Sleeping disease virus' of rainbow trout. While the first isolate was made in Ireland, it has also been isolated in Scotland and Norway. Preliminary studies would indicate that these isolates are closely related. Various groups have shown that PD can be reproduced experimentally via intraperitoneal and oral routes and, while mortality is not a huge feature of the experimental model, all the key histological lesions are reproduced.

Host range, geographic distribution

Atlantic salmon (*Salmo salar L*) is considered to be the most susceptible host but published experimental challenges have shown that brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) are also susceptible; however, the lesion profile is considerably less severe in these latter species. However, in in-house experimental challenge studies, we found that rainbow trout and Atlantic salmon had equally severe lesions. Pancreas Disease has been described in Scotland, Norway, Ireland, France and the west coast of the USA in farmed Atlantic salmon. Due to the difficulty of isolating SPDV from natural outbreaks of PD and the widespread distribution of Infectious Pancreatic Necrosis virus (IPNV), which can mask the SPDV and the disease in farmed salmon, PD is significantly under diagnosed in the field.

All stages of marine-reared Atlantic salmon are susceptible, including S0, S½ and S1 smolts. There is strong evidence of a high level of acquired life-long immunity to PD as recurrent outbreaks have not been recorded. While experimental PD can be reproduced in fresh water salmon parr, no natural outbreaks of PD have been recorded in freshwater.

Epidemiology

The epidemiology of PD was intensively studied in Ireland from 1989-1994 and PD was revealed to be the most serious cause of mortalities and production losses during that period. The study showed that the majority of cases occurred during the period of August to October following transfer of smolts in the spring. Mortality ranged from 0.1% to 63% and it was significantly higher

if the PD outbreak occurred early in the production cycle. Higher losses tended to occur in high energy off shore or tidal sites. The mean duration of a PD outbreak was 112 days. Since that study, the pattern and outcome of PD outbreaks has become less predictable with disease occurring during all months of the year and in all sizes of fish in Ireland, Scotland and Norway.

There could be a number of explanations for this observation. Since the mid 90's site management practices such as fallowing, single generation rearing, all in-all out stocking policies, slaughtering fish away from the growing site, vaccination and better sea lice control, have improved disease control generally. This may have contributed to the perceived reduced occurrence and losses from PD.

In general, the sea temperatures during recent summers in Ireland, Scotland and some parts of Norway have been higher than previously recorded, remaining above 15°C over the summer period. SPDV is similar to other fish viruses in that it does not multiply rapidly at temperatures over 15°C. It's preferred temperature range is 9-12°C. When PD occurs at winter temperatures, it is very insidious in nature, takes a long time to spread throughout a cage and site, and there is a prolonged recovery phase. From a production point of view, winter PD is potentially much more damaging than a PD outbreak shortly after transfer, as, while mortalities may be less, there is a significant loss of growth over a prolonged period.

Transmission is primarily due to direct fish to fish contact, but the involvement of other marine reservoirs such as molluscs, crustaceans and wild fish, or vectors such as sea lice, cannot be ruled out.

Clinical signs

The clinical signs of acute infection with SPDV can be difficult to detect, especially in winter conditions. Classically there is a sudden drop in feed intake, in some cases preceded by voracious feeding. A variable proportion of fish become lethargic and can be seen swimming around the edges and corners of the net pen. There may be a small increase in mortalities at this viraemic stage, but the most significant mortalities usually occur 3-6 weeks after the acute phase disease at temperatures of 12-

14°C. An increase in yellow cast-like faeces is often associated with PD. Other clinical manifestations include sudden death in large fish, fish lying motionless on the bottom of the pen, unusual swimming behaviour (including circling and spiral swimming) and spitting out feed pellets. A variable percentage of fish become runts.

Gross pathology

In acute phase PD, which is rarely detected, there will be no food in the gut, white or yellow casts in the gut and, occasionally, petechial haemorrhages over the surface of the pyloric caecae. In chronic phase PD, there will be a significant lack of abdominal body fat and poor condition factors in severely affected fish. Large good conditioned fish may show no gross lesions but rupture of the heart is occasionally observed.

