

# International Aquatic Veterinary Conference Athens, Greece 2010



World Aquatic  
Medical Veterinary  
Association



World Aquatic Veterinary Medical Association 2010 International Aquatic Veterinary Conference, Annual General Meeting, & CEPD/Family Cruise of the Greek Islands & Turkey

## *Proceedings of the*



## *2010 Aquatic Veterinary Conference*

**VETERINARY CONTINUING EDUCATION &  
PROFESSIONAL DEVELOPMENT (CEPD) PROGRAM**

**DIVANI PALACE ACROPOLIS Hotel – Athens, Greece  
July 11-14, 2010**



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## *Conference Sponsors*



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# 2010 Aquatic Veterinary Conference

## & Annual General Meeting



### VETERINARY CONTINUING EDUCATION & PROFESSIONAL DEVELOPMENT (CEPD) PROGRAM

<b>Sunday July 11</b>		<b>Location – Hotel Reception Area</b>
12:00 – 17:00	<b>Registration</b>	Hotel Foyer Area
18:00 – 20:00	<b>Informal Reception</b>	Hotel Reception Area/Bar
<b>Monday July 12</b>		<b>Location – Aristotelis A Conference Hall</b>
09:00 – 09:30	<b>Welcome to WAVMA, the 2010 Conference &amp; Greece Introduction of Dignitaries, Sponsor Recognition &amp; the Conference Program</b>	Fotini Athanassopoulou - 2010 WAVMA President; Dean of Veterinary Medicine, University of Thessaly J Menegatos DVM PhD. Hellenic Veterinary Medical Society
<b>Session: Emerging Diseases &amp; Integrated Management I</b>		<b>Moderator – David Scarfe</b>
09:30 – 10:00	<b>Emerging and Resurging Infections of Fish in the Great Lakes: Potential Threat to Conservation Efforts</b>	Faisal M*, Loch TP
10:00 – 10:30	<b>Viral Hemorrhagic Septicaemia Ecology and Disease Susceptibility</b>	Faisal M*, Kim R, Millard E, Weeks C
10:30 – 11:00	<b>Tea/Coffee break</b>	
11:00 – 11:30	<b>Vaccination Programs – A Practical Guide to Using Different Vaccination Methods and Technologies to Protect Fish Through the Production Cycle</b>	Wardle R*
11:30 – 12:00	<b>Use of Oregano Essential Oil for the Control of Parasite and Microbial Diseases of Mediterranean Fish</b>	Athanassopoulou F*, Yiagnisis M, & Bitchava K
12:00 – 12:30	<b>The Role of Intestinal Microflora under Acute Stress Conditions on the Health of European Sea Bass (<i>Dicentrarchus labrax L.</i>) Possible Translocation.</b>	Yiagnisis M* & Athanassopoulou F
12:30 – 13:30	<b>Lunch (provided)</b>	
<b>Session: Emerging Diseases &amp; Integrated Management II</b>		<b>Moderator – Julius Tepper</b>
13:30 – 14:00	<b>The effect of different oxygen levels on the European sea bass health under conditions of acute stress</b>	Yiagnisis M*, Alexis M, Govaris A, Bitchava K & Athanassopoulou F.
14:00 – 14:30	<b>Problems in Transporting 20 Tons of Live Eels from Egypt to the Netherlands</b>	Werkman P
14:30 – 15:00	<b>Pangasius Breeding Problems in India</b>	Werkman P
15:00 – 15:30	<b>Tea/Coffee break</b>	
15:30 – 15:45	<b>Limitations and Shortfalls in Parasite Control – Future Needs</b>	Athanassopoulou F*, & Bitchava K
15:45 – 16:00	<b>Controlling Chytridiomycosis in Aquatic Frogs in the Pet Industry</b>	Saint-Erne N*
16:30 – 17:00	<b>Panel Discussion – Optimal Approaches for Monitoring &amp; Managing Emerging Diseases</b>	<b>Panel</b> – Athanassopoulou F, Faisal M, Saint-Erne N, Wardle R, Werkman P & Yiagnisis M

<b>Tuesday July 13</b> Location – Aristotelis A Conference Hall		
<b>Session: Biosecurity – Preventing, Control &amp; Eradication of Aquatic Diseases I</b> Moderator – Peter Werkman		
08:45 – 09:00	Introductions & Housekeeping	
09:00 – 09:30	The Use of an Upflow Refugium and Phytoremedial Device for Water Purification in Clinical Pet Fish Practice	Tepper JM*
09:30 – 10:00	The Control of a Pathogenic strain of <i>Aeromonas sobria</i> Using a Refugium and Phytoremedial Device	Tepper JM*
10:00 – 10:30	Is KHV Zoonotic? Assessing the Zoonotic Potential of Aquatic Animal Diseases	Walster CI*
<b>10:30 – 11:00</b> Tea/Coffee break		
11:30 – 12:15	Bacterial Kidney Disease Dynamics and Control Efforts	Faisal M*, Schultz C
12:15 – 13:00	Common Tropical Fish Diseases, Diagnosis and Treatments in the Retail Pet Industry	Saint-Erne N*
<b>12:30 – 14:00</b> Lunch (provided)		
<b>Session: Biosecurity – Preventing, Control &amp; Eradication of Aquatic Diseases II</b> Moderator – Chris Walster		
14:00 – 15:00	Developing Efficient & Effective Biosecurity Programs for Ornamental and Commercial Aquaculture Clients	Scarfe AD*, Walster CI, Palić D.
<b>15:00 – 15:30</b> Tea/Coffee break		
<b>Session: Clinical Issues in Ornamental Pets</b> Moderator – Chris Walster		
15:30 – 15:45	Ova Retention in Female Koi	Saint Erne N*
15:45 – 16:15	Surgery in Pet Fish	Werkman P*
16:15 – 17:00	<i>Panel Discussion</i> – Optimal, Practical & Economic Biosecurity	<b>Panel</b> – Faisal M, Saint-Erne N, Scarfe AD, Tepper JM, & Walster CI
<b>Wednesday July 14</b> Location – Aristotelis A Conference Hall		
<b>Session: Round Table Discussion – Aquatic Veterinary Education &amp; Core Competencies</b> Moderator – David Scarfe		
09:00 – 09:30	Non-Indigenous Freshwater Crayfish Species in Greece and Risks for Biodiversity	Perdikaris C*, Paschos I, & Athanassopoulou F
09:30 – 10:00	Aquaculture Development in Tanzania Challenges And Future Prospects	Masurli B*
10:00 – 10:30	<i>An Introduction: Recognizing Aquatic Veterinary Competency – Education, Opportunities, and a Refined Process for Advancement</i>	Scarfe AD*
10:30 – 11:00	Aquatic Veterinary Competency & Accreditation – Sharing a Vision	Athanassopoulou F*
<b>11:00 – 11:30</b> Tea/Coffee break		
11:30 – 12:00	<i>Open Discussion</i> – Alternatives & Options for Recognizing Competency in Aquatic Veterinary Medicine	Open Attendee Discussion
12:00 – 12:30	<i>Open Discussion</i> – Prioritizing Options for Implementing Aquatic Veterinary Competency Programs	Open Attendee Discussion
<b>12:30 – 13:00</b> Lunch (provided)		
<b>Session: WAVMA Annual General Meeting</b> Moderator/Parliamentarian – David Scarfe		
13:00 – 14:30	<ul style="list-style-type: none"> <li>• Introductions &amp; Recognition of Sponsors</li> <li>• WAVMA Structure, Functions</li> <li>• WAVMA 2006-2010 Accomplishments</li> <li>• WAVMA Strategies and Plans for 2011 &amp; Beyond</li> <li>• Adjourn</li> </ul>	Chris Walster (Secretary) Fotini Athanassopoulou (2010 President) Julius Tepper (2011 President)
<b>14:30 – 19:30</b> Tea/Coffee, social interaction & free time		
<b>19:30</b> Meet in Hotel Lobby for Transportation to Banquet – Hotel Foyer/Reception Area		
<b>20:00 – 24:00</b> Banquet – Stamatopoulos Tavern		

***The purpose of the World Aquatic Veterinary Medical Association is:***

To serve aquatic veterinary medicine practitioners of many disciplines and backgrounds by developing programs to support and sustain members, and the aquatic species industries that they serve.

To identify, foster and strengthen professional interactions among aquatic medical practitioners and other organizations around the world.

To be an advocate for, develop guidance on, and promote the advancement of the science, ethics and professional aspects of aquatic animal medicine within the veterinary profession and a wider audience.

To optimally position and advance the discipline of aquatic veterinary medicine, and support the practice of aquatic veterinary medicine in all countries

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## *World Aquatic Veterinary Medical Association*



### ***Welcome to Athens***

It is my pleasure to welcome you to Athens. The World Aquatic Veterinary Medical Association is honoured to host this conference in such a great city and we hope you have a very productive and enjoyable time while you are here. The organizing committee has worked very hard over the past months to produce an attractive meeting and one that enhances scientific, technical and commercial value for all participants.

We have prepared an attractive program of sessions that we believe will be useful for scientists and practitioners alike. One additional initiative that was officially launched is the stimulation of discussions regarding the important issue of recognition of aquatic veterinary competency and education status. We trust you will find the conference venue convenient and we encourage you to have fun while in Athens both during the social events we have organized as part of the conference as well as on the cruise and on your own while touring the many attractions of the city and its surroundings.

We thank our sponsors for their support.

See you all in Athens!!

Prof. F. Athanassopoulou DVM, MSc, PhD, MRCVS  
*President of WAVMA*  
*Dean, Faculty of Veterinary Medicine,*  
*School of Medical Sciences*  
*University of Thessaly,*  
*Greece*

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## Presentation 1

### **Limitations and Shortfalls in Parasite Control-Future Needs**

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Fisheries and Aquaculture have a very important impact in the economy and ecology of Mediterranean countries and in particular of Greece. In 2000, the combined Mediterranean production exceeded 130,000 tons. Marine aquaculture industry is one of the largest industries in Greece, the leading producer of sea bream and bass. The sudden increase of production, however, over the last decade has been associated with pathological problems. Furthermore, as in the salmonid industry, with the development of marine net pen farming, new or unusual infections by parasitic pathogens have been observed. This is due to different hosts being reared in new geographic areas or by indigenous species being reared in a different condition, i.e. Net pen. Over the last decade, parasite species such as *Lernathropus kroyeri*, *Ceratothoa oestroides* (in sea bass), gill trematodes (in all cultured species) and Myxosporida (especially in *D. puntazzo*) have caused significant problems (mortalities, delay in growth rates and / or loss of fecundity). Therefore parasites requiring treatments are the Myxosporeans (*Myxidium. leei*, *Polysporoplasma sparis*) and Isopods and Copepods. In some areas there are also heavy mortalities due to Monogenea infections.

