Project no. SSPE-CT-2003-502329

PANDA

Permanent network to strengthen expertise on infectious diseases of aquaculture species and scientific advice to EU policy

Coordination Action

Scientific support to policies

Deliverable 10 - Environmentally safe control strategies

Due date of deliverable: Month 30
Actual submission date: Month 44

Start date of project:01/01/04 Duration:44 months

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Revision [1.0]

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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1. Executive summary

Aquatic animal health management is a wide complex area of research since diseases depends on species, farming systems, environmental conditions and pathogen characteristics. Good husbandry and management practices at farm level shift the balance in favor of cultured organisms versus opportunistic or real pathogens is in all cases the cornerstone of any successful health strategy. Appropriate water quality and stocking densities, correct feeding strategy and good hygiene standards as well as appropriate vaccination plans are factors that could play significant role in the improvement of farm health status. Quarantine involving thermal or chemical water disinfection is a necessary precaution during imports of live animals and gametes. Appropriate protocols for disinfection and sanitary handling and disposal of mortalities and appropriate methods for treatment of infected fish by products are essential to contain the disease in a farm or an area. Site selection and farm carrying capacity significantly influence disease patterns. Clean water supply or appropriate inlet water treatment as well as disinfection of the effluent water in land-based farms are important means of disease control. Quarantine protocols for imported disease free broodstock, disinfection of eggs prior to introduction from reliable sources, minimised handling induced stress, year class separation, species appropriate stocking densities are significant factors affecting farm health management that are not well understood and are not supported by applied research. Parasitic biological circle and intermediate hosts knowledge is paramount in effective prevention, containment and treatment of these pathogens. New options are made available and always the most environmentally friendly method should be utilised. Selection of disease resistant stocks does not always coincide with fast growing populations and selection for a disease might be associated with increased susceptibility to others. Fast growing families have also been proven more susceptible to disease outbreaks. Multifactor genetic selection is very important in order to be relevant for the industry. Disease prevention by the application of vaccines and immunostimulants as well as alternative treatments, where applicable, have in recent years advanced aquaculture as we know it resolving the risk from major especially bacterial diseases. Vaccines significantly reduce the need for other therapeutics, saving costs, and reducing problems such as antibiotic resistance and concerns over residual levels or environmental impact. Definition of 3uestionna vaccination strategies with the selection of the right type of vaccines, application method and schedule to adapt to the epidemiology of the disease in the farm is important to alleviate pressure especially in culture and health management of new species. While vaccination, strictly speaking, is not applicable in the case of mollusc diseases because of the lack of antibodies, the use of chemotherapeutics may be relevant for aquaculture in some particular conditions like in hatchery-nursery, but is not practical in the natural environment.

The aim of this report is to illustrate the main areas of interest in terms of prevention, containment and treatment not only of the diseases identified in WP2. While the focus in Aquatic Health Management is on the animal health and welfare, the compliance with current EU, state and regional legislation and the consumer safety it is closely interlinked with the environmental future sustainability in terms of effects on the aquatic environment and the wild fauna as well as the aquaculture industry's economic viability.

2. Introduction

The aim of this Workpackage is to:

- Consider the currently available methods for the prevention, containment and treatment of the most serious diseases
- Identify those which are applicable in different production systems as well as being environmentally safe
- Advise where research is needed to develop alternatives to those which may have adverse effects on the environment

This Report on Evaluation of current methods for controlling the disease hazards is the deliverable of this workpackage and includes:

- Assessment of their likely impact on the environment and
- Recommendations for their application in different production systems and for various aquatic animal species.
- Identification of training needs for scientists and fish farmers
- Identification of gaps in knowledge and research needs regarding development of these methods in European aquaculture.
- Recommendations for guidelines and policy / legislation options with regards to application of new methods for the control of the diseases

FEAP was allocated as leader of this workpackage due to it's relevance to the future and sustainability of the aquaculture industry. Dr. Panos Christofilogiannis FEAP consultant that was the WP5 coordinator selected the five main areas of interest in order to divide the work to fish health experts:

- Antimicrobial chemotherapy
- Vaccine technology
- Antiparasitic treatments
- Genetic resistance
- Alternative treatments

Table 1. Experts called to participate in the task force

WP5 Task force expert	Institute	Country	Field
Myriam Algoet	CEFAS	UK	Alternative treatments
Tony Ellis	FRS	UK	Vaccination Methods
Pete Smith	UIG	Ireland	Antimictobial Chemotherapy
Kurt Buchmann	RVAU	Denmark	Antiparasitic treatments
Pierre Boundry	IFREMER	France	Genetic Resistance
Panos Christofilogiannis	FEAP	Belgium	Health Management

The first task force meeting was realised in Luton and the second task force meeting was in Hydra island Greece. Due to lack of availability three members of the taskforce requested to be substituted after the second task force meeting.

The new WP5 task force included three new members in the areas of Alternative treatments, Genetic resistance and Antiparasitic treatments:

Table 2. Experts called to participate in the second task force

WP5 Task force expert	Institute	Country	Field
David Verner-Jeffreys	CEFAS	UK	Alternative treatments
Tony Ellis	FRS	UK	Vaccination Methods
Pete Smith	UIG	Ireland	Antimictobial Chemotherapy
Efi Athanasopoulou	UoThessaly	Greece	Antiparasitic treatments
Richard Paley	CEFAS	UK	Genetic Resistance
Panos Christofilogiannis	FEAP	Belgium	Health Management

Two more task force meeting were organised in Lelystad and Weymouth while the coordinator visited CEFAS in Weymouth two more times to work with CEFAS task force members and other experts on the finalisation of the WP5 report.
Task force meeting (Weymouth) - Involvement of external experts:

- Dr. Grant Stentiford (Crustacea)
- Dr. Matt Longshaw (parasites / molluscs)
- Dr. Isabelle Arzul (molluscs)
- Dr. Richard Paley (Resistance breeding)
- Dr. David Verner-Jeffreys (Biosecurity - Disinfection)
- Dr. Myriam Algoet (Alternative treatments)
-

WP5 organised a workshop on "Critical review of fish health strategies clinical efficacy and environmental impact" in Hydra, Greece May 2005 in extention of a PANDA consortium meeting and following FEAP Annual General Assembly. Please see details on the programme and presentations on http://www.europanda.net/m_area/docs/wp5/Hydraworkshop.xls

Prof. Pete Smith developed and sent a 5uestionnaire to PANDA members on "Current susceptibility testing practices of fish health diagnostic laboratories"

Prof. Efi Athanassopoulou wrote a chapter on an "Overview of the treatments for parasitic disease in Mediterranean aquaculture" that formed the basis for the antiparasitic treatment chapter.

Dr. Panos Christofilogiannis wrote a General introduction of the policy background of control of diseases and infections in aquatic animals in PROFET Policy for the Workshop on coldwater marine aquaculture Bergen, Norway 15-16th March 2007

The project co-oordinator (Dr Barry Hill) gave a presentation in the final PANDA Workshop in Weymouth in March 2007. For more detils on the programme and presentations please log on to: http://www.europanda.net/whatsnew.aspx

Finally the coordinator presented in the PANDA consortium meeting in June in Corfu the progress on the final report.

Aquatic animal health management is a wide complex area of research. This report while describes available options for the WP2 identified diseases briefly focuses on the principles for the prevention, containment and treatment of aquatic animal diseases that could assist pathologists, fish farmers, administrators and politicians to

understand the issues and the requirements for future steps in policy making and implementation.

3. Disease cards

3.1 Fish diseases

3.1.1 Epizootic haematopoietic necrosis

Epizootic haematopoietic necrosis virus is a member of the family Iridoviridae and genus *Ranavirus*, affecting a wide range of teleosts. The natural hosts of EHNV are uncertain, but as the virus is closely related to viruses that infect amphibians (and reptiles) it is possible that reservoirs of infection could exist outside fish populations. EHN disease has only been recorded from south-eastern Australia. Outbreaks in redfin perch *(Perca fluviatilis)* and trout *(Oncorhynchus mykiss)* typically occur in summer when the water temperatures range above 11°C to 17°C (Langdon *et al*, 1986; Whittington *et al* 1994). Rainbow trout are susceptible experimentally at temperatures ranging from 8°C to 21°C, while redfin perch are refractory to infection at temperatures below 12°C. Natural out-breaks in redfin perch typically involve all age classes when the virus is first introduced to an area but thereafter annual epizootics in juveniles are seen. In rainbow trout, mortalities may be seen in any age class, but are most noticeable in fingerlings. The incubation period is affected by environmental temperature (Whittington and Reddacliff, 1995). EHNV although heat sensitive, could survive for long periods in the aquatic environment and on fomites (Langdon, 1989). EHNV persists for a long time (more than 2 years in tissues at -20^{o}C and for more than 97 days in distilled water) without decrease in titre; resists desiccation for more than 115 days at 15^{o}C but is labile outside a narrow pH range either side of neutral but is not completely inactivated by 400 mg/L hypochlorite in the presence of organic material (Langdon, 1989). Outbreaks in trout farms may be related to epidemics in redfin perch in the watershed. The role that survivors play as carriers of infection is not fully understood. There is no effective treatment for EHN.

Vaccines:	• Not available
Chemotherapy:	• Not possible
Immunostimulation:	• Not tested
Resistance breeding:	• Not available
General husbandry practices:	• Implementation of hygiene practices at farms. • Low stocking densities and adequate water quality • Proper cleaning and disinfection of site. • Stocking with fish of known health status. It is uncertain whether a true carrier state occurs in rainbow trout. Vertical or egg-associated transmission cannot be ruled out • Avoid mixing of fish from different sites. EHNV is readily spread in water, but infection can be transmitted between aquaculture establishments with movements of fish. There is generally a very low prevalence of infection in rainbow trout so that the virus can easily be transmitted inadvertently with batches of fish. • Virus inactivation (CEFAS 2005) - As the virus is highly resistant it can be spread on fishing gear, other inanimate objects and by piscivorous birds - Langdon concluded that the virus could survive for long periods in the aquatic environment and on fomites (Langdon, 1989). - Sensitive to Temperature and High pH - Resistance to Acid ensiling • Prevention of spread of the disease - Strict quarantine with movement controls would be required to prevent spread. - Destruction of all infected and exposed fish. - Thorough cleaning and disinfection of site. The virus is transmitted through infected transport water and equipment. - Proper carcass disposal. The virus replicates to very high titre in infected fish and is shed in body fluids and from carcasses as they decompose in water. - Control is by strict isolation and hygiene measures.

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3.1.2 Infectious salmon anaemia

ISA is a serious and notifiable orthomyxo-like virus that is gradually spreading. Cases have been observed in wild Atlantic salmon in Norway, the UK and Canada, but other species might also carry the virus. It is believed that the disease is transmitted horizontally through movement of infected fish. True vertical transmission of ISA is believed unlikely, although transmission via ovarian fluids may be possible. So far, Atlantic salmon, sea trout/brown trout, rainbow trout and Atlantic herring (*Clupea harengus*) are the only species susceptible to ISA.

Vaccines:	• Inactivated, experimental, injectable monovalent and multivalent vaccines using Canadian and Norwegian isolates. (Jones et al 1999, Brown et al 2000, Biering et al 2005). • Commercial, killed, injection (Canada) during the past 5 years but efficacy levels in the field are not documented. • Efficacy under investigation (Carrier risk?). • A vaccine against ISAV has been developed by Aqua Health, and is undergoing field trials in Canada. The vaccine is currently prohibited in Europe, as ISA is a List 1 notifiable disease for which eradication is the only option.
Chemotherapy:	• Not available
Immunostimulation:	• Under investigation
Resistance breeding:	• Currently, major developments in ISA control include the use of genetic improvement programmes aimed at producing fish with increased resistance to diseases. The Akvaforsk Genetics Centre (AGC) and Aquagen in Norway have had a breeding program for ISA and furunculosis resistance since 1995 including IPN since 1997. (www.aquagen.no; Midtlyng et al. 2002)
General husbandry practices:	• Control movements of fish personnel and equipment, remove mortalities. • Set up Control & surveillance zones around the confirmed site. • Monitor stocks carefully. • Remove infected populations, by cull or harvest in containment. • Introducing pathogen free resistant juveniles, or non susceptible species. • Monitor wild stocks.

	• Implementation of hygiene practices including thorough fallowing of sites. Legislatory measures, such as restrictions on movements and transportation of fish, disinfection of offal and waste water from slaughterhouses, enforced sanitary slaughtering and restrictions on affected, suspected and neighbouring farms, have shown to be efficient in reducing the incidence of ISA in Norway. • Role of Slaughter house effluents / Well boats. Most ISA outbreaks occur in spring or early summer (Thorud, 1991). Epidemiological studies on ISA have demonstrated that poor biosecurity allowing passive transmission (proximity to other ISA affected sites and to salmon slaughterhouses) and active transmission (management practices which increase exposure to foreign biological material, such as the number of hatcheries from which smolts are obtained) are the major risk factors (Vågsholm et al, 1994; Jarp and Karlsen, 1997). • Virus inactivation at low pH and Temp>50^{o}C ISAV is sensitive to a range of chemical disinfectants (Smail et al 2004). • Currently the cornerstone of ISA control is the early diagnosis of the disease.

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3.1.3 Red sea bream iridoviral disease

RSBIV belongs to the Iridoviridae family. It is highly contagious causing variable mortality rate in cultured marine fish in Japan. It affects red seabream (Pagrus major), yellowtail (Seriola quinqueradiata), sea bass (Lateolabrax sp.), Japanese parrot fish (Oplegnathus fasciatus), as well as many other cultured marine fish. It is transmtted horizontaly through infected fish.

Legislatory measures such as restrictions on movements and transportation of fish as well as enforced sanitary slaughtering and restrictions on affected; suspected and neighbouring farms have been applied in order to control the spread of the disease.
No treatment is possible.

Vaccines:	• Commercial vaccine for red sea bream only is available. • Experimental formalin-killed injectable vaccine for red seabream, striped jack and seriola (Japan). Nakajima *et al.* (1997 and 1999). • Experimental, injectable DNA (Japan).(Caipang *et al*. 2006)
Chemotherapy:	• Not available.
Immunostimulation:	• Under investigation
Resistance breeding:	• Under investigation in limited form - Inami *et al* (2005) reported the first microsatellite mapping of red seabream and 2 QTL markers associated with resistance to RSIV
General husbandry practices:	• Introducing pathogen free juveniles. • Implementation of hygiene practices at farms. • Avoid practices that can decrease water quality and/or increase stress such as overcrowding and overfeeding. • Restrictions on movements and transportation of fish. • Enforced sanitary slaughtering and restrictions on affected, suspected and neighbouring farms

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3.1.4 Koi Herpervirus disease

KHV (CYHV3) is a member of Herpesviridae family, primarily affecting common and koi carp and it has been found to be widespread in some countries. The disease is temperature dependent, only expressing itself above 15°C and below 28°C. Like all herpes viruses, KHV can be latent within a host and difficult to identify using current testing techniques. There is no cure but early detection can help prevent further spread to unaffected fish stocks.

Vaccines:	• Autologous Commercial live attenuated in Israel (Perelberg *et al.* 2005). • Experimental killed (Yasumoto *et al.* 2006). • DNA by injection, immersion, oral (Japan, Korea, Israel, USA, Canada). • There are currently 7 groups reported to be working on vaccines for KHV. Immunizing "agents" are reported to include killed-virus, attenuated-live virus and DNA. Delivery techniques include oral, immersion and injectable. The groups include: - Yoshimura & Miyazaki, Mie University, Japan, - Aoki, Tokyo University, Japan, - Perelberg & Kotler, Hebrew University-Hadassah Medical School, Israel, - Shivappa & Levine, North Carolina State University, CVM, USA, - Ritchie, University of Georgia, USA, and - Novartis, Canada - Henderson Morley (UK)
Chemotherapy:	• Not available
Immunostimulation:	• Under investigation
Resistance breeding:	• Resistant strains available in Israel (Shapira et al. 2005). • There is a live carp gene bank (Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), Szarvas, Hungary) and strains are currently being tested for resistance to KHV within the EU project "Eurocarp"
General husbandry practices:	• Perchase fish from a reliable supplier who can provide assurances of disease-free status that adequately quarantine all fish arriving at their facilities. • Adequately quarantine all newly introduced stock at permissive temperature (21–25°C), with naïve sentinal fish (Haenen *et al.*, 2004), for four to eight weeks prior to (re) introducing them into the general fish populations. • Observe fish in the quarantine system, noting symptoms and behaviour, particularly those typical of KHV.

	• Use separate equipment to house and handle quarantined fish and thoroughly disinfect after use. • There are two major strategies for dealing with the disease when it has been identified: Depopulation or vaccination. The choice of strategy differs in each MS. • Disinfection. - Disinfection can be compromised (incomplete) if items are contaminated with debris and/or have rough or porous surfaces. Clean items prior to disinfection and increase the exposure time for rough and/or porous items. Care should be taken that disinfecting solutions are clean and active.

Knowledge Gaps

- Research on latency of infection and virus reservoir in the aquatic environment
- Research on the potential of vertical transmission
- In vitro chemical inactivation studies are problematic because the virus is difficult to grow to high titre in cell culture. These tests generally require demonstration of > 10^4 $TCID_{50}$ decrease following exposure to the disinfectant.

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3.1.5 Streptococcus agalactiae

Streptococcus agalactiae is a major bacterial pathogen that is the cause of serious economic losses in many species of freshwater, marine and estuarine fish worldwide. Streptococcus agalactiae is a Group B streptococcal bacterium that causes severe economic losses in a number of species of cultured and wild fish. This infectious bacterium is common in aquaculture facilities where fish are intensively cultured in fresh, brackish, or marine waters. The high densities of fish and the aqueous environment favor the rapid transmission of streptococcal disease. Moreover, infected cultured fish may transmit the disease to wild fish populations, or infected wild fish may transmit the disease to cultured fish. The disease affects rainbow trout, eel, yellowtail, tilapia and ayu (sweetfish) in Japan; golden shiners in the US and South Africa, and turbot, sea bass and rainbow trout in Mediterranean countries (eg Italy, Spain). Tilapia growers consider streptococcal diseases caused by S. iniae and S. agalactiae the most serious economic threat to profit loss (Klesius *et al*., 2000; Shoemaker *et al*., 2000; Shoemaker *et al*., 2001; Evans *et al*., 2002). Streptococcus agalactiae infection is responsible for severe economic losses in seabream and tilapia production (Evans et al., 2002; Glibert et al., 2002).

Vaccines:	• Commercial killed injection Tilapia (USA). • Experimental, killed, immersion/oral, Tilapia (USA). • Experimental bacterins have shown promising results and can therefore be considered an important and effective immunoprophylactic measure of disease prevention (Romalde et al, 1998).
Chemotherapy:	• Potentiated sulfonamides, oxytetracycline, erythromycin, ampicillin and amoxycillin. • Mcrolide and chloranphenicol (currently prohibited for use in food species) have been effective. • Reoccurring outbreaks
Immunostimulation:	• Under investigation
Resistance breeding:	• No information
General husbandry practices:	• Sanitary prophylaxis - Good biosecurity - Control importation measures for susceptible species - Eradication (?) • Broad-spectrum biocides effective - 0.5% Virkon S - 0.2% Proxitane 5 - 1000 ppm Chloramine T - Fam 30 (Cefas data) • Implementation of hygiene practices at farms. • Low stocking density.

	• Limit handling and stressful conditions • Role of wild fish carriers

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3.1.6 Lactococcus garviae

Lactococcus garvieae (syn= Enterococcus seriolicida) is an important emerging bacterial pathogen that affects a wide range of fresh water and marine fish species worldwide. The agent has also been implicated in diseae in Macrobranchium rosenbergii and has potential clinical significance in mammals, including man. L. garvieae is a particularly severe problem for southern European trout farmers where the disease causes high mortalities in the summer (at temperatures higher then 16^{o}C) In addition, seabream, seabass, yellowtail, amberjack, and rainbow trout producers suffer severe economic losses due to Lactococcus garviae infection (Kitao, 1993; Eldar *et al*., 1996, 1999; Schmidtke and Carson, 1999).

Vaccines:	• Commercial autologous killed injection (Applied in trout- Spain, Italy). • Commercial (Schering Plough), killed, immersion /oral (Tilapia).
Chemotherapy:	• Potentiated sulfonamides, oxytetracycline, erythromycin, ampicillin and amoxycillin. • Reoccurring outbreaks.
Immunostimulation:	• Under investigation.
Resistance breeding:	• No information.
Alternative treatments:	• Under investigation (Bacterial Phage Therapy - Japan). • Probiotics in trout (B. Austin).
General husbandry practices:	• Relatively resistant to heat (>65^{o}C required) • Relative resistance to low and high pH • Implementation of hygiene practices at farms. • Low stocking density • Limit handling and stressful conditions • Role of wild fish carriers

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Schmidtke, L.M., Carson, J. (1999). Induction, characterization and pathogenicity in rainbow trout Oncorhynchus mykiss (Walbaum) of Lactococcus garvieae L-forms. Vet Microbiol 69, 287–300.

3.1.7 Streptococcus iniae

Streptococcus iniae is an emerging bacterial pathogen in cultivated tilapia, hybrid striped bass, rainbow trout, yellowtail, eel, and turbot. This disease is recognized as one of the most problematic bacterial pathogens in intensively cultured fish and wild fish. Streptococcus iniae is a pathogen in fish, capable of causing invasive disease and outbreaks in aquaculture farms. Invasive S. iniae infection causing cellulitis of the hand or endocarditis has also been described in people who had recently handled fresh, whole fish from such farms.

Vaccines:	• Experimental and licenced bacterin. Good protection in field (turbot, Spain). Licenced in Indonesia by Intervet / SP for Asian sea bass - Tilapia (USA), turbot (Spain). • Experimental live attenuated vaccine. • Immersion and oral vaccine against Lactococcus garviae and Streptococcus iniae in tilapia.
Chemotherapy:	• Potentiated sulfonamides, oxytetracycline, erythromycin, ampicillin and amoxycillin. • Reoccurring outbreaks
Immunostimulation:	• Under investigation
Resistance breeding:	• No information
General husbandry practices:	• Implementation of hygiene practices at farms. • Low stocking density • Limit handling and stressful conditions • Role of wild fish carriers

3.1.8 Trypanosoma salmositica

Hemoflagellates are a group of internal flagellates. These organisms live in the blood stream of both salt water and freshwater fish. The two most common genera are Trypanosoma, an elongate organism with 1 flagellum and an undulating membrane, and Trypanoplasma, which has 2 flagella and no membrane. There are two hosts for these organisms. They are primarily transmitted by aquatic leeches, which attach to the fins or skin and occasionally to the gills. For this reason they are usually not a problem in cultured fish although they may be in marine cage culture of salmonids. Stress appears to increase susceptibility. Infections in wild fish are usually of little consequence to the fish. It has been reported that stressing infected fish with low levels of contaminants etc. can cause debilitation. Signs of heavily infected fish are lethargy, anemia and pale gills. Some species of these genera parasitize internal organs of fish. *Trypanoplasma spp*. and T*rypanosoma* spp. include parasites of the bloodstream and of tissues, with indirect life cycles (leeches are the main vectors).

Vaccines:	• Experimental attenuated, injection (salmonids).
Chemotherapy:	• None Available.
Immunostimulation:	• No information.
Resistance breeding:	• Heritability of innate resistance (complement) in brook char (Forward *et al*. 1995). • Resistant families of Atlantic Salmon identified (Chin *et al*. 2004).
General husbandry practices:	• Elimination of Leeches (Chlorine - Pond drying – Liming). • Eliminate Stress that appears to increase susceptibility. • Mortality is dependent on fish stocks and species and may be high in juveniles.

References

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3.1.9 Ceratomyxa shasta

Ceratomyxa shasta is an important pathogen, causing serious loses in cultured and wild populations of salmonids on the west coast of North America. Significant mortalities can occur, depending on fish species, as susceptibility is variable. A polychaete intermediate host (Manayunkia speciosa) has been demonstrated in the life cycle of this Myxosporean.

Vaccines:	• None.
Chemotherapy:	• Fumagilin and TNP470 (analog).
Immunostimulation:	• None.
Resistance breeding:	• Mapping of resistance loci in Rainbow trout (Nichols *et al*. 2003).
General husbandry practices:	• Target Intermediate host: Fresh water polychaete (Manayunkia speciosa). • Actinospore susceptible to UV (UV Filtration in open systems).

Reference

Nichols, K.M., Bartholomew, J., Thorgaard, G.H. (2003). Mapping multiple genetic loci associated with Ceratomyxa shasta resistance in Oncorhynchus mykiss. Dis Aquat Org. 56(2), 145-54.

3.1.10 Neoparamoeba pemaquidensis

Amoebic gill disease is a major problem for Australia's salmon aquaculture industry. Fish infected with AGD are safe to eat, but they lose condition. Amoebic gill disease develops after amoebae Neoparamoeba spp. colonises and replicates on the gills of susceptible fish. In turn, host epithelial cells adjacent to the amoebae proliferate unabated until death. Salmonids grown in Tasmanian waters are susceptible to AGD, but the condition has also been reported in Atlantic salmon, rainbow trout, Chinook salmon, coho salmon, turbot and seabass in North America and/or Europe. In Tasmania, Atlantic salmon smolts were first transferred to seawater during the mid-1980s. Subsequently, AGD outbreaks occurred and were responsible for demoralising losses. AGD is observed in association with 35 parts per thousand salinity and may be detected year-round in these conditions. However, outbreaks are most prevalent during the warmest months of the year.

Vaccines:	• Surveys of marine sites around Tasmania indicate that neoparamoeba spp. is ubiquitous, and therefore in the long run the industry would prefer to adopt preventative measures such as vaccination.
Chemotherapy:	• None
Immunostimulation:	• None
Resistance breeding:	• AQUAFIN CRS (CSIRO /University of Tasmania) Markers for resistance in AS (Wynne et al 2007)
General husbandry practices:	• Fresh water baths every 6 weeks**.** • Freshwater bathing is an effective treatment, and the development of commercial-scale freshwater bathing allowed the industry to prosper. Bathing involves moving all fish from a net-pen into fresh water contained within a canvas liner for three or four hours, then releasing them back into a net-pen. This is an expensive process and reportedly accounts for up to 20 percent of production costs. It is also limits production, as fish must be farmed within reach of a suitable source of fresh water. • Chlorine dioxide (at 25ppm) and Chloramine T (Halamid^{TM}) (at concentrations between 10 and 25ppm) are potntial candidate additives in freshwater baths to be more efective in killing Neoparamoeba and removing offen dig parasite from the gills of AGD affected salmon. A preliminary cost analysis suggests that Chloramine T is a favoured treatment due to lower cost. Chloramine T at 1 hour bath was effective even in seawater and was as effective as at 3 and 6 hours bath.

References

Munday et al (2001). Gill disease of marine fish caused by infection by Neoparamoeba pemaquidensis. Journal of Fish Diseases 24, 497-507.

Wynne, J., Cook, M., Nowak, B. and Elliott, N. (2007). MH polymorphism associated with resistance towards amoebic gill disease in Atlantic salmon (Salmo salar L.). Fish and Shellfish Immunology (in press).

3.1.11 Parvicapsula pseudobranchicola

In 2002 the first clinical disease outbreaks caused by *Parvicapsula* were diagnosed in five Atlantic salmon (*Salmo salar*) farms in northern Norway. In 2004 the parasite was detected in several farms in northern Norway as well as in Trøndelag and Møre and Romsdal. Spores resembling a *Parvicapsula* sp. have been identified in juvenile pink salmon (*Oncorhynchus gorbuscha*) and juvenile chum (*O. keta*) salmon.

Vaccines:	• None
Chemotherapy:	• Fumagilin and TNP470 (analog)
Immunostimulation:	• None
Resistance breeding:	• None
General husbandry practices:	• Target Intermediate host: Fresh water polychaete (Manayunkia speciosa). • Actinospore susceptible to UV (UV Filtration in open systems).

3.1.12 Gyrodactylus salaris

Gyrodactylus salaris represents a major threat to farmed and wild Atlantic salmon. The disease was first identified in Atlantic salmon in rivers and farms in Norway (caused by the introduction of the parasite on introduced Baltic strains of Atlantic salmon). GS also infects other salmonids (in declining order of susceptibility): Rainbow trout *(Oncorhynchus mykiss),* Arctic char *(Salvelinus alpinus)*, North American brook trout *(S. fontinalis)*, grayling *(Thymallus thymallus)*, North American lake trout *(S. namaycush)*, brown trout *(Salmo trutta)*.

Vaccines:	• None
Chemotherapy:	• None
Immunostimulation:	• None
Resistance breeding:	• Resistance breeding has not yet been tried. • SALMOGYRO programme identified markers associated with resistance (Gilbey *et al.* 2006) which would allow breeding programs. • Naturally resistant fish strains exist (eg Baltic Neva strain). • Parasite Genetic clades more pathogenic.
General husbandry practices:	• Previous extreme practice: Rotenone (Norway). • Current Practice: Acidified aluminium sulphate. • Virkon S / Formalin bath and other ectoparasiticides. • Avoid Mis-diagnosis with Gyrodactylus teuchis (France). • *Gyrodactylus salaris* has mainly been spread with transports of live salmon and rainbow trout. At least 30 fish from a production unit (e.g. a tank) should be examined under a dissecting microscope prior to a transport. • Several chemicals in bath treatments are effective against the parasite, e.g. formadehyde and sea water, but usually all parasites are not completely eliminated. • Recent results indicate that oral treatment with triclabendasole in feed (40 g per kg of feed for 10 days) and nitroscanate (>0.6 g per kg of feed per day) may eliminate an infection. • Sources of the Parasite include Infected fish, wet equipment, such as fishing tackle, net and waders, that has been used in an infected river or farm. Each production unit should have equipment for cleaning and handling fish not used in other units.