Histopathology

Careful interpretation of the key histological lesions is required. At 12-14°C, acute phase PD [0-10 days post infection (pi)] will reveal acute pancreatic acinar cell necrosis, with a rapid disappearance of the majority of pancreatic exocrine tissue. A variable inflammatory reaction is observed at this stage in the peripancreatic fat. Acute necrosis of heart fibres occurs concurrently with the above pancreatic lesions. Cardiac muscle cells in the atrium and the compact and spongy layers of the ventricle can be affected. Affected cells become very eosinophilic and have shrunken nuclei. Skeletal muscle lesions are rarely seen at this stage. Due to the difficulty in recognising early PD, this stage is rarely presented for histological diagnosis.

In mid-stage PD, from 10-21 days pi, there will be significant loss of exocrine pancreas in the majority of fish, with concurrent and variable cardiac myopathy. Skeletal muscle lesions appear around 14 days pi and affect both red and white muscle fibres. The lesions are typical of hyaline degeneration, as described in other species, with swollen fragmented eosinophilic sarcoplasm, central migration of myocytic nuclei and subsequent invasion of the sarcoplasm by phagocytic macrophages. The distribution and severity of these muscle lesions is very variable but can be a good indicator of the prognosis for the population. If you have severe red and white muscle damage, the prognosis for survival is poor.

In chronic phase PD, from 21-42 days pi, the pancreas will be in the recovery phase. In general, if there has been a minimal inflammation or disruption of the peri-acinar tissue, the pancreas has a significant ability to regenerate. In a small percentage of fish, where severe fibrosis of the peri-acinar tissue occurs, the pancreas does not recover and these fish become runts. At this stage, pancreatic acinar tissue will appear around pancreatic ducts and in small clusters around the periphery of the periacinar supporting tissue, and this distribution will be apparent for some time after the PD outbreak.

Relationship between lesions and sample point during a PD outbreak at 12-14°C.

Stage of PD	Pancreas	Heart	Muscle
Acute (1-10 d)	√	√	X
Sub-acute (10-21 d)	√	√	√
Chronic (21-42 d)	X	X/√	√

√ = lesion present; X = lesion absent

The heart has a significant capacity for regeneration of damaged cardiac cells in young smolts and numerous mitotic figures may be seen, especially at the junction of the compact and spongy layers; this is less common in growers. Recovering hearts will appear very cellular with large active nuclei. There will occasionally be focal proliferation of endocardial cells and mild epicarditis. In mildly affected hearts, no obvious changes may be seen at this stage. The skeletal muscle lesions will reach their peak severity at this stage and this often corresponds to peak mortality in the pen. In severely affected fish, all of the red muscle bundles are severely damaged, as are a significant proportion of the white muscle fibres. As fish must continue to swim to survive, there is no possibility of resting damaged muscle to aid recovery; therefore, severely affected fish normally die. If they are in a high energy, high current site, exhaustion will also contribute to the high mortalities usually observed on such sites. If samples are taken after 42 days, it may be difficult to confirm a diagnosis of PD, as the most severely affected fish will have died and the remainder will be in various stages of recovery.

No consistent lesions are seen in other organs of PD-affected fish. Occasionally, single cell necrosis is seen in the liver and focal gliosis in the brain. In some fish, the striated muscle of the oesophagus is damaged, resulting in an

inability to swallow; hence, the spitting behaviour.

Diagnosis

Histopathological examination still remains the principal method for diagnosing PD. Virus isolation remains difficult from natural outbreaks, as the wild type virus is very slow growing in cell culture and can be easily overgrown by IPNV if IPNV is not neutralised. Serological diagnosis can be used for retrospective confirmation of a PD outbreak with SPDV-neutralising antibodies appearing from 14-21 days pi. RT-PCR analysis is available at a limited number of laboratories. An EU research programme has just started to develop rapid diagnostic tests for this problematic disease and rapid specific immunohistochemical and serological tests will hopefully soon be available.

Differential diagnosis

It is important to distinguish PD from IPN in marine farmed Atlantic salmon. The main differences are that:

- IPN tends to occur within 3 months of transfer to sea.
- Mortalities occur during the acute phase of IPN.
- There is not the same degree of appetite loss with IPN.
- The histological lesion profile is different in IPN, with acute pancreatic necrosis (often focal in nature) and a distinct catarrhal enteritis, which is not seen in PD.
- No cardiac or skeletal muscle lesions are observed with IPN.
- Confirmation by immunoperoxidase studies is possible for IPN.

It is also important to remember that concurrent infection with IPNV and SPDV can occur.

In older fish, an important differential diagnostic consideration is Cardiomyopathy Syndrome (CMS), which has been described in Scotland and Norway. No cause has yet been determined but severe PD-related cardiac lesions are very similar to those described in CMS.