Future needs require research on:

- Alternative treatments and management interactions ( esp. sea lice)
- Wild fish epidemiology, interactions (Myxosporeans, Isopoda)
- Basic research on taxonomy, identification and life cycles of marine parasites
- Early & rapid diagnostic methods for other myxosporeans and protozoa
- Effective and environmentally friendly antifouling systems
- Correct feeding and correlation with parasitic disease
- Technical improvement in seed production, disease resistant stock

Management measures (quarantine, training, strategic therapies, veterinary presence and monitoring of diseases), regulation (importation, international trade, HACCP, notifiable diseases), minimization of importation of various fish and different fish species, biosecurity and international trade of live fishes and shellfishes are major issues to be addressed in handling successfully parasite control.

The combination of the correct treatments with other management and disease prevention strategies (vaccinations, water quality improvement, production of tolerant fish, alternative treatments) and further regulation will help ensuring the successful development of the sector in the future.

## Presentation 2

### **Use of Oregano Essential Oil for the Control of Parasite and Microbial Diseases of Mediterranean Fish**

Authors (\*indicates Presenter): Athanassopoulou F.,\* Yiagnisis M., Bitchava K.

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The recent growth of the aquaculture industry in Greece together with the introduction of new fish species (*Puntazzo puntazzo*, *Dentex dentex*, *Pagellus spp* and *Diplodus sargus*) in intensive rearing systems has led to an increased occurrence of different pathogens including Myxosporidia, that have caused serious problems in the last few years, constraining further development. The most common parasites affecting Mediterranean fish are the myxosporeans (especially *Myxidium leei*), often implicated in serious losses in cultured sharpnose sea bream (*Puntazzo puntazzo C.*) and sea bream (*Sparus aurata L.*). In contrast to mammalian therapeutics, the use of pharmaceutical substances, particularly antiparasitic drugs in fish is limited. Research on anti-myxosporean/microsporean treatments concern mainly salmonids there is little research on Mediterranean fish. There are no licensed antiparasitic compounds for Mediterranean species or official minimal residue levels (MRL's) currently available and all information is extrapolated from coldwater species, especially salmonids. This can cause problems as treatment conditions are very different in terms of environmental (temperature, pH, stability, toxicity to other aquatic animals) and individual fish factors (safety, metabolism, stress, residues, etc.). Therefore alternative, low impact substances are assessed for treatment and prevention of these diseases. Origanum essential oils have been found to have inhibitory effects on microorganisms and in spore forming organisms. For the first time these are tested against Myxosporean infections and microbial infections in Mediterranean fish.

Over the last 10 years oregano oil (OEO) was assessed and proved as a promising treatment of Myxosporidia infections in sea bream (*Sparus aurata* and *Diplodus puntazzo*). Treatments with oregano essential oils resulted in reduction of prevalence up to 50%. Carvacrol and thymol, the two major phenols that constitute about 78-82% of the essential oil, are principally responsible for this activity.

OEO was also tested against *Aeromonas sobria* experimental infections of common carp (*Cyprinus carpio*) where reduction of prevalence was 35%. The study was performed in order to examine the effect of dietary supplementation of oregano essential oil on the resistance to *A. sobria* infection in carp.

Results showed that dietary oregano oil at the inclusion level of 12ml/5g biomass has an important antibacterial effect and it also helps the growth rate. Larger fish were less susceptible to microbial challenge.

Two more studies were performed in order to examine the effect of dietary supplementation of oregano essential oil on combined infections by bacteria-parasites in farmed sea bass. The first study was carried out to examine the effect of dietary oregano essential oil on combined infection by the isopod sea lice *Ceratothoa oestroides* (Risso, 1826), and the oral challenged pathogenic bacterium *Listonella (Vibrio) anguillarum*. Results showed that dietary oregano oil at the inclusion level of 0.9-1ml/100gr food has an important antiparasitic-antibacterial effect.

The effect of dietary oregano essential oil supplementation on the development of the histopathological lesions caused by the pathogenic bacterium *Photobacterium damsela subsp. piscicida* in farmed gilthead sea bream (*Sparus aurata*) was also assessed. Fish fed the diet with OEO exhibited 24.46% improvement in the mean number of the percentage % of fish exhibiting necrotic lesions in the spleen. Multiple granulomas were observed only in the fish of the group B, suggesting that OEO could decelerate the development of the lesions.



### **Dr. F. Athanassopoulou**

#### Short biography:

Professor & Dean of the University of Thessaly, School of Health Sciences, Faculty of Veterinary Medicine. My teaching duties include teaching of fish biology, fish pathology, aquatic animal health and aquaculture to 3rd and 5th year students and zoology and ecology to 2nd year students. I am also involved in research on pathology and microbiology of aquatic organisms including aspects of public health. I am also responsible for the only MSc course of the faculty with two specializations: MSc course in “Aquatic Animal Health” and MSc in “Aquaculture”.

In 1995, after my return to Greece I worked as a consultant in fish pathology for three major aquaculture groups while I was waiting for my appointment to the National Agricultural Research Foundation. (NAGREF). From 1997-2001, I worked at the Department of Fish Diseases & Aquaculture of the Institute of Veterinary Research of Athens of NAGREF where I was involved in research on pathology and on immunology aspects of myxosporean parasites and on research on diseases of wild and new cultured marine and freshwater species of fish.

In 2001 I was elected Associate Professor at Faculty of Veterinary Medicine of the University of Thessaly where I am now Professor and Head of Laboratory of Aquatic Animal Health, Ichthyology & Aquaculture. I have also been appointed as an expert at the Committee for vaccine validation and licensing of the Food & Drug Organisation of Greece (4 years) and an expert on fish diseases for the European Union and the Consumers Association of Greece. There are currently 5 PhD students involved in research of marine fish/ aquatic animals in the Laboratory.

In the past years my research has been focused on pathology related to parasitic treatment, alternative treatments, toxicology of drugs/ plant extracts/ ethnobotany and environmental pollution in fish and vertebrates and immunological aspects of these treatments to cultured marine and freshwater fish.

For the last 2 years I have also developed an interest in aquatic mammals and wildlife pathology and diseases and I hope I will continue further this research in the future.

## Presentation 1

### **Flavobacterium columnare / Myxobolus tilapiae Concurrent Infection in the Earthen Pond Reared Nile Tilapia (*Oreochromis niloticus*) During the Early Summer**

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*Flavobacterium columnare* (*F. columnare*), the dermatropic Gram negative yellow pigmented bacteria was isolated from different sites of skin ulcerations in the Nile tilapia (*Oreochromis niloticus*) and Nile catfish (*Clarias gariepinus*) collected from an earthen pond located at an aquaculture station in Sharkiya Province, Lower Egypt during an acute episode of mass kills during the early summer of 2009. An acute infection with *F. columnare* was behind the emergent event of mass mortalities among both populations. Many of the Nile tilapias exhibited typical signs of hole - in- the head like lesions from which *F. columnare* together with the myxosporean spore, *Myxobolus tilapiae* (*M. tilapiae*) were retrieved. Most of the cohabitating infected Nile catfishes exhibited severe form of saddle back like ulcer. The identities of the retrieved isolates were confirmed using morphological, biochemical and molecular tools. The research leads us to conclude that the two diverse etiological agents (*F. columnare* and *M. tilapiae*) under the triggering effect of the abrupt change in the water quality measures (abrupt rise in the water temperature, ammonia, pH, sharp decrease in dissolved oxygen) have synergized together to induce the above mentioned pathology with the consequent re-emergence of fish mass mortalities.

## Presentation 2

### **First Record of Mycobacteriosis in the Dusky Grouper (*Epinephelus marginatus*) from Mediterranean Coastal Shores of Marsa Matrouh Province, Egypt**

Authors (\*indicates Presenter): Eissa A. E.,\* Zaki M. M., Abdel Aziz A., Saeid S.

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*Mycobacterium marinum* (*M. marinum*) was isolated for the first time from the dusky grouper (*Epinephelus marginatus*) collected during the early fall of 2008 emergent mass mortalities among the grouper populations of the Saloum bay and rocky coastal zones of Marsa Matrouh province, Egypt. The acid fast bacilli of the *M. marinum* were successfully detected within the Ziehl - Nielsen stained granulomatous reactions scattered within liver of the collected fishes. Laboratory diagnosis was based on the successful isolation of the pathogen on Lowenstein-Jensen medium after 14 days of incubation at 28°C. Further confirmatory scheme based on the conventional biochemical testing was adopted to ensure the final

identity of the retrieved isolates. Positive acid fast staining, negative PAS staining, Gram positive staining, no motility, growth at 25 °C, 30 °C and no growth at 37 °C, smooth yellow-lemon colored appearance of colonies were all among the criteria used for identifying the retrieved isolates. We hypothesized that, the emergence of the disease in this fish population was triggered by the high magnitude of environmental contaminants, abrupt rise of water temperature, predation, and presence of number of cohabitating reservoir wild animals in the surrounding aquatic environment.