	• Equipment used for fish transports or that has been in contact with infected fish should be dried, frozen or disinfected (e.g. in 1% Chloramine-T [Halamid]).

Reference

Gilbey, J., Verspoor, E., Mo, T.A., Sterud, E., Olstad, K., Hytterød, S., Jones, C., Noble, L. (2006). Identification of genetic markers associated with Gyrodactylus salaris resistance in Atlantic salmon Salmo salar. Diseases of Aquatic Organisms 71, 119-129.

3.1.13 Aphanomyces invadans

Vaccines:	• None
Chemotherapy:	• Lilley *et al*. (1997) and Campbell *et al*. (2001) investigated a range of potential fungicides, however little was of practical value.
Immunostimulation:	• None
Resistance breeding:	• None
General husbandry practices:	• Controls on importation of live fish. • Eradication when detected? • Liming water and improving water quality, together with removal of infected fish, can be effective in reducing mortalities.

References

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3.2 Mollusc diseases

3.2.1 *Candidatus* Xanohaliotis californensis

Candidatus Xenohaliotis californiensis is a Rickettsia-like organism and is responsible for the withering syndrome of abalone. It occurs along the south-west coast of North America in California, USA and Baja California, Mexico. However, as infected abalone have been transported to Chile, Japan, Israel and other countries, the geographical range of the aetiological agent is suspected to be broad where California red abalone, *Haliotis rufescens*, are cultured. It has been recently reported in Europe including Iceland (in cultured *H. rufescens*), Ireland and Spain (in cultured *H. tuberculata*) (Balseiro et al. 2006) . Withering syndrome (WS) is a chronic wasting disease responsible for mass mortalities in wild black abalone (*H.chacherodii*) populations. The disease has also been observed in farmed red abalone, *H. rufescens*, and this has prompted the California Department of Fish and Game to place a partial ban on movement of cultured red abalone from locations where WS is endemic to locations free of this disease (Andree et al, 2000). Early and accurate detection (gross pathology, histopathology PCR) of parasites in marine invertebrates is critical, because therapeutic approaches are limited. Withering syndrome occurs at elevated water temperatures (~18°C and above) . The incubation period of withering syndrome is prolonged and ranges between 3 and 7 months. Cumulative mortality has been recorded at over 99% in black abalone and over 30% in red abalone. The pathogen and disease (withering syndrome) may occur year round, but losses due to the disease occur most often in the summer and autumn, after a 3-4-month period when temperatures are elevated over 15°C (Moore et al. 2002).

Vaccines:	• Not applicable
Chemotherapy:	• Oxytetracycline medicated diet may reduce mortalities
Immunostimulation:	• Not applicable
Resistance breeding:	• Abalone breeding programs up to present have only addressed growth. Research into assessing resitance to WS in black abalone starting in US.
General husbandry practices:	• Ban on movement of Red abalone from infected locations. • Reducing water temperatures to about 15°C or less may decrease the severity of the disease. • Early and accurate detection (gross pathology, histopathology PCR) of bacteria in marine invertebrates is critical, because therapeutic approaches are limited. • Avoidance has therefore become a principal means of disease management and resource protection.

Reference

Andree, K.B., C.S. Friedman, J.D. Moore and R.P. Hedrick. (2000). A polymerase chain reaction assay for the detection of genomic DNA of a Rickettsiales-like prokaryote associated with withering syndrome in California abalone. Journal of Shellfish Research 19, 213-218.

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Moore J.D., Finley C.A., Robbins T.T. and C. Friedman (2002) Withering syndrome and restoration of Southern California Abalone populations. California Cooperative Oceanic Fisheries Investigations 43: 112-116

3.2.2 Pacific oyster nocardiosis (PON) *Nocardia crassostreae*

Nocardia crassostreae (Actinomycete bacteria) was reported from the west coast of the US, British Columbia and Japan in Pacific oyster (*Crassostrea gigas*) and in *Ostrea edulis* cultivated near infected *C. gigas*. It has been recently reported in a few flat oysters and Pacific oysters from The Netherlands (Engelsma *et al.,* submitted).

Vaccines:	• Not applicable
Chemotherapy:	• Not applicable
Immunostimulation:	• Not applicable
Resistance breeding:	• No information
General husbandry practices:	• Diseased oysters should not be moved • Moving oysters out of warm areas during summertime may reduce impact of the disease

Reference

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Engelsma, M. Y., Roozenburg, I., Joly, J-P. (submitted). Isolation of Nocardia Cassostreae from pacific oysters (Crassostrea gigas) in lake Grevelingen, The Netherlands. Proceedings of the EAFP conference, Copenhagen, September 2007.

3.2.3 Marteilioidosis

Marteilioidosis is caused by *Marteilioides chungmuensis* (phylum Paramyxea) that affects the Pacific cupped oyster, *Crassostrea gigas* and *C. nippona* when imported into enzootic area. This parasite has been reported from Japan and Korea. Parasites similar to *Marteilioides chungmuensis* were described in *Crassostrea echinata* (Australia) and *Ruditapes philippinarum* (Korea).

The parasite infects the cytoplasm of oocytes and can affect large areas of the reproductive follicles causing irregular enlargement of the infected gonadal tissues. Infected oysters lose their marketability due to the unaesthetic appearance and thus causes a serious economical impact.

The prevalence of infection increases in summer when oysters spawn and decreases in winter when spawning has finished (Imanaka et al. 2001).

Vaccines:	• Not applicable
Chemotherapy:	• There is no applicable treatment for molluscs
Immunostimulation:	• No information
Resistance breeding:	• No information
General husbandry practices:	• Diseased oyster should not be moved • Growing triploid oysters which are not susceptible to infection by the parasite in affected area has been recommended in Korea (Park, 2005).

References

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Park, M.S. (2005). Survey on the ovarian parasite, *Marteilioides chungmuensis* in the culture Pacific oyster, *Crassostrea gigas* in Korea. In: Walker, P.J., R.G. Lester, M.G. Bondad-Reantaso (eds.) Diseases in Asian Aquaculture V. Proceedings of the 5th Symposium on Diseases in Asian Aquaculture. Fish Health Section, Asian Fisheries Society, Manila. pp. 311-320.

3.2.4 Perkinsus olseni / atlanticus

Perkinsosis is an infection of marine molluscs, caused by protistan parasites of the phylum Apicomplexa, genus *Perkinsus*, *Perkinsus marinus* and *P. olseni*. *Perkinsus olseni* affects many abalone species (*Haliotis ruber, H. cyclobates, H. scalaris* and *H. laevigata*). and clam species (*Ruditapes philippinarumR. Decussatus, Anadora trapezia, Austrovenus stutchburyi, Pitar rostrata*) and is possibly harboured by some 50 other mollusc species. The pathogenis recorded in Australia, New Zealand, Uruguay, Korea, Japan, China and Europe including Italy, France, Portugal, and Spain . *P. atlanticus* has been found to be genetically indistinguishable from *P. olseni* (Murrell *et al*., in press; Robledo *et al*., 2000). There is international consensus for the determination of the one species incorporating *P. olseni* and *P. atlanticus*. The real impact of the pathogen on clam and abalone populations is under discussion in term of mortality. However, perkinsosis induces some tissue damages (pustules) which induces loss of marketability of affected abalone. The spatial distribution of *Perkinsus olseni* seems to be partly controlled by temperature, salinity and substrate type (Park and Choi 2001).

Vaccines:	• Not applicable
Chemotherapy:	• cyclohexamide, pyrimethamine, deferoxamine (DFO) and 2, 2-bipyridyl inhibit *P. olseni in vitro*, and DFO inhibits *Perkinsus olseni in vivo* (Elandalloussi et al. 2005). Bacitracin has been shown to reduce, but not eliminate *P. marinus* in infected oyster hosts (Faisal et al. 1999). This compound may be effective for *P. olseni* as well. However, their use may be relevant for aquaculture, but is not practical in the natural environment.
Immunostimulation:	• Not applicable
Resistance breeding:	• No information
General husbandry practices:	• Free countries, zones and aquaculture establishments. • Low density stocking may reduce transmission of the pathogen. • Policy and procedures for importation of life molluscs.

References

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Robledo, J., Coss, C., and Vasta, G. (2000). Characterization of the ribosomal RNA locus of Perkinsus atlanticus and development of a polymerase chain reaction-based diagnostic assay. Journal of Parasitology 86 (5), 972-978.

3.2.5 Perkinsus marinus

Perkinsus marinus (=*Dermocystidium marinum*, =*Labyrinthomyxa marina*) belongs to the genus *Perkinsus* which seems to be closely related to the Dinoflagellida (Goggin and Barker 1993, Perkins 1996, Siddall *et al*. 1997, Reece et al 1997). *Perkinsus marinus* causes disease of economic importance in *Crassostrea virginica* but while *Crassostrea gigas* and *C. ariakensis* can be infected but do not develop the disease.
This parasite is present along the eastern coast of the United States of America from Maine to Florida, and along the Gulf of Mexico coast to the Yucutan Peninsula.
The disease seems to be influenced by both temperature and salinity. Death usually occurs during or shortly after the warmest annual water temperatures and the parasite prefers low salinity.

Vaccines:	• Not applicable
Chemotherapy:	• Bacitracin and cycloheximide have been shown to reduce, but not eliminate *Perkinsus marinus* in infected oyster hosts (Calvo & Burreson, 1994; Faisal et al., 1999). Their use may be relevant for aquaculture, but is not practical in the natural environment.
Immunostimulation:	• Not applicable
Resistance breeding:	• The CROSBreed program at Virginia Institute of Marine Sciences has developed oysters resistant to *Haplosporidium nelsoni* and *Perkinsus marinus* (Ragone Calvo *et al*., 2003). • University of Maine/Industry co-operative oyster breeding program - further crossing the haplo/dermo resistant line with a juvenile oyster disease resistant line.
General husbandry practices:	• Dermo disease is easily transmitted from oyster to oyster so it is imperative to avoid moving infected oysters into an area containing uninfected oysters. • Holding oysters at salinities less than 9 ppt will retard disease development and restrict disease associated mortalities. • If possible, let grow out areas remain fallow for one to two years before planting seed stocks. • To date, eradication has proven impossible. • Methods used to reduce the commercial impact of the disease on infected populations consist of reducing the density of oysters and harvesting or moving oysters to low salinity areas (lower than 9 ppt) before water temperatures increase to 15-20 °C. However, infections may persist for years in low salinity areas (Burreson and Ragone Calvo 1996). • O'Farrell *et al*. (2000) suggested that transferring infected

	oysters to low salinity will result in strains of *P. marinus* acclimated to low salinity and thus able to withstand periodic events of extremely low salinity. Thus, caution is necessary when using low salinity areas to treat or control infection and/or disease. • Cold winter temperatures may limit the natural spread of this pathogen to northern areas. In enzootic areas, strategies designed to enhance and supplement natural recruitment of oysters, along with keeping growing areas free from *P. marinus* by limiting oyster transplantation, currently offer the most promise for maintaining commercially harvestable stocks (Krantz and Jordan 1996). • Fallowing beds after removing infected oysters has not proven effective in some areas but early harvest to avoid mortalities caused by *P. marinus* may be feasible (Butsic *et al*. 2000). Ford *et al*. (2000, 2001) demonstrated that juvenile oysters from nursery systems (seed) that use raw water pumped from an enzootic area are highly likely to be infected although infections may be very light in intensity and low in prevalence. • Disinfection - Treating raw water, by filtration to 1 µm and then ultraviolet light (30,000 µW s^{-1} cm^{-2} UV irradiation), will help protect hatchery produced seed from infection. - Standard bleach sterilization procedures which use chlorine concentrations of 10-25 parts per million as a method to kill *P. marinus*, prior to ocean disposal of *P. marinus* contaminated materials, are ineffective according to Bushek et al. (1997). Apparently one hour at 50 °C or one hour exposure to fresh water effectively killed the parasite (Bushek et al. 1997). - Organic N-halamine disinfectants (up to 25 mg/L for up to 12 h exposure, depending on the specific chemical formulation) can also be used to disinfect seawater contaminated with *P. marinus* (Delaney *et al*. 2003)
Pathogen spread modeling simulations	• Seasonal proliferation of *P. marinus* has been modeled to estimate time to critical levels and duration of infection in *C. virginica* (Hofmann *et al*. 1995, 1999; Soniat and Kortright 1998; Brewster et al. 1999, 2000; Ragone Calvo and Burreson 2000; Brousseau and Baglivo 2000a,b; Ragone Calvo et al. 2001). One model has been developed into an internet program that may assist in calculating the time to a critical level of disease (Soniat et al. 2000, Ray et al. 2001; see http://www.dermowatch.org/) • Simulations of the model developed by Hofmann et al. (1999) could be used to understand the causes underlying the

	northward spread of the disease and to restructure the practices of the oyster industry to maximize production under conditions where the life span of the commercial species is controlled by disease. The model of Ragone Calvo *et al*. (2001) suggests that a single transmission event may be sufficient for *P. marinus* to become enzootic in a specific year class of oysters located in moderate to high salinity areas, while periodic transmission events are required for the parasite to persist in low salinity areas. A model for managing the oyster fishery during times when disease is a controlling influence was developed and assessed using oyster populations affected by *P. marinus* (Klink *et al*. 2001).

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3.3 Crustacean viral diseases

Viral crustacean diseases have emerged during the last decade as serious economic impediments to successful shrimp farming. Nine viruses (or groups of closely related viruses) are known to be enzootic in Western Hemisphere penaeids, five of which have emerged as serious pathogens to one or more species of cultured shrimp. Viral diseases have also severely impacted the shrimp farming industry in the Eastern Hemisphere. In the shrimp growing regions of the Indo-Pacific and East Asia, at least 12 viruses (or groups of closely related viruses) have been recognised. Out of these, five have been documented to be responsible for serious epizootic diseases regionally, and two as having caused panzootics throughout much of the Indo-Pacific and East Asian industries. Although a significant number of different viruses have been discovered in penaeid shrimp, only four are considered important to the international shrimp farming industries (Lightner, 1999). These are the white spot syndrome virus (WSSV), yellow head virus (YHV), taura syndrome virus (TSV), and infectious hypodermal and haematopoietic necrosis virus (IHHNV). All the identified viral diseases are exotic to EU

Vaccines:	• Limited experimental vaccines available
Chemotherapy:	• No proven chemotherapeutants available
Immunostimulation:	• No information
Resistance breeding:	• Only use certified SPF stock
General husbandry practices:	• Avoid movement of live stock between sites. • In case of outbreaks - Strict isolation of outbreak ponds with movement controls and control of personnel movement - Destruction of all infected and exposed shrimp by incineration or burial. - Thorough cleaning and disinfection of outbreak ponds.

Reference

Lightner, D.V. (1999). The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods, and management strategies. Journal of Applied Aquaculture 9, 27-52.

3.3.1 YellowHead disease

YHV (Genotype 1) is highly infectious for most known species of cultivated penaeid shrimp naturally infecting black tiger shrimp *(Penaeus monodon)* and *P. setiferus.* Lethal experimental infections have been achieved with other cultivated penaeids (e.g. *P. vannamei, P. stylirostris, P. aztecus* and *P. duorarum*). It is reasonable to assume that most cultured penaeids would be susceptible to infection. Natural infections also occur in other shrimp without signs of disease (e.g. *P. merguiensis, Acetes* spp., *Palaemon styliferus, Metapenaeus ensis).*

The yellow head virus (YHV) from South East Asia and the morphologically similar lymphoid organ virus (LOV) and gill associated virus (GAV) from Australian giant tiger prawns, are rod-shaped, enveloped viruses that replicate in the cytoplasm of infected cells. While LOV and GAV are not causing mortalities on their own presence of YHV in the affected shrimp is always linked with mortalities. The causative agent of YHV is thought to be a member of the Rhabdoviridae family and, most recently, the Coronaviridae (Walker, cited in Lightner, 1999). YHV seriously affects giant tiger prawns in intensive culture systems in South East Asian and Indo-Pacific countries (Thailand, China, Malaysia, Indonesia, India and Texas, United States of America) while GAV affects those cultured in Australia. YHV and GAV infections typically occur in juvenile and sub-adult prawns. American penaeids are highly susceptible to experimental infection by YHV. While post-larvae stages are refractory to infection, white leg prawn, blue shrimp, white shrimp (Penaeus setiferus), brown shrimp and pink shrimp juveniles are susceptible to challenge by the virus and suffer significantly from the disease. Disease outbreaks may occur at all seasons but usually at 50–70 days after pond stocking when environmental conditions are poor.

Vaccines:	• Sign of resistance development as subsequent outbreaks are less acute • No consistently effective vaccination methods • Experimental, injection, dsRNA
Chemotherapy:	• None Available
Immunostimulation:	• No information
Resistance breeding:	• No information
General husbandry practices:	• Purchase specific pathogen free (SPF) or PCR-negative seed stocks. Vertical, transmission has not been found. However, if it exists, its frequency is very low. • Horizontal transmission may be direct or vectorial, water being the major abiotic vector therefore: - Biosecure water and culture systems are required. - Proper cleaning and disinfection of ponds before stocking, including removal of potential carrier crustaceans because rapid transmission occurs from infected shrimp through the water and by cannibalism of weak or moribund shrimp - Animate disease vectors include infected but nondiseased carrier crustaceans are the major source of infection for

	rearing ponds so elimination or screening of potential carriers from exchange water is required. - Avoid stress induced by chemical and insecticide residues. - Avoid poor widely fluctuating environmental conditions - Avoid exchange of equipment amongst ponds - Avoid use of fresh aquatic feeds. • In case of an outbreak: - Strict isolation of outbreak ponds with movement controls and control of human traffic. - Continuous removal and destruction of moribund and dead infected shrimp and exposed shrimp by incineration or burial because although the mode of virus shedding from infected shrimp has not been established, cohabitation results show that transfer from carriers to shrimp occurs rapidly - Thorough cleaning and disinfection of outbreak ponds, intake water, nets and other equipment - Treating the pond and equipment with chlorine and iodine has proved effective.

Reference

Lightner, D.V. (1999). The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods, and management strategies. Journal of Applied Aquaculture 9, 27-52.

3.3.2 Whitespot virus

White spot virus (WSV) or white spot syndrome virus (WSSV) is Whispovirus. At least five viruses in the white spot syndrome complex have been identified. They appear to be very similar viruses in morphology, replicating in the nuclei of infected cells (Lightner, 1996). White spot disease (WSD) has been recorded from most Asian countries were penaeid shrimp are pond reared. Original outbreaks were reported from the People's Republic of China in 1993 and they spread rapidly thereafter to Japan, Taipei China and the rest of Asia, but not Australia. Since early 1999, it has been widely reported from shrimp farms in the southern United States of America, Central America and northern South America. Affected shrimp cease feeding and may be observed near the surface of the water; 100% mortality can occur within 7 days. The WSS complex infects and causes serious disease in many species of penaeid shrimp and a variety of other decapod crustaceans. Species of Asian penaeids infected by WSS-complex viruses include: giant tiger prawn, green tiger prawn, Japanese kuruma shrimp, fleshy prawn, red tail prawn, Indian white prawn, banana prawn, Trachypenaeus curvirostris (southern rough shrimp) and Metapenaeus ensis (greasy back shrimp). Western penaeids are also susceptible to WSS infection and disease.

Vaccines:	• Commercial (Bioshield, Thailand) injection, vibrio bacterin. • Experimental, formalin killed virus, injection (Namikoshi *et al.* 2004) or oral (Bright Singh *et al*., 2005) offered limited short term (10day) protection. • Experimental, recombinant coat protein, injection (Namikoshi e*t al.* 2004; Witteveldt *et al.*, 2004) or oral (Witteveldt *et al* 2004; Witteveldt *et al* 2006) or immersion, oral and injection (Jha *et al.* 2006) - maximum 3 weeks protection. • Experimental, Glucan, oral. (Chang *et al.* 2003) • Experimental dsRNA injection (Robalino *et al.* 2004) • Experimental DNA vaccine (Rout *et al.* 2007) up to 7 weeks protection
Chemotherapy:	• None Available
Immunostimulation:	• No information
Resistance breeding:	• Limited success so far, very low heritability (Gitterle *et al.*, 2005; Gitterle *et al.,* 2006; US Marine Shrimp Farming Program 2004 report - www.usmsfp.org/research)
General husbandry practices:	• Transovarial transmission has been confirmed and intra-ovum transmission has not been ruled out. However, if it exists, its frequency is very low. The major source of infection for rearing ponds is grossly healthy carrier fry that have acquired the virus from spawners in shrimp hatcheries.therefore PCR prescreening of wild or pond-reared broodstock and/or their spawned eggs / nauplii - Discarding of positives is required.

	• Disease outbreaks may occur at all seasons and at all phases of pond rearing but since is favoured by the following predisposing factors is important to avoid: - widely fluctuating environmental conditions. - stress induced by chemical and insecticide residues. • Sanitary Prophylaxis - PCR Screening and elimination of infected broodstock from the hatchery. - Stocking of ponds with post larvae (PL) of known health status or screened as negative for WSSV by PCR. - Proper cleaning and disinfection of water (major abiotic vector) and of ponds before stocking, including removal of potential carrier crustaceans. - Animate vectors include over 40 known species of crustaceans. Elimination or screening of potential carriers from exchange water is very important. - The mode of virus shedding from infected shrimp has not been established, but cohabitants with infected carriers acquire active infections within 36–48 hours. Rapid transmission occurs from infected shrimp through the water and by cannibalism of weak or moribund shrimp therefore continuous removal and destruction of moribund and dead shrimp whenever they appear is essential. - Avoidance of exchange of equipment amongst ponds. - Avoidance of the use of fresh aquatic feeds • In case of outbreaks: - Strict isolation of outbreak ponds with movement controls and control of human traffic. - Destruction of all infected and exposed shrimp by incineration or burial. - Thorough cleaning and disinfection of outbreak ponds.

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3.3.3 Infectious hypodermal and haematopoietic necrosis (IHHNV)

Infectious hypodermal and haematopoietic necrosis virus is a parvovirus that affects several commercial species only of penaeid shrimp in both Asia and the Americas. The virus invades all tissue cells of ectodermal and mesodermal origin. It causes acute epizootics and mass mortalities in blue shrimps, green tiger prawns and giant tiger prawns. Although white leg prawns are relatively resistant to this virus, cultured specimens can become chronically infected with IHHNV. The chronic condition is known as runt deformity syndrome (RDS). RDS is an economically important disease of cultured white leg prawns characterised by lower overall crop production and shrimp with increased size variability, reduced growth rates, and cuticular deformities and it can be observed all over North and South America, and wherever the species is cultured (Lightner, 1999). RDS may also occur in *P. monodon* and in stocks of *P. stylirostris* that have moderate resistance to IHHNV. Natural infections of IHHNV have been observed in blue prawns, white leg prawns, western white shrimps, yellow leg brown shrimp, giant tiger prawn, green tiger prawn and Japanese kuruma tiger prawn, although Indian white prawn and banana prawn seem to be refractory to IHHNV. The juvenile stages are the most severely affected. Where it may cause high mortality rates (~ 90%) in some geographic strains of farmed or wild *Penaeus stylirostris* (Pacific blue shrimp). While some individuals clear the virus, IHHNV typically establishes life-long persistent infections in populations of *P. stylirostris, P. vannamei*, and *P. monodon,* and these carriers may pass the virus onto their progeny by vertical and to other populations by horizontal transmission. Although IHHNV has been found in some farms in the regions, the virus is not known to occur in wild penaeid species in the Gulf of Mexico or the Caribbean, or Western Atlantic. In the Eastern Hemisphere, IHHNV has been documented in wild and cultured penaeid shrimp from the Philippines, Japan, Thailand, Singapore, Indonesia, the Middle East, French Polynesia, New Caledonia, and Guam. An IHHNV-like agent (and the possibly related virus, lymphoidal parvo-like virus or LPV) has been reported from penaeid prawns in Australia.

Vaccines:	• None Available.
Chemotherapy:	• None Available.
Immunostimulation:	• No information.
Resistance breeding:	• *L. stylirostris* resistant IHHNV stocks successful application in shrimp farms. No increased resistance to white spot syndrome virus (WSSV) (Limited use).
General husbandry practices:	• Vertical transmission from parents to offspring is important in the transmission and spread of IHHNV, but its mechanism has not been determined. • Vertical transmission by contamination of spawned eggs by virus containing follicular materials, haemolymph, or faeces from infected adults (from females in most culture situations). therefore: - PCR prescreening of wild or pond-reared broodstock and/or their spawned eggs / nauplii - Discarding positives - Very successful development of specific pathogen free (SPF) stocks (*L. vannamei - L. stylirostris)* - *L. vannamei* influx to Asia through trade is challenging

	P. monodon • Sanitary measures at the farm level - Avoid horizontal transmission by cannibalism of weak or moribund shrimp is rapid much more efficient than water transmission. - Disinfect contaminated culture system water, tanks, plumbing, nets, and other equipment.
Eradication methods can be applied to certain aquaculture systems:	• Complete depopulation of all culture stocks, • Disinfection of the culture facility, • Avoidance of re-introduction of the virus (from other culture facilities, wild shrimp, etc.), • Re-stocking with IHHNV-free postlarvae that have been produced from IHHNV-free broodstock. • Use of certified SPF (specific pathogen free) domesticated shrimp stocks. • Use of IHHNV-resistant (specific pathogen resistant or SPR) domesticated stocks of *P. vannamei* or *P. stylirostris*.

Reference

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3.3.4 Taura syndrome

Since its recognition in 1992 as a disease of cultured white leg prawn in Ecuador it was spread up to 1995 throughout most shrimp farming regions of the Americas with shipments of infected PLs and broodstock. Taura syndrome virus (TSV) became enzootic in cultured and some wild penaeid shrimp stocks on the Pacific coast of the Americas from Peru to Mexico. It is reported in cultured penaeid stocks from the Atlantic, Caribbean, and Gulf of Mexico coasts of the Americas, but not in wild stocks. The disease and the virus were reported in Taiwan and China, imported with infected *P. vannamei* from Central America. This syndrome is caused by a virus classified as a picornavirus based on its virion structure (Bonami et al, 1997). Survivors of TSV infections remain persistently infected by the virus, perhaps for life, providing the virus with an opportunity for both horizontal and vertical transmission. TSV is highly infectious for the Pacific white shrimp *Penaeus vannamei*. but the disease is less common in blue shrimp *P. stylirostris* . TS disease may occur in postlarvae from ~PL12, in juveniles and adults but most epizootics at farms are in early juveniles. Horizontal transmission by cannibalism of weak or moribund shrimp is rapid and efficient. TSV can be passed from shrimp to shrimp via the water, but with far less efficiency than by cannibalism. Vertical transmission from parents to offspring is important in the transmission and spread of TSV, but its mechanism has not been determined.

Vaccines:	• No consistently effective vaccination methods • Experimental, injection, dsRNA (Robalino *et al.* 2004)
Chemotherapy:	• None Available
Immunostimulation:	• No information
Resistance breeding:	• TSV-resistant domesticated stocks of *L. vannamei* and *L. stylirostris* • Domesticated lines of TSV-resistant *L. vannamei* are used throughout Americas / South-East Asia • After TS appearance in Central America, improved TS resistance was reported in wild caught *L. vannamei* PLs used to stock shrimp farms • United States Marine Shrimp Farming Program (linkage map for *L.vannamei* – Shrimp map, ShrimpESTbase)
General husbandry practices:	• PCR prescreening of wild or pond-reared broodstock and/or their spawned eggs / nauplii - Discarding positives • Very successful development of specific pathogen free (SPF) stocks • (L. vannamei - L. stylirostris) • L. vannamei influx to Asia challenging P. monodon • Attempts to control virus sources

	• Prevent Cannibalism of infected shrimp. • Avoid Cohabitation of infected and uninfected individuals. • Disinfect Infected transport water, intake water, nets and other equipment. • Use antipredator nets because faeces from gulls and other seabirds that have fed on TSV infected shrimp. • Sanitary Prophylaxis - Proper cleaning and disinfection of ponds and supply reservoirs and canals and before stocking, including removal of potential carrier crustaceans, especially shrimp. - Elimination or screening of potential carriers from exchange water. - Avoid exchange of equipment amongst ponds. - In case of outbreaks - Strict isolation of outbreak ponds with movement controls and control of human traffic. - Destruction of all infected and exposed shrimp by incineration or burial. - Thorough cleaning and disinfection of outbreak ponds.

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3.3.5 Coxiella cheraxi

There are few reports of pathogenic RLOs causing serious diseases in crustaceans (Vago *et al*. 1970, Frederici *et al*. 1974, Lightner *et al*. 1985, Brock *et al*. 1986, Owens *et al*. 1992), and even fewer have been reported from crabs. Mass mortality of shrimp has been associated with RLOs (Krol *et al*. 1991, Lightner *et al*. 1992, Loy & Frelier 1996), and infections in blue king crabs *Paralithodes platypus* (Johnson 1984, Meyers *et al*. 1990), golden king crabs *Lithodes aequispina* (Meyers *et al*. 1990), and green shore crabs *Carcinus mediterraneus* (Bonami & Pappalardo 1980) were thought to be fatal. Prevalences from aquaculture systems have rarely been reported. In blue crabs *Callinectes sapidus*, the prevalence of RLO was 2.3% in a Maryland shedding facility, but heavy infections were not fatal (Messick & Kennedy 1990), and were associated with little pathology (Messick 1998). However, the infection in the Chinese mitten crab is significant due to the high prevalence in pond systems and the rapid mortality associated with the disease. These conditions are similar to those reported for epizootics in shrimp aquaculture systems (Frelier et al. 1993).