Management and Prevention

SPDV is most certainly endemic in Atlantic salmon farming regions in Europe and possibly world-wide. Good management practices, such as fallowing and all in-all out stocking, have undoubtedly reduced the impact of this disease in recent years but there is no doubt that there

would be significant savings to farmers if an effective vaccine was coupled to these measures.

Dietary management has also been applied to reduce stress and aid recovery. Many farms withhold feed from all fish on site for 5-10 days upon suspicion of PD, as there was some circumstantial evidence that this reduced the impact and losses. However, in our experience, it may take 2-3 months for all pens on a site to become infected and this strategy may be causing unnecessary losses in production due to days off feed. If possible, dietary management should be on a cage by cage basis to optimise any effect and reduce consequent growth penalties. Additional vitamin E and C in the diet may aid recovery.

A PD vaccine is in development at Intervet and, after initial trials with a multivalent product, a two-stage vaccination strategy is now being tested as the most effective way of protecting salmon from PD. It has proven to be very effective in small-scale trials and is currently being tested on commercial farms with promising results to date.

Written by Dr. Marian McLoughlin, Veterinarian, RCVS Specialist in Fish Health & Production, Belfast, U.K.

Extra reading:

MF McLoughlin, RN Nelson, JI McCormick, HM Rowley, DG Bryson. Clinical and histological features of naturally occurring pancreas disease in farmed Atlantic salmon, *Salmo salar* L. J. Fish Diseases, 2002, 25:33-43.

PRODUCT NEWS

Chorulon® for Fish

Successful fish farming depends on controlling the entire life cycle of the cultivated fish. This starts with timely management of the fish's reproduction and spawning schedule according to the hatchery requirements. Most fish species are seasonal breeders, and only a few breed continuously. Among the seasonal breeders, there is variation in the time of year when breeding occurs. Fish integrate their reproductive activities with seasonal environmental cycles. Certain environmental factors, such as temperature, photoperiod, rainfall and water quality parameters, act as

cues for the approaching season which is favourable for reproduction. Signals from the environmental cues and endogenous physiological cycles input to the neuroendocrine system, which in turn regulates pituitary and gonadal function. One of the main reasons for the lack of ovulation and spawning in a number of cultured fish is failure of the pituitary gland to release gonadotrophin, one of the hormones involved in the regulation of fish reproduction.

Chorulon® is a purified, sterile and highly effective preparation of chorionic gonadotrophin, which has been routinely used for nearly two decades to induce and synchronize the ovulation and spawning of various cultured fish species.

The high efficacy and standardized properties of **Chorulon®** offer fish farmers the interesting prospect to clear their way to a more efficient, safe and standardized induced breeding performance of their fish.

Chorulon® also provides the fish farmer with a tool to eliminate losses due to partial ovulation and spawning, as well as to enhance egg quality, milt production, sperm volume and motility, and resulting fertilization rates. In comparison to the traditionally used crude fish pituitaries, **Chorulon®** has several further significant advantages (see table) which have become increasingly important in today's modern and intensive fish farming industry:

Product:	Readily available.
Purity:	Highly purified.
Sterility:	Sterile.
Disease risk:	No risk of disease transmission.
Tolerance:	Does not induce antibodies in fish.
Usage:	Easy and ready to use.
Activity:	High standardised biological activity.
Efficacy:	Known dose response.
Response:	Highly predictable latency time.
Fish loss:	No need to kill donor fish.

Chorulon® is the only hormonal spawning aid approved by the FDA in the USA for use in finfish (NADA no. 140-927). It is a freeze-dried preparation of human chorionic gonadotrophin (hCG) for administration after reconstitution in the accompanying sterile diluent. It has luteinizing hormone (GtH-II)-like activity with little or no follicle stimulating hormone (GtH-I) or oestrogenic activity.

After reconstitution, Chorulon® should be administered via intramuscular injection just ventral to the dorsal fin for one (1) to three (3) injections. Any single injection should be administered, depending on the fish species, at a dose of 110 to 1120 I.U./kg body weight (bw) for males and 150 to 4000 I.U./kg bw for females. Summaries of doses tested in representative fish species are contained in the following tables. The dose to be used in other species of finfish may differ from those species listed in the tables, but should generally fall within the ranges given above. For new species, estimated doses should be first tested on a small number of fish.

Table 1: Tested fish species/dose combinations found to be effective.