**Dr A. E. Eissa**

Short Biography:

- Alaa Eldin Eissa, D.V.M PhD. (Michigan State University – Dissertation title: Bacterial Kidney Disease in Michigan Salmonids).
- Holder of a PhD. in Microbiology and Molecular Diagnostics of Aquatic Animals from Michigan State University, USA.
- Holder of a Masters in Vet Sciences “Fish Diseases” from Cairo Univ.
- Holder of a BVSc degree from Cairo Univ. accredited to DVM degree from Michigan State University.
- Extensive teaching and research experiences in Veterinary Medicine, Veterinary Pathology, Microbiology, Aquatic Animal Medicine, Aquatic Animal Pathology, Aquatic Toxicology, Environmental Pollution, Molecular Diagnostics, Molecular Pathology and Immuno-histochemistry.
- Speaks , writes English and Arabic very fluently
- Passed the academic writing skills of Michigan State University
- Reviewer at Journal of Wildlife Disease (JWD), Cairo University Journal of Advanced Research (JAR) and Chinese Journal of Oceanology and Limnology (CJOL) and African Journal of Biotechnology (AJB).
- Associate Editor at African Journal of Pharmaceutical Sciences and Pharmacy (AJPSP); Nature and Science.
- Principle investigator of an ongoing 200 K Cairo University Research Fund project “Investigating the roles played by shrimp, freshwater crayfish and fish in the spread of Avian Influenza H5N1 under the Egyptian Territory”.
- Published more than 15 international articles since the end of 2005.
- Attended, lectured and organized a large number of international conferences since 2003.

## Presentation 1

### **Emerging and Resurging Infections of Fish in the Great Lakes: Potential Threat to Conservation Efforts**

Authors (\*indicates Presenter): Faisal M.,\* Loch T. P.

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In recent decades, numerous fish kills have occurred within the Laurentian Great Lakes Basin (LGLB), several of which were attributed to infectious agents, such as the Viral Haemorrhagic Septicaemia Virus, *Renibacterium salmoninarum*, and *Flavobacterium columnare*. As a result, it became apparent that some novel fish pathogens had invaded the LGLB, while others resurged, leading to both explained and idiopathic fish kills. These epizootics and the associated socio-political repercussions brought LGLB fish health programs to the forefront of priorities and many federal and state agencies included fish health metrics as indicators of ecosystem health. In this context, in the last few years, three OIE –reportable fish viral infections appeared in the USA causing devastating losses. The Viral Haemorrhagic Septicaemia (VHS) disease resulted in the issuing of a Federal order that placed restrictions on the movement of fish in the Great Lakes states, a matter that caused economic losses to an already ailing economy. Spring Viraemia of Carp (SVC) also emerged in the United States in 2002 killing thousands of fish. In 1998, Koi Herpesvirus (KHV) has also emerged in the USA killing thousands of common carp (*Cyprinus carpio*) in the State of New York. Novel bacterial infections have also been detected such as *Pantoea agglomerans*, *Carnobacterium maltaromaticum*, *Chryseobacterium piscum*, and over 200 new Flavobacteria spp. that were never reported from North America prior to 2009. Finally, serious outbreaks of epizootic shell disease in American lobster (*Homarus americanus*) have generated concern along the southern New England coast and eastern Long Island Sound. The prevalence and severity of shell disease have increased within inshore areas of southern New England and resulted in significant decreases in lobster catches and marketability. Potential explanations for the emergence of new diseases will be presented with examples from case studies.

## Presentation 2

### **Viral Hemorrhagic Septicemia Ecology and Disease Susceptibility**

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The Viral Hemorrhagic Septicemia virus (VHSV) has invaded the Laurentian Great Lakes basin leaving behind trails of ecologic and economic devastation. This OIE-notifiable disease has been associated with serious infection of 28 freshwater fish species and its range has extended to include the watersheds of four of the Great Lakes. Since none of the

other VHSV-strains have shown such tendency to freshwater fish species, there are mounting concerns that VHSV may continue its devastation outside the Great Lakes basin. As a result, there is an urgent need to better understand the biological characteristics of this emerging VHSV strain, the host-virus interactions in particular. The original isolation of VHSV in the Great Lakes was from adult muskellunge (*Esox masquinongy*) and sequence analysis of the isolated virus proved that it forms a novel sublineage within VHSV genotype IV, designated IVb. Experimental infection studies using multiple species demonstrated that mortalities can be induced in more than 10 species, however, the most susceptible species was the muskellunge, followed by the largemouth bass. The least susceptible were several salmonid species. Depending on the dose of exposure, VHSV infection can run peracute, acute, subacute, or subclinical course of infection. Survivors can harbor the virus for up to nine months with or without shedding into the surrounding water. Moreover, VHSV IVb could be isolated from leeches and amphipods in infected lakes.

### Presentation 3

#### **Bacterial Kidney Disease Dynamics and Control Efforts**

Authors (\*indicates Presenter): Faisal M.,\* Schultz C.

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Since the first discovery of Bacterial kidney disease (BKD) in the Spey and Dee rivers of Scotland in 1933, the disease has spread through Europe, North and South America, and Japan. In 1952, BKD emerged in the Laurentian Great Lakes Basin and within five decades, the disease became enzootic affecting native, introduced, propagated and wild fish of the genera *Oncorhynchus*, *Salvelinus*, *Salmo*, and *Coregonus*. BKD is believed to contribute to natural mortalities of wild and feral fish stocks in the basin. In the late 1980s, BKD is believed to have contributed in the widespread mortalities of Chinook salmon in Lake Michigan that was associated with dramatic declines in salmon fisheries.

The Great Lakes Fishery Commission, through its Fish Health Committee, developed policies and protocols to minimize losses of diseases including BKD. The combination of infected broodstock culling, reduced stocking levels, and improved rearing practices in hatcheries has had an apparent positive effect in reducing BKD-mortalities in hatchery production of both Coho and Chinook salmon, and may have contributed to increased survival of stocked fish in the Great Lakes.

Despite considerable research on BKD, we continue to lack the basic understanding of how its causative agent, *Renibacterium salmoninarum*, spreads throughout the Great Lakes basin. We also lack knowledge about reservoirs of *R. salmoninarum* within the basin, and the biotic and abiotic factors related to *R. salmoninarum* survival and BKD prevalence. Filling these gaps of knowledge is essential for the design and development of strategies for the effective control and prevention of BKD. Recent surveys demonstrated that a number of non-salmonids, wild fish species harbor *Renibacterium salmoninarum* and may contribute to its spread basin wide.

Given the complexity of Great Lakes systems, current research utilizes a reductionist approach to disentangle ecosystem-level processes of importance to disease prevalence and persistence. In particular, host-pathogen-environment interactions involved in BKD are being studied through a large-scale analysis of altered gene expression profiles. Host genes associated with disease resistance are being sequenced, and their degree of expression, in existing natural stocks and hatchery strains determined. The ultimate goal is to develop a protocol for broodstock selection utilizing among others, genetic markers of resistance to BKD.



**Dr. M. Faisal**

Short Biography:

Dr. Mohamed Faisal is a professor of aquatic animal medicine at the Department of Pathobiology and Diagnostic Investigation of the Michigan State University (MSU)-College of Veterinary Medicine and the Department of Fisheries and Wildlife of MSU-College of Agriculture and Natural Resources. Dr. Faisal joined MSU in March of 2001 and prior to that he was a professor at the School of Marine Science, The College of William and Mary, Virginia, USA. He received his doctoral degrees from the University of Ludwig-Maximilian, Munich, Germany. As a veterinarian, he devoted his career to the study of diseases affecting aquatic animals (both freshwater and Marine). In specific, he is interested in deciphering the mechanisms of the pathogen-host interactions; how the pathogens invade its host and overcoming its immune system and how the host reacts and combat the intruding microbes. Over a career that extended 37 years, Professor Faisal has made several milestone discoveries that advanced his field of specialization. During his relatively short tenure at MSU, he was successful in establishing the Aquatic Animal Medicine Program that integrated MSU, Michigan Department of Natural Resources, Michigan Department of Agriculture, and the Great Lakes Fishery Commission. Dr. Faisal's research enabled him to develop several important tools that facilitated the diagnosis of emerging infections and finding promising targets for the biological control of invasive species. He is instrumental in providing guidance to the Great Lakes states on how to manage their fishery resources in the presence of emerging pathogens.

At the international level, Dr. Faisal is the Lead Scientist and Co-Founder of the Living Oceans Foundation, which is undertaking important research to mitigate the effects of diseases on coral reef biodiversity. He is also on the International Pollution Responses in Marine Organisms Association and is Consultant to the Secretary General of the UN on pollution issues. He established a central laboratory for fish disease diagnosis in Senegal 2008. Dr. Faisal has been recognized by a number of national and international awards and honorary degrees.

## Presentation

### **Fish Health Situation in Tanzania**

Authors (\*indicates Presenter): Maly R.

Principal Aquaculture Officer  
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#### 1. Introduction.

Diseases occurrence in fish is not common in Tanzania, owing to the facts that domestication of fish and fish related species are not yet advanced in the country. Also the method applied for fish farming does not open chances for diseases. The Development of diseases in fish is mainly facilitated by environmental pollution in water, bad weather and the overall poor farm management. Once management is poor chances for disease attack is obviously.

#### 2. Fish diseases in Tanzania.

Although Tanzania has a vast water masses which include marine (Indian Ocean) and fresh waters (lakes, rivers and natural and man-made ponds) disease in fish is not a common phenomena. However, internal parasites like nematodes and external ones like argulus can be found in fishes caught in the main water bodies without showing any signs of disease or poor health conditions. In farmed fishes nematodes are rather common particularly when culture establishment is earthen ponds.

#### 3. (a) Possible conditions for disease occurrence in fisheries.

Surface injuries are common in fisheries especial during fishing; hence the situation can cause lesions in fish skin and later on open chances for fungi and bacterial growth. Abrasion of skin happen when water level decreases, this incidence could occur in the water bodies without spread out effect, due to natural buffering especially in the marine ecosystem, but in fresh water microbial growth has been experienced after abrasions.

#### 3. (b) Aquaculture.

In the aquaculture system the incidence of disease occurrences is not common because activities are not well pronounced for the time being. The situation of fungi infections had occurred in the outdoor farms for a while, but fungi were cleared by immersing the affected fish in potassium permanganate solution. In fresh water if this situation appears sodium chloride can be used for treatment. Currently disease in fishes is not a problem in the country, so Tanzania has no particularly veterinarians for aquatic animals, but when fish is subjected to diseases, aquaculturists do take care of the fish health.