A rickettsia-like organism isolated from infected, farm-reared Cherax quadricarinatus was cultured in the yolk sac of developing chicken eggs, but could not be cultured in 3 continuous cell lines, bluegill fry (BF-2), fathead minnow (FHM), and Spodoptera frugiperda (Sf-9). The organism was confirmed by fulfilling Koch's postulates as the aetiological agent of mortalities amongst C. quadricarinatus. When C. quadricarinatus was inoculated with the organism, mortality was 100% at 28°C and 80% at an ambient temperature of 24°C. Horizontal transmission with food and via the waterborne route was demonstrated, but mortalities were lower at 30 and 10% respectively over a 4 week period. The suggested classification of this organism is Order Rickettsiales, family Rickettsiaceae, tribe Rickettsieae, within the genus Coxiella. They suggested it should be named Coxiella cheraxi sp. Nov.

Two forms of rickettsiosis have been reported in freshwater crayfish. One rickettsia-like organism (RLO) is systemic, whilst the other RLO infects only the hepatopancreatic tubule epithelium. The systemic RLO, recently named Coxiella cheraxi, has been associated with serious mortality in *Cherax quadricarinatus* (redclaw) in Australia.

Vaccines:	• None
Chemotherapy:	• Not known
Immunostimulation:	• None
Resistance breeding:	• None
General husbandry practices:	• No information

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3.4 Amphibian diseases

3.4.1 Amphibian Iridoviridae Ranavirus

Iridoviruses are animal viruses that infect only invertebrates and poikilothermic vertebrates, such as fish, insects, amphibians, and reptiles. They have been implicated as causative agents of serious systemic diseases among cultured and ornamental fish, as well as wild fish. A ranavirus linked epizootic, systemic disease causing death within wood frog Rana sylvatica tadpoles and leopard frog Rana pipiens metamorphs was identified at 3 different locations within Southern Ontario, Canada. Clinically normal, laboratory-raised wood frog egg broods were also found to test weakly positive for ranavirus. The population effects of disease on these amphibian communities have not yet been conclusively associated with population declines, but warrant more focused consideration.

Vaccines:	• None
Chemotherapy:	• None
Immunostimulation:	• None
Resistance breeding:	• None
General husbandry practices:	• Prevent its introduction into susceptible stocks

3.4.2 Batrachochytrium dendrobatidis (Amphibian Chytridiomycosis)

One of the major contributors to global amphibian declines and extinctions is the emerging infectious disease, chytridiomycosis. Chytridiomycosis is caused by the fungus Batrachochytrium dendrobatidis. It causes very high mortality (90-100%) in some species of amphibians, and is responsible for catastrophic die-offs of entire frog communities and extinction of species. Over 94 species of amphibians from 15 families from Australia, New Zealand, South America, North America, Central America, Europe, and Africa have been found infected with B. dendrobatidis. Infected individuals typically die within 2-3 days after the onset of clinical signs. The mechanisms by which chytridiomycosis becomes fatal to frogs is unknown. The thin, well-vascularized skin of frogs is critically important as a respiratory organ and a direct pathway for taking up water to maintain hydration. Epidermal changes caused by chytridiomycosis seriously impair water, electrolyte, pH, and blood gas balance in infected amphibians. Pounds *et al* (2006) linked the disease epidemics with global warming due to gradual shift of temperatures in highlands of Central America to the growth optimum of Batrachochytrium. This is a good example of the human related activity environmental impact and the significant increase of the significance of certain diseases to an epidemic level.

Vaccines:	• None Available
Chemotherapy:	• None Available
Immunostimulation:	• No information
Resistance breeding:	• No information
General husbandry practices:	• Control of importation of susceptible amphibians. • Good biosecurity level. • Depopulation when outbreaks occur. • Sensitive to temperature, extremes of pH , chemical disinfectants: - Physical treatments: heat (60 °C; 5 min) - UV light - Desiccation - Chemical treatments: 70% ethanol - 1 mg/ml Virkon - 1 mg benzalkonium chloride or 1% sodium hypochlorite for 20 sec killed cultures - Field use: Path-X TM - Quaternary ammonium compound 128 - TriGene and F10 (Johnson *et al* 2003)

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extinctions from epidemic disease driven by global warming. Nature Vol 439, 161-167.

4. Prudent Antimicrobial Chemotherapy

Veterinarians and fish health specialists need a variety of efficacious, cost-effective and safe chemotherapeutants to enable prompt the therapeutic intervention to battle infectious fish disease outbreaks. Many important questions are raised regarding the integration of bacterial resistance monitoring, pharmacokinetics, metabolism, clinical efficacy, drug residue depletion, target animal safety, consumer safety, and environmental impact linked to antibiotic application.

There are a group of therapeutic agents, loosely known as antimicrobials or antibiotics, which have a specific application that is limited to diseases associated with bacterial infection. Thus, from the perspective of their control and management, the diseases of aquatic animals that have been identified by WP2 as being associated with bacterial infection, represent a distinct class. Work Package 2 has identified three bacterial species *Streptococcus agalactiae, Streptococcus iniae* and *Lactococcus garvieae* as being associated with diseases that may emerge as significant causes of loss and mortality in European aquaculture.

Were any of these bacteria to become endemic in European aquaculture, current experience would suggest that the most effective method for their control would be the development of vaccines. However the development, testing and licensing of new and effective vaccines is an expensive and lengthy process. Two situations can be identified where antimicrobial therapy will represent either the most appropriate or the only available response.

i) The diseases are epidemic and sporadic and do not occur with sufficient frequency to justify the investment of time and money in the development of vaccines.

ii) The diseases become endemic and significant but effective vaccines have not yet been developed, tested, licensed and marketed.

Thus, at least in the short-term, the initial defence against the emergence of diseases associated with these bacteria would have to rely on antibiotics. How long this initial antibiotic-dependent phase will last cannot be accurately determined but prudence would argue that would be reasonable to plan on the basis that the dependence on antibiotics would persist through a number of production cycles.

Any consideration of the appropriate antibiotic therapy of these diseases would have to consider issues associated with regulatory constraints, constraints imposed by our understanding of the efficacy of treatment protocols and the constraints imposed by the incidence of clinically significant resistance in the target bacteria.

Current regulatory situation

A large number of antibiotics are available for the control of disease in human medicine and in the land-based food producing industry. In contrast, the number of antimicrobials with full regulatory acceptance for use in aquatic animals is very small and severely restricts the choices that a veterinarian can make.
In Europe the regulation of antimicrobial use is highly complex. The following summary only provides a rough guide to a very intricate legal process. The FAO document prepared by Subasinghe and Alderman

(www.fao.org/docrep/004/AC343E/AC343E00.HTM) provides a reasonable starting point for those interested in gaining more detailed understanding. Full regulatory acceptance involves two stages. The first is the setting a maximum residue level (MRL) and the second the granting of a Marketing Authorisation (MA). The governing of the legality of residues via the generation of MRL values is not a species-specific process and, therefore, specific studies in fish and/or other aquatic animals are not required. The data required by an MA, however, depends on the claims being made by the company (the label). Claims must include both the species of aquatic animal in which the agent is to be used and the diseases against which it is recommended for use. The data required for an MA includes those relating to efficacy, host safety, consumer safety (residue and withdrawal to MRL) and environmental safety. As a result, generating the data necessary for an MA is a very expensive process. These costs, which are exacerbated by the need to regularly renew any MA, place major constraints on the availability of antimicrobial agents for aquaculture. A company must be sure that the size of any potential market would be sufficient to justify the costs of obtaining an MA. A number of situations can be envisaged where the size of the potential market is unlikely to provide economic justification for the expenditure necessary to apply for an MA.

i) Treatment of diseases of new aquatic animal species
In this context a new aquatic species may mean a species where the husbandry and production conditions are under development or one that is new to a specific country. It could also include the farming of a fish species in novel conditions such as the development of marine culture of a species previously cultured in fresh-water. In each of these conditions there will be a significant period during which the size of any production is insufficient to justify expenditure on obtaining an MA for any therapeutant. There is an irony here. It is exactly during these development phases that disease and therefore the need for antimicrobial therapy will be at its greatest.

ii) Treatment of new or emerging diseases.
To the extent to which any MA specifies the diseases that the agent can be used to treat, then the emergence of diseases associated with new or emerging diseases will not have been addressed. Given the time required to prepare the data necessary for an MA there will always be a significant time delay between the emergence of a new disease and the authorisation of any antimicrobial to treat it. Diseases that, although not new, only occur rarely can also be included in this group.

iii) Treatment of diseases in well-run industries.
As any industry develops improved husbandry practices and obtains effective vaccines, the incidence of disease associated with bacterial infection and, therefore, the sales of antibiotic agents to that industry will decline. As sales decline the relative cost incurred by the requirements to re-new MA increases. Under situations of reduced sales and increasing regulatory cost companies will, and in some countries already have, let the MA lapse and cease to make certain antimicrobial products available.

Agents currently authorised in Europe

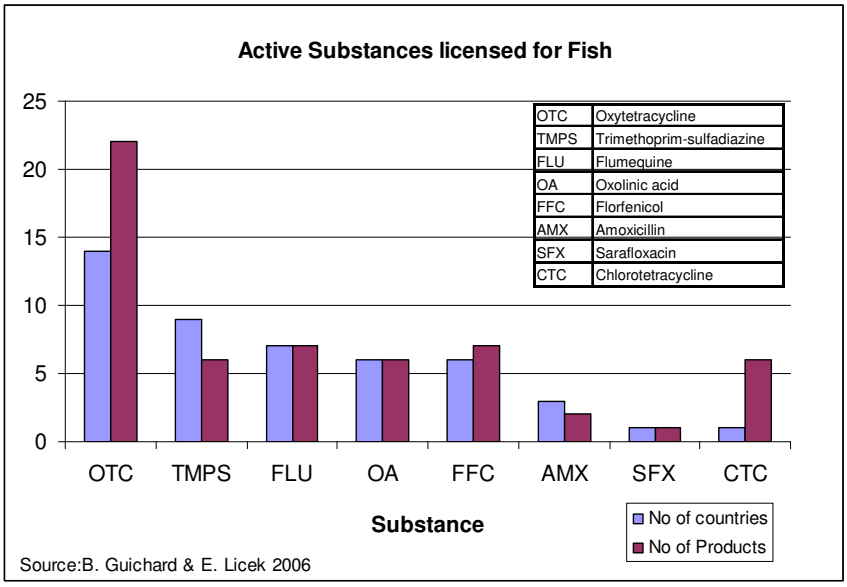
Guichard and Licek (2006) have recently established the number of antimicrobial agents that currently possess MA for use in aquaculture in 31 counties in the European region. These data are summarised in the Table 3 below.

Table 3: Number of products authorised for aquaculture use in European countries.

No of active substances	Countries
0	9
1	7
2	5
3	8
4	2
5	0

Active substances that demonstrate high levels of cross-resistance (such as flumequine and oxolinic acid) are grouped together and are treated as a single agent in this Table.

Figure 1. Active substances licensed for fish



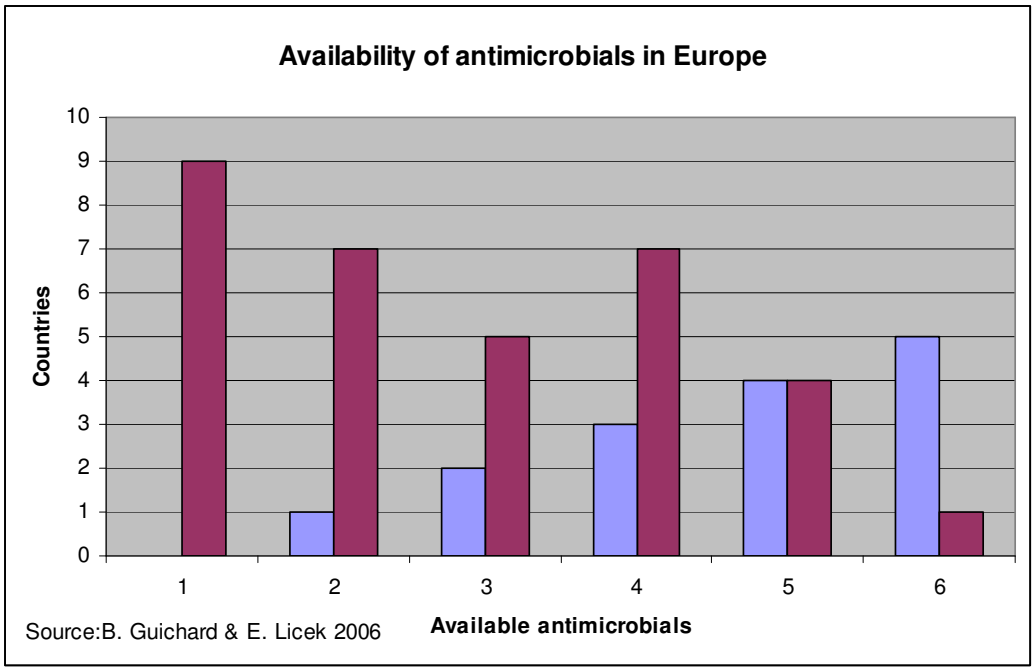
The data in Table 3 is limited to the agents that are authorised for use in different countries. Information on the conditions (host species and diseases) that relate to these authorisations has not been collected. There is no clear relationship between the size of the aquaculture production of any country and the number of agents that have full regulatory acceptance. For example, of the 5 European countries with production in the range 50,000 – 100,000 tonnes, one has approved 4 agents, one has approved 3 and another 2 agents but two of these countries have not approved a single product. Overall a very small range of antimicrobials has been granted MA for use in European aquaculture (see Table below).

Table 4. Agents authorised for use in European aquaculture

Antimicrobial family	Countries

Tetracyclines	16
Quinolones	12
Potentiated sulphonamides	9
Florfenicol	7
Amoxycillin	3

Figure 2. Availability of antimicrobials in Europe



The regulations outlined in summary above place very severe restriction of the use of antimicrobials in aquaculture. The impact of many of these regulations are particularly significant in aquaculture because of the size of these industries, in many if not all European countries, relative to the costs of full regulatory compliance. Two factors can, however, be considered that mitigate the full impact of these regulations. One is the cascade principle and the second is national non-enforcement.

Cascade

The cascade system is one that governs the conditions under which off-label use can be permitted. Essentially it allows that, where there is no suitable product to treat fish, a suitable product approved in other food animal species may be prescribed. There are considerable constraints on the application of the cascade principle and these are detailed in EU Directive 2001/81/EC. Even the details provided in this directive leave ambiguities on certain issues, such as, with respect to aquaculture, what is meant by a "small number" of animals.

Given the delays that would be necessary to obtain a relevant MA for specific the use of any agent in the treatment of any emerging disease, it is almost certain that any treatment will have to occur under the cascade system.

National non-enforcement.

With respect to the enforcement of regulations governing the use of antimicrobials in aquaculture, Europe does not present a level playing field. Some countries enforce the relevant regulation with rigour whereas others appear to place little emphasis on enforcement. The data collected in preparing Table 1 above revealed that some

countries with significant production (>50,000 tonnes) have not authorised the use of any antimicrobial agents. As there is ample anecdotal evidence that antimicrobials are used in these countries this situation can only be sustained by systematic non-enforcement.

Efficacy

Choice of agents
There is no evidence that *S. agalactiae, S. iniae* or *L. garvieae* present any special problems with respect to any inherent resistance to antimicrobials. A number of broad-spectrum antimicrobials have been suggested as suitable therapeutic agents.
In a technical note to Greek fish farmers Varvarigos (2001) suggested that, potentiated sulfonamides, and oxytetracycline are usually effective and that, erythromycin, ampicillin and amoxycillin represented good alternatives. In the US a technical note by Yanong and Francis-Floyd (2002) suggested that erythromycin was the drug of choice. Dawish and his co-workers (Darwish, et al 2002. Darwish & Ismaiel, 2003, Darwish 2006.) have reported a series of laboratory-scale trials aimed at determining optimum treatment protocols for *S. iniae* infections. These studies suggest that amoxicillin, oxytetracycline and florfenicol can all have therapeutic value.

Availability of agents
Of the antimicrobial that have been suggested for the treatment of *S. agalactiae, S. iniae* or *L. garvieae* infections all with the exception of erythromycin and ampicillin have an MA for three or more European countries. It can therefore, be assumed that MRLs have been set for these agents. It is, however, probable that none of these MAs specifically cover infections by Gram-positive cocci. Were disease to emerge as a consequence of infections by these bacteria, it is possible that a reasonable argument could be made for the use of oxytetracycline amoxicillin or florfenicol under the Cascade principle. This use of the Cascade principle, therefore presents a short-term solution to the problems that the emergence of these diseases would represent.

Choice of treatment protocols.
The optimisation of treatment protocols is an essential component of the prudent use of antimicrobials. Not only should treatments be optimised to maximise their efficacy but also to minimise the chance of resistance emerging during the therapy (Lees et al, 2006). With respect to optimising therapies in aquaculture it is probable that, with respect to bacteria capable of systemic infection, the properties and pharacodynamics of the agent in any fish species have a greater importance that the specifics of the infecting bacterium. Thus. It is reasonable to suggest that any errors in applying a treatment protocol developed for one systemic infection to the therapy for another would be minor.

Acquired resistance.

A major factor that limits the value of antibiotic therapy is the emergence of resistant variants of the target bacteria. Although it is correct to state that there is a general tendency for the frequency of resistance to a specific agent in the bacteria inhabiting a particular environment to reflect the frequency of its use in that environment, this relationship is neither simple nor direct. In some cases resistance emerges rapidly (Kim et al, 1993) and in others (Michel et al. 2003) the emergence of resistance is quite slow.

Any attempt to treat an infection caused by a resistant bacterium is not only certain to fail but it also represents a very imprudent use of a valuable resource.

Antibiotic Resistance Mechanisms

Antibiotic Resistance is always a relative term. A strain can be classified as resistant to a specific antibacterial agent if it is able to function, survive and persist in concentration of antibacterial higher than the members of its parental population. A species on the other hand can more loosely be classified as resistant when its members can function, survive and persist to higher concentrations of an antibacterial agent than the members of other species (Smith, 1995). Bryan (1989) suggested that there are two forms of antimicrobial resistance: positive function resistance and bacterial persistence. Positive function resistance is the form where a microbe gains the capability to resist one or more antimicrobial agents by the acquisition of a gene function. Usually this results from the transfer of plasmid DNA into the bacterial strain. The gene functions are for the most part concerned with inactivation or modification of the antimicrobial agent, the addition of an efflux system or the specification of a new target or the enzymatic modification of the target. In each of these circumstances there is relatively little disadvantage to the cell in possessing these additional gene functions. Thus there is a tendency for these mechanisms to be relatively stable in a strain and for bacterial populations derived from these to contain predominantly resistant organisms. Humans for a substantial period of time and from individual, institutional and community reservoirs may carry the resistant strain. Control can be achieved in two major ways, either by infection control or by the development of new agents or inhibitors, which are insensitive to or overcome the resistance mechanism. It is also possible that control could be achieved in the long term by restriction of a particular agent, as the strains are not likely to be perfectly stable. This is the main mechanism by which bacterial pathogens acquire resistance to antimicrobials like Oxytetracycline.

In relation to courses of antimicrobial therapy positive function resistance is unlikely to appear during therapy unless acquired by cross-infection during a course of treatment or more rarely by selection of a minority population of the pathogenic strain. It is likely that this form of resistance will be readily detected by susceptibility testing because of the relatively high levels of resistance normally specified and the high frequency of resistance in the particular bacterial population. Therefore, positive function resistance would usually be found at the onset of therapy. Plasmid-mediated resistance to antimicrobials has been identified in a number of bacterial fish pathogens. Plasmid-mediated resistance to 4-quinolones has not been reported in fish pathogens, presumably because they appear to effectively inhibit the process of conjugative plasmid transfer (Nakamura et al., 1976), so that the spread of quinolone resistance by gene transfer is unlikely.

The second form of resistance can be termed 'persistence' and the mechanisms responsible, mechanisms of persistence. In these circumstances persistence is the result of resistance from mutation or from temporary resistance due to gene regulatory events or phenotypic changes that would only become evident during exposure of a bacterial population to an antimicrobial agent. Thus persistence is only seen during courses of therapy. These changes allow a microbe to persist during therapy by giving some levels of antimicrobial resistance. However, they are frequently deleterious to the organism in some manner causing growth impairment to a variable degree and in some

cases a temporary reduction in virulence. Mechanistically, these changes operate by reducing permeability or target affinity for a drug or by turning on chromosomally specified enzymes capable of degrading the drug. In contrast to positive function resistance these changes result in loss or reduction of gene function or a functional change that provides a metabolic load to the organism. Such forms of resistance are most likely to be effective where there is a local or general deficiency of host defences, where drug entry is poor or where the local environment is antagonistic to the action of the drug. Persistence may impair the organism but after removal of the antibiotic there are strong selective forces for the parental form to return or for loss of the regulatory or phenotypic changes because of better growth and virulence characteristics. The effect of persistence is to slow therapeutic response, establish colonization, or produce outright therapeutic failure. This is the mechanism by which bacterial pathogens acquire resistance to Antimicrobials like Oxolinic acid.

Methods for determining clinically significant resistance are, therefore, a critical component of any rational, prudent and responsible use of these agents. Determining resistance is a two-step process. The fist is the generation in the laboratory of a measure of the susceptibility of the bacterium. The second is the interpretation of the clinical significance of that measure.

In vitro susceptibility measurements

In recent years there has been significant progress in developing, validating and harmonising laboratory methods for determining susceptibility. This process started with an EU funded Concerted Action (FAIR CT 97-3760) that generated the protocols published by Alderman and Smith (2001) and has reached fruition with the publication by the Clinical and laboratory Science Institute (CLSI) of the M42-A (CLSI, 2006a) and M49-A (CLSI, 2006b) protocols. Although, the CLSI protocols must be seen as a work in progress, there would appear to be no justification for the use of any other methods or protocols. With respect to the bacteria being considered here (*S. agalactiae, S. iniae* and *L. garvieae) the CLSI* the protocols are still in an early stage of development. Work is required to set the appropriate growth conditions, the appropriate control strains and the acceptable range of measures for these control strains. Programmes addressing some of these issues are in hand but it will be years rather than months before the complete protocols for these Gram-positive bacteria are finalised.

Interpretative criteria and breakpoints.

The development of interpretative criteria that would allow the determination of the clinical significance of data generated by the CLSI protocols must necessarily follow the development of the protocols themselves. The approved version of the protocols only appeared in 2006 and, therefore, there are, as yet, no interpretative criteria that have been validated for any group of bacteria. Thus with respect to *S. agalactiae, S. iniae* and *L. garvieae* we lack not only the detailed laboratory protocols for measuring susceptibility but we also lack the means to interpret any data we do generate.

Breakpoints in current use

As a component of the work of WP5 a survey of the current susceptibility testing practices of laboratories was performed. The details of this survey have been published (Smith in press$_{a,b}$) and the following major conclusions can be drawn from the survey are;

1. The majority of responding laboratories used disc diffusion protocols to measure susceptibility of field isolates.
2. The majority of responding laboratories were using either Alderman and Smith (2001) or CLSI test protocols.
3. There were wide disagreements in the breakpoints being used to attribute clinical meaning to the susceptibility test data generated in different laboratories (see Appendix 1). Studies of epidemiological cut-off values (EUCAST, 2000) from the distribution of susceptibility measures of *Aeromonas salmonicida* (unpublished data) provide strong support for the idea that many of the breakpoints currently in use are erroneous and seriously misleading.

Fish – Human Bacterial Pathogen interactions / resistance transfer

There are 3 possible threats to human health from use of drugs in aquaculture: a) residues of drugs in fish destined for human consumption, b) development of drug resistance in human pathogenic bacteria and c) direct toxic effects on human from handling the drugs (Bernoth, 1991) Four ecological compartments may be considered as important for the transfer of resistance to antimicrobials; humans, animals, plants and soil-water. The common factors between the four ecological compartments are the antimicrobials, the bacteria, and the genes that code for resistance. Some resistance genes have been shown to move between bacteria in each compartment and it is possible for bacteria to move between the compartments. Bacterial gene transfer is now thought to occur not only in the human and animal intestine but throughout the biosphere, especially in nutrient-rich sites such as aquatic systems, sediments, soils, in the vicinity of plant roots, and in the sludge of the biological sewage treatment systems. Antimicrobial resistant bacteria have been isolated from all of these sites. Resistance may also be spread from plants and vegetables treated with antimicrobials or fertilised with wastes containing animal or human faecal residues. Thus resistance should be considered as a phenomenon of global genetic ecology. The crucial questions are whether resistance genes are transferable between environmental microorganisms and mammalian pathogens, and whether there are cascades of exchanges between related species or genera. The chain of resistance transfer is probably much more complicated and longer from plant pathogens to mammals than from animals to man. At present, no definitive antimicrobial resistance rates and predictive models are available. The Advisory Committee on the Microbiological safety of food in UK (2001) suggested that three are the potential routes of spread of antibiotic resistance from fish to human pathogens. The drinking water pathways despite the barrier between temperate environment fish pathogens and human pathogens, dilution factors and complex formation / reduced antibiotic bioavailability availability, refers only to antibiotics where transferable resistance occurs. In kitchen contamination route involves all viable bacteria that could contaminate uncooked food and wound infection is the last route involving handling hazards for fish farm and fish processing unit personnel. There is considerable evidence to support the view that antimicrobial use in animals, both in the therapy of infections and as feed additives, is associated with an increasing prevalence of bacteria exhibiting resistance to the agents used. Many drugs used in animals can select for bacteria, which are resistant to antimicrobials used in man. An important question is to what extent the increasing prevalence of antimicrobial resistance in animals contributes to the increasing prevalence of resistance among human pathogens. Transmission to man of zoonotic agents such as Salmonella spp. and Campylobacter spp. is of particular importance in

assessing this relationship. Humans will acquire both pathogenic and non-pathogenic antibiotic resistant organisms from animals. This can be partly controlled but not entirely prevented by good food hygiene. Zoonotic bacteria like salmonella therefore have to be controlled primarily during food production, according to the concept of pre-harvest pathogen control (WHO, 1983). Austin (1985b) in experiments with 4 fish farming sites found that during treatment with oxolinic acid, oxytetracycline, or a potential sulfonamide, the total count of bacteria in the effluent was not markedly altered, but the spectrum slightly sifted to Gram-positive species and resistances increased, returning to usual levels with 9 days after cessation of treatment (Bernoth, 1991). During a second OTC treatment in a microcosm study a significant increase in the number of sediment OTC resistant bacteria was observed highlighting the need for prudent use of antibiotics in aquaculture (Vaughan et al., 1996). Resistance in microflora has been reported in several studies (VanHouweling, 1971; Jacobsen and Berlind, 1988; Bjorklund et al., 1990; Samuelsen et al., 1992a; Coyne et al., 1994; Kerry et al., 1994; 1995; 1996a; 1996b; 1997). The quantification of resistance to antimicrobial agents in the fish farm environment is based on the study of frequency of resistance in simulated environments (McPhearson et al., 1991; Nygaard et al., 1992; Samuelsen et al., 1992a,b; Hansen et al., 1992a; Spaanggaard et al., 1993; Ervik et al., 1994a; Kerry et al., 1994). While some researchers generally suggest that antibiotic use in fish farming may add to the environmental resistance pool (Spanggaard et al., 1993) we have to remember that bacterial isolation using a limited range of culture methods and media, gives us only small percentage of the actual flora present and of any changes brought about by the antibiotic use or ecological changes induced in the fish farm environment (Smith et al., 1994). Problems arise in simulated environments because culture methods are fundamentally inefficient (Buck, 1979) supporting growth on any medium, in a fraction below 1% of the total viable count (Hoppe, 1976) or for other researchers around 0.3% (Buck, 1974). McPhearson et al., (1991) identified aquaculture ponds in south eastern US as potential reservoirs of antibiotic resistant bacteria. Regardless of whether resistance was mediated by exposure to antibiotics or by other mechanisms that pool of antibiotic resistance bacteria may have public health significance. Health risks and implications associated with plasmids that were detected in a strain of Salmonella, other members of the Enterobacteriaceae and several Pseudomonas species were discussed after the occurrence of these antibiotic-resistant human pathogenic bacteria in integrated fish farms in India (Twiddy and Reilly, 1995). During OTC treatment periods, bacterial populations in sediment markedly declined but recovered once treatment once treatment had ceased. No increase in resistance to antibiotics was recorded among isolates screened during the first treatment period with OTC. However, during the second treatment period a significant increase in numbers of sediment bacteria resistant to OTC was observed, highlighting the need for prudent use of antibiotics/chemotherapeutants in aquaculture (Barker and Alvarez, 1993)

Recently FAO, OIE and WHO organised a Joint Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance in Seoul, Republic of Korea (13–16 June 2006) to evaluate the usage patterns, and public health impact of this use, and to develop strategies to minimize the risk. Although data on quantities of antimicrobials used in aquaculture are not available in most countries, available evidence suggests that the amount of antimicrobials used in aquaculture in most developed countries is limited and in some countries the quantity has been decreasing. Nevertheless, large quantities of antimicrobials are used in aquaculture in some countries, often without professional consultation or supervision. Furthermore, an important proportion of aquatic animals raised for the global aquaculture industry are raised in countries with

insufficient regulations and limited enforcement for the authorization of antimicrobial agents used in animals. In some countries availability of registered antimicrobials is insufficient which contributes to illegal use. The public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial resistant bacteria and resistance genes, and the occurrence of antimicrobial residues in products of aquaculture. The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in bacteria in aquatic environments from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens. However, a quantitative risk assessment on antimicrobial resistance in aquaculture is difficult to perform due to lack of data and the many different and complex pathways of gene flow. Prevention and control of bacterial diseases in aquatic animals is essential to minimize the use of antimicrobials and avoid the negative impact of antimicrobial resistance. Efficacious vaccines and improved systems for mass vaccination of finfish should be developed, and an optimization of vaccine licensing procedures should be promoted. The Joint Consultation suggested that Programmes to monitor antimicrobial usage and antimicrobial resistance in bacteria from farm-raised aquatic animals and their environment should be implemented and national databases should be developed to achieve efficient communication.