Common Name, Genus & Species, Family	Tested dose(s) (I.U./kg bw/injection)		No. of injs.	Inj. interval (h)
	Males	Females		
Yellow perch, <i>Perca flavescens</i> , Percidae	nt ¹	150-660	1	--
Striped bass, <i>Morone saxatilis</i> , Percichthyidae	110-1100	165-555	1	--
White bass, <i>Morone chrysops</i> , Percichthyidae	143-1120	200-1650	1	--
Razorback sucker, <i>Xyrauchen texanus</i> , Catostomidae	nt	220	3	24
Walleye, <i>Stizostedion vitreum</i> , Percidae	165-880	320-1820	1-3	72
Red snapper, <i>Lutjanus campechanus</i> , Lutjanidae	550	1100	1	--
Sauger, <i>Stizostedion canadense</i> , Percidae	1100	1100-2200	1	--
Chinese catfish, <i>Clarius fuscus</i> , Clariidae	nt	4000	1	--

Bighead carp, <i>Hypophthalmichthys nobilis</i> , Cyprinidae	nt	360-600	2	8
Silver carp, <i>Hypophthalmichthys molitrix</i> , Cyprinidae	nt	275-2475	2	24
Rohu, <i>Labeo spp.</i> , Cyprinidae	120-150	200-360	1-2	(5-7)
Japanese flounder, <i>Limanda yokohamae</i> , Pleuronectidae	nt	2600-12600	1-2	(72)
Grey mullet, <i>Mugil cephalus</i> , Mugilidae	nt	20000-40000	2	24-28
Gilthead seabream, <i>Sparus aurata</i> , Sparidae	nt	200-400	1-2	(48)
Groupers, <i>Epinephelus sp.</i>	nt	200-500	2-3	24
Sea bass, <i>Dicentrarchus labrax</i> , Mononidae	nt	1000	1	

nt=not tested

Table 2: Tested fish species/dose combinations found to be safe

Common Name, Genus & Species, Family	Tested dose(s) (I.U./kg bw/injection)		No. of injs	Inj. Interval (h)
	Males	Females		
White bass, <i>Morone chrysops</i> , Percichthyidae	1650	3300	1	--
Walleye, <i>Stizostedion vitreum</i> , Percidae	1650	3300	1	--
Grass carp, <i>Ctenopharyngodon idella</i> , Cyprinidae	5500	11000	1	--
Channel catfish, <i>Ictalurus punctatus</i> , Ictaluridae	5500	11000	1	--

METHODOLOGY

Fish and shellfish vaccination IV. Strategy for Reduction of Side Effects with Injection Vaccination - 1

This is the first of four sections in an article on the importance of water temperature, fish size and light (photoperiod) on the development of side effects after intraperitoneal injection vaccination of Atlantic salmon. These are the results of a collaborative research project involving Matre Aquaculture Research Station and Intervet Norbio, both located in Bergen, Norway. The key people involved at Matre Aquaculture Research Station were Arne Berg (Researcher), Tom Hansen (Manager of Matre Aquaculture Research Station) and Eva-Kristine Hansen (Master's student, University of Bergen).

Focus on vaccination strategy

Current vaccination strategies are primarily based on vaccine properties associated with the development and duration of immunity. Little research has been done to formulate a comprehensive vaccination strategy that also attempts to reduce the risk of side effects after vaccination.

As expected, the transition from water-based to oil-based vaccines lead to an increased incidence of side effects. From the first vaccines until those used today, optimisation work has been carried out in an attempt to reduce side effects. However, field experience indicated that vaccine-independent factors also played a significant role in the development of side effects. It was felt that new knowledge about each factor and their interaction(s) was needed to ensure a further reduction in the risk of unacceptable side effects after vaccination.

In 1997 Intervet Norbio initiated research collaboration with the Institute of Marine Research's Matre Aquaculture Research Station. The research project is still going on, and is scheduled to end in 2003. The project aims to identify and generate new knowledge

about risk factors associated with the development of side effects. Based on the new knowledge, we hope to arrive at an optimal vaccination strategy to achieve both high efficacy and reduced side effects.

The results so far are derived from work carried out on S1 smolts vaccinated with the same vaccine (which is no longer in distribution). Industrial scale trials were carried out at Matre Aquaculture Research Station, located on the west coast of Norway. A large number of fish were used in order to make the results as representative as possible of normal production conditions. On-going and new trials involving both S0 and S1 smolts will eventually complete the data already collected.