#### 4. Conclusion.

In Tanzania diseases in fish is limited and the country is still emphasizing in the policy on the need of avoiding diseases in fishes, even fisheries regulation of 2009 is strongly supporting and sensitizing the production of quality fish products for fetching better market price and profit realization. However, since aquaculture is expanding we need to train aquatic veterinarians for development and sustainability of aquaculture

## Presentation 1

### **Red Mark Syndrome in Rainbow Trout**

Authors (\*indicates Presenter): Metselaar M,\* Thompson K, Adams A.

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#### **History**

Red-mark syndrome (RMS), a disease seen mostly in rainbow trout (*Oncorhynchus mykiss*) and possibly in some other salmonid species, is of unknown aetiology. The disease has been observed in continental Europe (Switzerland, Austria, Germany, France and other countries). A disease with similar clinical signs has been reported in the USA, referred to as Strawberry Disease (SD USA) (4).

#### **Symptoms**

RMS is a skin disease resulting in lesions on the flank of the fish. The wound is typically elevated and red. The severity of the lesion is graded on a scale 1 to 3 (5). The disease occurs when the water temperature is below 15°C. Fish with RMS do not appear sick and continue to feed and grow. Morbidity is high, but mortality is usually very low. This results in a downgrading of stock, which in turn results in substantial economic losses for the trout farmer (5, 6).

#### **Aetiology**

Although RMS has now been studied for several years, the cause of the disease has not yet been established. Several agents have been linked to the disease, such as *Flavobacterium psychrophilum* (1, 2), Rickettsia-like organism (RLO) (4, 5), and an Adenovirus (3), while a hypersensitivity reaction has also been proposed. The general belief is that it is a bacterial disease because it is possible to transmit RMS by stocking non-infected fish in the same tank with diseased fish, and treating the disease with antibiotics reduces healing time(6). As of yet, no definite agent has been isolated from diseased fish.

#### **Treatment of RMS**

It is possible to treat RMS with antibiotics and disinfectants, but left untreated RMS will resolve without major effects or mortalities. There are some reports of systematic fallowing and disinfection of the ponds helping to irradiate the disease from the farm.

#### **Prevention**

Prevention is better than treatment, however this requires knowledge of the causative agent. Current research in the Aquatic Vaccine Unit at Stirling is focusing on this.

This PhD project is funded by Intervet Schering Plough Animal Health.

## Presentation 2

### **Advanced Diagnostics**

Authors (\*indicates Presenter): Metselaar M., \* Westerhof I., Kik M.

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**Disease:**

Mycobacteriosis, fish tuberculosis

**Signalment:**

Moenkhousia with negative buoyancy/ abnormal pitch

**Differential Diagnosis:**

Rupture of swim bladder

Collapse of swim-bladder

Filling with fluid

Intra-coelomic space-occupying lesions

Heavy foreign bodies

**Diagnostics:**

X-ray, Ultrasound, biopsy of swim-bladder, Bacteriology

**Treatment Options:**

Mycobacteriosis is usually the result of suboptimal environmental conditions. Once the mycobacteria are established they are hard to control. Subclinical infections are very common and so far hard to detect in the living fish. This makes it hard to control. Antibiotics have been tried in experimental trials before, but improvement of management is mandatory. The bioavailability of the medicine is unknown, and in small fish the medicines are difficult to administer.

**Resolution:**

Stamp out and disinfection and/or improved management and increased monitoring.



**Dr M. Metselaar**

Short Biography:

I'm a Dutch veterinarian currently working as a PhD student at the Institute of Aquaculture in Stirling. During my first degree I work as a fish vet in the Netherlands, mainly with koi but also other ornamentals. My PhD is on a new disease in rainbow trout, called Red Mark

Syndrome. The disease causes high economic losses through its high morbidity but has a low mortality. The main symptom is lesions on the side of the fish, there is no clinical sickness as the fish eat and grow normally. The cause of the disease is not known and the essence of my project. To find the causative agent I use a wide range of techniques but the main ones are immuno-histochemistry, PCR in situ hybridisation and sequencing to make the agent visible and cell-line and agar isolation to try and grow the agent. The main objective is to develop a vaccine to prevent the disease.

Presentation

**Effect of Modified Atmosphere Packaging on the Sensory Quality of Mussels (*Mytilus galloprovincialis*) During Refrigerated Storage**

Authors (\*indicates Presenter): Pantazis, P.A.\*, Bitchava, K., Athanassopoulou, F.

Laboratory of Ichthyology & Fish Pathology, Faculty of Veterinary Medicine, University of Thessaly, Greece.

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The present work aimed to study the effect of modified atmosphere packaging on the sensory quality of mussels (*Mytilus galloprovincialis*) during refrigerated storage. Mussels were packed under two modified atmospheres MAP 1 (50% CO<sub>2</sub> / 50% N<sub>2</sub>), MAP 2 (80% CO<sub>2</sub> / 20% N<sub>2</sub>) and aerobic (CONTROL) conditions. The samples were then stored at 4°C for 12 days. Sensory attributes (odour, appearance, taste and overall acceptability) of the samples were evaluated by a trained panel at 3 days intervals up to the end of refrigerated storage. The sensory attributes showed that CONTROL samples remained acceptable up to the 6th day of storage at 4°C. MAP 1 and MAP 2 samples remained acceptable up to the 9th day of refrigerated storage. MAP 1 and MAP 2 samples presented not significantly different ( $P > 0.05$ ) organoleptic properties up to the 6th day, while MAP 2 samples presented significantly higher ( $P < 0.05$ ) scores for odour and overall acceptability than the MAP 1 samples on the 9th day of storage at 4°C.

Presentation

**Non-Indigenous Freshwater Crayfish Species in Greece and Risks for Biodiversity**

Authors (\*indicates Presenter): Perdikaris C.,\* Paschos I., Athanassopoulou F.

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Non-indigenous freshwater crayfish species (NICS) thrive in European waters. Currently, NICS in Europe represent 62% of total species, although endemism is 75% in Greece. The occurrence of signal crayfish in at least one satellite population in the artificial Lake of Agra (Macedonia), the uncontrolled importation of aquarium pet species and the increasing farming interest could impact in the future the remaining populations of indigenous crayfish species (ICS) in the country. The current contribution aims to stress out issues related to NICS and ICS in Greece and to propose actions needed to minimize-prevent possible negative impacts on biodiversity.



**Dr C. Perdikaris**

Short biography:

Dr Costas Perdikaris (BSc Ichthyology, MSc Aquaculture, PhD Astacology) works as an Ichthyologist in the Fisheries Department of the Prefecture of Thesprotia (NW Greece) and as visiting lecturer in the Department of Aquaculture & Fisheries, Technological Educational Institute of Epirus (Greece). He is actively involved in freshwater aquaculture research.

## Presentation 1

### **Controlling Chytridiomycosis in Frogs in the Pet Industry**

Authors (\*indicates Presenter): Saint-Erne N.

PetSmart Inc.  
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Dwarf Frogs (*Hymenochirus curtipes*) are popular aquarium pets, but they can be carriers of a devastating fungal infection, Chytridiomycosis (*Batrachochytrium dendrobatidis*). This fungus has been spreading across the globe since 1939 and is linked to the decimation of wild frog populations. The global transportation of frogs and other amphibians for scientific research and the pet trade brings with it the risk of spreading this fungal infection. The frogs can be treated for the fungus with a variety of topical antifungal medications (Betadine, Benzalkonium chloride), as well as through heating the water to a temperature that kills the fungus but is safe for the frogs. *Batrachochytrium dendrobatidis* grows within a wide range of temperatures (4–28°C) and grows optimally at 17–25°C. But the fungus is highly sensitive to heat, and will die within 4 hours at 37°C. Preventive treatments using one of these methods will reduce the risk of spreading this serious fungal infection, and reduce the losses associated with the fungus in aquatic frogs kept as pets.

## Presentation 2

### **Common Tropical Fish Diseases, Diagnosis and Treatments in the Retail Pet Industry**

No abstract available.

## Presentation 3

### **Ova Retention in Female Koi (*Cyprinus Carpio*)**

Authors (\*indicates Presenter): Saint-Erne N.

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Koi are a popular ornamental pond fish. The normal reproductive cycle has breeding occurring in the spring as the pond water temperature increases. Due to various factors, in some instances older female koi may not express their eggs (ova or roe). Retention of the egg mass can cause compression of the abdominal organs, kidney dysfunction that produces osmoregulation abnormalities (edema), and egg necrosis. Gonadal sarcomas also occur in koi and may be associated with egg retention. Abdominal distension and abnormal swimming behavior are some of the signs associated with egg retention, and the disease can progress to causing death. Diagnosis of egg retention can be made through radiography and sonography, as well as through laparoscopy and exploratory surgery. Treatment can be

provided through environmental manipulation to stimulate egg laying, or by use of hormonal stimulant injections and manually stripping the ova. In some cases surgical removal of retained ova has been successful. Environmental conditions should be monitored to ensure appropriate conditions for future normal reproduction cycles.

#### Presentation 4

### **Diagnosis and Treatment of Diseases in Guppies and Angelfish**

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PetSmart Inc.  
USA

Email: [Saint-Erne@Q.com](mailto:Saint-Erne@Q.com)

#### **Disease:**

External protozoa, Monogenean Flukes, Intestinal Protozoa, Intestinal Nematodes

#### **Signalment:**

Weight Loss, Poor Growth, Sudden Death

#### **Differential Diagnosis:**

Poor Water Quality

#### **Treatment Options:**

Medicated food, Medicants added to the water

#### **Resolution:**

These species are two of the most commonly sold tropical fish, yet have among the highest loss rate of aquarium species. Treatment protocols have been shown to significantly reduce the mortality in these two popular species of tropical fish. Unfortunately, the fish breeders and wholesalers do not routinely provide treatments to these fish, so it falls on the retail pet store or the purchaser of the fish to provide the treatment. Diagnostic tests and treatments by a veterinarian examining the fish at the store or after purchased by and aquarist will greatly reduce losses associated with the described diseases.



**Dr. N. Saint-Erne**

Short biography:

Nick Saint-Erne, DVM has been continuously involved with pet fish since setting up his first aquarium as a child in 1968. His major field of study was Fisheries Biology for his Bachelor of Science degree. In 1984 he received his Doctor of Veterinary Medicine degree from Kansas State University. He practiced small animal and exotic pet medicine in Las Vegas, Nevada for 15 years (1984-1999).