Smith et al. (1994) on a comprehensive review the real problem dimensions and suggested that there was no evidence of a continuing increase in the frequency of resistance in most human pathogens in the developed world. The level of resistance in these pathogens is primarily a function of the use of antimicrobial agents by the medical profession. With the exception of the case of the Salmonella there is little compelling evidence that the use of antimicrobial agents in veterinary medicine has had an adverse effect on the therapy of human pathogens. Epidemiological and ecological considerations of pathogenic bacteria associated with fish suggest that resistant variants are unlikely to cause any impact on human pathology. In developed countries, the possibility that antibiotic resistant bacteria deriving from fish farm application of antimicrobial agents might reach the human consumer through the drinking water chain appears to be remote. Factors that need to be taken is to consideration include very high dilution factors and the fact that most fish pathogens present in temperate climates are not capable of is affecting humans. In addition, antimicrobials (although more available in fresh water than in salt water) are complexed by calcium and magnesium ions readily available in the aquatic environment and therefore are poorly bio-available. There is also a multiplicity of other anthropogenic sources of antibiotics in ordinary river water, including those derived from land run-off and, more especially, the presence of effluents from sewage plants that may be derived from human antibiotic usage. Against all of these factors and sources the comparative input from such farming antibiotic use must be recognized to be small. The treatment processes ending in chlorination routinely practised in treatment of drinking water should ensure that there are no viable microorganisms in that water when it leaves the water treatment plant. Thus, the likelihood of transfer of any transferable antibiotic resistance to the drinking water consumer from fish farming use should not be regarded as a significant possibility (Alderman and Hastings ,1998).

Antimicrobial Chemotherapy - Practical considerations

The practical aim for the farmers is to identify if the applied treatment protocols and related achieved tissue concentrations *in vivo* are sufficient to control the bacterial pathogens and on the other hand which is the period that should be allowed in order to make sure in practical terms that antibiotic concentration falls bellow the MRL level. An important issue for the containment of antibiotic resistance in the aquaculture industry is to define the problem and apply good practices and codes of conduct. Several issues should be addressed: Research has indicated that many factors are involved in the selection and spread of antimicrobial-resistant bacteria. Most important amongst these appears to be the extent of usage since the relationship between the amounts of antibacterials used and the prevalence of resistance is broadly quantitative. Thus, the control and containment of resistance is likely to be successful only if the measures employed include a reduction in the use of antimicrobials in all spheres of current application. The measures, which need to be considered for control and containment of antibiotic resistance, include: Improved prescription use, reduction of need and provision of new antimicrobials as well as education of prescribers and fish farmers. In order to preserve the ability to treat bacterial infections in fish, action must be taken to reduce the overall use through the implementation of preventive measures and by prudent use. In veterinary medicine, antimicrobials may be prescribed for prophylactic or therapeutic purposes. In addition, complications arise in aquaculture where disease epidemiology and treatment always refers to fish populations in different production systems. In those situations it is necessary to administer antimicrobials to the whole group even though all fish do not yet demonstrate clinical signs of infection at the time of administration, but it is likely that most of them will get the disease in the next days. In that sense the "cascade principle" for aquaculture, as mentioned, before should be reviewed and amended. Such use of antimicrobials is often referred to as metaphylactic use. The veterinary surgeon / fish pathologist prescribing antimicrobials should have knowledge of the disease history including disease epidemiology, preventive and other measures undertaken. To achieve an optimal and prudent use of antimicrobials, guidelines for the use of antimicrobials may be established as a help to the veterinarian. Such guidelines would also support the veterinarian against demands for antimicrobials by fish farmers. These guidelines should be to achieve three goals; optimal therapeutic effect and/or protection and welfare of fish at risk, control of antimicrobial resistance and provision of practical, affordable treatment that avoid risks of residues in or damage to aquaculture products for human consumption. Emergence and spread of resistance is a serious effect of antimicrobial use in animals, so facilities to monitor and analyse regularly the prevalence and patterns of resistance should be developed as a priority. Antimicrobial susceptibility data should be quantitative and produced under strict quality assurance. Interpretation and reporting of results from different laboratories must be harmonised. More detailed knowledge about the usage of antimicrobials and the impact on epidemiology and prevalence of antimicrobial resistance in different environments will assist a greater understanding about the forces behind the development and dissemination of antimicrobial resistance. Registration of the amounts of veterinary medicines sold by each company should be available as well as the recording of indications and use of veterinary drugs and pre-medicated feeds by means of a logbook on each farm and in each veterinary practice. Such a recording system of health status and use of medicines will make it not only feasible to monitor the usage of antimicrobials on a farm and by prescription, but also the indications for antimicrobial usage and efficacy of antimicrobial therapy under practice conditions. It is understood that such studies are undertaken by pharmaceutical companies but we believe that they should be made public. All antimicrobials administered on farms should be used only as part of a comprehensive veterinary health programme. In

contrast to the situation in human medicine more drastic and effective management and other disease preventive methods can be undertaken in animal production. Batch-wise, year class-segregated production using all-in/all-out systems with biosecurity routines together with optimal nutrition, environment, management routines and vaccination strategies can greatly improve fish health and decrease the need for antimicrobials. The introduction of vaccines against furunculosis in fish farming in Norway has eliminated an alarmingly large use of antimicrobials. Recent research has been devoted mainly in the field of Probiotics as means to prevent colonisation of the intestine by bacterial pathogens mainly Vibrios. It is difficult to find or create truly novel agents, which are patent, able. On the other hand there may be a finite number of appropriate targets in bacteria. Finally it is commercially unattractive to invest in research having little chance of producing a return. The cost of research, development and testing of a novel antimicrobial is now probably in excess of US$350 million and the time required for effective marketing is at least 6-7 years (Cohen, 1992). As a result, the number of companies investing in antibacterial research declined even before the recent trend towards company mergers occurred. Moreover, there is the risk that a costly new antimicrobial drug may well become obsolete within a few years, reducing the economic returns that can be expected to a level that is insufficient to justify the investment. The thirteen-year industrial-property protection period for veterinary medicinal products for fish should be extended to 15 years in order to enable the pharmaceutical industry to derive full benefit from such products thus initiating renewed incentives to invest and achieve marketing authorisations for fish products. The time it takes to obtain registration and to develop the data required for the extension of a marketing authorisation to different species and to different diseases should be considerably shorter. Both centralised and decentralised procedure should be maintained providing the necessary flexibility in terms of faster launching of new products in the aquaculture market. Evaluation of the operation of the procedures for the granting of market authorisation has revealed the need to revise, in particular, the mutual recognition procedure in order to increase the scope for cooperation between Member States. Different levels of competence in different Member States as well as different level of vigilance often leave more options for treating or preventing fish diseases in some countries while leave none in others. A simple example is that a few Member states deny acting as reference Member states in the Mutual recognition procedure. A basic degree of harmonisation should be included in the enforcement of the new legislation in order to avoid the creation of indirect trade barriers. The large number of aquaculture species and relevant pathologies complicate the procedure of extension of use or species and emphasis should be made into making this process faster at a Member state level as well as at EU level. Systematic extrapolation of MRL values between species would make new drugs available faster for minor species. The ‘cascade’ principle needs to be modified. In the case of absence of authorised veterinary medicinal product for a condition affecting fish, a familiar case for fish veterinarians, the veterinarian is allowed to treat that fish population under his/her direct personal responsibility on a particular fish farm, in order to avoid causing unacceptable suffering to the fish population, with a licensed product in another Member state for fish or for other food producing animal The reference however to a “small number of animals” in the legislation is not applicable for aquaculture because fish are reared in cages or tanks in large numbers and these are treated as units in terms of epidemiology, prevention or treatment of fish diseases. The licensing and application of Fish vaccines should be encouraged in order to support the European Aquaculture industry trend to limit the use of antimicrobials and to ensure high product quality and safety. New searches for drugs that interfere with virulence factors or

mechanisms of resistance, or which seek to modify the molecular biology of multiple resistant pathogens are being developed. However all these “new “ technology drugs should be carefully audited in terms of consumer safety especially when applied to food animals and will take a considerable time, if ever, to reach the aquaculture industry. Aspects of antimicrobial therapy are poorly addressed in under-graduate and post-graduate education. Thus, there is scope for expanding education of health professionals both in terms of good prescribing and how to minimise antimicrobial use by preventing infection occurring. For veterinary practitioners this might include updating on organised health control programmes mentioned above.

Chemotherapy in Aquaculture and potential environmental Impact

The environmental problems caused by fish farming are different in the different countries due to geographical, topographical, and physical conditions (Braaten, 1991). Environmental Impact Assessment is preclusive in order to lease the site and obtain permission for the operation of a fish farm, together with production management details and a description of the specific area in terns of physical, chemical and biological parameters of the water body (Karakassis et al., 1998). Unfortunately all data needed are usually obtained from published sources, rather than from specific monitoring programmes at individual sites. Annual predicted production, stocking density/m3 or production per water surface unit and carrying capacity calculations require site-specific environmental data. The criteria for land-based farms are covered by existing legislation, which requires an approved special water discharge study, which includes recommendations for the treatment of effluents before their discharge. Superficial sediment layer beneath sea cages fluctuates between 2cm (February) to 5-6cm (July), suggesting a recovery procedure during the winter months when feeding rate is reduced and the wind generated currents that shake up the sediment surface. Loose top segment of the sediment consists of 95% water, it has increased chlorophyll due to phytobentic organisms or due to the elevated phytoplankton production beneath the cages due to ammonia and phosphorus increase. However, most of the phosphorus is organically bound and not bioavailable, which is why the risk of phytoplankton blooms is less in Mediterranean where phosphorus is the limiting factor. In comparison to studies of sediment recovery following different activities the sediment recovery in aquaculture activities is faster because the scale impact in space is confined, the precipitation material (fish feed, fish waste) is more bioavailable than all other urban or industrial waste. A series of factors affect these bio-geo-chemical procedures (Karakassis et al., 1998). Karakassis et al., 1998 suggested the following measures for the reduction: a) avoidance of sites with muddy substance, b) avoidance of low degree of water exchange or high level of fluctuation of environmental parameters and c) selection of at least 500m minimum distances between farms in order to avoid transfer of diseased, parasites etc. (Figure 3). HPLC and bioassay methods have been utilised for the detection of the impact of the antimicrobials in the aquatic environment (Hansen et al., 1992a; Ervik et al., 1994a,b).

Large fraction of antibacterial agents, typically in the form of in feed treatment is not absorbed and retained, but is released to the environment through any of three routes. First, some fraction of the medicated feed is not ingested and instead falls directly to the substratum. Estimates of this feed loss in salmonid net-cage culture range from 15% to 40% and are likely to be highest during a disease outbreak when the cultured fish tend to feed less. This proportion of uneaten medicated feed is 30% in another study. It is reasonable to assume that 20% of the feed administered to fish is not eaten

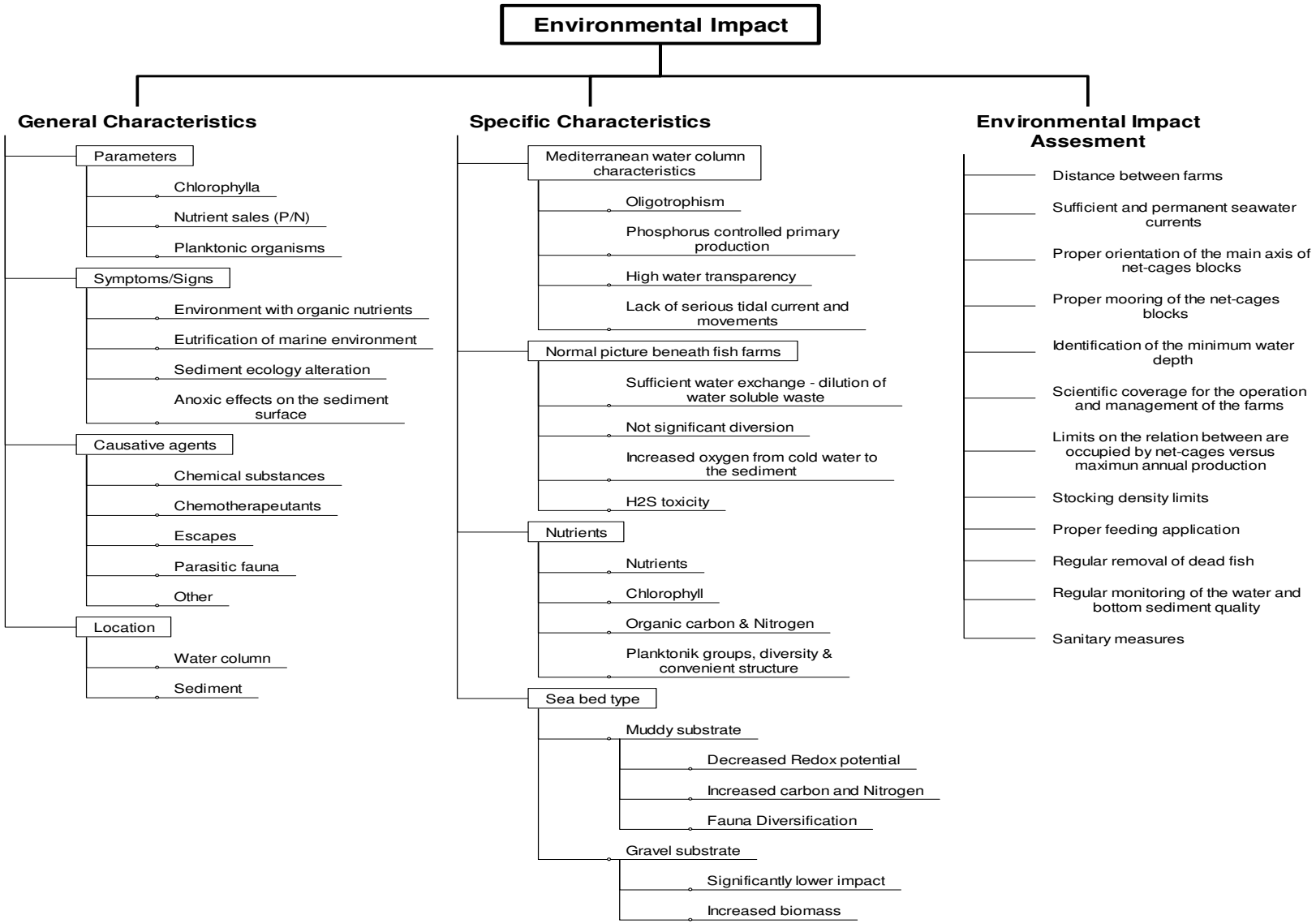
and passes through the cage and will be deposited on the sediment in the vicinity of the cages despite recent advances in underwater cameras and feeding monitoring. Sandaa et al. (1993) have suggested that during a disease outbreak this percentage may rise. A proportion of the antibacterial agent leaching from the pellets, and a further proportion is excreted by the fish. OTC and OA are practically un-degradable in sediments, and 60-98% of these chemicals are not absorbed in the gut of the fish (Braaten, 1991). Fate of uneaten medicated feed depends on fish farm site characteristics. The nature of the environment may significantly alter the biological activity, and therefore the extent of the environmental impact, of a particular concentration of an antibiotic. The significance of a chemical in the environment is not a function of its presence but its impact on processes in that environment (Smith et al., 1993). In marine cage sites with fast currents and large tidal flows there will be greater dispersal of solid and particulate material than in sites with poor flows. In addition the nature of the agent affects it's fate in the marine environment. The area of sediments containing measurable oxytetracycline residues was very localised, under the cages and to a distance of 30 m, but absent 100 m from the site (Capone et al., 1996). At the end of OTC treatment elevated frequencies of resistance were detected up to 75 m from the edge of the cage block and ceased one-month later (Kerry et al., 1994).

OTC is poorly absorbed especially in marine fish where the intestinal uptake is substantially reduced due to complex formation in seawater as compared to fish in fresh water (Smith, 1996). A major portion of the OTC administrated to farmed salmonids inevitably ends up in the environment, especially in the sediments under aquaculture facilities. No mechanism is known for biodegradation of OTC and thus it can remain in the sediments long enough to affect the indigenous bacterial flora and induce resistance (Lunestad and Goksoyr, 1990; Smith and Samuelsen, 1996).

Aquaculture operations are often linked with increased densities of wild fish and invertebrates in the vicinity due to the shelter provided by the culture structure or enhanced food resources (Carss, 1990). Fish and invertebrate in the vicinity of the farm may also consume uneaten medicated feed pellets (Nausbaum and Shotts 1981; Samuelsen et al., 1992a). Several studies on the impact of medicated diets on wild fauna around fish cages include studies in blue mussels *Mytilus edulis* (Moster, 1986; Ervik et al., 1994b; Coyne et al., 1997), sea trout Bjorklumd et al. (1990) coalfish, *Pollachius virens*, cod *Gadus morhua*, mackerel *Scomber scombus*, *ballan wrasse Labrus bergylta* and haddock *Melanogrammus aeglefinus* (Carss, 1990) oysters (*Crassostrea gigas)* (Capone et al., 1996), Dungeness crab (*Cancer magister)*and red rock crab *(Cancer productus)* and scallop (Capone et al 1996; Skjoelstrup et al., 2000). OA toxicity to freshwater crustacean Daphnia magna indicates the potential to cause adverse effects on the aquatic environment (Wollenberger et al, 2000). A fraction of the antibacterial is not absorbed during gut passage and is released to the environment via the faeces. The fraction of antibacterial released through this route may be higher that 90% for oxytetracycline (Cravedi et al., 1987). Finally, some antibacterials such as oxytetracycline are excreted via the urine and bile fluid in an unmetabolized, microbially active form (Bjorklund and Bylund, 1990). The presence of *Aeromonas salmonicida* and resistant strains in sediments beneath Norwegian fish farms illustrate the potential danger from persistent antibiotic concentration as selection factor in the sediment (Husevag and Lunestad, 1995). The area of sediments containing measurable oxytetracycline residues was very localized, however, detectable residues existed only under the cages and to a distance of 30 m, but were absent from a 100 m site (Smith et al., 1994).

The persistence of OTC in fish farm sediments may depend significantly on the type of sediment, chemical characteristics (O_2, H_2S, pH, etc), temperature and bacterial activity in the sediment. Compared to OTC, OA showed a faster loss of antibacterial effect in the fish farm sediments. Sediment porosity due to bioturbation by *Capitella 'capitata'* may accelerate antibiotic elimination while in other cases a white carpet of the blue/green algae *Beggiatoa* may decrease the antibiotic diffusion (Coyne et al., 1994; Smith et al., 1994), but in cases of extreme enrichment even this species may be eliminated. Quinolones may not be broken down because there are no enzyme systems to target these synthetic molecules. In that case any breakdown will depend on physical factors such as photolysis. Oxytetracycline is very soluble in seawater and solution and diffusion process is most likely a major mechanism of OTC escape from the sediment. OTC degradation in water is much faster than in sediment. Tetracyclines in water solutions are degraded by photodecomposition and half-lives depend on temperature, pH, air saturation and light intensity (Samuelsen, 1989a). Coyne et al., (1994). Oxytetracycline, Oxolinic acid and Flumequine photodegrade in the upper levels of the water column (Lunestad et al., 1995) while they exhibit no degradation in the sediments (Samuelsen et al, 1992a,b). Only OTC and not OA showed reduced antimicrobial activity when exposed to underwater light intensities. Photo-degradation is the process in which a component is directly altered by the action of light or indirectly altered by the action of the product of another component, which has absorbed light. An prerequisite for direct photo-degradation is that the chemical absorbs light in the UV – visible region (190-800 nm). Substances that do not absorb in this region cannot be directly photo-degraded. Therefore, photo-degradation will only have a limited effect on the drugs when administered as medicated feed. However, applying bath-treatment (O'Grady et al, 1988) where drugs are dissolved in water and kept in tanks, photo-degradation can be an important tool to decompose the drug and thereby decrease the environmental impact (Lunestad, 1995). OTC, whose binding to mineral and organic compounds of the bivalve tissues is stronger than OA, was eliminated more slowly from the bivalve tissues than OA. The biotransformation of the antibacterials agents by the bivalves did not seem to play a prominent part in the contamination and decontamination of the bivalve tissues (Pouliquen et al., 1996). New methods of monitoring overfeeding with underwater cameras and automatic feeding systems as well as collecting surplus feed and dead fish have been developed using Lift up feed collector systems or video and hydroacoustic feed detectors. This equipment can significantly reduce the environmental impact of antibacterial agents used in fish farming.

Figure 3: Environmental Impact Considerations



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5. Biosecurity Considerations

Introduction

Good husbandry and biosecurity should be central to the operation of all aquaculture operations and the implementation of effective national disease control strategies. The use of particular medicines and vaccines to control or prevent the development of particular diseases should be considered only as part of a general, integrated, disease preventative approach.

This is particularly the case for the diseases identified as of high risk to European aquaculture by the other PANDA work packages. In the absence of, at best limited, treatment options for the majority of these diseases, good husbandry and biosecurity are essential practical preventative measures . This could be considered to include:

- Identification and management of the risk reduction of introduction and transmission of disease
- Educate key personnel on management and those risks
- Containment of a disease once an outbreak has been identified. This would include treatment (where appropriate), quarantine or culling and disposing of affected stocks (depending on the National policy in place for the control of the particular disease), safe disposal of affected stocks,cleaning and disinfecting the affected farm or unit and equipment, paying attention to health and safety considerations and environmental implications of treatments.
- In the case of a serious disease outbreak, where animals of harvestable size may be marketed, containment from farm to process remains under statutory control, including effluent treatment

For all scenarios, implementation of good biosecurity procedures is essential in minimising transmission of disease agents..

Biosecurity

Biosecurity can be defined as the exclusion, eradication or effective management of risks posed by pests and diseases to the economy, environment and [animal] health. A Veterinary Health Plan should be an essential consideration for all companies.
Biosecurity involves good work practices and incorporates:

- A sensible approach to husbandry (appropriate stocking densities and water quality for the species under cultivation).
- Only sourcing eggs or fry or spat from sources that are free of disease (e.g. SPF stocks or have Certificate of Origin). Live fish, shellfish and ova-associated spread are the major mechanisms by which disease gains entry to production sites.
- Taking particular care about shellfish and finfish health
- Taking careful notes. Logs of mortalities should be kept, as well as notes of abnormal fish and shellfish behaviour.
- An awareness of disease risks, particularly during transfer of shellfish and finfish.
- Consideration of cleanliness
- Appropriate waste disposal

- Awareness of the need for good communication & training
- Avoid escapes or undue stress on cultured animals.
- Vets/FH experts should be called in immediately to investigate problems.

Hazard Analysis Critical Control (HACCP) principles

The inherent risks in the process of cultivation should be identified and systems then designed to control them, rather than identifying and reacting to particular problems after they arise. This entails:

- Hazard analysis, risks should be identified and assessed and the information used to create a flow chart of the process, listing the risks and available control measures
- Identification of the critical control points. The specific points where risks can be controlled should be identified , e.g. brood stock testing
- Critical limits should be established. This can be guided by EU/NRL/OIE/PANDA to develop measurable controls, such as Legislation or disinfectant standards.
- Procedures should be monitored –SOPs should produced and compliance surveyed with quality assurance.
- Corrective actions should be identified. This requires production of contingency and eradication plans, along with the appropriate controls
- There should be an emphasis on accurate record keeping, that should ideally be audited to a standard
- Ensure compatibility with, e.g. quality assurance systems

Internal biosecurity barriers

Internal barriers can prevent the transfer of disease agents around a site or operation. For this it is necessary to

- Define sanitary units
- Isolate separate units
- Display notices
- Produce disinfection points at all entrance & exits
- Restrict site access to production areas
- Provide external car parking and biosecure essential vehicle access
- Implement hygiene procedures between units and within each unit
- Provide vehicle baths

As well as physical or operational barriers that can be used within a site, there are other measures that staff can undertake internally to minimise the chances of disease spread within a site.

- Fish and shellfish health should be regularly monitored.
- Moribund and dead fish should be removed promptly and be disposed of by an approved manner
- Morts should not be returned to production areas (e.g. shellfish beds, cages or tanks).
- If necessary, diseased stock should be culled to remove the source of infection, as well as a for welfare reasons.

Good Biosecurity Between Facilities

With much of aquaculture production now undertaken by large consolidated companies, which operate across a range of sites, both nationally and internationally, there is a heightened risk of disease transfer between different production sites. As well as the high risk of disease movement associated with transfer of diseased broodstock, fry or spat from hatcheries to ongrowing facilities, there is also the risk of transfer of disease by mechanical transfer associated with staff and equipment (e.g. well boats or transport lorries). Measures should be put in place to minimise the chances of transfer of pathogens between sites. This can be done by:

- Providing site-specific protective clothing to personnel.
- Using separate gear between facilities
- Provide cleaning & disinfection points between the different sites (Footbaths / Hand wash)
- Disinfecting all equipment before using it on a different site.

Risk Identification

There are a range of potential risks in fish farming operations including, the stock, fish farm vessels, ancillary equipment, pallets, nets, tanks, cages, moorings, nets, facilities, harvesting and grading equipment, plant effluent, personnel and waste disposal.

For equipment, nets and tanks should be air dried prior to being cleaned& disinfected. Care should be taken to keep harvesting areas, vessels and equipment stores clear of rubbish.

Disposal and Containment of Shellfish and Finfish Products

Mortalities and moribund fish and shellfish should be removed daily and disposed of in accordance with EU legislation. Such material is classed as Category 2 waste under Animal Bioproducts Regulation EC1774/2002. Approved disposal methods at the present time include:

- Rendering
- Incineration
- or landfill.

Disinfection

A disinfectant is an agent that destroys infection-producing organisms. They are usually applied to inanimate objects and are toxic & harmful to living tissue, particularly shellfish larvae. Disinfectants are vital tools for effective farm biosecurity, used to inactivate potentially pathogenic micro-organisms on surfaces of equipment, tanks and clothing, or suspended in effluent. They are also used in aquaculture to disinfect gametes, principally ova. Disinfectants are required for decontamination of facilities and premises after outbreaks of notifiable diseases, such as infectious salmon anaemia (ISA), spring viraemia of carp (SVC), or viral haemorrhagic septicaemia (VHS) (Office International des Epizooties, 2007)

FRS Aberdeen have published a disinfectant guide that details the recommended steps to cleaning and disinfection of aquaculture facilities and equipment, including information as to how disinfectants should be applied (Fraser et al., 2006). It should be emphasised that cleaning is the most important step in disinfectant (removing a high percentage of infectious agents), allied to choosing an appropriate disinfectant, effective against a broad spectrum of disease agents. It should also be emphasised that manufacture or official instructions into appropriate dilutions and contact time, method of application for particular disinfectants should be consulted before using a particular product. Health and safety is also very important, both to users as well as the environment to which the disinfectant may be discharged.

Table 5. Recommended disinfectants for use in aquaculture operations - Disinfectants, doses and applications

Disinfectant	Example*	Dose	Application	1 Comments
Sodium hypochlorite	Klorsept (Jencons Scientific, UK)	100 ppm, 10 min 1000 ppm, 10 min 1000 ppm, 6 hrs	Boats, cages, tanks, hand nets, harvest equipment Processing plant effluent Cage nets	Reported effective against ISA (Torgersen, 1998 and Smail *et al*., 2004) and IPN (Elliott & Amend, 1978) Ensure an active free chlorine level of at least 5 ppm after treatment.
Chloramine T	Halamid® (Axcentive, France)	1% (w/v), 5 min	Foot bath, non-porous surfaces	Reported effective against ISA (Smail *et al.,* 2004) (www.halamid.com)
Chlorine dioxide	Zydox AD-05 activated by DRA-2 (Zychem Technologies, Norway)	100 ppm, 5 min	Processing plant effluent	Effective against ISA (Smail *et al*., 2004)
Iodophor	Buffodine, FAM30 (Evans Vanodine, UK) or Tegodyne (DiverseyJohnson, UK)	100 ppm, 10 min	Foot bath, clothing, diving gear, hand nets, salmonid ova, non-porous surfaces	Reported effective against ISA (Smail et al., 2004) and IPN (Elliott & Amend, 1978) Fading colour from brown to yellow indicates inadequate concentration. Not suitable for nets treated with antifoulant.
Peroxy compounds	Virkon S (Antec international, UK)	1% (w/v), 10 min (IPN) 0.5% (w/v), 30 min (ISA)	Foot baths, non-porous surfaces	Reported effective against IPN, ERM and BKD. Reported effective against ISA and furunculosis (www.antecint.co.uk).
Peracetic acid, hydrogen peroxide and acetic acid mix	Proxitane® 5 (Solvay Interox, UK)	0.4% (v/v), 5 min	Non-porous surfaces	Reported effective against ISA (Smail *et al.,* 2004).
Quarternary ammonium compounds	Cetrimide (FeF Chemicals A/S, Denmark)	125 ppm, 5 min	Plastic surfaces	Reported effective against VHS & furunculosis (Dorson & Michel, 1987). Not effective against IPN at 12,500 ppm.
Formic Acid		pH < 4, 24 hours	Ensiling fish waste	Reported effective against ISA (Torgersen, 1998). Also, effective against BKD & furunculosis but not against IPN. (Smail *et al.,* 1993)
Ozone		8 mg/l/min, 3 min (Corresponding to redox potential 600-750 mV)	Water – intake and effluent	Reported effective against IPN, furunculosis, ERM and *Vibrio anguillarum* (Liltved *et al*., 1995). Filtration, pre-treatment is recommended.
Heat		70°C, 2 hours (IPN) 60°C, 2 minutes (ISA) 37°C, 4 days (Noda)	Cage nets, diving gear, steam cleaning non-porous surface	Reported effective against IPN (Whipple & Rohovec, 1994). Reported effective against ISA (Torgersen, 1998). Reported effective against nodavirus (Frerichs *et al.,* 2000). Heat treatment above 71°C may reduce nylon net breaking strain.
UV		122 mJ/cm^2/sec (IPN) 290 mJ/cm^2/sec (Noda)	Freshwater intake supply	Reported effective against IPN (Liltved *et al*.,1995). Reported effective against Nodavirus (Frerichs *et al*., 2000). Efficacy compromised by organic loading. May be combined with ozone for treating effluent from processing plants.