Extracts from five completed trials will be presented in this and three future Newsletters. The importance of the following vaccine-independent factors was investigated:

- Time after vaccination
- Temperature shortly after vaccination
- Temperature a long time after vaccination
- Size after vaccination
- Light (photoperiod) after vaccination

What is a Speilberg score?

The assessment of side effects after vaccination is based on a scoring system developed and described by veterinarian Lars Speilberg. The side effects are graded on a scale from 0 to 6, where 0 stands for no side effects and 6 indicates the most serious degree of side effects. The degree of seriousness is described in relation to the extent of adhesions of inner organs, pigmentation and formation of granulomas in the abdominal cavity. The assignment of a Speilberg score is based on a subjective assessment that should be performed by a trained person. It is also important that the same person performs all the assessments (without knowing which group the fish comes from), whenever repeated sampling is necessary in a comparative study.

Time of vaccination

Six fish groups from the same population were vaccinated at different times (see table). In the period from August 1998 to March 1999 the groups were vaccinated at 6-week intervals. This trial does not focus on individual factors, but the experiment shows the composite effect of all factors that vary as a function of time. The time period is relevant when vaccinating S1 smolts.

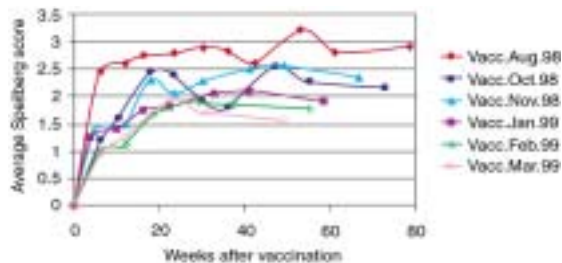
Objective of trial	Investigate the importance of vaccination time with respect to the development of side effects					
	Aug '98	Oct '98	Nov '98	Jan '99	Feb '99	Mar '99
Time of vaccination						
Weight (g) at vaccination	43	85	102	127	154	179
Average temp. (°C) the first 2 weeks after vaccination	14	11	7	7	7	7
Time of sea transfer	May 1999					
Evaluation of side effects	Up to and including 48-79 weeks after vaccination					

The findings show that vaccination in August 1998 generated the most severe side effects, while vaccination in March 1999 generated the least side effects. There was a gradual decline in the side effect profiles from the first to the last vaccinated group. This trial indicates that groups from the same population, but vaccinated at different times, develop significantly different side effect profiles.

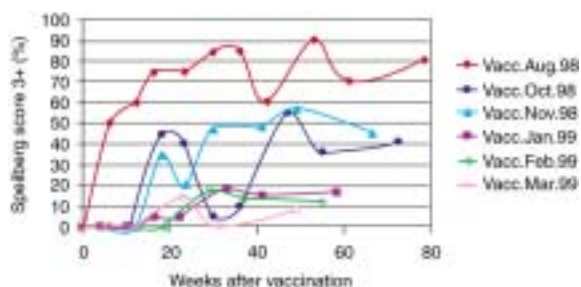
In order to move the project forward, focus was then shifted to the factors showing the largest natural variation as a function of time, namely, temperature, fish size and light (photoperiod). These are all factors that to a large degree can be controlled with current technology in the fish farming industry.

Summary

Vaccination at six different points in time from August 1998 up to and including March 1999 resulted in a gradual decline in side effects, with the lowest side effect profile in the group vaccinated in March 1999.



Development of side effects in relation to time of vaccination.



Frequency of severe side effects (Spielberg score of ≥ 3) related to time of vaccination.

SUMMARIES OF SCIENTIFIC PUBLICATIONS

Recent developments in fish vaccinology

Veterinary Immunology and Immunopathology. 72:203-212, 1999.

Gudding, R, Lillehaug, A, Evensen, O (Norway)
During the last 10 to 20 years vaccination has become established as an important method for prevention of infectious diseases in farmed fish, mainly salmonid species. So far, most commercial vaccines have been inactivated vaccines administered by injection or immersion. Bacterial infections caused by Gram-negative bacteria such as *Vibrio* sp., *Aeromonas* sp., and *Yersinia* sp. have been

effectively controlled by vaccination. With furunculosis, the success is attributed to the use of injectable vaccines containing adjuvants. Vaccines against virus infections, including infectious pancreatic necrosis, have also been used in commercial fish farming. Vaccines against several other bacterial and viral infections have been studied and found to be technically feasible. Pasteurellosis, streptococcosis (lactococcosis) and infections with iridoviruses are candidate diseases for control by immunoprophylaxis in the near future. The overall positive effect of vaccination in farmed fish is reduced mortality. However, for the future of the fish farming industry it is also important that vaccination contributes to a sustainable biological production with negligible consumption of antibiotics. A potential side-effect associated with injectable vaccines is local reactions in the peritoneal cavity. The paper presents recent developments in immunoprophylaxis of fish and some problems that should be addressed by the research community in the years to come.