Since 1999 he has lived in Phoenix, Arizona where he is the Technical Services Veterinarian for PetSmart Inc. PetSmart is the largest North American pet and pet supply retail company. His current role is to supervise the care of the fish sold at 1200 PetSmart stores. This includes annual visits to fish breeder's facilities, and supervising the health care of the tropical fish in PetSmart's 5 distribution facilities, containing in total almost 20,000 aquariums in buildings ranging in size up to 50,000 square feet of state-of-the-art aquarium systems.

## Presentation

### **Developing Efficient & Effective Biosecurity Programs For Ornamental and Commercial Aquaculture Clients**

Authors (\*indicates Presenter): Scarfe A. D.,\* Walster C. I., Palić D.  
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“Biosecurity” is a relatively recent term gaining prominence and is appropriately applied to describe procedures associated with preventing infectious and contagious diseases. In most situations it may be impossible to totally isolate most livestock or aquaculture operations from any and all diseases at all times. Consequently biosecurity programs must also include contingencies for inadvertent disease outbreaks. Appropriate biosecurity programs therefore including prevention, control and possible eradication measures.

To be effective, useful and justifiable to all stakeholders (including producers, industries, service providers and government agencies) biosecurity programs need to include: 1. a series of integrated and documentable processes and procedures that focus on optimal disease prevention, control and eradication practices; 2. be founded in sound science; 3. use practical approaches that are easily understood; 4. be as economical as possible; and, 5. have strong incentives to encourage compliance. These requirements are equally important for all programs, whether voluntarily initiated at farm or industry level, or mandated through government legislation and regulations.

This presentation will describe how veterinarians can assist clients in utilizing these principles to develop and implement biosecurity programs in any epidemiological unit (from individual tanks or ponds, whole farms, compartments, zones or watersheds, or any geopolitical areas such as states/provinces, nations, or regions of the world) and meet legislative or regulatory requirements and OIE standards.

Typical for biosecurity plans to be functional, effective and justifiable they need to involve several formal processes, including: hazard and risk analysis (hazard identification and prioritization, risk assessment/evaluation, risk management/mitigation and risk communication); analysis and remediation of critical control points (including evaluation and mitigation plans for correcting practices where disease could enter or leave the epidemiological unit); epidemiological principles (including necessary diagnostics, surveillance, monitoring and determining the status or freedom of diseases in the epidemiological unit); emergency preparedness (contingency protocols for disease control and eradication); and, auditing of procedures and records, and certification (providing assurance of disease freedom and useful as compliance incentives). Each of these processes and procedures will need to be integrated into aquaculture biosecurity plans and programs.

## Presentation

### **Molecular Epizootiology of Streptococcosis/Lactococcosis in Iran Aquaculture**

Authors (\*indicates Presenter): Soltani M.,\* Haghigi S.

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*Streptococcus* sp. is gram- positive coccus that causes streptococcal infections in fish due to intensification of aquaculture and cause significant economic losses in fish farm industry worldwide including Iran. In the present work the molecular epizootiology of streptococcal/lactococcal infections was studied in the cultured rainbow trout (*Oncorhynchus mykiss*) farms in seven provinces of Iran located in the north-west, north-east and south-west during 2006- 2008. Phenotypical tests as well as single and multiplex PCR using the universal and specific primers for detection of *Streptococcus* sp., *Enterococcus* sp., *Streptococcus iniae*, *Lactococcus garvieae* and *Streptococcus parauberis* were used. From total 113 cocci gram positive bacterial isolates recovered from the diseased fish with typical clinical signs exophthalmia sometimes together with haemorrhage and cataract, darkening of body, abdominal distension and prolapse of anus, 51 samples were identified as *S. iniae* and 40 samples were identified as *L. garvieae*. The rest 22 bacterial isolates were identified as a miscellaneous species of streptococcus genus including *S. agalactiae*, *S. parauberis*, *S. dysagalactiae* and *S. facieum*. The obtained results showed that disease outbreaks by *S. iniae* and *L. garvieae* are dominant aetiology of streptococcosis/lactococcosis in rainbow trout aquaculture in Iran. However, infections by other *Streptococcus* species are sometimes involved with the aquaculture industry in the country.

Key words: *L. garvieae*, *S. iniae*, PCR assay, *S. parauberis*, *S. agalactiae*, *S. dysagalactiae*, *S. facieum*

## Presentation 1

### **The Control of a Pathogenic Strain of *Aeromonas sobria* Using a Refugium and Phytoremedial Device**

Authors (\*indicates Presenter): Tepper J. M.

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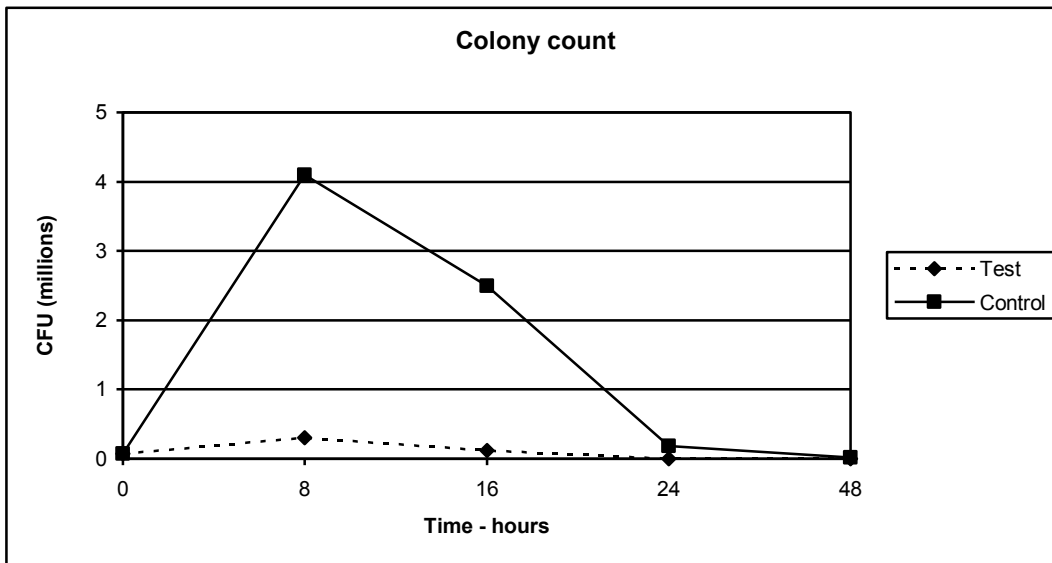
Based on clinical evidence suggesting superior control of ulcer disease in hospitalized koi, the hospital quarantine setup first described in 2004, using a refugium with upflow filtration and a phytoremedial device, was tested to prove its ability to control the growth of a pathogenic strain of the bacteria *Aeromonas sobria*.

After an outbreak of naturally occurring ulcer disease in a koi pond, a specimen was hospitalized for treatment. A culture was taken upon admission. The results were a marked growth of *Aeromonas sobria*. The bacteria was grown and maintained in the lab in tryptose soy broth. An experimental setup based on one previously described<sup>1</sup> was constructed using two identical 10-gals. (37.85l.) tanks. Each was fitted with a 2-gal. (7.56l.), 4" (10.16cm.) deep refugium. Water was circulated to the refugium via a submersible pump valved to deliver 100 gal. /hr. Each tank was fitted with a 200 watt heater and maintained at 24°C. The water in each setup was enriched with 18 ml of tryptose soy broth and inoculated with 5 ml of the *Aeromonas sobria* culture to give a starting dilution (0hrs.) of  $7.7 \pm 0.1 \times 10^4$  colony forming units/milliliter of tank water (CFU/ml.). A floating, phytoremedial device, unplanted and inert, was placed in the control unit refugium. A biologically active unit, consisting of a live *Impatiens* (*Impatiens sp.*) plant grown in a koi pond for several weeks prior to testing, was placed in the test unit. Sampling was done of the effluent of each refugium at 8, 16, 24 and 48 hrs. post- inoculation. Viable bacteria were determined by tenfold serial dilution and colony count method by plating on blood agar plates. The results are given below in CFU/ml (x million)

Results: The control unit allowed exponential growth of the bacteria. This would be expected in enriched water environments under ideal growth conditions, as might be found in a newly set up quarantine- treatment unit. In the test unit, the device controlled bacterial growth near starting levels and impeded the explosive growth seen in the control unit.

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<sup>1</sup> Tepper, J. M. Proceedings IAAAM 2000, 2004  
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## Presentation 2

### The use of an Upflow Refugium and Phytoremedial Device for Water Purification in Clinical Pet Fish Practice

Authors (\*indicates Presenter): Tepper J. M.

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A phytoremedial device<sup>1</sup>, coupled with an upflow refugium, functioned efficiently for water purification in koi ponds and in tanks with high stocking density over extended periods of time (Figure 1).

The design of this system utilized the biofiltration capabilities of the device to convert ammonia to nitrite to nitrate with uptake and utilization by the terrestrial plant *impatiens*, *impatiens* sp. Coupled with this function was the abundant microfauna of protists, rotifers and daphnia removing small, suspended particulates. Key to the effective function of the refugium is the elevated production of agglutinins. These substances are likely produced by the abundant nematodes found in the device and are transferred to the water (Figure 2). Removal of small particulates is accomplished by their agglutination into larger masses, with an increase in density allowing for settlement against the upward water flow. This allows the refugium to function efficiently with these devices placed anywhere in the aqua system and with no filtration media to require cleaning.

In hospital quarantine tanks used for treatment in pet fish practice, this system presents a significant cost savings by reducing a) initial equipment cost; b) floor space needed to house and treat large specimen koi; c) time spent on capture and restraint; d) time spent on cleaning filters and changing water.

Figure 1.

