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WORKSHOP REPORT

Diseases of eels in an international perspective: Workshop on Eel Diseases at the 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, 2011

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Introduction

In recent years, significant attention has been assigned to the decline in the wild freshwater eel stocks worldwide. Possible causative factors include fisheries (overfishing), habitat loss, migration barriers, and chemical pollution, and/or a combination of these factors. Infectious diseases however, as associated causal factors, have received little attention to date. In eel farming systems, diseases are lethal under certain stressful conditions and high stocking densities. Similar environmental or infectious disease conditions may also negatively affect

wild eel populations. Eel stocks also declined in Japan, where eel culture began in 1879 in Fukagawa, Tokyo. Farmed eel gradually decreased from 40,000 to 20,000 tons in 1999 to 2008, whereas eel imports increased year by year from 1995. Following traditional Japanese eel culture, where open ponds were frequently used, from 1972 pond culture inside a green house was introduced, at a water temperature of about 28°C. This change has been considered a main trigger to the appearance of new diseases.

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To address these issues of concern, a workshop on "Eel Diseases" was organized at the 2011 EAAP Conference, at Split, Croatia. In this workshop, with approximately 40 participants from various countries, field observations and research findings on eel pathogens and diseases were presented, with the aim to summarize the information on the status of known and emerging pathogens and novel techniques in diagnostics and therapy, and build a network on eel disease experts.

Parasitic diseases

A 25-years study comprising wild and cultured eel populations in the Netherlands identified eel infection with species specific parasites (*Anguillicoloides crassus*, *Myxidium giardi*), blood parasites *Trypanosoma* spp., and common ectoparasites (*Trichodina* spp., *Ichthyophthirius multifiliis*, *Ichthyobodo* spp., *Chilodonella* spp., *Pseudodactylogyrus* spp. and *Gyrodactylus* spp.). For example, *Myxidium giardi* affected the lateral line system of wild yellow eels, causing a high mortality in 2005; *Trypanosoma* spp. were found in blood in high percentages, mostly in wild silver eels, in parallel to the introduction of *A. crassus* into the Netherlands since the 1980s.

In Austria, the parasite community of eels (N=1044, 1994-2009) investigated in a shallow lake (Neusiedler See, a closed water body) showed characteristics similar to other natural eel populations in Europe, with only six species comprising the component community, and a maximum infra community richness of four species. The intestinal community was dominated by acanthocephalan *Acanthocephalus lucii* or *A. anguillae*, both exhibiting higher infestation levels in larger eels and oscillations of infestation parameters, depending on seasons and

sampling year. Interestingly, after 2004, *A. lucii* was not found in the two sampling sites, which coincided with a drastic decline of its main final host the European perch (*Perca fluviatilis*), whereas *A. anguillae* infestation remained at similar levels as before.

Comparing parasite communities between a typically brackish and a freshwater sampling site in Croatia showed structural and quantitative differences reflected by differences in salinity and season. Component community numbered in total nine helminths and one copepod, three myxozoans and one protozoan species (coccidian *Eimeria anguillae*). While *A. crassus* was more abundant in brackish environment, the freshwater eel population was dominated by *Ergasilus sieboldi*, whilst monogenean *Pseudodactylogyrus* spp. oscillations were observed in both eels populations.

Wild European eels from two different Spanish ecosystems in Western Mediterranean Sea, an oligohaline and a hypersaline (43-46.5 g/l) coastal lagoon (N=454, 2008-2011) as well showed differences in parasitological status. Oligohaline eels were infected by *M. giardi*, *E. anguillae* and two cestode species (*Bothriocephalus claviceps* and *Proteocephalus macrocephalus*), while hypersaline eels showed prevalence of *E. anguillae* and three trematode species (*Deropristis inflata*, *Lecithochirium* sp. and *Bucephalus* sp.). *A. crassus* prevailed in oligohaline environments (82%), while only 3% prevalence was observed in the hypersaline lagoon, suggesting a recent arrival to the latter environment or a lack of adequate intermediate hosts. L2 larvae were the most abundant life-stages indicating persistence of a chronic infection.

In Portugal, apart from many other eel parasites, *Pseudodactylogyrus bini* and *P. anguillae* were isolated from gills of wild eel since 1989. High infection levels have been observed specially in the autumn, coinciding with the period of glass eels arrival to the European west coast. This suggests that these very small eels might acquire a high infection and could suffer mortalities when entering continental waters due to these parasites.