*Inclusion of brand names is for illustrative purposes only and does not imply endorsement by Fisheries Research Services. Other products may be equally efficacious. Routine monitoring and logging of treatments is highly recommended.

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6. Vaccination strategies

In every intensive culture system where a single species is reared at high population densities, infectious disease agents are able to transmit easily between host individuals and large economic losses can result from disease outbreaks. All vertebrates have innate and acquired defences against infectious agents and when environmental conditions are good for the host, these defence mechanisms will provide protection against most infections. Vertebrates can be distinguished from invertebrates in their ability to respond "immunologically" in a specific manner to a pathogen or vaccine. Fish possess a well functioning adaptive immune system involving antibodies, lymphocyte sub-populations, T-cell receptors, cytokines and major histocompatability complex (MHC) molecules. Crustacea lack the conventional immune system associated with mammals and fish they possess an effective non-specific immune system based on agglutinins, lysins, preciptins, clotting agents and phagocytosis by haemocytes. This system can be stimulated for a short period of time, but does not appear to have a memory component. Invertebrates, such as shellfish, only possess non specific defense mechanisms. In the strict definition, vaccines are used only as a prophylactic measure in vertebrates because a particular vaccine against a particular disease induces protection that is specific for that particular disease and does not protect against other diseases. Vaccination mimics the invasion of pathogens and primes the immune system for another encounter with the pathogen without causing disease. Vaccination does not necessarily eradicate the fish carriers. However, vaccines also have nonspecific immunostimulatory properties that can also activate many nonspecific defense mechanisms. These can increase disease resistance levels but only for a short period of time. Thus, in their capacity to induce such responses they are also used in shellfish culture, especially of shrimps, but strictly speaking they should be termed in these species "immunostimulants".

Vaccines continue to be a major growth area within the aquaculture sector, saving costs, reducing the need for other therapeutics, problems such as antibiotic resistance and concerns over residual levels or environmental impact. They are also helping to control some significant viral diseases. The first commercial vaccines for aquaculture were formalin-killed bacterial preparations for immersion against ERM, Vibriosis and Furunculosis and were licensed in the US in the 1970s and in Europe and Scandinavia during the mid-1980s. However the list of diseases that can currently be controlled through vaccination is steadily increasing. The potential of fish vaccines as a control strategy for disease has attracted a number of vaccine companies to the aquaculture market. Investment in vaccine development for aquaculture is increasing as the industry grows and new vaccine technologies become available. The major market is salmon, but trout is also significant, and there are expanding opportunities in marine fish such as sea bass, sea bream, turbot, halibut, yellow tail and cod).

Immunoprotection and vaccination strategies on fish farms

Protection induced by a vaccine is specific to the disease agent used to make the vaccine. The mechanism of protection is dependent upon the production of antibodies or cytotoxic cells which specifically recognize molecules characteristic of a pathogen (antigens) and which lead to destruction of the pathogen. The immune response depends upon a great deal of cell division and production of proteins. There are two main factors which decrease the rate of cell division and protein production; namely

stress and low temperature. Stress shuts down these aspects of metabolism in favour of providing carbohydrate energy sources which might be required for fight or flight reactions. Animals under stress have less energy available to combat infections and are therefore more prone to disease. While some farm facilities may be able to exclude the entry of pathogens, for example hatcheries with disinfected water supplies, it is impossible to exclude pathogens in an open marine situation. Under these conditions, stress reducing management practices are paramount in maintaining the health of cultured animals. Even then, because of the close proximity of individuals in a farm, if certain pathogens do gain entry they are able to spread and multiply extremely rapidly and such massive infectious burdens can overcome the defences of even healthy animals.

Low temperature reduces the rate of chemical reactions and thus slows cell metabolism but the optimal temperature for a given species of fish is related to its normal temperature range. Thus for Atlantic salmon the optimal temperature for protein production and cellular activity is about 12°C and is extremely slow at 4°C, while for sea bass the optimum temperature is about 22°C and at temperatures of about 10°C the immune system of such warm-water species is suppressed. Nevertheless, the rate of induction of an immune response is faster in warm-water species. At their optimum temperature, Atlantic salmon will take about 6 weeks to start producing antibodies and the response will reach a peak at about 10 weeks post-vaccination, while sea bass start producing antibodies within 1 week. Animals under stress have less energy available to combat infections and are therefore more prone to disease. While some farm facilities may be able to exclude the entry of pathogens, for example hatcheries with disinfected water supplies, it is impossible to exclude pathogens in an open marine situation. Under these conditions, stress reducing management practices are paramount in maintaining the health of cultured animals. Even then, because of the close proximity of individuals in a farm, if certain pathogens do gain entry they are able to spread and multiply extremely rapidly and such massive infectious burdens can overcome the defences of even healthy animals.

It is important to vaccinate only healthy fish and to avoid stress during the vaccination process, such as over crowding, extreme changes in temperature and poor water quality. Handling should be kept to a minimum, and some companies suggest vaccination while grading the fish. It is also advisable to treat parasitic infections before vaccination and take special precautions when handling previously infected fish or known carriers of the disease. Fish should be starved prior to vaccination and oxygen should be supplied throughout immersion vaccination.

A number of factors need to be considered when deciding on a vaccine strategy, such as the disease status of the farm, the species of fish cultured, and the size of the fish and the water temperature at the time of vaccination. Booster vaccination can also be given either by injection, or orally once the fish have been moved to sea water so as to protect them in their second year. Research has shown that fish size and water temperatures are two of the most significant factors in determining vaccine effectiveness. Immunity is only achieved in vaccinated salmonids once they are over 1g in weight. However, this immunity declines after about 3 months, whereas fish vaccinated at 4g retain immunity at a higher level for at least a year. Minimum size for vaccinating sea bass/sea bream by immersion is between 1g and 2g Temperature

has a significant effect on the rate at which the immune response against the vaccine develops; the lower the water temperature the progressively slower the response.

Vaccine Design

Many antigens expressed by pathogens induce a specific immune response in the vertebrate host but these responses do not necessarily confer protection. It is important therefore for a vaccine to induce immune responses to specific antigens, termed "protective antigens", which block the ability of a pathogen to invade or proliferate in the host or to destroy the pathogen following infection. Many bacterial pathogens have developed ways of protecting themselves against various components of the host response and information about these mechanisms can assist in identifying which antigens form part of the bacterial armour and which are the "Achilles heel". The latter are likely to be important in inducing protective immunity. Further information is then required on how to produce large quantities of such antigens in commercial fermenters so as to include immunogenic doses in a vaccine. As many environmental or nutrient conditions affect antigen expression by bacteria, special culture conditions/media may be required to achieve this.

Methods of Vaccination

There are three main methods of administering vaccines to fish, immersion in a dilute suspension of the vaccine, oral vaccination and injection into the body cavity. For practical reasons the latter method requires the fish to be over about 15 grams in weight.

Immersion vaccination
This is effective for some, but not all vaccines. The vaccine against the bacterial disease vibriosis is highly effective when administered by immersion. It is used widely in salmonid and sea bass farming and probably could be administered by this route to most marine fish species. The vaccine against Pseudo-tuberculosis (Pasteurellosis) can also be administered by immersion to sea bass. Immersion vaccines are not formulated with any adjuvant and comprise a whole bacterial culture inactivated by addition of formalin. The vaccine is usually diluted about 1:10 with water and small batches of fish held in a hand net are immersed for about 30 seconds. Each litre of undiluted vaccine is sufficient to immunise about 100kg of fish. The method is ideal for vaccinating large numbers of small fish. With the exception of the vaccine against Enteric Redmouth, which is delivered by immersion to fish in freshwater hatcheries, all the other vaccines must be delivered by injection in order to achieve effective protection. In practice immersion vaccines are easier to administer and cause slightly less stress than injectable vaccines; they are also suitable for use with smaller fish. A dip treatment commonly consists of a tank of diluted vaccine (eg 1:9) which is sufficient for a known number of fish. Fish are taken from the stock tanks using a dip net, which is then immersed in the vaccination tank for a period of 30-60 seconds. The dip net is then lifted and the vaccine drained back into the vaccination tank, before the fish are returned to another holding tank. The main drawbacks of this method are that the fish are still stressed and damaged through handling, and no adjuvant can be used.

Injection vaccination

This is the most effective route of vaccination and as it allows the incorporation of adjuvants they induce long-term protection. However, it is obviously stressful to the fish, very labour intensive and cannot be used for fish much below 15g. Fish must first be anaesthetised and then passed to an operator equipped with a repeater syringe or through a vaccination machine. Atlantic salmon are usually vaccinated several months before transfer to sea water so that the protective immunity has time to develop before the stress of transportation to sea and exposure to the pathogens encountered in the marine environment. While the incorporation of oil adjuvants into injectable vaccines prolongs the duration of protection and acts as an immunostimulant, they have also been associated with some side effects. These can result in the viscera adhering to the body wall of the fish making evisceration during the slaughter process difficult and time consuming.

Oral delivery of vaccines

While this is an ideal method of vaccinating fish logistically, unfortunately is not as effective yet as injection. Oral vaccination is attractive since it imposes no stress to the animal, and has no additional labour requirement. In the early 1970's both vibrio and ERM oral vaccines were shown to be fairly effective but they required much higher doses than the immersion method and so were discontinued. There is still much research being conducted on development of effective oral delivery of vaccines, spurred on by the fact that the hind part of the intestine of fish is able to take up antigens and if a vaccine is given by anal intubation a strong immune response is induced. However, it appears that antigens administered orally are partly digested in the foregut and methods of protecting them as they pass through this area and releasing them in the hindgut are still being researched. It is only recently that techniques have become available to protect the vaccine from degradation in the stomach of the fish before they reach the immune sensitive areas of the lower gut. Antigen protection vehicle (APV) in the form of a stable emulsion containing vaccine and fish oil and a special emulsifying agent has been developed by Schering Plough. The emulsion is then added to fish feed pellets either at the feed mill or on fish farms. This type of oral vaccine is cheap to produce, it protects the antigens in the stomach of the animal, is highly palatable to fish and can be applied to any vaccine. A new type of vehicle, (poly D,L-lactic-co-glycolic acid microparticles) has been used in mammalian oral vaccines and is currently being evaluated experimentally for use in fish. The basis of oral vaccination is to protect the vaccine components from destruction by the fish digestive tract so that the antigens are able to penetrate the intestinal lining and stimulate an immune response. PerOs Technologies, Inc, has developed its patented Oralject TM technology that prevents the degradation of the vaccine's components by digestive enzymatic function and decreases the gastric pH of the fish intestine. Currently, the ARS patented Streptococcus iniae vaccine was incorporated into Oralject TM and fed to tilapia. The S iniae Oralject ^{TM} vaccine was efficacious following challenge with live S iniae in the oral-immunised tilapia.

Vaccine Technology

The ideal fish vaccine is global in scope (i.e., composed of antigens common to all strains) and capable of stimulating specific, long-lasting, humoral and cellular immune responses that result in solid protection. There are 4 main technologies used to produce vaccines for fish: Culture of the pathogen and inactivation with formalin (killed vaccines); Attenuated vaccines, recombinant vaccines; and DNA vaccines.

Traditional fish vaccines have used killed preparations of bacteria, viruses, fungi, or parasites delivered by waterborne exposure or by injection with adjuvants and today, this strategy forms the basis for most of the fish vaccines that have become commercially successful. A new generation of killed vaccines is expected as better delivery systems and new adjuvants become available. A significant advantage of killed vaccines is their high degree of safety and ease of development. This has also made them relatively easy to license. In fact, the main requirements for autogenous vaccines (killed cultures of a pathogen isolated from animals at an individual farm and restricted to use on that farm) is that they be produced in a licensed facility and used under the supervision of a veterinarian. The disadvantages of killed vaccines are that they are sometimes only effective when delivered by injection in the presence of an adjuvant and that protection is often less solid or of shorter duration than that resulting from natural infections. In addition, killed viral vaccines that must be produced in cell culture are probably not cost-effective for small fish.

Killed vaccines : Bacteria.

Most of the commercial vaccines are against bacterial diseases because these are relatively cheap to produce. Obviously the cost per dose of vaccine for use in aquaculture must be very low and it is inexpensive to culture most bacteria in large fermenters and to inactivate the bacteria and their toxins chemically (usually with formalin). Most of the economically important bacterial diseases in salmonid culture have been brought under control by the use of vaccination over the past three decades. For Atlantic salmon these are vaccines against furunculosis, vibriosis, cold-water vibriosis and enteric redmouth (ERM). These vaccines are usually administered by injection into the body cavity three to six months before the fish are transferred to sea water. The vaccines are formulated as an emulsion in a mineral or non-mineral oil which is known as an adjuvant. This enhances the level and duration of the immune response and a single injection is sufficient to provide protection for the rest of the production cycle. Furthermore, the adjuvant slows the rate at which the bacterial components of the vaccine (the antigens) are degraded in the body cavity. This allows the vaccine to be administered during the cold months of winter when the water temperature is usually too low to allow growth of most pathogenic bacteria. However, the immune response of the fish is also temperature dependent and when the vaccine is administered at low temperatures in winter (about $4^{o}C$) the antigens will be degraded by the time the temperature rises in spring, unless they are protected by an oil-adjuvant. The use of some oil adjuvants have some safety and welfare issues, namely the occasional induction of visceral adhesions/granulomas which may have adverse effects on growth of the fish or carcass quality (Midtlyng, 1997). Killed vaccines against ERM and vibriosis are often administered by immersion to rainbow trout, particularly if they are grown on in brackish water where the fish may become infected with vibriosis. Immersion vibrio and pasteurella vaccines are used for sea bass and sea bream fry.

Streptococcus iniae and Streptococcus agalactiae are major pathogens that cause serious economic losses in tilapia and numerous species of freshwater, marine and estuarine fish worldwide. Efficacious S iniae and S agalactiae vaccines were developed using formalin-killed cells and concentrated extra-cellular products. Extracellular products of these Gram-positive streptococci are important immunogens

that confer protective immunity following immunisation. This is a notable advancement in the development of efficacious killed vaccines. Indeed, commercial vaccines are now on the market.

Killed vaccines: Viruses

It is much more expensive to culture viruses in tissue culture and this has been a major obstacle in commercialising vaccines against virus diseases of fish. Inactivated virus vaccines have given moderate to high protection when administered by injection, but they lack efficacy when administered to fry by immersion, thus reducing their usefulness. However, a number of killed vaccines against viral diseases of Atlantic salmon are now becoming available. While some progress has been made in producing IPN and ISA vaccines by optimising the culture conditions and development of new cell lines which increase virus yield, more promising ways of reducing the production cost of viral vaccines have utilised biomolecular techniques, namely recombinant and DNA vaccines.

Attenuated vaccines

Attenuated vaccines for fish have been developed by traditional methods of serial passage in culture, by using naturally occurring mutants and cross-reacting strains or by mutagenesis of wild-type organisms. The development of attenuated bacterial vaccines was a biotechnological breakthrough. Attenuated vaccines are made by changing virulent pathogens so they retain the ability to infect and cause the host to mount an effective immune response without causing mortality, adverse reactions or reverting to the virulent form. Attenuated vaccines can be successfully administered by bath immersion, a cost-effective method of mass immunisation of large numbers of fish. Equally important, attenuated vaccines can be successfully used to immunise fingerlings and fry as young as seven to 10 days after hatching. Booster vaccinations are often needed with killed vaccines to maintain immunological protection within the host. Attenuated vaccines have the advantage in that they have transient growth within the host animal and as a result, provide prolonged stimulation of the animal‘s immune response. Often one vaccination is sufficient with these types of vaccines, eliminating the need for booster vaccinations. This immunisation will last the life of their production cycle, as opposed to a shorter duration of about six months for a killed vaccine. They also tend to induce a cell-mediated response, which is better suited for providing protection against viral infections. The response against these types of vaccines tends to produce good antibody responses but are less effective at inducing cell-mediated immunity or enhancing the mucosal immune response. The major concern with attenuated vaccines is the possibility that they may revert back to their virulent form. This has prevented experimental attenuated vaccines from developing into commercial products. While effective in laboratory trials, acceptance has been slow due to concerns about the release of live organisms into the environment and the perceived potential of the vaccine strain to revert to virulence, to replicate in the fish in unwanted ways, or to infect non-target species. This means that the testing required for attenuated vaccines can be extensive, making development costs relatively high. In addition, without a genetic marker to distinguish the vaccine strain from natural isolates, diagnostic examinations may be compromised and the vaccine will be difficult to protect commercially. More recently, molecular approaches have been used to circumvent some of these concerns. Following initial

development, attenuated vaccines for fish can be produced inexpensively and delivered efficiently by waterborne exposure. Because the attenuated strain replicates in the fish, it will stimulate both humoral and cellular immunity that is often superior to that provided by killed vaccines. It has recently become possible to irreversibly attenuate the pathogen through genetic engineering, thus removing concerns about vaccine safety. Until the early 1990s, most salmonid vaccines were based on inactivated (whole cell) cultures of the pathogenic organism, usually inactivated with formalin. Some vaccines, both commercial and experimental, are now based on purified macromolecules derived from the pathogen. These may be in the form of inactivated exotoxins, capsular polysaccharides or recombinant surface antigens. More recent generations of vaccines are based on whole cells grown under near in vivo conditions and additional cell extracts, providing recognised antigens. Near in vivo conditions include iron-restriction and glucose-rich media to induce capsule formation.

Recombinant vaccines.

Molecular biology techniques have made it possible to transfer viral genes to bacteria and yeast, which are inexpensive to culture and produce large amounts of viral vaccine cheaply. These so-called recombinant protein vaccines produced in *E. coli* or yeast are now becoming available for IPN. However, attempts to use these techniques for some virus vaccines, namely VHS and IHN, failed to induce protective immunity because of glycosylation problems.

DNA vaccines

A recent development in fish vaccinology is DNA vaccination, whereby plasmid DNA encoding foreign antigenic proteins is injected directly into the muscle of the host. The vaccine gene is expressed by the host muscle cells to produce the vaccine antigen, which in turn stimulates the host immune system to provide protection against the pathogen. DNA vaccination induces both a humoral and cellular response to the foreign protein and generates a significant immunological memory. It is possible to clone genes encoding immunogenic proteins from pathogens, and insert them into bacteria, yeast or mammalian expression systems, where they are expressed in large quantities. The cells used to express the proteins are then disrupted in order to release the recombinant pathogen protein, which can be purified using conventional biochemical techniques. There is an increased use of recombinant DNA technology in vaccine development for aquaculture for a number of different reasons:

- Conventional vaccines simply do not work
- Pathogens are intracellular and antibody responses elicited against them are ineffective
- Inappropriate antigens are expressed by the pathogen (bacteria cultured in vitro not express the same antigens in vivo)
- Some antigens cause immunosuppression of other antigens
- Protective antigens are masked by other antigens, or pathogens are difficult to bulk culture, eg Renibacterium salmoninarum these kind of vaccination programmes economically feasible It is possible to insert the genes encoding major pathogen antigens into attenuated viruses or bacteria. The attenuated host then acts as a vector for expression of these genes. The use of attenuated fish pathogens such as Aeromonas salmonicida and Yersinia ruckeri as

expression systems for recombinant antigens have, so far, only been used experimentally.

Commercial vaccines against viral fish pathogens are not particularly well established. Culturing the causative agent under defined conditions in fish cell lines then deactivating it is not always feasible. An approach often used for viral vaccines is to select protective antigens and then genetically engineer a recombinant vaccine against them. It is likely that DNA vaccines will have a significant impact on the viral vaccine market in the future.

The first viral vaccine to be produced was in Norway, against the IPN virus, in 1995 by Intervet Norbio. This vaccine was a recombinant subunit vaccine directed against the major capsid protein of the virus (VP2 protein), produced in Escherichia coli and administered in an oil adjuvant. This was followed by another oil-adjuvant vaccine made from inactivated virus.

The development and commercialisation of viral vaccines has, however, been hampered by the lack of an reproducible experimental challenge model, making their level of efficacy difficult to assess. The protective epitopes appear to be located on the surface glycoprotein of the virus, and antibodies directed against this neutralise viral infectivity. Both live and recombinant protein approaches have therefore been pursued and have proved effective, although they have not as yet achieved widespread marketing authorisation. There may be concerns about the possible reversion of attenuated virus to a virulent state and the possible formation of vaccinated carriers (ie apparently healthy fish infected with a potential pathogen).

More recently, DNA vaccines have been developed and these are remarkably effective against VHS and IHN. These vaccines are most effective when administered by intra-muscular injection and provide non-specific viral protection within 1 week due to stimulation of an interferon response. Specific protection appears later as the specific immune response develops. If methods of delivering DNA vaccines to first feeding fry could be developed, these vaccines could be useful in protecting small fish, even before maturation of the specific immune response, early protection being provided by the interferon response. This constitutes the next challenge for researchers. DNA vaccination offers the potential of producing highly protective and cheap vaccines against other viral diseases and possibly parasite diseases. Trials with DNA vaccines are promising, particularly against viruses. A number of other DNA vaccines for use in aquaculture are in progress worldwide.. Intramuscular injection of DNA vaccines against the major viral diseases of salmon, such as infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) has resulted in protection in laboratory trials. Moreover, a commercial variant of the former was approved by the CFIA in Canada. However, there is a problem with the public‘s perception of DNA vaccination, as it confuses the vaccination process with that of creating genetically modified organisms

Vaccines against parasites.

One area where vaccines have not been successful in aquaculture has been against parasites, although immunity has been described and while there is potential for vaccination to work little success has been achieved to date . A vaccine against sea

lice (Lepeophtheirus salmonis) remains a high priority for the aquaculture industry and a number of experimental vaccines are currently being tested. PKD, the causative agent of which is Tetracapsula bryosalmonae, is a particularly economically damaging disease for the trout industry in Europe. MAbs have been developed to identify antigens present on the parasite. Passive immunisation studies have identified antigens that may be protective and research is now concentrating on developing a recombinant vaccine. The most successful vaccines developed so far have been against Ichthyophthirius multifiliis using inactivated and live attenuated parasites, and Cryptobia salmositica using live attenuated parasites. However, the potential of DNA and recombinant technologies to play a role in the development of vaccines against parasites is huge. Already there has been several studies that show, at least experimentally, that these technologies may lead to the development of effective vaccines against some fish parasites. Recently advances have been made in preparation of a killed vaccine against scuticociliate protozoan, histophagous ciliate, Philasterides dicentrarchi one of the most important pathogen in turbot farming (personal communication CETGA).

Adjuvants

Adjuvants are substances that, when mixed together with antigen in a vaccine preparation, enhance the immunogenicity of the antigen. The exact mechanism of their action is not well established, but they appear to act as a depot for the antigen, prolonging the release of antigen at the injection site and stimulating the animal‘s immunity over a long period of time. They also seem to enhance macrophage/lymphocyte interactions and non-specifically stimulate lymphocyte proliferation. The main effect of adjuvants appears to be on humoral immune lipopolysaccharide antigens, which are considered to be T-independent antigens. Many of the recognised adjuvants can only be used with injectable vaccines due to their physical nature. Unfortunately, some adjuvants are unacceptable for commercial application due to severe local tissue responses and other side effects which result from their use. One of the most widely used experimental adjuvants is Freund‘s Complete Adjuvant (FCA), which is a mixture of killed Mycobacterium tuberculosis in a mineral oil. Other injectable adjuvants include aluminium hydroxide gel mixed with saponin, and certain oily emulsions of confidential composition. Potassium aluminium sulphate has been used as immersion and oral adjuvants in Vibrio vaccines, whilst dimethyl sulphoxide (DMSO) has been used in immersion vaccines for *Yersinia ruckeri in rainbow trout. Other possible adjuvants with potential use in immersion or oral vaccines include muramyl dipeptide (MDP), Levamisole and Ete (a soluble extract of the marine tunicate Ecteinascidia turbinata).* Many salmonid vaccines use an oil-based adjuvant as these are more effective. However, a number of side effects are particularly associated with mineral oil adjuvants:

- Reduced carcass quality (pigmentation at the site of injection, tissue adhesion and peritonitis)
- Reduction in growth rate and fertility of broodstock
- Difficulty in administration due to high viscosity
- Can result in hydrocarbons being passed to human consumers
- Potentially hazardous to vaccinators if accidentally self-injected

Alternative non-mineral oil based adjuvants are being tried. Many water-based vaccines for salmonids contain either glucan or aluminium-based adjuvants, which are

only partially effective, although they have relatively few side effects. Some companies are now marketing non-mineral oil, adjuvant injectable vaccines, which appear to have fewer side effects or use an oil-in-water rather than water-in-oil formulation, which reduces vaccine viscosity and makes it easier to administer, with reduced pigmentation and adhesion problems. New approaches in the area of vaccine adjuvants are under development, for example using interleukin-1 (IL-1), a cytokine involved in the regulation and control of the immune response. It has been possible to extract the gene encoding mammalian IL-1 and express it using recombinant DNA technology. Recombinant IL-1 is a highly effective adjuvant for vaccines. Bacterial genomic DNA, and other prokaryotic and protozoan DNA, are known to stimulate the vertebrate immune system.

Future of Fish Vaccines

In the last decade, the techniques of molecular biology have been used to develop a novel set of vaccines for fish. While several of these new approaches have been effective in stimulating specific immunity against challenge in the laboratory, only a few have been tested in field trials, and more work is needed to develop better delivery systems or to overcome potential regulatory concerns. For fish, the most promising of these new approaches include subunit vaccines, recombinant vectors, and genetic immunization.

Subunit vaccines consist of only a portion of a pathogen that will stimulate protective immunity. This is typically a protein that bears a protective antigen or the immunogenic region of such a protein. While subunit vaccines can be created by purification of the native antigen directly from cultures of the pathogen, development of a subunit vaccine by recombinant DNA technology usually involves inserting all or part of the gene coding for the appropriate antigen into a bacterium, yeast, or virus that can produce large amounts of the protein in vitro. Because no infectious agent is present, subunit vaccines are regarded as having a high level of safety, and the cost of producing the vaccine can be quite low after initial research and development costs. Although relatively easy to produce and license, the subunit vaccines that have been tested in fish have generally been less effective than desired. Subunit vaccines can also be made by chemical synthesis of peptides that mimic the antigenic epitope. However, like other subunit vaccines, they are more effective when delivered by injection with an adjuvant and may require coupling to a carrier molecule. Another drawback is that creation of peptide vaccines requires a detailed knowledge of the epitope structure of the protective antigens of the pathogen.

Recombinant vectors make use of DNA technology to insert copies of the genes from protective antigens into a virus or bacterium that will be able to infect the host and replicate without causing disease. While replicating, high levels of the recombinant antigen are produced by the vector that will stimulate the host immune system. One concern is that the method may be somewhat difficult to license owing to the use of a replicating recombinant organism. However, the strategy promises to be highly effective, and several human and animal vaccines are being developed with this approach. A significant advantage of recombinant vectors is that they readily lend themselves to the creation of multivalent vaccines that could stimulate protection against several pathogens simultaneously. Also, because of the replication of the vector, other aspects of host immunity may be co-stimulated.

Genetic immunization by injection of plasmid DNA coding for pro-tective antigens represents an exciting new area of vaccine development. Demonstrated in laboratory trials to be effective for viral, bacterial, and parasitic diseases of higher animals, the major advantage of this approach is that cells transfected with the plasmid produce the antigen in its authentic form. To the immune system of the animal, these cells appear to be infected and a full immune response is produced resulting in strong, long-lasting protection. Currently, these preparations have to be delivered by injection or by "gene gun" making them either more labor intensive or somewhat less efficient than desired; better delivery methods are needed if this technology is to become widely used in fish. Finally, because this technology is new, the potential for licensing these vaccines is difficult to evaluate; however, most concerns are expected to ease as DNA vaccines are developed for human and animal diseases. While substantial progress has been made in the molecular biology of fish pathogens and in the construction of novel vaccines that are effective in the laboratory, much remains to be done. The high cost of development and licensing of these vaccines is a major problem, especially if the market for the vaccine is limited to a small geographic area or to a minor species in aquaculture. Other significant problems that need to be overcome are the lack of optimal adjuvants and mass delivery systems for killed and subunit vaccines and an incomplete understanding of critical elements of the fish immune system.

Although it is difficult to predict the future of fish vaccination, recent experience suggests that there will be a trend toward the use of attenuated vaccines, recombinant vectors, and genetic immunization. The genetic engineering of stable attenuations with essentially no likelihood of reversion, no antibiotic resistance genes, and simple genetic markers will provide attenuated vaccines with significantly improved safety and acceptance. The low cost and high efficiency of such attenuated vaccines make them attractive candidates for commercial development. Genetic immunization, likewise, appears highly promising, especially for complex, conformation-dependent epitopes that serve as single protective immunogens; however, improved delivery systems will be required before this method can gain widespread use. Work will also continue on the development of recombinant vectors that can provide mass immunization. Finally, there will be a trend toward use of multivalent vaccines in an effort to lower costs.