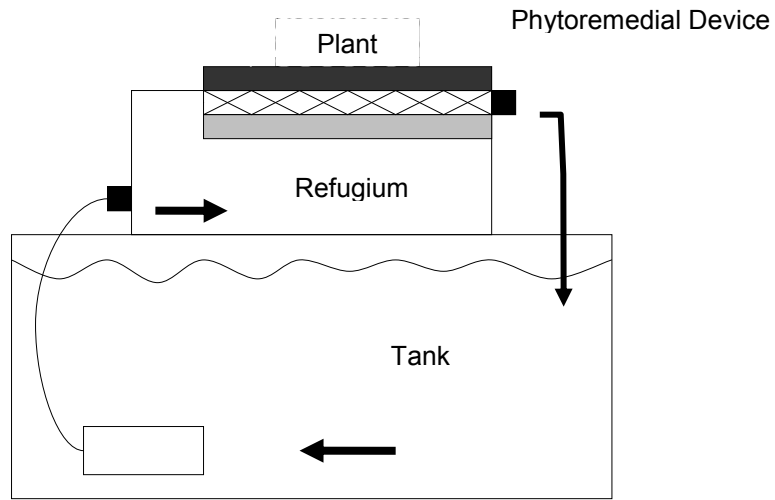
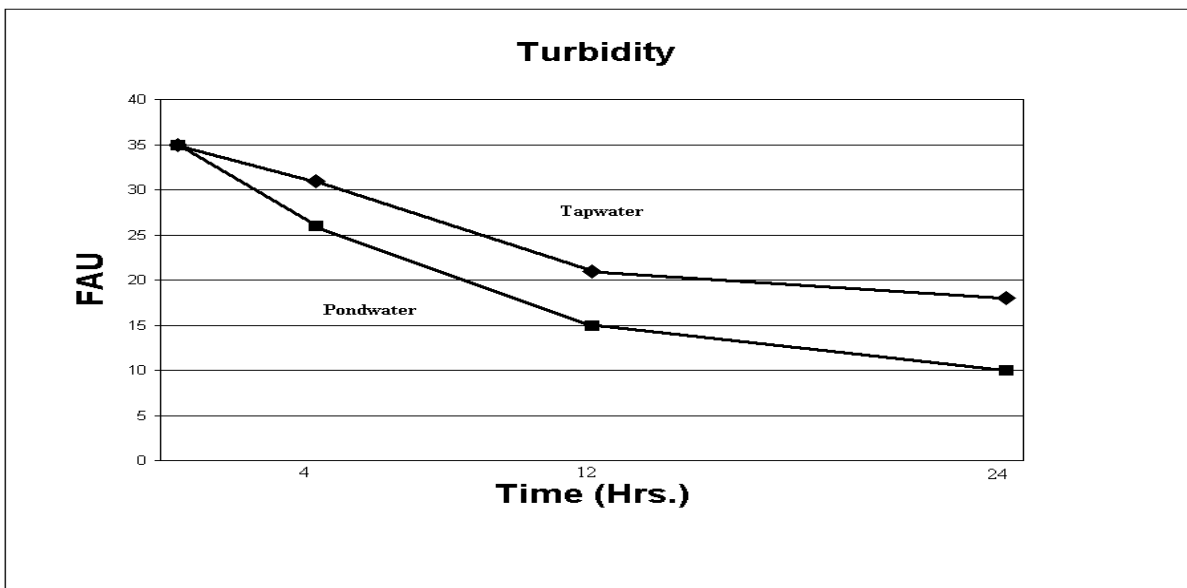


Figure 2.





**Dr. Julius M. Tepper, DVM**

Short Biography:

Born and raised in NYC, Dr. Tepper graduated from York College of CUNY in 1971. He then went to Europe to study veterinary medicine, graduating with honors from the University de Liege in Brussels, Belgium in 1976. Returning that year to NYC, he began practicing and held a special interest in birds and exotic animals. After many requests from clients, he opened the Long Island Fish Hospital in 1998 to care for the health of pet fish.

Dr. Tepper served as Treasurer of the International Association for Aquatic Animal Medicine from 2006- 09, as an Officer on the Executive Board of the World Aquatic Veterinary Medical Association since its formation in 2006, and is currently President-Elect. He is a member of the Association of Reptile and Amphibian Veterinarians, Association of Avian Veterinarians, the AVMA and state and local veterinary associations.

With a special interest in ponds, watergardens and koi, Dr. Tepper developed a phytoremedial device for water quality improvement and received a U.S. patent for this invention. Known as “Pond’toons”, these are currently in use in ponds, watergardens and in quarantine and treatment tanks for koi. Dr. Tepper has authored many papers on fish health and pondkeeping.

## Presentation

### **Is KHV Zoonotic? Assessing the Zoonotic Potential of Aquatic Animal Diseases**

Authors (\*indicates Presenter): Walster C. I.

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A zoonotic disease is a disease that can be transmitted from animals to people or vice versa. In general the disease usually exists in animals but can infect humans either directly or via a vector. Within aquatics the perception is that there are few zoonotic diseases considered globally as import and for those that are recognised the number of cases per year is small compared to other zoonotic diseases such as campylobacteriosis or salmonellosis. Whilst this might be correct, there is a possibility that this is an underestimate, due to poor awareness and surveillance. However, for those that are diagnosed the consequences can be severe including death.

Many diseases found in aquatics can be classified as emerging diseases, defined by WHO (The World Health Organisation) as, "An emerging disease is one that has appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range". One attribute of emerging diseases is that information on the zoonotic potential is limited, yet where a potential exists it is essential to ensure that information is disseminated to other professionals and the public effectively and quickly. This can be done by a qualitative risk assessment. Questions which need to be answered in carrying out the assessment are:

- What is the distribution?
- What is the prevalence?
- What is the aetiology?
- What is the epidemiology?
- What clinical disease is caused?
- Are diagnostic tests available?
- Is there any zoonotic potential?
- What are the potential sources of human exposure?
- Would zoonotic disease be detected?

Using a risk assessment algorithm after Spearman (see figure 1) the zoonotic potential of Koi Herpes Virus Disease will be assessed and some of the difficulties highlighted.

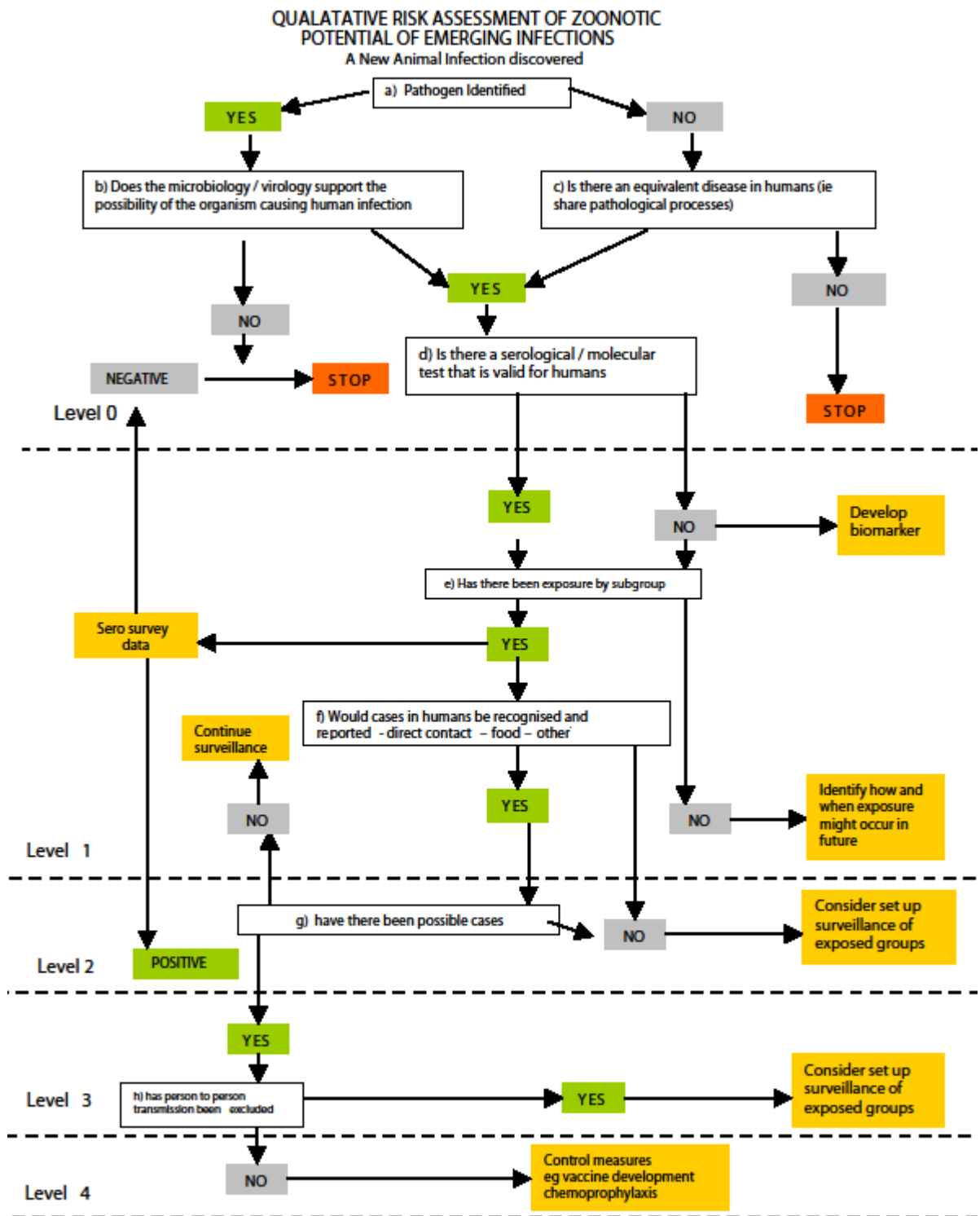


Figure 1



**Dr. C. I. Walster BVMS MVPH MRCVS**

Short Biography:

Dr. Chris Walster lives near Stafford and is the senior partner of an eight centre veterinary practice based in the English Midlands and has been involved in aquatic animal medicine for over 20 years. In 2007 he was awarded a Masters in Veterinary Public Health which stimulated an interested in epidemiology.

## Presentation

### **Vaccination Programs - A Practical Guide to Using Different Vaccination Methods and Technologies to Protect Fish Through the Production Cycle**

Authors (\*indicates Presenter): Wardle R.,\* Gould C., McLoughlin M.