Infection of the glass-eel swimbladder with the nematode *Anguillicola crassus*

Parasitology. 121:75-83, 2000.

Nimeth K, Zwerger P, Wurtz J, Salvenmoser W, Pelster B (Austria)

The ability of the nematode *Anguillicola crassus* to infect eel larvae (glass-eel stage) was tested. The results show that glass-eels fed on infected copepods, the natural intermediate host of the nematode, can be infected. Light microscopical examination of the infected developing swimbladder tissue revealed that the infection results in a significant thickening of the connective tissue. The basolateral labyrinth of gas gland cells is very much reduced in infected swimbladders, and the distance of gas gland cells to blood capillaries is enlarged. Critical swimming speed, defined as the speed where the larvae were no longer able to swim against the current, was similar in infected and uninfected animals. At intermediate speeds (about 60-80% of critical swimming speed) infected eels showed a slightly higher swimming activity than control animals. Resting oxygen consumption, measured as an index of metabolic activity, within the first 2 months of infection was higher in control animals, which may be due to a reduced rate of activity in infected glass-eels. By 4-5 months after the infection, however, it was significantly higher in infected animals. This may indicate that at this stage a higher activity of the animals is required

to compensate for the increase in body density, but swimming performance of infected and non-infected glass-eels was not significantly different. Oxygen consumption during swimming activity, measured in a swim tunnel at 50% of maximal swimming speed, also was not affected. The results thus show that even glass-eels can be infected with *A. crassus*, and this probably contributes to the rapid spread of the nematode in Europe. While aerobic metabolism during swimming activity is not affected at this stage of infection, the swimbladder tissue shows severe histological changes, which most likely will impair swimbladder function.

Ceratomyxa seriolae* n. sp. and *C. buri* n. sp. (Myxozoa: Myxosporea) from the gallbladder of cultured yellowtail *Seriola quinqueradiata

Syst. Parasitol. 48:125-130, 2001.

Yokoyama H, Fukuda Y (Japan)

Ceratomyxa seriolae n. sp. and *C. buri* n. sp. (Myxozoa: Myxosporea) were found in the gallbladder of cultured yellowtail *Seriola quinqueradiata* [Temminck & Schlegel (Carangidae)] in Japan. Mature spores of *C. seriolae* n. sp. were elongate and 6.5 (6.0-7.5) micron long and 33.7 (28.0-41.5) micron thick. *Disporous plasmodia* of *C. seriolae* n. sp., 40-100 micron in size, were amoeboid to spherical. *C. buri* n. sp. were elliptical with a flattened posterior end, 6.5 (5.5-7.5) micron long and 14.3 (11.0-16.5) micron thick. Spherical plasmodia of *C. buri* n. sp., 15-20 micron in diameter, were disporous. In periodical sampling of yellowtail bile from August, 1999 to February, 2000, the two new species of *Ceratomyxa*, as well as *Myxobolus spirosulcatus* Maeno [Sorimachi, Ogawa & Kearns 1995], first appeared in October, and the prevalences were very variable (20-100%) during the study period.

The efficacy of exogenous hormones in stimulating changes in plasma steroids and ovulation in wild black bream *Acanthopagrus butcheri* is improved by treatment at capture

Aquaculture. 191:351-366, 2002.