Marketing authorisation /Regulators of vaccines and their use

In order to ensure that vaccines are safe and efficacious, the marketing of vaccines is closely regulated. The aim of the regulations is to protect animal health and welfare, users of the products and the environment. Usually, government departments set out the requirements for the quality, safety and efficacy of vaccines and companies must meet these requirements before receiving authorisation to market the product. For EU countries, the European Pharmacopoeia includes guidance on the type of research and developmental studies which should be carried out to help establish that the vaccine will meet the minimum standards. These standards are detailed and exacting and the whole process takes time and great expense. For conventional killed vaccines, market authorisation can be considered by a national authority and if granted applies to other EU member states. However, if a vaccine is derived from a biotechnology process eg a recombinant, live genetically modified or DNA vaccine, application for marketing must be made through the centralised EU Committee for Veterinary Medicinal

Products which is part of the European Medicines Evaluation Agency (EMEA) (Lee, A.1997). The supply of vaccines to fish farmers is also regulated. It is usually pharmacies or agricultural merchants who are authorised to supply vaccines and records must be kept of the products supplied by them and to whom they are supplied. Fish vaccines are not usually classified as prescription only medicines. However, in the EU, the control methods for different diseases have different specifications and diseases exotic to the EU are classified into Category 1 diseases. Such diseases, like ISA must be controlled by eradication policies and vaccination is currently not allowed. However, contingency planning to allow some level of vaccination such as the vaccination of farms surrounding an outbreak (ring vaccination) is in progress (Anonymous, 2000).

Vaccine producers

The first company to produce a commercial vaccine for fish was Wildlife Vaccines Inc in Colorado, USA in 1976. This followed on the discovery that formalin-killed cultures of *Yersinia ruckeri* and *V.anguillarum* could effectively protect fish from ERM and vibriosis, respectively, by a simple immersion delivery. Initially, injection delivery of vaccines was considered to be impractical until the devastating losses from furunculosis in Atlantic salmon farms in Scotland and Norway during the late 1980's. Immersion vaccination against this disease was ineffective but an injectable vaccine was highly effective and soon the industry developed means of using this, with great success. By this time, as the salmon farming industry was growing at a fast rate and disease outbreaks were potentially economically disastrous, other small companies came into being, including AquaHealth in Canada, Aquaculture Vaccines Ltd, AVL (a successor of Wildlife Vaccines Inc) in the UK, and Apotech Laboratorium and Norbio in Norway. During the 1990's, aquaculture has experienced a worldwide growth and vaccination is seen to be integral to a sustainable industry. Also, with the advent of biotechnology and the potential of producing vaccines with a low cost but with high research and development investment, and the high cost of obtaining market authorisation, larger companies have become involved. Norbio was taken over by Intervet and recently Novartis bought AquaHealth and Shering-Plough acuired AVL and recently Intervet. With the backing of these pharmaceutical giants, the development and use of fish vaccines should be ensured for the long-term future.

Immunostimulants – Immunomodulators

Various vaccines and therapeutics have been successful in controlling many of the diseases. However, there are occasions when specific treatments may not be available or may be ineffective due to stress-induced immunosuppression or the immaturity of the animal. Routine fish husbandry is potentially very stressful, particularly during transportation, when fish are subjected to stressors such as handling, crowding, and changes in water quantity and water temperature. Carriers of disease may act as a reservoir for infection, and when subjected to stressful events, their immunosuppressive state allows the pathogen the chance to multiply and cause disease. As well as disease associated mortalities, immunosuppression can lead to decreased growth and condition. The recent use of immunostimulants by the aquaculture industry appears useful in combating stress-associated immunosuppression. Such substances are reported to boost the immune system of the animal in the short term, when applied either on their own or in vaccines as

adjuvants, and are apparently very affective at stimulating the non-specific defence mechanisms of the animal. Although many different immunostimulatory compounds have been investigated, a number are now commercially available, of which beta-1,3/1,6-glucan is the most well known of the immunostimulants currently used. Both soluble beta-glucans (eg scleroglucan, schizophyllan and lentinan) and microparticulated beta-glucans from yeast (M-Glucan,Macroguard) have been shown to function as immunostimulators in fish. They enhance both non-specific and specific immunity in the animal, reduce mortality due to opportunistic microorganisms, reduce the risk of disease by microbial pathogens, and enhance resistance to parasites and the efficacy of vaccines and antimicrobials. The exact mode of action of glucans remains unclear, but enhanced protection against microbial pathogens, observed after administering glucans to fish, correlates with increased blood lysozyme and complement since it has been shown that Atlantic salmon macrophages possess receptors for beta-glucan.

Booster feeds enriched with nucleic acids could increase resistance to infection, reduce the effects of physical stress, improve immunity from vaccination and improve feed conversion rates. Nucleotides are the units of nucleic acid DNA and RNA and are important constituents of enzymes and co-enzymes such as ATP, NAD, FAD etc. When endogenous supplies are insufficient for normal cell function, nucleotides become semi-essential nutrients, for example during periods of limited nutrient intake, during disease episodes, or during periods of rapid growth.

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7. Alternative treatments

In general there are no or, at best limited treatment options for the majority of the Panda listed diseases. There are even fewer so-called 'alternative treatments' then the other options already covered (chemotherapy and immunoprophylaxis). Good biosecurity (see earlier) remains the frontline defence against both the importation and spread of these diseases. There is an effort by a number of researchers worldwide to develop alternatives and these will be briefly reviewed, even though there are no alternative treatments that are presently authorised for the control of the diseases identified as particular risk to European aquaculture.

Immunostimulants

Immunostimulants are designed to boost the non-specific defences of the subject to which they have been administered (Anderson, 1992). Numerous naturally derived and artificially synthesised products have demonstrated a broad range of immunostimulatory activities and can be administered either alone, or combined with a vaccine to increase its efficacy. For instance Peddie and Secombes (2005) list more then 30 product types tested for aquaculture use; these included nutrients such a vitamin C, E and polyunsaturated acids as well as alginates and β –glucans. As well as control of bacterial diseases, immunostimulants are also being developed to help control viral infections, e.g. Jensen et al (2002) showed an increase in Mx protein in organs of treated salmon and some protection against ISAV following treatment with synthetic ds RNA (poly I:C). However very few of the products that have showed promise experimentally are available to the farmer for control of any diseases, with none, to date, authorised for the control of any of the diseases listed by PANDA WP2 and 3. Legislative considerations, product efficacy in the field and economic barriers are some of the common barriers to commercialisation of novel immunostimulants.

Pro and pre-biotics

There has been a wide range of research aimed at developing probiotics (that can be defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance"(Fuller 1989),for aquaculture this can also be extended to include addition of microorganisms that improve the environment external to the animal. Microorganisms used with reportedly beneficial effects include Gram negative and positive bacteria, yeasts and unicellular algae. In terms of Panda listed diseases, xx report that L. garvieae infections in rainbow trout can potentially be controlled by addition of xxx in feed. However despite this abundant literature there are, to date, no authorised probiotic treatments for control of any of the diseases listed by Panda WP2 and 3. Pre-biotics are a more recent concept, that can be defined as ' nutrients added to the feed that can selectively stimulate already present and established bacteria in the intestine , which are known to have a beneficial effect on the host; (Gibson and Roberfroid 1995).Probiotics are generally non-digestible dietary supplements, including a variety oligosccahrides, lactulose, lactinol and inulin. Current research mainly takes place in human medicine, with only limited reports of their use in aquaculture. There no authorised prebiotic treatments for control of any of the diseases listed by Panda WP2 and 3.

Bacteriophage therapy

Another alternative control strategy that has received limited attention for aquaculture use, so far, is phage therapy. With phage therapy, bacteriophages are administered to control the bacteria responsible for infections. Phage therapy is a long-standing concept first developed in 1918 by D'Herelle (Douglas, 1975). Subsequent work focused on the testing, with mixed success, of phage therapy against a range of medical conditions, such as anthrax, bronchitis, scarlet fever (Douglas, 1975). With increasing problems of antimicrobial resistance emerging (Anon, 2006), attention has returned to phage therapy, with a number of studies and reviews now describing their use for control of diseases in veterinary and human medicine (Barrow, 2001; Bradbury, 2004; Duckworth & and Gulig, 2002; Merril et al., 2003; Pirisi, 2000; Stone, 2002; Thiel, 2004). Bacteriophage have also been isolated and characterised for a number of fish and shellfish pathogens. Many of these studies (e.g. Rodgers, 1981) were mainly concerned with identifying bacteriophage for use in bacterial typing schemes, or characterisation of bacteriophage properties (Matsuzaki et al., 2000;Yuksel et al., 2001), including their potential role in virulence (Munro et al., 2003).

At present, there are also a few studies describing the use of phage therapy in aquaculture including the treatment of red-fin disease in loach (Wu et al., 1981) and milkfish vibriosis (Wu et al. 1984). Survival of yellowtail (*Seriola ialandi*) and ayu (*Plecoglossus altivelis*), experimentally infected with Lactococcus garvieae (identified by WP2 and WP3 as a disaease of significant threat to European aquaculture) and Pseudomonas plecoglossicida respectively, was improved by oral and i.p. administration of specific phage (Nakai et al., 1999; Park et al. 2000; Park and Nakai, 2003). Other workers have looked at the use of bacteriophage to control bacterial disease in invertebrates such as molluscs (e.g. Tai-wu, 2000). However, there no authorised bacteriophage treatments for control of any of the diseases listed by Panda WP2 and 3.

Non conventional methods of parasite control.

Non-conventional tools for the control of parasites rely on a thorough understanding of the biology and lifecycle of each parasite. Methods include biological and chemical control strategies.

Regulatory barriers

Many of these potential treatments pose a very limited risk to the environment, thus representing possible sustainable treatment strategies for the future. It would be helpful if the present regulatory barriers to their commercialisation were reviewed at a European level.

Conclusions

It is widely recognised that intensive farming of animals goes hand in hand with culture of their pathogens. Aquaculture has had severe problems from time to time as a consequence of infectious diseases. In Atlantic salmon farming, Norway was initially plagued with vibriosis diseases and Scotland and Norway suffered badly

from furunculosis in the late 1980's. These diseases were combated with large amounts of antibiotics which reached a peak in Norway in 1987 with over 50 tonnes of antibiotic used in an industry producing about 70 thousand tonnes of salmon. The widespread use of vaccines began in the early 1990's and by 1996 less than 1 tonne of antibiotics was used in the production of over 320 thousand tonnes of salmon (Markestad and Grave 1997).

There is little doubt that these bacterial diseases have been very successfully brought under control by vaccines. However, there are still many diseases for which vaccines are not available and the susceptibility of Pacific salmon to Bacterial Kidney Disease has markedly restricted the development of the culture of these fish species on the Pacific coast of North America.

As new industries grow, new diseases come to the foreground, for instance Piscirikettsia in Chilean salmon culture, Paramoebic gill disease in Tasmanian salmon culture and Spanish Turbot farming , Streptococcosis in Mediterranean sea bass and sea bream. Old diseases find new hosts, for example IPN, long known to affect salmon hatcheries, has in recent years caused high mortality in salmon post-smolts and has devastated several halibut hatcheries.

To combat these diseases and to ensure the sustainability of aquaculture great attention must be paid to sanitation and good husbandry (including nutrition). The treatment of disease by chemotherapy, which was performed widely in the 1970-80's, resulted in the induction of antibiotic resistant strains of bacteria and chemo-resistant lice. Furthermore, the growing concern for the environment and the consumer about the increasing usage of chemicals and antibiotics in aquaculture, led to increasing control and restrictions on their usage. This stimulated much research in the 1980-90's into development of more environmentally and consumer friendly methods of control such as vaccines and immunostimulants. These have achieved remarkable success and the pace of current research in this area using biotechnology to produce vaccines more cheaply, suggest that this approach will allow continued growth and sustainability of fish culture into the future.

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8. Antiparasitic Treatments

Protozoan infections

The most common substances used to treat protozoa and monogenean parasites in fish are shown in Table 1 . Most of these drugs concern freshwater fish and only a limited number of these drugs have been tested in Mediterranean fish mainly in experimental trials.(Athanassopoulou, Karagouni, Dotsika, Vatsos, Tavla, Christofilloyanis, Lytra and Dourala, 2003). Although some of these have been proved effective, the commercial use of drugs requiring a bath application is very limited to marine cages due to the labour involved. Therefore, drugs applied per os are preferred.

Marine protozoa and especially *Oodinium* and *Trichodina* spp. are usually treated with disinfectants. *Oodinium* is one of the most difficult parasites to treat and this treatment only concerns land-based systems (tanks). Cooper sulphate as constant drip is the only drug of choice. Trichodinids can be treated successfully with a combination of malachite green and formalin but, again, only in tanks (hatcheries). Formalin (saturated 37% aqueous solution of formaldehyde gas) is a widely used chemical for parasite treatments or disinfections in both freshwater farms and marine hatcheries. It is also used as anti- bacterial and anti-fungal agent and has become popular because its recent licensing as an approved therapeutic for farmed finfish in USA and Canada (Speare et al., 1997). Mebendazole and Levamisole have been used to treat monogeneans in marine fish at doses similar to those applied in freshwater fish with no great success. Levamisole in bath treatments was used also to treat amoebic gill disease (in Atlantic salmon (Zilberg et al., 2000). No successful treatment was noticed when levamisole was given in the food. Ivermectin is very effective against monogeneans and isopod/copepod infections (Athanassopoulou et al., 2002b). However, this drug has a lot of disadvantages as it produces residues in tissues and environment, it is very toxic in low temperature (Roth et al. 1993) and inhibits respiration in gills (Toovey et al., 1999).

Formalin

Formalin (= saturated 37% aqueous solution of formaldehyde gas) is a widely used chemical for parasite treatments or disinfections in both freshwater farms and marine hatcheries. It is also used as anti- bacterial and anti-fungal agent and has become popular because its recent licensing as an approved therapeutic for farmed finfish in USA and Canada (Speare et al.,1997). Intermittent use in salmon at prophylactic treatments is effective and safe at this species (Powell & Speare, 1996; Speare & McNair, 1996). The literature on the safety of formalin is conflicting (Rucker, 1962; Smith & Piper, 1972). The doses however, have never been tested in Mediterranean fish or even seawater and if not at appropriate level can cause necrosis and serious gill damage (Wedemeyer, 1971; Hammell, unpubl. data). The toxic effects of formalin is increasing with increasing temperatures (Alderman and Michael,1992). Formalin solutions will degrade with precipitation of parafolmadehyde, which is toxic to salmonids and must be removed prior to use. Formaldehyde is a potential carcinogen and proper safety precautions should be taken by staff administrating this agent. (Burka et al., 1997).

Hydrogen peroxide

Hydrogen peroxide has been used as bactericidal agent (Derksen, Ostland and Ferguson, 1999; Itou , Iida and Kawatsu, 1997) and as fungicidal for eggs (Burka et al, 1997), but, its main use has proved to be against sea lice in the treatment of chalimus and mobile stages . Treasurer and Grant (1997) found that under commercial farming conditions lice treated with hydrogen peroxide at 1500ppm for 20 minutes at 10°C did not reattach to salmon. Treated egg strings did not produce viable copepodids. (Mc Andrew, Sommerville and Bron, 1998). Hydrogen is a strong oxidizing agent and causes the parasite to separate fron its host possibly by generating lethal oxygen emboli inside the sea lice (Thomassen, 1993). Unfortunately, the chemical has a very narrow safety margin and becomes narrower with increasing temperature (Bruno and Raynard, 1994). Also, a tremendous volume of the hydrogen peroxide is required to treat sea pens which is costly. Hydrogen peroxide can damage the gills of fish in experimental treatments and some mortalities have been reported when farmed fish are treated. (Burka et al., 1997). For all these disadvantages, the chemical is not likely to be used in Mediterranean fish.

Chloramine T

Chloramine-T is an organic chlorocompound, which slowly breaks down to free chlorine. It is most commonly used as a antibacterial disinfectant and as a mucous stripping agent. It is also used for treatment of protozoa and for skin and gill flukes in a variety of fish species (Meinertz et al., 1999). Mixed infections can simultaneously treted with this chemical (Ostland et al., 1995). Treatment using low doses (2.5-10 ppm) up to one hour is normally without problems and not stressful in both freshwater and marine environment (Powell et al., 1994; Athanassopoulou, unpublished data).

Potassium permaganate ($KMnO_3$)

Potassium permanganate is very effective against cichlidogyriasis of tilapia fish (*Oreochromis hornorum*) (Flores-Crespo et al., 1995) and ectoparasitic ciliate (*Ambiphrya ameiuri*) channel catfish (*Ictalurus punctatus*) (Goncharenko et al., 1985).

Copper sulphate ($CuSO_4$)

It is used as a source of copper ion (Cu^{++}) for the treatment of marine fish diseases caused by the protozoan parasites *Amyloodinium (Oodinium*) *ocellatum* and *Cryptocaryon irritans* (Cardeilhac et al., 1988). It is also used for controlling fresh water fish diseases caused by *Costia*, *Chilodonella*, *Epistylus* and *Trichodina* (Plumb, 1991) and Ichthyophthiriasis (Schlenk, Gollon and Griffin, 1998). Total suspended solids are normaly correlated to the efficacy of the chemical and is normally toxic to fish especially in soft water (Schlenk et al., 1998). Its use therefore in marine farms is very limited.

Iodophores

Iodine is an effective disinfectant for most egg pathogens and has been widely adopted by the aquaculture industry (Evelyn et al., 1986). Iodophor treatment may prevent *Loma salmonae* infections of the endothelial cells of Chinook salmon (*O. tshawytscha*, Walbaum) by reduction of viability of spores. Some spores survive even when used high concentrations of iodophor (Shaw et al., 1999). Iodophor toxicity varies with species, parental stock, pH, egg condition and state of development (Alderman, 1984; Fowler and Banks, 1990).

Malachite green

Malachite green is used as an ectoparasiticide and external fungicide on fish and fish eggs (Culp and Beland, 1996) in freshwater. The dye is seems to act as an irreversible respiratory enzyme poison (Alderman, 1985). The tissue levels of malachite green especially when co-administrated with formalin enters and accumulates in exposed fish to levels greater than in the initial exposure concentration (Clifton-Hadley and Alderman, 1987). It is effective against proliferative kidney disease (PKD) of rainbow trout, *Salmo gairdneri* Richardson, in established clinical or sub-clinical infection caused by the ciliate *Ichthyophthirius multifiliis* and exposed to malachite green under laboratory conditions (Clifton-Hadley and Alderman, 1987). Because of its long term persistence in tissues (Alderman and Clifton Hadley (1993) and its possible teratogenic and carcinogenic effects, it is now banned (Meyer and Jorgenson, 1983).

Monogenean infections

Benzimidazoles

Benzimidazoles act either by disruption of energy metabolism in helminths (inhibition of fumarate reductase), or disruption of polymerization of tubulin in cellular microtubules (Manger, 1991). Albendazole, mebendazole and fenbendazole have significant deleterious effects on uni- and multinucleate merots, sporogonial plasmodia, sporoblasts, and later sporogonic stages of microsporidian species *Glugea anomala*, Moniez, 1887 (Schmahl and Benini, 1998). Fenbendazole and triclabendazole were effective without signs of toxicity against *Gyrodactylus* sp. infecting rainbow trout. In contrast, oxibendazole and albendazole were effective but were toxic as well. Other benbimidazoles, such as thiabendazole, oxfendazole and flubendazole were totally ineffective (Tojo et al., 1992). Mebendazole and levamisole have been used to treat monogeneans in marine fish at doses similar to those applied in freshwater fish with no great success. Levamisole in bath treatments was used also to treat amoebic gill disease (in Atlantic salmon (Zilberg et al., 2000). No successful treatment was noticed when levamisole was given in the food.

Levamisole

Levamisole has been demonstrated, in some fish species, to be good as enhancer of non-specific or specific immune responses when given alone or with a vaccine (acting as an adjuvant), respectively (Anderson, 1992). *In vitro* treatment of fish leukocytes with levamisole enhanced phagocytic cell activities (chemotactic activity, phagocytosis, respiratory burst and myeloperoxidase activity) or natural cytotoxic activity in carp (Siwicki, 1987, 1989; Baba et al., 1993), rainbow trout (Kajita et al., 1990), coho salmon (Olivier et

al., 1985) and gilthead seabream (Meseguer et al., 1997a, Mulero et al., 1998a, 1998b; Cuesta et al., 2002). Levamisole acts on nicotinic receptors of *Ascaris suum* and other large nematodes (Martin, 1993). Levamisole is found to be effective in a freshwater bath against the nematode *Anguillicola crassus*, pathogenic in eels under *in vivo* conditions (Tarachewski et al., 1988). Levamisole was effective against monogenean *Gyrodactylus aculeate* parasitizing the skin of sticklebacks (*Gasterosteus aculeatus*) causing damage of the parasite tegument and *Diplozoon paradoxum* parasitizing the gills of chubs (*Squalius cephalus*) and breams (*Abramis brama*) causing severe damage along the midbody (Schmahl and Taraschewski, 1987).

Niclosamide

Niclosamide is a chlorinated salicylamide that has been for many years the drug of choice for the treatment of most human and animal tapeworm and trematode infections. It interferes with the energy metabolism of helminths, possibly by inhibiting adenosine triphosphate (ATP) production as well as by uncoupling oxidative phosphorylation in the mitochondria of the parasite during electron transport from NADH to flavoprotein (James and Gilles, 1985). Niclosamide is found to be effective against the histophagous ciliate *Philasterides dicentrarchi* that causes fatal scuticociliatosis in farmed turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*) (Iglesias et al., 2002). Niclosamide was also effective against monogenean *Gyrodactylus aculeate* parasitizing the skin of sticklebacks (*Gasterosteus aculeatus*) and *Diplozoon paradoxum* parasitizing the gills of chubs (*Squalius cephalus*) and breams (*Abramis brama*) causing damage of the parasite tegument and severely affected along the midbody, respectively (Schmahl and Taraschewski, 1987).

Bithionol

Bithionol is halogenated diphenylsulfide and it was the drug of choice for the treatment of fascioliasis and paragonimiasis until praziquantel became available. Its mechanism of action is related to an inteference in ATP production in parasites. The succinic dehydrogenase system of liver flukes is very vulnerable and it is selectively inhibited by bithionol and niclosamide. Bithionol has an uncoupling effect on oxidative phosphorylation thus prohibiting the formation of ATP in parasites (James and Gilles, 1985). Bithionol sulfoxide is found to be effective against the histophagous ciliate *Philasterides dicentrarchi* that causes fatal scuticociliatosis in farmed turbot (*Scophthalmus maximus*) and sea bass (*Dicentrarchus labrax*) (Iglesias et al., 2002).

Isopoda and Copepoda

The most common substances used to treat these parasites in marine fish are shown in Table 6. The most frequently used treatments against salmon lice *Lepeophthieirus salmonis on* commercial farms are various bath treatments including hydrogen peroxide , dichlovos and cypermethrin *(Burka et al., 1997,* Pike & Wadsworth, 1999; Toovey & Lyndon, 2000). All of these treatments are effective in removing of adult or pre-adult stages, but, only cypermethrin has been shown to reduce larval stages. Recently hydrogen peroxide has also been found to affect eggs (Mc Andrew et al., 2000). Among the systemic treatments for lice Ektoban (teflubenzuron) and Slice (Emamectin benzoate) have shown good results (Stone et al;, 2000;Amstrong et al., 2002; Ramstad et al, 2002). However, all these drugs are tested against the copepod *Lepeophthieirus salmonis*. (salmon sea lice*).* Mediterranean fish are mainly infected by the isopod *C. oestroides* and *Caligus* sp., but, it is mainly the isopod that causes

problems and for which treatments are needed. Therefore, data related to the efficacy of the above drugs cannot be used in the case of the isopod parasite. In vitro treatments with deltamethrin against *C. oestroides* has been proved effective (Athanassopoulou et al., 2001a), but, preliminary trials with emamectin has been disappointing (Athanassopoulou, upubl. data). Experimental treatment of sea bass with Diflubenzuron also showed good results (Athanassopoulou, unpubl. data)

Organophosphates

Organophosphates inhibit many enzymes, especially acetylcholinesterase by phosphorylating its esterification site. They cause a blockade of cholinergic nerve transmission in the parasite, resulting in spastic paralysis.Organophosphates are used as to treat crustacean parasites as sea lice, *Ceratothoa gaudichaudii* in salmon and *Argulus* sp. In freshwater salmonids as well as gill and skin flukes although the use of these compounds as anti-trematodes has decreased because of the development of resistance (Burka et al., 1997). Organophosphates (dichlorfos, trichlorphon) have been used as standard treatments for mobile stages of sea lice (*Lepeophtheirus* sp and *Caligus* sp) by the immersion bath method in salmon. Their therapeutic index is low and affect the cholinesterase both the host and parasite alike as well as other organisms in the aquatic environment. They represent a safety risk for chemical handlers as they administer treatments and parasite resistance is well documented. (Roth et al., 1993 ; Burka et al., 1997). Azamethiphos, a newer organophosphate, has recently used in Atlantic salmon because is shown to be more effective against chalimus, pre-adult and adult stage of the sea lice (O'Halloran et al., 1996). The clinical application is 0.1ppm for 30-60 min but this is very difficult to achieve in cages. Effects of azamethiphos on the environment have not been published (Roth et al., 1993).

Pyrethrins & Pyrethroids

The main mechanism of action of the synthetic pyrethroids, such as deltamethrin and cypermethrin, involves the slowing down of the sodium channels of the nerve cells (Blagburn and Lindsay, 1995) and interaction with GABA receptors of the flukes. They have a broader spectrum of activity than the organophospate compounds since they also decrease the numbers of the chalimus in addition to the pre adult and adult stages of sea lice. Pyrethroids are synthetic analogues of pyrethrins with similar pharmacological properties. Pyrethroids can also inhibit brain AChE in both juvenile and adult fish (Reddy et al., 1991). Pyrethrins and pyrethroids are very safe to use for arthropod infections in mammals, but, in fish the safety margin is considerably reduced. And fish toxicity has been reported (Roth et al., 1993). Deltamethrin under laboratory conditions has high potential toxicity to fish. It has been reported that deltamethrin influences the ontogenesis of fish causing changes in hatching rate, abnormalities in development, and a decrease in body length (Roth et al., 1993).

Chitin synthesis inhibitors

Chitin synthesis inhibitors, lufenzuron, diflubenzuron and triflubenzuron, are effective against larval stages of sea lice and less effective against adult sea lice. Teflubenzuron was used for treatment of farmed Atlantic salmon *Salmo salar* L. infected with sea lice *Lepeoptheirus salmonis,* Kroyer, 1838. Maximum efficacy was observed toward chalimus and pre adult stages of *L. salmonis* at approximately 26 d post-medication without no adverse drug reactions or palatability problems (Ritchie et al., 2002). Because of binding to marine

sediment and remain in the environment for prolonged periods of time are currently a limited, temporary approval in Norway. Diflubenzuron showed good results against the isopod *C. oestroides* of sea bass (Athanassopoulou, unpubl. data) and it is a promising drug because it is administered in feed.

Avermectins & Related drugs

Avermectins have been used in aquaculture mainly to control sea lice infestation. Ivermectin is used extensively in livestock and companion animals and is safe drug to use in mammals, but, in fish the blood brain barrier is not as impervious as it is in mammals and CNS depression and deaths have been reported in salmon at therapeutic doses (Burka et al., 1997). It has also a prolonged residence time and the drug is not metabolized (Hoey et al., 1992). Sea lice invluding chalimus stages can be killed by ivermectin but the therapeutic index is rather narrow (Johnson and Margolis, 1993). In contrast to ivermectin, emamectin benzoate is recently registered for the use in European countries and proved to be very effective in salmon against sea lice (Stone et al., 1999, 2000a, 2000b, Armstrong et al., 2000, Ramstad et al., 2002). It is found to interfere with GABA receptor of nervous system leading to flaccid paralysis. Ivermectin is very also effective against monogeneans and isopod/copepod infections (Buckmann & Bjerregaard, 1990;Athanassopoulou et al., 2002). However, this drug has a lot of disadvantages as it produces residues in tissues and environment, it is very toxic in low temperature (Roth et al.,1993) and inhibits respiration in gills (Toovey et al., 1999).

Treatments for endoparasites

Anticoccidial drugs

Anticoccidials are generally given to poultry in the feed to prevent acute disease and the economic loss often associated with subacute infection. Prophylactic use is preferred because most of the dosage occurs before signs become apparent, and delayed treatment may not benefit the entire flock. There are a lot of drugs developed including amprolium, clopidol, halofuginone, ionophores, nicarbazin, tetracyclins and sulphonamides (Croft, 1997) but only a few are used in fish for infections of parasites producing similar spores (ie. Myxosporea and Microspores) and these are described below:

Fumagillin

Fumagillin, an antibiotic isolated by *Aspergillus fumigatus*, was used primarily for treating *Nosema apis* (Microsporea) infections in honey bees *Apis mellifera* (Ketznelson and Jamieson, 1952) and for treating patients with amebiasis (Killough et al., 1952). The molecular mechanism of fumagillin action on microsporidial replication is poorly understood. In *in vitro* ultrastructural studies, *Encephalozoon cuniculi* organisms treated with fumagillin were irregularly shaped. Proliferation stage organisms were typically swollen and contained irregularly shaped cytoplasmic vesicles (Shadduck, 1980). In microsporidian *Octosporea muscaedomesticae*, fumagillin treatment caused a decrease in total RNA, suggesting that fumagillin inhibited RNA synthesis (Jaronski, 1972). In fish, fumagillin was effective against the microsporean *Pleistophora anguillarum* in eels *Anguilla japonica* (Kano et al., 1982) and against *Myxidium giardii* infections by blockade the development and prevention the formation of new spores in European eels *Anguilla anguilla* (Székely et al., 1988). It is used to control *Enterocytozoon salmonis* (Hedrick et al., 1991) and *Loma salmonae* (Kent and Dawe, 1994, Speare et al, 1999a) infection in chinook salmon (*Oncorhynchus tshawytsha*). It is effective against haemorrhagic thelohanellosis caused by *Thelohanellus hovorkai* and *Sphaerospora renicola* in common carp (Cuprinus carpio, L.) when administrated during the infective period (Yokoyama et al., 1999, Yokohama et al., 1990, Molnár et al., 1987), early intracellular trophozoites and more developed plasmodia of *Hoferellus carassi* (Yokoyama et al., 1990), *Myxobolus cerebralis* in rainbow trout (El-Matbouli and Hoffman, 1991) and against the myxosporean *Sphaerospora testicularis* in sea bass (Sitja-Bobadilla and Alvarez-Pellitero, 1992). TNP-470 is a semi-synthetic analogue of fumagillin, that acts possibly as fumagillin by inhibition of RNA synthesis (Jaronski, 1972) as well as by affecting host cell function or growth by reduction of levels of mRNA encoding for cyclin D1, which plays a role in regulating cell division at the mid-G1 phase, in human umbelical endothelial cells (Hori et al., 1994). TNP-470 is effective *in vitro* against the Microsporidia: *Encephalitozoon intestinalis* and *Vittaforma corneae* (Didier, 1997). Fumagillin is known to reduce growth in rainbow trout and sea bass (*Dicentrarchus labrax*) when feeding 1% fumagillin for one month accompanied by mortality and decrease in haematopoietic tissues (Wishkovsky et al., 1990; Sitja-Bobadilla and Alvarez-Pellitero, 1992) and depletion of the renal interstitium in chinook salmon (Hedrick et al., 1988).