Robin Wardle, Director Global Technical Services and Market Support, Intervet/Schering-Plough Animal Health

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Vaccination is the preferred method of disease control in many aquaculture sectors. This paper reviews some of the issues relating to vaccination and the latest trends in vaccination programs using a variety of different technologies and vaccine types to achieve the best overall disease control against single and multiple pathologies.

Controlling disease by vaccination has historically depended upon choosing a single vaccine and using it within the constraints that the method of administration and the possibility of handling the fish population imposed upon the farmer and vet prescribing the vaccine.

Intervet/Schering Plough

Animal Health has been working in many different disease areas and using different vaccine technologies, has developed vaccination strategies to overcome these restrictions.

This talk will provide insight for implementing a vaccination program and the science behind these developments, and, using examples from farmed salmonids and marine fish species, will highlight the ways to develop vaccination programs for most effective use of these vaccines in the field.

The talk will also cover recent developments in salmon vaccination in Norway, trout vaccination in Europe and vaccination of sea bass. These cover the major variables in vaccination including protection against viral and bacterial pathogens and protection for both short and long- term immunity.

## Presentation 1

### **Problems in Transporting 20 Tons of Live Eels from Egypt to the Netherlands**

Authors (\*indicates Presenter): Werkman P.

Netherlands

Email: [werkman2@zonnet.nl](mailto:werkman2@zonnet.nl)

Problems arose when 20 tons of live silver eels (eels fully grown and on their way to the Sargasso Sea) arrived after a 14 day journey by boat and truck from Alexandria, Egypt to Breda, The Netherlands.

Fishes showed abrasions, wounds and infections on arrival. I was asked to guide this transport and try to find out the cause of this quality problem. A rather easy solution was found.

## Presentation 2

### **Pangasius Breeding Problems in India**

Authors (\*indicates Presenter): Werkman P.

Netherlands

Email: [werkman2@zonnet.nl](mailto:werkman2@zonnet.nl)

Breeding Pangasius in a region North of Kolkata, India is being done on a small scale. Problem: Eight days after hatching, five days after being put in a pond 80% of the fry died. I made suggestions for lowering the mortality rate.

## Presentation 3

### **Surgery in Pet Fishes**

Authors (\*indicates Presenter): Werkman P.

Netherlands

Email: [werkman2@zonnet.nl](mailto:werkman2@zonnet.nl)

Some tips of do's and do not's are discussed with examples of performing simple surgical removal of tumours and eyes in fishes



**Dr. P. Werkman**

Short biography:

Peter Werkman graduated in 1972 at the University of Utrecht, Netherlands to become Dierenarts (DVM). After 8 years of farm animal practice, he then worked as a small animal and fish practitioner in The Netherlands for 27 years. Since 2009 he is a practitioner for fishes only.

Fish farmers and pet-fish owners, wholesalers and zoo aquarium fishes have been seen by him not only in The Netherlands, but also in Egypt, Malawi, Nigeria and recently in India.

## Presentation 1

### **The Role of Intestinal Microflora under Acute Stress Conditions on the Health of European Sea Bass *Dicentrarchus Labrax L.* Possible Translocation.**

Authors (\*indicates Presenter): Yiagnisis M.,\* Alexis M. N., Athanassopoulou F.

Marine Microbiologist, Hellenic Center for Marine Research, Aquaculture Institute.  
Fish nutrition and Pathology Lab. Agios Kosmas, GR 16777, Helliniko, Athens. Greece

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Acute stress caused by netting and transportation operations gives the chance to main bacterial pathogens, as *Listonella anguillarum*, to invade fish intestine and create pathogen carriers or to cause disease. Also acute stress permits in other dominant vibrio bacteria of intestinal microflora to be passed from the gastrointestinal tract to out-intestinal positions like blood circulation and cause mortalities. Bacteriological examinations of intestine and kidney were performed in groups of sea bass before and after acute stress handlings. Vibrio bacteria, pre-existing as dominant in healthy fish microflora, were isolated 1-4 days after handling, from the kidney of handled diseased fish.

## Presentation 2

### **The Effect of Different Oxygen Levels on the European Sea Bass Health under Conditions of Acute Stress.**

Authors (\*indicates Presenter): Yiagnisis M.,\* Govaris, A., Bitchava K., Athanassopoulou F.

Marine Microbiologist, Hellenic Center for Marine Research, Aquaculture Institute.  
Fish nutrition and Pathology Lab. Agios Kosmas, GR 16777, Helliniko, Athens. Greece

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Three experimental groups of sea bass, with average weight of 110 grams, were reared for two months under different dissolved oxygen levels (2.2, 3.5 and 7 ppm O<sub>2</sub>) but under the same sea water flow 30 l kg<sup>-1</sup> h<sup>-1</sup>. The higher food intake in normal oxygen level compared with the other two levels, resulted in an increase in the total aerobic intestinal microflora load of at least 5 times, compared to the lowest level. The occurrence of mortality, after acute stress in the form of intense handling, by transferring them to small tanks of 10 litres for five minutes without aeration and water renewal and then returned to their own rearing tanks, was higher (26.6%) for the group of normal oxygen, 13.3% for the group of 3.5 ppm O<sub>2</sub> and nil for the lowest level of 2.2 ppm O<sub>2</sub>. The additional stress due to change of environment (tank) caused an increase in the average bacterial infection of the bass at all levels of oxygen, especially in the group of lowest oxygen.

### Presentation 3

#### **Large Marine Fish Kill due to Infestation by Toxic Alga *Chatonella Sp.***

Authors (\*indicates Presenter): Yiagnosis M.,\* Pagou K., Prapas A., Rigos G., Bitchava K. Nikoloulaki C., Athanassopoulou F

Marine Microbiologist, Hellenic Center for Marine Research, Aquaculture Institute.  
Fish nutrition and Pathology Lab. Agios Kosmas, GR 16777, Helliniko, Athens. Greece

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**Disease:**

Toxicity from phytoplankton

**Signalment:**

Inflated swim bladder, necrotic foci to gills with a lot of mucus, a lot of the alga present in fresh smears of gills.

**Differential Diagnosis:**

Oxygen depletion, toxic gases

**Treatment Options:**

None

**Resolution:**

None



**Dr. M. Yiagnosis**

Short Biography:

**Mary Yiagnosis:** Hellenic Centre for Marine Research. Aquaculture Institute. Marine Biologist, M.Sc. in Biological Oceanography, PhD candidate, Laboratory of Ichthyology and Fish Pathology, Veterinary School, University of Thessaly. Speciality: Fish and Water Microbiology.

Presentation 1

**Study on Viral Nervous Necrosis (VNN) as a new Invasion and Epizootic Disease in Caspian Sea**

Authors (\*indicates Presenter): Zorriehzahra M. J.,\* Gasemi M., Ghiasi M., Karsidani S. H., Nakai T., Sharifpour I, Shau-Chi C., Soltani M., Saeifouri P., Najafi J., Asghar Said A.

Iranian Fisheries Research Organization (IFRO), Coldwater Fishes Research Center (CFRC), NACA Regional Lead Center in Iran ( RLCIr ) for Coldwater Aquaculture, 18th Km of Dohezar Road, P. O. Box: 46815-467, Tonekabon, , Mazandaran, Iran.

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Unknown acute morbidity occurred in wild golden grey mullet (*Liza auratus*) of Caspian Sea in the Guilan province of Iran in February, 2004. First announcement of the morbidity was reported in Ziba-Kenar region of Rasht city, in 10.4-10.8<sup>o</sup>C water temperature and fish size of 15-20 cm. Viral Nervous Necrosis (VNN) or Viral Encephalopathy and Retinopathy (VER) caused by piscine nodavirus (Nodaviridae). It is a worldwide disease that affecting many species of marine fish, and causing high mortalities of affected larvae and juveniles. VNN was first described by Yoshikoshi & Inoue (1990) in Japanese parrotfish (*Opelegnathus fasciatus*) in Japan. Disease has been reported as a serious viral disease of larval and juvenile and sometimes older marine fish that occurs worldwide except for Africa (OIE, 2003). In order to determine the cause of the mortality, examinations were carried out from various angles. These include environmental surveys, bacteriological, hematological, molecular biological and histopathological examinations. Results suggested that the present mortality of golden grey mullet in Caspian Sea is associated with viral nervous necrosis (VNN) caused by piscine nodavirus. Affected fish weighting 200-250 g were collected for examinations. Hematology, Histopathology, Bacteriological, Parasitological, Ecological and Virology survey were applied. Necropsy was done in aseptic condition on fish frozen at -20<sup>o</sup>C. Tissues such as liver, kidney, spleen, eye, and brain, were removed, homogenized with PBS, and centrifuged at 2,000 rpm for 10 minutes. The supernatant was passed through 0.45 µm membrane filter. These supernatants were sent to Hiroshima University (O.I.E. Reference Laboratory for VNN) Japan and National Taiwan University. New primer sets (Nakai, unpublished) were used for RT-PCR and nested PCR. The sequence data obtained was compared with representative coat protein gene sequences of piscine nodaviruses which belong to different genogroups; SJNNV, TPNNV, BFNNV and RGNNV (Nishizawa et al. 1997, Iwamoto et al. 2001). For the pathogenicity test, sevenband grouper (*Epinephelus septemfasciatus*) weighting average 45 g was challenged with the filtered homogenate of pooled brains of the diseased golden grey mullet (water temperature: 21<sup>o</sup>C). Clinical signs of moribund fish were erratic swimming behavior such as spiral and belly-up at rest, lethargic appearance without any surface erosion. The gross pathological changes were characterized by gas accumulation and high distension in swim bladder, yellowish liver, liquefaction of gall in gall bladder, presence of excess micro sands accumulation in caecum with hyperaemia of intestine. Examination of brain sections revealed hyperemia, degeneration and necrosis in external granular layers as well as focal vacuolation. No predominant bacteria were isolated from any internal organs of the affected fish. Neither ectoparasites were not found, nor were protozoans observed in the blood smears. The coat protein gene of piscine nodavirus was detected in all 8 brain homogenates of diseased golden grey mullet by RT-PCR and nested PCR, though the RT-PCR amplicons weakly appeared in the agarose gel electrophoresis.