Haddy, JA, Pankhurst, NW (Australia)

Sexually mature female black bream were captured by rod and line and injected with saline, human chorionic gonadotropin (**hCG**) or luteinizing hormone releasing hormone analogue (LHRHa) at capture, or 24 h post capture (saline and LHRHa treatments only). All

fish were bled and checked for ovulation for 5 days post injection. Plasma levels of oestradiol (E_2), testosterone (T), 17,20 β -dihydroxy-4-pregnen-3-one (17,20 β P) and cortisol were determined by radioimmunoassay. Saline-injected fish ovulated only on day 1, whereas treatment with LHRHa or **hCG** resulted in fish ovulating throughout the experiment. Treatment with LHRHa at capture resulted in a better ovulatory response than treatment with **hCG** at capture or LHRHa 24 h post capture. Plasma E_2 levels in saline-injected fish were high at capture and had significantly dropped 1 day after capture. Injection with **hCG** or LHRHa at capture resulted in plasma E_2 levels remaining significantly elevated for 2 days post injection. Injection of LHRHa 24 h post capture failed to significantly elevate plasma E_2 levels over controls. Plasma T levels were similar to E_2 profiles. Plasma levels of 17,20 β P were not significantly different between any treatments, but showed a tendency to increase after capture. Plasma cortisol levels showed no treatment effects and were initially low at capture before becoming elevated between days 1 and 2 post capture. These results show that capture and handling stress reduce the responsiveness of fish to exogenous hormone treatment and that best results are obtained if hormonal treatment is administered at the time of capture.

Off-season spawning of sunshine bass (*Morone chrysops* × *M. saxatilis*) exposed to 6- or 9-month phase-shifted photothermal cycles

Aquaculture. 167:67-83, 1998.

Tate, AE, Helfrich, LA (USA)

Annually-spawning sunshine bass (*Morone chrysops* × *M. saxatilis*) were exposed to 6-, 9- or 12-month photothermal regimes. Abbreviated (phase-shifted) cycles were produced by excising photothermal conditions corresponding to May 17 to August 17 (9-month cycle), or May 17 to November 17 (6-month cycle), from a simulated annual cycle. Phase-shifted cycles reduced the interval between spawning and ovarian recrudescence, advancing gonadal maturation 2 to 3 months, and permitting off-season (December and January) spawning. Rapid gonadal growth began in June (6-month), July (9-month) and August (12-month cycle), coincident with decreasing artificial photoperiods and water temperatures. The offset in initiation of maturation persisted through final maturation, as females in the 6-, 9- or 12-month cycles matured after different

periods of photothermal exposure (206, 240 and 268 days, respectively). Maximum oocyte diameters (855, 893 and 844 μ m) were similar among females on all cycles. All females underwent final oocyte maturation (FOM) and ovulated after human chorionic gonadotropin (hCG) injection. Testicular growth among males on the three cycles began simultaneously (June). Maximum testicular diameters (11.2, 11.9 and 11.5 mm, respectively) were achieved after 153, 174 and 199 days in the 6, 9 and 12-month cycles, respectively. Milt was produced by >90% of the males in each treatment, and the duration of production was proportional to the cycle length, lasting 38, 42 and 91 days in the 6, 9 and 12-month cycles, respectively. Sperm concentration, duration of motility, and seminal fluid pH differed among males on the three cycles, but these differences produced no changes in fertilities, which averaged 69, 59, and 68% for fish on the 6-, 9- or 12-month cycles, respectively. All sunshine bass held on a second, sequential 6-month cycle developed mature gonads and were induced to spawn. However, peak oocyte and ovarian diameters were reduced and fertility was low (29%), suggesting additional time was needed for gonadal maturation. The good reproductive performance of fish exposed to 6- and 9-month phase-shifted periods indicates that shortened cycles are effective for inducing off-season maturation of sunshine bass, and suggests merit in evaluating the efficacy of phase-shifted cycles to advance maturation of higher-value striped bass broodstock.

Induction of out-of-season spawning in walleye (*Stizostedion vitreum*)

Aquaculture. 163:151-161, 1998.

Malison, JA, Procarione, LS, Kayes, TB, Hansen, JF, Held, JA (USA)

Simple environmental and hormonal treatments were used to induce out-of-season spawning in walleye *Stizostedion vitreum* up to 10 weeks prior to the normal reproductive season. Wild walleye were captured in the autumn, held in earthen ponds, and in late January, February, and March (approximately 10, 6, and 3 weeks prior to natural spawning), 16-20 female and 4-8 male walleye were recaptured and transferred to indoor tanks. Water temperature was raised from 2°C to 10°C over a one week period, and photoperiod held at 12 h light: 12 h dark. The females were injected with either human chorionic gonadotropin (hCG), des-Gly¹⁰ [D-Ala⁶] LHRH-ethylamide (LHRHa), hCG and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20-P),