Toltrazuril

Toltrazuril displays both anthelminthic and antiprotozoal activity. It is a symmetric triazine derivative, is capable of entering the host cell as well as the intracellular or tissue-inhibiting parasitic stages in chicken-parasitizing coccidia *Eimeria* sp. (Mehlhorn et al., 1984).

Incubation of *Glugea anomala*, which parasitizing the connective tissue of sticklebacks (*Gasterosteus aculeatus*), led to complete destruction of multinucleate meronts and to fragmentation of sporogonial plasmodia suggesting an inhibitory effect of the drug on nuclear division (Schmahl et al., 1990) possibly *via* inhibition of enzymes involved in pyrimidine synthesis and nuclear division (Harder and Haberkorn, 1989).It is active against pre-spore stages of *Myxobolus* sp. in the gills of bream (*Abramis brama*) as well as against developmental stages of *Henneguya* sp. parasitic in the gills of the tapir fish (*Gnathonemus petersi*) (Schmahl et al., 1989b, 1991). It is also effective against parasitic ciliates such as *Ichthyophthirius multifillis*, *Trichodina* spp. and *Apiosoma* spp. (Mehlhorn et al., 1988, Schmahl et al., 1989a, From et al., 1992) and fish-infecting members of the Coccidia, Microsporidia and Myxozoa (Mehlhorn et al., 1988). It has been suggested that in Mediterranean fish, it is effective for treatment of Myxosporean infections including *Myxidium leei* (Lytra, pers. comm.). It is also known to be effective against the monogenean *Gyrodactylus aculeatus* (Schmahl et al., 1988). Similar results in fish and crustacean parasites has been shown with the asymmetric triazinone derivative HOE 092 V when dissolved in medicinal baths against the gill-parasitic *Henneguya* sp. in tapir fish (*Gnathonenus petersii*) and against the skin-parasitic *H. laterocapsulata* in hybrids in clariid catfish (*Clarias gariepinus X Heterobranchus bidorsalis*) (Schmahl et al., 1992, 1993).

Amprolium

Amprolium is a structural analogue of thiamine (vitamin B1) causing a competitive inhibition to thiamine utilization by the parasite. It acts upon the first generation in the cells of the intestinal wall, preventing differentiation of the metrozoites. It may also suppress the sexual stages and sporulation of the oocysts. Amprolium has been proved ineffective against *Hexamita salmonis*, *Gyrodqctylus* sp. and *Ichthyobodo necator* in rainbow trout (Tojo and Santamaria, 1998a, 1998b, 1998c).

Quinine

Quinine acts on skin-inhabiting trophozoite stages of *Ichthyophthirius multifiliis* in ornamental fish causing severe alterations in the parasite's structure, mainly consisting of an enlargement of the alveolar sacs, a partial destruction of the nephroplasm, and a sloughing off of the membranes bordering the contractile vacuoles (Schmahl et al., 1996). It is also shown to be effective against the plasmodial developmental stages of *Henneguya* sp. in tapir fish (Zegula, 1997). Quinine administrated orally led to malformation of the presporogonic and pansporoblastic stages of *Henneguya* sp. parasitizing the gills of tapir fish (Dohle et al., 2002).

Metronidazole

Metronidazole is a nitroimidazole primarily used in the treatment of infections caused by anaerobic bacteria and protozoa such as *Trichomonas vaginitis* and *Giardia lambilia* (Lau et al., 1992, Tally and Sallivan, 1981). Metronidazole has a low molecular weight, resulting in easy penetration of the cell membrane of both aerobe and anaerobe microorganisms. Inside the cell metronidazole reduced, *via* a ferri-doxin system that exist in anaerobic microorganisms, to reactive intermediates that damage the DNA of the microorganisms (Tally and Sullivan, 1981). Its effect on fish parasites is not proven. Experimental use against *Loma salmonae* did not show good results (Speare et al., 1999a).

Salinomycin

Salinomycin is an antibiotic belonging to ionophorous polyethers and acts as a chelator with monovalent cations, especially for potassium, disturbing the intracellular concentration balance for monovalent ions (Kinashi et al., 1973). It has a strong activity against many microorganisms including coccidia. It causes in *Eimeria* sp. shrinking of the pellicula, vacuolisation of the cytoplasm and destruction of mitochondria (Raether et al., 1991). Salinomycin administrated orally causes deleterious effects on the trophozoite cytoplasm and on the presporogonic and pansporoblastic stages of *Henneguya* sp. parasitizing the gills of tapir fish. It was also observed a severe shrinking of the plasmodia and an enlargement of the sutural ridges in the pansporoplasts and malformation of the polar capsules (Dohle et al., 2002).

Benzimidazoles

In monosporidia, benzimidazoles cause a decrease in the number of ribosomes, gross enlargement of the SER, vacuolization of the cytoplasm and complete disintegration of the nuclear structures (Schmahl and Benini, 1998). Albendazole acts on *Encephalitozoon cuniculi* by inhibition of the formation of the intranuclear spindle (Colbourn et al., 1994) and on *Nosema bombysis*, a silkworm parasite, by clumbing of chromatin in the nuclei, inhibition of spindle formation and spore malformation (Haque et al., 1993). Experimental use against *Loma salmonae* showed good results (Speare et al., 1999a).

Sulfonamides

Sulphonamides have been used as chemotherapeutic agents against bacterial diseases in mammals and fish for decades. (Alderman, 1988). They compete with para-aminobenzoic acid (PABA) in the biosynthesis of tetrahydrofolic acid in the pathway to form folic acid. Some drugs of this group can have anticoccidial action especially when combined with other compounds such as Toltrazuril (Laczay, Voros and Semjen, 1995; Haberkorn,1996).

Oregano Oils

In oregano (*Oregano vulgare*) essential oil the most abundant compounds were also γ-terpinene, *p*-cymene, carvacrol and thymol (Daferera et al., 2000). These components are found to have inhibitory effects on microorganisms (Athanassopoulou,, Kotou, Watsos & Giagnisi (2000) especially in spore forming organisms (Sivropoulou et al., 1996; Mejiholm & Dalgaard, 2002).

Recent Research, Developments and constrains in Antiarasitic Treatments

The main parasitic diseases of Mediterranean fish causing problems (mortalities, delay in growth rates and / or loss of fecundity) and requiring treatments are the Myxosporeans Myxidium. leei, Polysporoplasma sparis and Ceratomyxa sp. and Isopods and Copepods. In some areas there are also heavy mortalities due to Monogenea infections. Some preventive methods are currently available for Isopoda infections but, there is an urgent need for research in anthelminthic treatments as well as background information on life cycles and early diagnostic procedures, as, in many cases, this is non- existent for Mediterranean parasites.

In view of the EEC policies concerning reduction of chemical use in the water environment, the strategic and effective use of chemotherapeutants becomes essential. As in the case of salmonid aquaculture, the combination of the correct treatments with other health management and disease prevention strategies, such as vaccinations, water quality improvement, production of tolerant fish, alternative treatments this will help ensuring the successful development of the sector in the future.

In recent years, a few major research projects have been undertaken in Greece, funded by the Greek Ministry of Research and Technology concerning the efficacy of different antiparasitic drugs in Mediterranean fish. The results of these experiments are under publication and are discussed briefly below. In addition, a joint EEC project involving most of Mediterranean countries dealing with early diagnosis, epidemiology and prevention methods for the pathogen Myxidium leei in P. puntazzo and S. aurata has just started.

Research on Monogenea

There is limited funded work done in recent years concerning the treatment of Monogenea in Mediterranean cultured fish. Preliminary experimental treatments with ivermectin at levels used for sea lice in salmon in sea bass has been promising (Athanassopoulou, unpubl. data).

Research on Isopoda/Copepoda

Due to local funding a little more information exists on drugs used for these infections.

Ivermectin

The toxicity and histopathology of ivermectin was studied in 3 and 35 g healthy sea bass, *Dicentrarchus labrax* L., following in-feed, oral intubation and injection administration at dose rates ranging from 0.5 to 3.5 mg/kg. Estimated LD50 values for 3 g fish were 0.335 and 0.106 mg/kg following oral intubation and injection administration respectively for fish reared at 11^{o}C and 0.839 and 1.023 mg/kg following oral intubation and injection administration respectively for fish reared at 20^{o}C. For 35 g fish reared at 11^{o}C, the estimated LD50 was 0.523 and 0.361 mg/kg following oral intubation and injection administration respectively. No signs of toxicity were observed when the compound was administered via the feed at 0.5 and 0.7 mg/kg. However, toxicity (> 10%) was observed at dose rates of 0.2 mg/kg and

higher when the compound was administered via oral intubation and at 0.5 mg/kg when administered via injection. The compound was found to be significantly more toxic to fish reared at 11°C than 20°C. Further, ivermectin was found to be more toxic to 3 g than 35 g sea bass when administered via injection. Histopathological examination of the major organs revealed pathology was largely restricted to gills and intestinal tissue. In 3 g sea bass, lesions were also found in the kidneys. (Athanassopoulou et al., 2002). The therapeutic effect of the drug was very satisfactory in copepod (*Lernathropus kroyeri)* infections of sea bass (Athanassopoulou et al., 2001). However, due to the problems associated with this drug, research is required to find other alternative treatments that will be both economical and easy to administer. Emamectin benzoate could be an alternative drug if adequate research is undertaken to assess toxicity and dose ranges and tissue residue levels in Mediterranean fish.

Deltamethrin

Experimental treatments of 30 min duration were undertaken in both sea lice (*Ceratothoa oestroides*) *in vitro* and in infected with *C. oestroides* sea bass of average weight 5.73 and 20.06g, kept in experimental tanks at temperature 20^0C. In both experiments different concentrations were used and evaluated. For the sea bass tests the following concentrations of the drug were tested: 10μg/L, 5μg/L, 3μg/L, 0.15μg/L, 0.1μg/L, 0.05μg/L. The results were assessed in one hour and 24 hours and 48 hours. The best results were achieved at the dose **of 10μg/L (0.01mg/L)** where the prevalence was reduced from 100% to 0% over 24 hrs in both large and small fish. The parasites were dead also at 48 hours. The dose of 5μg/L reduced the prevalence from 100% to 11.74% and from 85.7% too 0% for large and small fish respectively. Finally, the dose of 3μg/L the results were respectively from 88.2% to 37.5% (large fish) and from 87.5 to 13.3% (small fish). Smaller doses did not have any effect at 24 or 48 hours on the parasites.

Diflubenzuron

Experimental treatments were undertaken in infected with *C. oestroides* sea bass of average weight 5.73 and 20.06g, kept in experimental tanks at temperature 20^0C. The results were very promising and the toxicity of the drug very limited (Athanassopoulou et al., unpublished data).

Research on Myxosporea/ Microsporea

Extensive research was undertaken in years 1997-2001 concerning the immunology and treatment of different myxosporeans of cultured *Sparus aurata*, D. *puntazzo* and sea bass and *Sargu*s sp.

The purpose of the first study was to test experimentally different drugs and therapeutic schemes in order to find as efficient commercial treatment for fish infected with myxosporeans. Two series of land based experiments and one experimental cage trial were performed for this purpose. In the first land- based experiment, 10g and 30g *Sparus aurata* naturally infected in the kidneys with *Polysporoplasma sparis* were used. Initially, six different doses of Fumagillin, two doses of Toltrazuril, one dose of Amprolium, ESB_3 and Salinomycin were tested. In

the second land based experiment 25g and 50g fish infected with the same parasite were treated with Origanum essential oils, Toltrazuril with propylene glycol, Amprolium, and a combination of Salinomycin 12%+Amprolium(SA). In the field trials, 15 and 155g *Sparus aurata* infected with the same parasite were treated with SA, Origanum essential oils and Fumagillin. In all trials the drugs were incorporated in food and administered according to the selected schemes, while their efficacy was evaluated in terms of mortality (acceptable level <2%), pathology and prevalence rate of *Polysporoplasma sparis*. According to our results the combination of SA proved the most effective treatment for *Polysporoplasma sparis*. infection in *Sparus aurata* as (1) the therapeutic scheme and commercial product used was not toxic and (2) a significant reduction of % in the prevalence rate was observed. (Athanassopoulou et al., 2004a,b).

Oregano oils also have been found effective in treating bacterial (*Vibrio anguillarim and alginolyticus) in vitro* as well as in treating myxosporean infections in Sparidae (Athanassopoulou et al., 2000; 2003).

In a recent study some of these drugs were also tested against *E. leei* infections in D. *puntazzo*. Two medicated diets were applied: a) Salinomycin and Amprolium (AS) and b) Fumagillin (F). Significant drug effect on reduction of prevalence, intensity of all developmental myxosporean stages and mortality rate was observed at the end of both treatments, in comparison to untreated fish. The effect was most prominent in case of Salinomycin and Amprolium combination, exhibiting a significant reduction in intensity, prevalence and mortality rate in treated fish without any histopathological evidence for toxic side effects or reduction on growth. Furthermore, sporocysts and mature spores with distorted structure were observed in both tested treatments, but their prevalence was higher in the case of Salinomycin and Amprolium medication than Fumagillin, showing the effectiveness of the drug directly on the parasite. This data suggests that Salilomycin with Amprolium may be a promising treatment for myxosporean infections in intensively cultured warm-water fish, leading to parasite elimination. (Golomazou et al, 2006).

The potential antiparasitic and immunomodulatory effect of the above medications against myxosporean parasites on the innate immune system of sharpsnout sea bream (*Diplodus puntazzo*) was also investigated. Fish naturally infected with *Myxobolus* sp. (Bivalvulida/Platysporina), a histozoic parasite mainly affecting the renal interstitial tissue, were treated by oral administration of a combination of salinomycin with amprolium, *Origanum* essential oil or fumagillin in a small-scale field trial. Various leucocyte functions influenced by myxosporean infection were examined in order to determine treatment effects on leucocyte immunocompetence of treated fish. One month post medication all treatments caused a significant decrease in prevalence and intensity of infection in comparison to untreated, infected fish. The effect was most prominent in salinomycin with amprolium treated fish, which one month post medication contained either no cysts at all or a few spores free in melanomacrophage centres revealing almost total elimination of the parasite and the antiparasitic action of the treatment. There was no histopathological evidence of drug toxicity. Antiparasitic action was accompanied by a significant enhancement of phagocytic activity demonstrated by ingestion of large numbers of latex beads and the secretion of high levels of reactive nitrogen intermediates by phagocytes *in vitro*. Complete restoration of the diminished mitogenic responses and serum lysozyme secretion was

also detected in salinomycin with amprolium-treated fish compared to untreated, infected fish. These data suggest that salilomycin with amprolium may be a promising treatment for myxosporean infections in intensively cultured warm-water fish, exhibiting action partially via the enhancement of host, innate immune functions and leading to parasite elimination. (Karagouni et al., 2005a)

The impact of a successful anti-myxosporean medication on the innate immune system of juvenile and adult gilthead seabream (*S. aurata* L.) naturally infected with *Polysporoplasma sparis* in the kidney was also investigated in a small-scale field trial. The infected fish were treated orally with the combination of salinomycin and amprolium, two drugs well known for their anti-coccidial effect in other animals. Drug efficacy and safety was evaluated in terms of changes observed in histopathology, mortality and *P. sparis* intensity and prevalence rate. Phagocytic functions of head-kidney leucocytes were also investigated at the end as well as one month post the medication. Salinomycin with amprolium exhibited a significant reduction in intensity and prevalence rate in both juvenile and adult fish, and no histopathological evidence for toxic side effects was observed. In addition, the successful treatment was closely correlated with a complete restoration of the diminished phagocytic ability and capacity as well as NO, and lysozyme secretion in a time dependent manner. This data suggests that salilomycin with amprolium can be an alternative treatment for myxosporean infections in warm-water fish, possibly exhibiting their action through the enhancement of host innate functions. (Karagouni et al 2005b).

Table 6: Summary of the chemotherapeutants used in aquaculture

Parasites/use	Compound	Administration
Isopoda and Copepoda	Ivermectin	Per os
	Emamectin (Slice)	Per os
	Chitin synthesis inhibitors	Per os
	(Teflubenzuron).	Per os-
	Organophorphates	
	Dichlovos	Bath>17C
	Azametipos	Bath
	Pytherthin & pyrethroids	bath
	Hydrogen peroxide	bath
	Chloramin-T	bath
	Deltamethrin-*C. oestroides*	bath (Alphamax)
	Levamisole	bath
	Ivermectin	bath, per os
Monogeneans and other helminths	Formalin	bath
	Organophorphates	bath
	Amprolium	per os
Protozoa-Internal		bath
	Oregano essential oils	per os
	Quinine	per os
	Salinomycin	per os
	Sulfonamides	Bath
		Per os
	Toltrazuril	Per os
	Fumagillin	Per os
	TNP-470	
Protozoa-external		
Cryptocaryon sp.		bath
Oodinium sp.	Formalin	bath
	Reduced salinity	bath
	Formalin	
	Copper sulphate	bath
		bath

Disinfectants	Benzalkonium chloride Chloramin-T Copper sulphate Formalin Hydrogen peroxide Iodophores Potassium permaganate	Prolonged immersion Prolonged immersion-12hrs Bath Bath Dip

Table 7: Compounds used in experimental treatments for Myxosporea in Mediterranean fish *(S. aurata & P.puntazzo)*

Compound	Dose	Scheme
1.1.1.1.1.1 Fumidil-1	2 -up to 25mg/kg	3 and 6 weeks
1.1.1.1.1.2 Toltrazuril-2	600ml/T biomass	2 days on, 3 days off, 2 days on, repeat after 15 days
Toltrazuril-2	600ml/T biomass	2 days on, repeat after 15 days
Amprolium	190g/T biomass	30days
EsB-3	200g/T biomass	3days on, repeat after 15 days0
Ampr+EsB-3	100+100g/T biomass	30 days
Ampr+Salilomycin12%	100+70g/T biomass	30days
Oregano oils (Ecodiar)	8-12ml/5Kg biomass	30 days

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9. Advances in Genetic Resistance

Introduction

Compared to static global capture fisheries production (at around 95.5 million tonnes) aquaculture continues to increase globally reaching an estimated 45.5 million tonnes in 2004 with value of US$ 63.3 billion (FAO 2007). The increased culture densities, decreased quality of environment and mixing of populations associated with aquaculture which leads to increased incidence and associated impacts of outbreaks of disease threatens to hamper the ability of the aquaculture industry to supply the demand for high quality protein required by global population expansion. Thus the culture of aquatic species with increased disease resistance represents one of the tools, alongside strong bio-security, early disease diagnosis, use of vaccines and antimicrobial treatments, available to the aquaculture industry today to combat such disease outbreaks.

Progress towards improved disease resistance

Genetic selection in aquaculture could arguably be said to have started over 3000 years ago when the first fish were kept in ponds, and progressed to involve directed selection as the Japanese developed Koi varieties in the 1800s. Whilst the "re-discovery" and further development of Mendelian genetics in the early 1900s allowed directed selective breeding to be applied to plant and terrestrial animal production, the technology largely bypassed the aquaculture industry at the time. However some notable early selective breeding programs include selection for increased survival to furunculosis (67% over 3 generations) in brook trout (*Salvelinus fontinalis*) (Embody and Hyford, 1925) and selection for increased growth and fecundity in rainbow trout (*Oncorhynchus mykiss*) (Donaldson and Olson, 1955). Full-scale genetic breeding enhancement programmes in aquaculture only really began in earnest in the 1960s. The molecular biology revolution of the 1980s started to be implemented in aquaculture in the mid 1990s and selective breeding programs are now becoming established in many areas of the industry with the initial efforts based on finfish. There have been numerous reviews on the development of genetic improvement in aquaculture over the past two decades (e.g. Chevassus and Dorson, 1990; Gjedrem, 1997; Hulata, 2001; Dunham *et al*., 2001) and to attempt another full review here is beyond the scope of this project. A précis of the extent to which genetic improvements have or are being made with respect to improved disease resistance in general and to the diseases specified by Panda Work Package 2 is presented below.

Disease resistance (or tolerance) from innate and immune responses is under polygenic control. The pre-requisite for effective selective breeding is sufficient genetic variability with heritability (the proportion of phenotypic variation that is attributable to genetic variation). Genetic variation within species for resistance to diseases has been established in fish, molluscs and crustacea (Chevassus and Dorson 1990; Fjalestad *et al*., 1993; Nell and Perkins 2006; Gaffney and Busheck 1996; Argue *et al*., 2002).

The high fecundity of many aquacultured species means that traditional mass (or individual) selection programs taking only a few of the largest individuals form each generation or, in the case of disease, those surviving challenge can lead to rapid

inbreeding and associated loss of desired traits. Since mass selection is relatively "low tech" and low cost and thus available to a wider range of breeders research is ongoing to develop mass selection strategies that minimise inbreeding (eg. Chevassus *et al*., 2004; Dupont-Nivet *et al*., 2006). The more advanced approach to breeding programs is family based selection. Family based selection programs involve the keeping of a breeding nucleus for which individual broodstock are selected based on the performance of full or half siblings assayed for particular traits. Family based breeding programs require either the keeping of individual families in separate tanks until large enough to be tagged or the use of DNA fingerprinting for parentage assignment, both of which have significant cost implications. Mass selection is suitable for traits with medium to high heritability (such as growth), whereas family selection is more efficient for traits such as disease resistance, with lower heritability. However because of the cost implications the number of breeding programs using family based selection in aquaculture is still quite limited. Since the added value brought from breeding programs is still not generally recognised, producers are often not willing to pay the extra price for improved genetics thus it is often only the larger companies, with integrated breeding and production facilities that can realise the benefits of such selection programs. Improved education and transparent benchmarking systems for the various available genetic resources would allow producers to see the benefit of breeding programs and buy into the technology.

Modern molecular DNA based technologies also now offer significant potential improvements to selective breeding programs (see reviews by Hulata, 2001; Liu, 2001; Dunham *et al*., 2001; Chistiakov *et al.,* 2006). The development of polymorphic molecular markers in aquacultured species is central to this process. Molecular markers are divided into Type 1 and Type II according to their association with genes of known function or unknown genomic sequence respectively. The range of molecular markers available includes allozymes (type I), restriction fragment length polymorphism (RFLP - type I or II), random amplified polymorphic DNA (RAPD - Type II), amplified fragment length polymorphism (AFLP - type II), microsatellites (type II mostly unless identified within expressed sequences), expressed sequence tags (EST - type I) and single nucleotide polymorphism (SNP – type I or II). Molecular markers have a wide range of applications including individual identification and parentage assignment, characterisation of population genetics, construction of genetic maps, mapping and identification of economically important quantitative traits. Liu and Cordes (2004) comprehensively reviewed the relevant power (based on polymorphic information content) and various applications of the different markers in aquaculture genetics. AFLP, microsatellite and more recently SNP and EST markers are increasingly the markers of choice. Identification of polymorphic molecular markers in a species allows the generation of genetic linkage maps by assigning (or mapping) markers to chromosome configurations based on their segregation between families. Co-segregating markers are placed into linkage groups and percentage recombination defines the distance between markers. Linkage maps can then be used to identify regions of the genome (unidentified genes) linked to desired, measurable, inheritable traits (e.g. disease resistance) - such regions are known as quantitative trait loci (QTL). The more markers available (high density) the better will be the map resolution. Markers that are closely associated with the QTL can then be used in breeding programs through marker assisted selection (MAS). Linkage maps have been generated for a number of aquacultured species (eg. rainbow trout (Young *et al.,* 1998; Sakamoto *et al.,* 2000; Nichols *et al.,* 2003); Atlantic

salmon (Moen *et al.,* 2004a; Gilbey *et al*., 2004); brown trout (Gharbi *et al.*, 2006); carp (Sun and Liang, 2004), tilapia (Lee *et al*., 2005); channel catfish (Waldbieser *et al*., 2001; Liu *et al*., 2003) sea bass (Chistiakov *et al*., 2005) red sea bream (Inami *et al*., 2005); Japanese flounder (Coimbra *et al*., 2003); Eastern oyster (Yu and Guo, 2003); Pacific oyster (Li and Guo, 2004; Hubert and Hedgecock, 2004); blacklip abalone (Baranski et al., 2006); black tiger shrimp (Wilson *et al*., 2002); and Pacific white shrimp (Perez et al., 2004; Zhang *et al*., 2007) , though in many cases the marker density is still only low to medium. Furthermore potential QTLs have been identified for resistance to IPN in rainbow trout (Ozaki *et al*., 2001); IHNV in rainbow / cutthroat trout crosses (Palti *et al*., 1999), and with reference to the diseases list in WP2 of this project, to ISA in Atlantic salmon Moen *et al*., 2004b); RSIV in red sea bream (Inami *et al*., unpublished 2005); *G. salaris* in Atlantic salmon (Gilbey *et al*., 2006), to *C. Shasta* in rainbow trout (Nichols *et al*., 2003); *P. marinus* in the Eastern oyster (Yu and Guo, 2006) and TSV resistance in Pacific white shrimp (Doidge *et al.,*unpublished, 2006). As yet there are no reports of identification of candidate genes for these QTLs.

The anchoring of type 1 markers (from known coding genes) to linkage maps, the integration of linkage and physical maps and eventual generation of high resolution physical maps represent the next stages in mapping genomes of aquacultured species. The most comprehensive physical map of course is an annotated full genome sequence. Genome sequencing is currently considered to be too expensive (though new technologies, e.g. 454 Sequencing, may eventually offer more cost effective routes). In the absence of full genome sequences physical maps can be generated from sequencing large insert libraries e.g. bacterial artificial chromosome (BAC) libraries and from radiation hybrid analysis. BAC libraries and in some cases physical maps have been generated for a number of important aquaculture species e.g. channel catfish (Xu *et al*., 2007), Atlantic salmon (Thorsen *et al*., 2005; Ng *et al*., 2005), rainbow trout (Katagiri *et al*., 2001; Palti *et al*., 2004), carp and tilapia (Katagiri *et al*., 2001), Eastern and Pacifc oyster (Cunningham *et al*., 2006) Kuruma shrimp (Koyama *et al.,* 2006 unpub.) and Pacific white shrimp (Zhang *et al*., 2007, unpub.) Physical mapping increases the ability to identify candidate genes controlling QTL which can then be used for specific directed gene assisted selection (GAS). To date only one radiation hybrid physical map has been generated for an aquacultured species, the gilthead seabream (Senger *et al*., 2006).

The relative lack of detailed extensive genomic information for many aquacultured species can in part be combated by comparative mapping. The comparison of map rich or indeed fully sequenced model species with cultured species allows researchers to home in on QTLs or known genes through the identification of conserved synteny (the preserved order of genes) between species. Initial comparative mapping in aquacultured species includes comparisons between rainbow trout, Atlantic salmon and Arctic charr linkage maps (Danzmann *et al*., 2005); between EST derived microsatellites from rainbow trout and human, mouse, zebrafish, fugu and tetraodon (Rexroad ^{3}rd *et. al*., 2005) and between gilthead seabream radiation hybrid maps with tetraodon and zebrafish (Sarropoulou *et al*., 2007). Varying degrees of synteny were determined throughout indicating that the possibility of identifying candidate QTLs or genes through comparison with model species does at least exist as a medium term goal.

Again, for reasons of cost the use of modern molecular techniques and marker or gene assisted selection in breeding strategies still remains to be taken up to any degree by the aquaculture industry. Less costly methods of genotyping would significantly impact in this area.

Selection for, or research towards resistance to the Panda WP2 listed diseases

Of the viral diseases affecting fish listed by WP2, there is as yet no published evidence for research into EHNV resistance. ISAV and KHV have seen the most efforts to develop selectively bred resistant lines. ISAV, though exotic to the EU, is endemic in Norway and AquaGen in collaboration with AKVAFORSK has been running a family based selective breeding program for Atlantic salmon since 1995 (Mityling *et al*., 2002) to include selection for resistance to ISA as well as to furunculosis and infectious pancreatic necrosis virus (IPN). Resistance to ISA was found to be heritable but is perceived to be medium to low at $h^2 = 0.13$ (Gjoen *et al*. 1997). The same group has also identified genotypes of the major histocompatability complex (MHC) genes conferring resistance (and susceptibility) to ISA (Kjøglum *et al*., 2006). Moen *et al*., (2004) identified two putative QTLs for ISA resistance in Atlantic salmon.