The sequence analysis on the nested PCR products (177 bases) indicates that the present virus is closely related to reported piscine nodaviruses. Mortality at %100 was produced in sevenband grouper by intravitreal injection of the filtrate of pooled brain homogenates. Fish lost balance and died 4-6 days after injection. The brains of the dead sevenband grouper were strongly positive in RT-PCR test. The sequence analysis of the RT-PCR amplicon (345 bases) revealed again genetic relatedness between the present virus and RGNNV. Moreover, trials to isolate viruses using E-11 cells have not succeeded. Similarity of clinical signs, RT-PCR with primers specific to piscine nodaviruses identified the coat protein gene in the affected brains of golden grey mullet and the nucleotide sequence analysis revealed that the present mullet virus is genetically most related with RGNNV among known 4 betanodavirus genogroups.

## Presentation 2

### **Aetiologic Agents of Fry Mortality Syndrome in Rainbow Trout (*Oncorhynchus mykiss*) in Iran**

Authors (\*indicates Presenter): Zorriehzahra M. J.,\* Mohd Daud H. H., Bejo M. H., Soltani M., Gholizadeh M.

Iranian Fisheries Research Organization (IFRO), Coldwater Fishes Research Center (CFRC), NACA Regional Lead Center in Iran ( RLCIr ) for Coldwater Aquaculture, 18th Km of Dohezar Road, P. O. Box: 46815-467, Tonekabon, , Mazandaran, Iran.

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An investigation was conducted in order to find out the etiological factors of Fry Mortality Syndrome (FMS) that causes serious economic loss in rainbow trout farms in Iran. In recent years obscure fry mortalities have been observed in many hatchery farms in Iran. It was reported that the rate of fry and juvenile mortality increased dramatically in some provinces e.g. 23 million fry were produced in hatchery centers of Chahar Mohal Bakhtiary province in 2002 but nearly 21 million fry (91.3%) in different stages of growth died before distribution to farmers. Also close to 23 million fry were produced in Mazandaran province, but 12 million fry equivalent to 52.12% of total fry production died mysteriously. This investigation was carried out with objectives of detecting and confirming the main causative agent that contribute to the occurrence of Fry Mortality Syndrome in Iran. During 32 months, from October of 2001 until May of 2004, 52 different hatchery centers and rearing farms of rainbow trout (*Oncorhynchus mykiss*) which were located in Tehran, Mazandaran, Guilan, Fras, Markazi, Kerman and Kohkiluyeh Boyerahmad provinces, were visited and various samples from affected farms were collected. Collected samples consisted of ovarian fluid, milts, eggs, eyed-eggs, larvae, fry < 1 g and 1-3 g as well as internal organs from adult fishes. A total of 2,107 samples were collected from farms in six provinces and were examined by five methods such as virology (410 samples), bacteriology (899 samples), serology (consisted of IFAT: 392 samples and ELISA: 44 samples), histopathology (160 samples) and hematology (202 samples). Some of the mentioned approaches such as fish cell culture, ELISA and IFAT techniques were set-up and optimized for the first time in Iran. The clinical signs of suspected fishes were darkening, exophthalmia, ascites, abnormal swimming and whirling. From 410 samples that of tissues inoculated on to cell cultures two samples showed CPE in EPC and BF-2 cell lines which were inoculated with ovarian fluid from broodstock obtained from hatchery farms in Mazandaran province. The CPE was similar to IHN virus induced. The CPE foci revealed dying cells congregated as grape-like clusters (ballony performance with

cytolysis). TEM findings in infected cells showed bullet-shaped particles having sizes of 130-180 nm in length and 65-70 nm in diameter. From the virion morphology it was suggested that observed particles were similar to Rhabdovirus. FAT examination revealed that all samples were examined with MAbs and PAbs against IPNV and VHSV were negative. On the other hand, two samples were positive when examined with MAbs and PAbs against IHNV. These smears were originated from samples that had showed CPE in EPC and BF-2 cell lines and bullet shaped particles in electron microscopy. ELISA findings (cut-off value, optical density and detection-level percentage) showed that IHNV had higher percentage of detection with 23.25% in comparison with other relevant viral diseases i.e. IPNV with 7.31% and VHSV with 14.29%. Hematological findings revealed that total white blood cell count, i.e. lymphocyte and neutrophil in investigated fish showed significant increased compared with the control fish ( $p < 0.05$ ). On the contrary, all the samples showed a decreased in RBC, Hb and HCT values. In addition, MCHC and total protein plasma showed a marked decreased ( $p < 0.05$ ). In the blood serum components analysis, similarly it was revealed LDH and AST showed a significant decreased ( $p < 0.05$ ). In conclusion, with marked clinical signs, cell culture observation and TEM findings, ELISA and IFAT results, histopathology and hematological findings (blood and biochemical parameters) seen in the current investigation lead to possibility of a viral disease agent infection as the cause of fry mortality syndrome in the hatchery and rearing trout farms in Iran. From findings of the current study, it is concluded that IHNV-like virus could be most probable etiologic of fry mortality syndrome in Iran.

Key words: Fry Mortality Syndrome, Rainbow trout, Cell culture, TEM, ELISA, Hematology, IHNV, IPNV, VHSV, Iran



Dr. J. Zorriehzahra

Short Biography:

- Employed at the Ministry of Jihad-e-Agriculture since 1985 as expert in the different positions.
- Member of Scientific Board in Ministry of Jihad-e- Agriculture since 1991 up to now (Assistant professor with more than 20 years experiences).
- Head of Coldwater Fishes Research Center (CFRC) 21<sup>st</sup> July 2008 - up to now
- Project Manager of five research projects that last of them has title as follows:

- Conservation and Restoration of Caspian salmon (*Salmo trutta caspius*) stocks in the Southern Caspian in Caspian Sea Environment Protection (CEP) institution. Jan.2010 up to now.
- Advisor of six academic theses in Master of Science and PhD degree.
- Authors of more than 40 scientific publications (Articles and abstract in Conference proceeding) and six handbooks in fish disease subjects.
- Major interests: identification of viruses isolated during the surveillance, use of laboratory techniques including; serology affairs such as FAT, ELISA, IHC, virology assay, Cell culture, and PCR.

Poster Presentation

**Effects of EM (Effective Microorganisms) Cocktail's on Growth of Fish, Body Weight, Survival in Symphsodon Discus**

Authors (\*indicates Presenter): Bilal T., Aytemiz I., Danyer E.\*

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EM (effective microorganisms) is a cocktail consisting of a microorganism such as phototropic bacteria, lactic acid bacteria, actinomycetes, fungi and yeast. It has been used in Food, agriculture, animal husbandry, fisheries, environmental cleanliness and in the medical field; also some of countries are using this in their environmental policies. Aquarium fisheries is a big industry by nearly \$ 300.000.000 worth of budget in worldwide. The fishes which are in the aquarium have been threats like bacterial, viral, parasitic, fungal diseases that are experiencing significant economic losses.

In our study we are working with four aquariums which were designed in static system containing ten Symphsodon Discus fishes. In breeding we used a ration which was accepted in the world and for the prophylaxis, a week after separating from mother we used metranidazole (250 mg/100 lt) and praziquantel (250 mg/100 lt) and EM (1 ml/1000 lt/day). In study to the first group (control) we used standard diet + metranidazole + praziquantel, 2. group, standard diet + metranidazole + praziquantel + EM, 3.group, standard diet + EM, 4.group, not spirulina exists standard diet + metranidazole + praziquantel + EM was applied. The aim in the not exist spirulina group is to investigate relation between spirulina and EM. Because spirulina is used for protein and colour supplement in Discus breeding. Our purpose is to find the answers that "How effect EM on growth of fish, body weight, survival and to investigate the effect of parameters such as pH and conductivity of water?"

Key words: Symphsodon Discus, aquarium fish, breeding, effective microorganisms, spirulina.



**E. Danyer**

Short biography:

I was born in 1986 in Turkey. I will graduate from Istanbul University Veterinary Faculty this July. I have undertaken studies about marine mammals' pathology and parasitology I have undertaken studies about nutrition of pet fishes.



**I. Aytemiz**

Short biography:

Işıl Aytemiz is a fourth year student at Istanbul University Faculty of Veterinary Medicine and also studies as a student assistant in Department of Nutrition and Nutritional Diseases. She has been focusing her studies on aquatic medicine for two years.

Her study in Necropsy Techniques in Small Marine Mammals was considered worthy of Poster Presentation Award in 11th International Scientific Research Congress. Also, she has endeavored to point out the importance structuring of the Search and Rehabilitation Centers for small marine mammals and sea turtles. She has done research about nematodes in Harbour Porpoises' stomach contents in Western Black Sea and Effects of EM (effective microorganisms) cocktail's on growth, body weight and survival of fish in Symphsodon Discus.

She served in various positions at Istanbul University Scientific Research Club and finally served as the president of the Club and 11th International Veterinary Medicine Students Scientific Research Congress in the year 2009. She is continuing her studies in Istanbul University Faculty of Veterinary Medicine.

Poster Presentation

**Toxicity of Raw Oil on Great Sturgeon (*Huso huso*)**

Authors (\*indicates Presenter): Khoshbavar-Rostami H., \*Soltani M., Mokarami A., Yelghi, S.  
Center research of inland waters of aquatic animals stocks, Iran

Email:

Toxicity of raw oil was assessed in great sturgeon provided at water temperature of 22°C, dissolved oxygen 7-9 mg/l, pH 7.9, and total hardness of 145 mg/L. Also, some hematological and biochemical variables were measured in the survival fish after 96 hours. The LC50 96 hours of 4 mg/L was obtained. The levels of RBC and Hb were lower in test fish compared with control fish, while PCV, MCH, MCHC and MCV levels were higher in test fish. Also, leucocytes, lymphocytes and eosinophils counts were lower in test fish compared with control fish, while neutrophils and monocytes contents were higher in test fish. Furthermore, contents of ASP, ALT, and ALP, LAD and total protein were lower in fish exposed compare to control fish, while levels glucose was higher than control fish.

Key words: Toxicity, raw oil, great sturgeon, hematology, biochemistry

## **Previous WAVMA Conferences and Meetings**

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