or saline as a control. Each month, at least some females in each treatment group were successfully induced to ovulate. No control fish ovulated at any time. In January, hCG was the most effective treatment at inducing ovulation (3/5 fish). In February and March, all but one hormone-injected fish ovulated. In general, the eggs collected from fish treated with either hCG or LHRHa were of good quality with overall survival highest in hCG-treated fish. Eggs collected from 17,20-P-treated fish were small and had very low survival. In February and March, serum levels of estradiol-17 β and testosterone were different between fish treated with 17,20-P and those treated with either hCG or LHRHa. Out-of-season spawning could be used to provide walleye fry for intensive culture systems at multiple times of the year, thereby facilitating research on indoor fry culture. In addition, walleye fingerling production could be initiated as early as January, allowing public and private hatcheries to produce larger age-0 walleye fingerlings for stocking than would otherwise be possible.

Interactions between monogenean parasites and their fish hosts

International J. for Parasitology. 32:309-319, 2002

Buchmann, K, Lindenstrøm, T (Denmark)

Parasite factors associated with recognition and selection of the host and the mechanisms in the host responsible for acceptance or rejection of the invading organism were evaluated. Sensory structures in parasites are able to detect differences between different fish species and this ability to discern between fishes may be based on both chemical and mechanical stimuli on the host surface. Complex glycoproteins, proteins, carbohydrates and simple molecules attract parasites or modify their behaviour. Furthermore, attachment of the monogenean parasite to a host is dependent on both mechanical structures and chemical factors in the parasite. These systems comprise anterior pads, posterior haptors, gland secretions, and muscular elements. The parasite needs access to appropriate nutrients which can be absorbed and used for reproduction and in this context signals from the host are needed for an optimal physiological response of the parasite. The innate and adaptive immune systems of the host are important elements in this question. Investigations have indicated that innate host factors (complement, lectins, acute phase reactants, macrophages) can bind to monogeneans and elicit severe damage to the

parasites. The targets for these hostile products are not only the monogenean tegument, but may involve the gastrodermis and glands. However, the parasite's ability to avoid and even exploit the wide array of immunological elements of the host may be an important player in the dynamic interactions between host and monogenean determining host specificity. Even fish hosts susceptible to a certain parasite show an ability to mount a protective response at post-infection periods. Elevation of the host's production of adaptive and non-adaptive factors following monogenean infections of a certain duration may explain the acquired response.

challenge was performed 10 wk post-vaccination.

Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*, and evaluation of a vaccine against SJNNV

Diseases of Aquatic Organisms. 45:33-44, 2001.

Húsiga, S, Grotmol, S, Hjeltnes, B K, Rødseth, OM and Biering, E (Norway)

Immunisation by intraperitoneal injection of an oil-emulgated recombinant partial capsid protein (rT2) from striped jack nervous necrosis virus (SJNNV) was performed on adult turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*. A specific humoral immune response was recorded in both species, and the levels of rT2-specific antibodies increased markedly in all groups during the 20 wk experiment. A challenge model for SJNNV was established by intramuscular injection of juvenile turbot. The turbot developed viral encephalopathy and retinopathy (VER), also known as viral nervous necrosis (VNN), with cumulative mortality in the range of 25 to 66%, after intramuscular inoculation with SJNNV propagated in the striped snake head cell line (SSN-1). Although neither clinical signs nor mortality were registered, SJNNV was neuroinvasive after bath exposure. The infection after both modes of challenge was verified by means of immunohistochemistry and RT-PCR, and SJNNV was reisolated in cell culture. The results indicate that SJNNV may have entered the central nervous system (CNS) by axonal transport through motor nerves after intramuscular inoculation. A vaccine efficacy test was performed on juvenile turbot, employing oil emulsified rT2 as a test vaccine and intramuscular inoculation of SJNNV. Significant protection was observed when the



PHOTO

Some of the research and development (R&D), and technical and marketing support (TMS) staff of Intervet's aquatic animal health team recently met at Intervet International bv, Boxmeer, The Netherlands. From left to right: Luc Grisez (R&D, Singapore), Marian McLoughlin (TMS Consultant – UK & Ireland), Eric Rijke (R&D, Boxmeer), Annick Bolland (TMS, Boxmeer), Dag Knappskog (TMS, Bergen), Zilong Tan (TMS, Singapore), Odd-Magne Roedseth (R&D, Bergen), Y. Wada (R&D, Japan), Arild Tangeraas (R&D, Bergen), Alistair Brown (TMS, Bergen/Boxmeer), Emiel Hendriks (R&D, Boxmeer), William Enright (TMS, Boxmeer).

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