In carp previous research has demonstrated the relatively low variability of domesticated strains of carp compared to wild strains (reviewed by Vandeputte, 2003). Hines *et al*. (1974) and Kirpichnikov *et al.* (1993) demonstrated crossbreeding of domesticated carp with wild carp could increase disease resistance. Based on this experience to attempt to improve resistance to KHV Shapira *et al.* (2005) crossed wild Sassan strain (C. carpio haematopterus) from the Czech republic with Nasice (Croatian) and Dor-70 (Israeli) domesticated strains of carp. A significant increase in survival to KHV challenge was exhibited in the Dor x Sassan cross (60.7% survival) relative to all other crosses tested (8-33.7%), with no loss in growth rate. Unfortunately the pure Sassan strain was not tested in these experiments. A crossbreeding program with common carp also exists in Hungary and within the EU project "EUROCARP" families of 4 different carp strains from the live carp gene bank (HAKI – Research Institute for fisheries, Aquaculture and Irrigation, Sarvas, Hungary) are being tested for resistance to KHV (and A. salmonicida). Of the four fish viral pathogens in the list KHV is the only one that is not exotic in the EU and since its rapid spread around the globe from first identification in 1998 it has become endemic in many countries. Development of resistant stocks would represent a significant advance in safeguarding the most important freshwater aquaculture species in the world (FAO 2007).

There is very limited information available on resistance to red sea bream iridovirus. Inami *et al*. (unpublished 2005) reported the first microsatellite mapping of red sea bream and identified two possible QTL markers associated with resistance to RSIV.

For the three bacterial pathogens in the list there is currently no information available on either breeding schemes or laboratory research into resistance. There is however evidence available for successful selection for resistance to other bacterial pathogens. As previously noted Embody and Hyford (1925) successfully improved resistance to Aeromonas salmonicida over 3 generations of selection in brook trout. Selective breeding programs for resistance to the same bacterium in Atlantic salmon are on-

going (Midtyling *et al*., 2002). Henryon *et al*., (2005) demonstrated additive genetic variation in rainbow trout to the two bacterial pathogens *Yersinai ruckeri* (causing enteric redmouth disease), *Flavobacterium psychrophilum* (causing rainbow trout fty syndrome) and to viral haemorrhagic septicaemia virus. They also found that the correlations between the resistances to the bacterial and viral pathogens were weak indicating it should be possible to improve resistance to each disease simultaneously. Developing resistance to *S. agalactiae, S. iniae and L. garviae* ought therefore to be possible.

Of the five parasitic diseases affecting fish listed by WP2, *Gyrodactylus salaris* represents the only disease currently not exotic to the EU. There are currently no reports of resistance breeding programs for *G. salaris.* Baltic strains of Atlantic salmon are known to be relatively resistant, whereas East Atlantic (Norwegian and Scottish) strains are highly susceptible (Bakke *et al*., 1990, 2002; Bakke and MacKenzie, 1993; Dalgaard *et al.,* 2003). As part of the "SALMOGYRO" EU project aimed at understanding the genetic basis for resistance in salmon to *G. salaris*, Gilbey *et al.,* (2006) identified 10 microsatellite based QTLs associated with resistance from backcrosses of Baltic and Scottish salmon suggesting that future marker assisted selection programs would be successful.

Many species of teleost and some strains within species show differences in resistance and susceptibility to *Trypanoplasma (Cryptobia) salmositica* (see Woo, 2003 for review). The plasma of resistant brook charr lyse the parasite via the alternative pathway of complement fixation (Forward and Woo, 1996). This innate resistance was suggested to be controlled by a single dominant Mendelian locus and was shown to be heritable (Forward *et al.,* 1995) leading to the possibility of breeding pathogen tolerant fish. In infected tolerant brook charr the metalloprotease secreted by *T. salmositica* (an important virulence factor) was effectively neutralised by α_2 macroglobulin a natural anti-protease and as such has led Woo to suggest the possibility of producing transgenic resistant fish. Acquired immunity to *T. salmositica* has also been demonstrated and antibody titre was shown to differ relative to parasitaemia in full sib families of Atlantic salmon showing association between increased resistance and earlier antibody response suggesting exploitable genetic variation in humoral response to resistance (Chin *et al*., 2004).

Variation in resistance to *Ceratomyxa shasta* has been demonstrated both between and within species in farmed and natural populations of salmonids (see Bartholomew, 1998 for review) and this variation has been shown to be genetically controlled (Ibarra *et al.*, 1992, 1994; Bartholomew, 2001). More recently Nichols *et al.* (2003) using AFLP markers mapped multiple genetic loci associated with resistance (and susceptibility) to *C. Shasta* from crossed clonal lines of rainbow trout. The authors invitingly note that one of the linkage groups displaying significantly associated markers is the same linkage group on which a novel immune-type receptor gene and an immunoreceptor tyrosine-based motif-bearing C type lectin are found but the association of these potential candidate genes requires further detailed study.

Little information is available on resistance to *Neoparamoeba pemaquidensis* since its discovery as the causative agent of amoebic gill disease (AGD) was only relatively recent (Munday *et al.*, 2001). Wynne et al., (2007) observed high variation (0% to 85% gill filaments infected) in 30 full sib families of Atlantic salmon and identified

major histocompatability marker alleles associated with reduced and increased AGD severity.

There is no information available on resistance to *Parvicapsula pseudobranchicola.* Similarly, other than relative species susceptibility there is no information available on resistance to the fungal fish pathogen *Aphanomyces invadans.*

For the molluscan diseases, there is no information available on resistance to the bacterial diseases *Candidatus Xanohaliotus* californiensis in abalone or *Nocordia crassostreae* in bivalves. Similarly there is no information available on resistance to the non-exotic parasisite *Perkinsus olseni/atlanticus*.

A significant body of research is building on resistance to *Perkinsus marinus* (causative agent of Dermo) in *Crassostrea virginica*. Resitant strains of *C. virginica* have been developed. The CROSBreed program at Virginia Institute of Marine Sciences has developed oysters resistant to both *Haplosporidium nelsoni* and *Perkinsus marinus* with up to 61% reduced mortlity compared to control strains (Ragone Calvo *et al.*, 2003). The University of Maine/ Industry co-operative oyster breeding program is further crossing a haplo/dermo resistant line with a juvenile oyster disease (JOD – caused by *Roseovarius crassostreae*) resistant line (unpublished). Yu and Guo, (2003) generated a medium to low density genetic linkage map in *C. virginica* (using AFLP markers with a few microsatellites and ESTs) and further demonstrated genetic variation and divergence between selected and wild strains of *C. virginica* (Yu and Guo, 2005). The same authors recently identified 12 potential QTL markers for Dermo resistance in selected lines (Yo and Guo 2006). Other genomic resources such as BAC libraries (Cunningham *et al.*, 2006) and the generation and analysis of expressed sequence tags and genes involved in resistance (Tanguy *et al.,* 2004; Quilang *et al*., 2007 and references therein) are in development for the eastern oyster.

Fewer attempts have been made to selectively breed molluscs with resistance to *Marteilia* species. However mass selection of surviving *Saccostrea glomerata* after *M. sydneyi* infection in Australia has led to resistant lines with up to 40% reduction in mortality after three generations (Nell *et al.* 2006 and references therein). Resistance in these oysters has been correlated with variations in the phenyloxidase cascade an important invertebrate defence mechanism. Newton *et al.,* (2004) showed that *M. sydneyi* resistant oyster had higher phenyloxidase activity whilst Bezemer *et al.,* (2006) identified 5 distinct forms of phenyloxidase in wild and selected oysters and found one form to be significantly reduced in the selected resistant oysters suggesting possession of this form makes oysters susceptible and has been negatively selected against in the resistant lines.

The crustacean viruses listed by WP2 are currently all exotic to the EU. Apart from relative species susceptibility there is no information on selection for resistance to Yellow head virus.

Maritech (Yuma, Arizona) currently market Super Shrimp® a selected line of *Litopenaeus stylirostris* resitant to IHHNV that showed greater than 80% survival after 20 generations of mass selection (Clifford H.C., 1998; Tang *et al.,* 2000). Hizer *et al.,* 2002 identified a number of RAPD markers from the resistant Super Shrimp

line compared to wildtype *L. stylirostris* which may be associated with resistance but this remains to be determined.

A number of domesticated lines of TSV-resistant *L. vannamei* and TSV-tolerant *L. stylirostris* have been developed and are in production globally although peer reviewed publications on their performances are limited. High Health Aquaculture Inc (and the Oceanic Institute Hawaii) by 2000 had developed a fourth selected generation of *L. vannamei* that reportedly averaged 92% survival compared to the 31% for unselected stocks (Wyban, 2000). However, Argue *et al*., 2002 demonstrated a negative correlation between growth and resistance in the early generations of these selected lines prompting a change in direction of the selection strategy. A number of genetics and genomics resources are under development for shrimp aquaculture. Intital linkage maps for *L. vannamei* based on AFLPs (Perez *et al*., 2004) and both AFLP and microsatellites (Zhang *et al.,* 2007) have been developed. The United States Marine Shrimp Farming Program is developing a shrimp EST database and a linkage map for *L. vannamei* from which a number of potential markers associated with resistance to TSV have been identified (Doidge *et al.,*unpublished 2006). Linkage mapping (Wilson *et al.,* 2002) and EST database development is also ongoing in Australia for *P. monodon.*

In contrast, efforts to improve resistance to WSSV in shrimp have seen little success to date. Gitterle *et al*., 2005 reported additive genetic variation but very low estimates of heritability (h^2= 0.03 and 0.07) for resistance to WSSV in experiments with two batches of full sib families of *L. vannamei*. The experiments also demonstrated a negative correlation of resistance with growth. Further refining the challenge methodology in similar experiments the same authors reported even lower heritability estimates but both negative and positive correlations of resistance to growth (Gitterle *et al*., 2006).

There is no information available for developing resistance to the crustacean bacterial disease Coxiella cheraxi.

Amphibian diseases generally relate to global declines observed in natural amphibian populations as opposed to problems encountered in culture. As such there is limited information available on resistance to *Ranaviruses* or *Batrachochytrium dendrobatidis.* A literarture search identifies a rapidly expanding list of amphibians susceptible to *ranaviruses* (see Daszak *et al.,* 1999; Greer *et al.,* 2002). Mortlity rates are generally high but can be as low as 40% (Daszak *et al*., and references therein). Pearman *et al*., (2005) showed significant variation in susceptibility between diverse populations of *Rana latastei* in Italy when challenged with Frog Virus 3 and correlated the increased resistance to higher genetic diversity within the population. Production of protective and long lasting antibodies to ranaviruses has been demonstrated in toads (Zupanovic *et al.* 1998) and frogs (Maniero *et al*., 2006). Together this suggests variation in susceptibility/resistance exists within and between amphibian species that could be exploited for conservation purposes.

Inter- and intraspecific as well as between-life-stage variation in susceptibility (and conversely resistance) of various amphibian species to infection by *B. dendrobatidis* has been demonstrated (Blaustein *et al.,* 2005; Garcia *et al.,* 2006). Retallick *et al.* (2004) reported establishment of remnant post decline populations co-existing with

endemic infection of *B. dendrobatidis* without obvious continued mortalities in the Eungella Torrent Frog (*Taudactylus eungellensis*) in Australia. *B. dendrobatidis* probably avoids the adaptive immune response by residing in the non-vascularised keratinised epithelium hence a significant amount of research has been directed to the innate immune response to *B. dendrobatidis* in amphibians and in particular to antimicrobial peptide defences. A number of peptides have been identified from several families showing varying effectiveness (see Rollins-Smith and Conlon, 2006 for review). Different species possess different natural peptide repertoires and this may be correlated with different resistances to *B. dendrobatidis* (Rollins-Smith *et al*., 2006) however, variation within species remains to be assessed. Assessment of this variation in antimicrobial peptide innate response in natural populations may allow some prediction for determining which species may be at greatest risk of future population decline.

Environmental impacts of breeding for disease resistance.

It is clear then that selective breeding combined with genetic technologies, if properly managed, can be an effective tool for developing disease resistance, at least in economically important species for aquaculture. Aquaculture impacts on the environment, the extent of which is constantly in debate. These impacts range from pollution with effluent, eutrophication from excess feed, habitat destruction, use of natural stocks for feed or wild seed, altered aquatic biodiversity and interaction with wild conspecifics affecting genetic conservation. With reference to the genetic impact of aquacultured species until relatively recently there has been little evidence to inform this debate, the exception perhaps being for Atlantic salmon. A recent EU project GENIMPACT was initiated to address this issue (Svåsand *et al*., 2007) and provides an excellent review of the data available (or not) for 12 commonly aquacultured species with respect to domestication, culture and breeding and impact on wild populations; genetically modified organisms; monitoring and modelling tools for genetic impact evaluation and recommended management options.

Potential genetic impacts are very species dependent, with the degree of wild population genetic structuring; the degree of domestication of stock in aquaculture (and thus difference from wild genetic makeup); the extent of opportunity for interaction (e.g. number of escapees) and the relative fitness of escapees being the major factors involved. Bivalve farming in the EU For example still currently relies heavily on wild captured spat, and though commercial hatcheries are increasing the difference of cultured stock from wild populations, some of which are relatively homogeneous, is still relatively small. In Atlantic salmon on the other hand extensive population structuring exists within wild stocks, which have experienced severe decline and local extinctions over recent history (WWF, 2001). Production of farmed Atlantic salmon, mostly based around a few breeding strains, exceeds by over 300 fold production of wild salmon and it has been estimated that up to 2 million farmed salmon escape each year into the North Atlantic, representing about 50% of the wild pre fishery numbers (Atlantic Salmon Federation, 2002). Escaped farm salmon have been shown to breed with wild salmon (Crozier, 1993, 2000; Clifford *et al.,* 1998) although their breeding performance is reduced (Flemming *et al*., 1996, 2000). McGinnity *et al*., 2003 investigated estimated lifetime success over two generations of wild, farmed, hybrid (F1 and F2) and backcross (F1 hybrid x wild and F1 hybrid x farm) groups of Atlantic salmon. The authors demonstrated faster juvenile growth in

the hybrids and backcrosses allowing for displacement of wild parr; showed reduced survival of hybrids and backcrosses leading to reduced recruitment and, given repeated escapes, indicated an overall fitness reduction of the wild population.

Such interaction studies are lacking for many cultured species (Svåsand *et al*., 2007). Furthermore the effects of genetic interaction of cultured species with specific enhanced disease resistance with their wild counterparts has received even less attention. For many cultured species information on wild population genetic structure is lacking. If the genetic impact of aquaculture is to be assessed this information should be obtained, furthermore it provides a basis from which to direct conservation efforts for the various populations. Significant live and cryo-gene banking should then also be pursued. Identification of escaped farmed animals will assist in assessment of genetic impacts, but this will require significant financial input (tagging or DNA fingerprinting).

The selection for improved survival on disease challenge may result in either disease resistant or disease tolerant individuals with the ability to either prevent access and remove the pathogen or restrict replication and live with the pathogen respectively. Tolerant, as opposed to resistant, farmed individuals represent potential carriers for infection of wild populations. The environmental impact of resistant or tolerant fish will differ depending on the status of the disease for which resistance is required (i.e. exotic or endemic). Selective breeding programs with defined goals to improve disease resistance should assess the basis of that resistance.

The prevention of cultured species from genetically interacting with wild counterparts has historically been attempted by physical containment or the production of sterile animals. Total physical containment for the prevention of escapees is unlikely to be achieved in most aquaculture systems however containment should be regularly reviewed and improved where possible. Sterile animals can be derived by a number of methods and has been demonstrated in a number of species (see Hulata, 2001 and Dunham *et al*., 2001). Generation of sterile animals by triploidy is currently the most amenable for use on a commercial scale, but is not suitable for all species and development of sterile animals has still to be achieved for many species (Hulata, 2001; Svåsand *et al*., 2007).

Given current public scepticism and consumer resistance about genetically modified organisms, although a number of experimental transgenic aquacultured species have been created, there are no such animals in production (for consumption) and it seems likely to be some time before they are. Meanwhile continued research into the impacts these organisms will have on native species is required alongside research and development to mitigate negative impacts.

Gaps - Recommendations

It is difficult to see how extensive Euro funding can be spent on researching diseases that are exotic to the EU with possible exceptions for *Gyrodactylus salaris* and where a significant export trade exists of species to areas where the listed diseases are encountered. Continued development for resistance to the pathogens currently affecting aquaculture in the EU (e.g. IPN, KHV, VHS, sea lice, bonamiosis, marteliosis) should of course continue. Though still some way off, the development

of genetic and genomic resources for species of importance will in time lead to the identification of candidate resistance genes, which can then be selected for by breeding programs. It remains to be seen how many of these resistance genes will be applicable to multiple pathogens but careful management of selection programs could provide aquacultured species with a well defined and broad genetic base for resistance to current and future encountered pathogens. Collaborative international research on the WP2 listed pathogens should be encouraged to provide access to resources that may be of use in the future.

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10. Identified Knowledge Gaps and Recommendations

Antimicrobial chemotherapy

- There is a definite lack of good quality information on prevalences of antimicrobial resistance, amounts of antimicrobials used, the applied modes of use, and the outcomes of use.

- MIC method harmonisation should continue to kinetics as well as on environmental impact methodologies.

- More research is needed into factors that influence the selection of resistant microorganisms is needed, particularly at the *in vivo* and epidemiological levels.

- More research and epidemiological data are needed to clarify the issue of inter-species transfer of antimicrobial resistance.

- It is very important to evaluate the environmental impact from the antibiotic use in terms of deposition of residues in the sediment, water column and wild fauna as well as to describe the mechanisms and the locations for the selection of resistant environmental bacteria. The population dynamics of these bacteria their impact to the natural bacterial flora and the likelihood to affect the human flora along with all the relative implications should be evaluated because there are a lot of misperceptions and aquaculture is being blamed without any data when environmental groups extrapolate data from poultry farms and other terrestrial farmed animals with no concern on the enormous differences that the aquatic environment pauses.

- Clinical trials of efficacy are presently almost entirely funded by the pharmaceutical industry and have objectives largely confined to satisfying regulatory authorities. Thus trials, which seek to optimise the dose, dose interval and duration of treatment are rarely done. We have to elevate the service provided to the industry from an empirical to scientific level where we will take all aspects of chemotherapy into account.

- Veterinary prescription although compulsory, should be re-vitalised in many countries because this the way by which at all times a scientist has the responsibility of the treatment applied.

- Focus is needed on methods of reducing the need for antimicrobials by lowering the prevalence of infection. Widespread implementation and enforcement of methods for reducing transmission of pathogens as well as detailed epidemiological studies are needed in Aquaculture.

- More studies are needed on the impact of changes of farming conditions and management practices on fish stress and welfare.

- Antimicrobial treatments should be aimed directly at the infectious agent and the need for broad spectrum or combined antimicrobial usage will be more limited.

- Immunological, molecular techniques of increased sensitivity should be made available in the field to be able to monitor fish immunological status and to plan vaccination strategies accordingly.

- Determination of the optimum mode of oral administration of chemoterapeutants is very important in order to avoid shortfalls.

- For hatcheries vehicle drug delivery studies should continue in order to increase affectivity and decrease environmental impact.

- Investigation of optimum uptake and residue kinetics will require incorporation of population distribution studies where current practice is audited and then suggestions are made to ensure increased clinical relevance and efficacy.

- Real antibiotic resistance level monitoring in commercial conditions

Biosecurity

- Basic research on resistance of fish pathogens to various chemicals and methods of disinfection

- **Alternative methods of disposing of fish and shellfish waste:**
  Meeting the present ABP (EC1774/2002) requirements poses a significant challenge to European fish farmers, that often operate at remote sites some considerable distance from approved disposal centres. The amount of waste material itself can also be very considerable (in the case of a large-scale die-off or cull following an epizootic). Rendering, incinerating such material with its high water content can also be a challenge to licensed operators.
  In the case of shellfish shell, disposal of clean shell back to sea (natural environment returning calcium to the ecosystem) is not currently allowed under waste legislation causing issues for processors. Shell also acts as culch, acting as a settlement surface for recruiting molluscs and other animals in support of the ecosystem and maintainance of the substratum.
  Recognising these problems, FRS and Cefas have undertaken work to identify alternate hygienically safe methods of disposing of such ABP category 2 fish waste, that are both more practical for farmers and potentially able to generate useable by-products (Cefas, 2005). The work involved testing the effectiveness of acid and alkali ensiling processes and composting at inactivating resistant pathogens in fish material. *Lactococcus garvieae* and infectious pancreatic necrosis virus (IPNV) were shown in initial laboratory-scale tests to be more resistant then a range of other fish-pathogenic bacteria and viruses, so were selected for larger scale composting and alkali-ensiling trials (initial tests showed acid ensiling was not an efficient method of disposing of these pathogens). Both composting and alkali ensiling were

shown to be able to inactivate test pathogens (which included exposing Atlantic salmon mortalities from field IPNV epizootics to the processes). As both processes result in a high organic content by-product that can then potentially be used in agriculture, it is recommended that further work needs to be done at a European level to both further validate the results of this initial testing exercise at a pan-European level to potentially allow a derogation to EC1774/2002 to allow alternative methods then those presently approved to dispose of aquaculture mortalities.

- **Benchmarking - Disinfectant standards**
  Although the use of disinfectants may be well established and very effective in most cases, the criteria for selection and application for particular purposes may be less well understood.
  Under the provisions of the Animal Health Act 1981, the United Kingdom's Department for Environment, Food and Rural affairs (Defra) maintains a list of disinfectants approved for the control of diseases of terrestrial animals (http://www.defra.gov.uk/animalh/diseases/control/testing_disinfectants.htm).
  At the present time this system of approvals does not extend to pathogens of fish or shellfish.
  Among already-available authorisation or listing schemes, Norway operates a disinfectant approval scheme based on their Regulation 194 (Anon, 2002). Their dossier requirements are based on a draft of the EU's Biocides and Pesticides Directive (1998/8/EC), with the test conditions to be used (temperature and organic load) and test organisms specified and mandatory (Anon, 1997). The actual standards to be used are not clearly specified in their guidelines though, and may not be appropriate to the needs of other countries.
  However, the methods used in these different studies were not always standard, making realistic comparison of the effectiveness of the different products, or active ingredients, investigated problematical.
  There is also limited information about effective methods for control of some notifiable diseases. Many of the published studies referred to are either old or are primarily concerned with inactivating a single agent (e.g. Smail et al., 2004; Graham et al. 2007). Disinfectant guides (e.g. Fraser et al., 2006) are consequently also reliant on published data aimed at specific pathogens for specific purposes or manufacturer-supplied data for information on the effectiveness of particular products, particularly with regard to information as to the effectiveness of newer biocides. In support of a proposed Defra aquaculture-disinfectant listing scheme, work has been undertaken to design practical and robust standards for manufacturers to demonstrate their products' effectiveness against aquaculture pathogens (Cefas, 2006). Two Phase 2 Step 1 CEN chemical disinfectants and antiseptics – quantitative suspension test standards were selected and modified according to advice from a committee of UK aquaculture health professionals and scientists responsible for implementation of the Defra terrestrial and the Norwegian aquaculture disinfectant approval schemes. For testing a product's likely virucidal effectiveness it was recommended that a version of EN 14675:2006 (e.g. British Standards Institution, 2006) should be used, substituting bovine enterovirus for IPNV. IPNV is a very resistant non-enveloped virus of significance for European salmonid culture. The ability to inactivate IPNV will likely show the disinfectant will also inactivate other viruses at the same

or higher dilutions. For testing a product's bactericidal activity, it was recommended that EN1656:2000 be used, with *Yersinia ruckeri*, *Aeromonas salmonicida*, *Carnobacterium piscicola* and *Lactococcus garvieae* used as the substituted test organisms. For both standards it was recommended that challenging conditions be used (high soiling and a temperature 4 °C. To pass as acceptable for use in standard treatment, a dilution of disinfectant should be required to show a 4 log reduction for virus and 5 log for bacteria following 30 min exposure.

There is now a need to integrate these proposed testing standards with other, e.g. EU systems, and develop standards for testing against other particularly important pathogens.

- **Disinfection standards for molluscs and crustceans**

  This work currently excludes shellfish, however the EU reference laboratory see a need for development of disinfection standards for molluscs and good biosecurity practices. There is very little information as to what disinfectants are effective against crustacean and mollusc parasites, bacteria and viruses.

- **Egg disinfection**
- Another important application of disinfection is sanitation of eggs. This is a very important method of restricting the movement of pathogens that can potentially be carried on the surface of eggs or in ovarian fluid, both within and between facilities and member states. At the moment there are differences between EU member states in terms of what is permitted to surface sterilise finfish eggs, with some member states treating iodophores and other egg disinfectants as permitted sanitizers with no medicinal claim attached while others do not allow their use for this purpose, presumably requiring the company marketing such a product to obtain an authorisation for that particular use. It is recommended that this problem be tackled at an EU level so farmers wishing to import or export ova are able to sanitise them using safe and effective treatments.

Vacciantion strategies

Several diseases, especially those caused by viruses, cause major mortalities in larval fish. Following hatching, the specific immune system takes some time to develop and the larval fish relies entirely on its innate defense mechanisms. Vaccines are only effective if the immune system is developed enough to respond to the antigens being presented. In larval fish, especially of the marine cold water species such as halibut, cod and haddock, there is a significant live feeding stage during which the immune system develops. During this period vaccines would be ineffective and a more suitable treatment may be with immunostimulants. These, however, have only a short effective time span. Vaccines should be targeted at the broodstock in order to eliminate vertical transmission.

- Further research on innate non specific components of the fish and crustacean immune system.
- Research on increased efficacy of vaccine oral delivery system.
- Research on monitoring parameters of fish immune response level (specific antibody levels etc.) are required for further customization and fine tuning of vaccination strategies.
- Basic research on protective antigens of various important pathogens.
- Further research on vaccine technologies for viral pathogens.
- Further research on vaccine technologies against parasitic pathogens.
- Transfer of technologies from terrestrial animals in improving fish vaccine adjuvants.
- Evaluation of potential environmental impact of attenuated and DNA vaccines.
- Research on reduction of side effects of oil based adjuvants.
- Research on subunit vaccines, recombinant vectors, and genetic immunization.
- Further research on immunostimulants and immunomodulators.
- Facilitation of faster licensing procedures in order to attract more pharmaceutical companies in investing on development of effective fish vaccines.

Antiparasitic treatments

- Research on reduced environmental impact of currently available antiparasitic treatments.

- Research on alternative antiparasitic treatments.

- Increased effort on treatments and control strategies for isopod parasites.

- Increased effort on treatments and control strategies for myxosporidian parasites.

- Increased effort on treatments and control strategies for monogenean parasites that recently acquired increased importance due to high associated fish mortalities.

- Increased effort on treatments and control strategies against histophagous ciliate, Philasterides dicentrarchi one of the most important pathogen in turbot farming.

- Research on strategies for reduction of resistance building of sea lice treatments.

- Investigation of potential antiparasitic and immunomodulatory effect of alternative medications against myxosporean parasites on the innate fish immune system.

Alternative treatments

- **Regulatory constraints**
  WP5 identified that the major barrier to implementation of alternative treatments for both the diseases identified as being a particular risk to the EU, as well as a range of other diseases that already causing problems for EU farmers, was the time and cost associated with gaining marketing approvals for veterinary medicines. It is obviously very important that marketed medicines are both safe and effective. However, if means could be found to streamline the regulatory process it is likely that a greater range of effective treatments will be made available to farmers and veterinarians.
  As well as constraining the development and application of new treatments, the present regulatory climate also constrains the use of generic chemical treatments. As already identified for the use of iodophores for sanitation of salmonid eggs, there are other chemical treatments, such as Chloramine T, hydrogen peroxide and formalin dips, that have been used successfully by farmers and veterinarians for number of years to treat external parasites and other infections in a range of fish and shellfish species. There is potential for these to become so-called ‘orphan’ treatments as no company will be willing to bear the costs of obtaining the appropriate Marketing Authorisations to allow their use to continue. If a company were to obtain an MA for a fish medicine based on one of these generally available bulk chemicals they would not be able to readily defend their product from competition by farmers illegally obtaining and using cheaper generic versions.
  The US have developed a scheme whereby the regulatory packages for such orphan products are partially sponsored by the US government, reducing the costs for companies seeking to gain FDA marketing authorisations for such products. The EU should investigate whether there should be general support

given to helping to retain some of these particularly cost-effective, widely available treatments.

Crustacean Pathology

- Environmental impact evaluation of five identified warm water exotic crustacean diseases in temperate EU waters. Evidence suggests that WSSV viruses will replicate and cause disease at low temperatures, affects all decapods will have severe impact.

Genetic resistance

- Develop and improve knowledge of the physiological basis of disease resistance (and other important traits)
- Develop high density and high resolution integrated genetic maps for the species of importance
- Further explore comparative mapping from map rich to map poor species
- Consider full genome sequencing of important aquaculture species
- Establish international collaborations assisting in research into the diseases currently exotic to the EU
- Identify sources of funding and investment on research of exotic species and diesases
- Improve knowledge of genetic diversity of wild populations
- Further research the environmental impact of aquaculture stocks genetically selected for disease resistance (and other traits)
- Develop live- and cryo-banks of all available wild strains maintaining access to genetic diversity for conservation and selective breeding purposes.
- Minimise the environmental effect of escapees on wild populations – develop better reproductive manipulation for production of sterile animals
- Improve collaboration between research and industry to facilitate strategic research and technology transfer for establishment of improved selective breeding strategies.
- Establish clear and transparent National and International benchmarking processes to allow comparison of the different genetic resources available to producers on the market to encourage uptake of improved genetic resources

11. Epilogue

Aquatic animal health management largely affects the sustainability of aquaculture industry. Available options for the prevention, containment and control of all the fish, mollusc, crustacean and amphibian diseases diseases identified in WP2 are briefly described in terms of antibacterial treatments, biosecurity and disinfection strategies, vaccination strategies, antiparasitic treatments, alternative treatments and advances in genetic resistance. Environmental implications of currently available strategies are identified and research gaps and recommendations on future work are addressed in a report that aims to assist pathologists, fish farmers, administrators and politicians to understand the issues and the requirements for future steps in policy making and implementation in order to protect European aquatic resources from the introduction and spread of important diseases.