Fish health assessments of newly captured glass eels (*Anguilla rostrata*) in Canada from Maritime Rivers (N=1205, 2006-2010) have been done for inspections of glass eels transferred from Nova Scotia and New Brunswick to the Great lakes in Ontario, where these animals were intended for restocking. In 2006-2009, 4 million eels were restocked, after the testing of 340 elvers (10 individuals per pool) has been done during a 3 weeks pre-transfer quarantine period. The study revealed trophozoite stages of the ciliate *I. multifiliis*, and necrotizing hepatitis with an associated intranuclear microsporidian morphologically consistent with a *Nucleospora* sp. On one occasion, older elvers were found harbouring larval and pre-adult nematodes associated with the wall and lumen of the swimbladder, which morphologically were consistent with *A. crassus*. In addition, this single lot was also infected with an yet unidentified myxosporidian, parasitizing the urethra and urinary bladder.

Bacterial diseases

In the Netherlands two potential zoonotic bacterial species were isolated: *Vibrio vulnificus* (twice related to a zoonosis) and *Edwardsiella tarda*, as well as *Aeromonas sobria*, *A. hydrophila*, *Pseudomonas anguilliseptica* (causative agent of red spot disease), *Flavobacterium* spp., and, in brackish

and marine kept eels other *Vibrio* spp..

Because of the shift to a higher water temperature in the eel culture system in Japan, bacterial infections like *Vibrio anguillarum*, *P. anguilliseptica*, *A. hydrophila*, *E. tarda*, *Flavobacterium columnare* and atypical *A. salmonicida* have been expressing an increased infection rate. Conversely, such changes in rearing systems resulted in reductions of disease caused by *Saprolegnia diclina* and the microsporidium *Heterosporis anguillarum*.

During a health survey of glass eels in Canada between 2006-2010, bacteria (*Yersinia ruckeri*) were only isolated from 1 of 10 samples cultured in 2009.

Viral diseases

In the Netherlands, several viruses infecting European eels have been identified, and in the case of farmed eels these outbreaks were mostly stress induced. The viruses regularly caused a severe hemorrhagic disease with increased mortality, but nonclinical cases were seen as well. Three viral species frequently isolated from both wild and farmed eel are the aquabirnavirus *Eel virus European* (EVE), the rhabdovirus *Eel virus European X* (EVEX), and the alloherpesvirus *Anguillid herpesvirus 1* (AngHV1). EVEX belongs to a group of fish vesiculovirus-like isolates which includes the closely related eel rhabdovirus *Eel virus American* (EVA) and the three historical French eel rhabdovirus isolates, B_{44'}, C_{30'} and D₁₃. Two other French isolates (B₁₂ and C₂₆) are most likely eel novirhabdoviruses. The viruses were found as single or double infections, and were coincidentally associated with one or two bacterial and/or severe parasitic infections. While EVE and EVEX outbreaks generally occurred at

eel farms with water temperatures ranging from 15 to 20°C, AngHV1 disease usually occurred around 26°C. In the Netherlands, EVE has not yet been detected in wild European eel. Other pathogenic European eel viruses have been described and partially characterized (see also Munro et al., 2011).

In Germany, the AngHV1 virus was found to be one of the main threats for both cultured and wild eels, since the virus is widespread in the wild and the majority of eel farms are supposedly latently infected. Elvers may carry different viruses without any clinical signs. After metamorphosis from glass eels via elvers into silver eels, those latently or persistently present viruses may cause disease with high losses. Besides AngHV1, also birnavirus (type Sp), picornavirus, reovirus and different rhabdoviruses (EVEX, SVCV-like, Perch Rhabdovirus-like) were detected and confirmed by cultivation in Eel Kidney (EK-1) cells, PCR, RT-PCR, electron microscopy, immunofluorescence assay (IFAT), serum neutralisation, *in situ* hybridisation (ISH), and/or antibody ELISA. Inactivated vaccines against AngHV1 and birnavirus have been developed and used in a farm for already 5 years, whilst a vaccine against reovirus is currently in preparation.

In Japan, viral endothelial cell necrosis (VECNE) has been a serious threat for Japanese culture of *Anguilla japonica*. Histopathology of diseased eels showed intense congestion and dilatation in the central venous sinuses of gill filaments, and hemorrhages in liver and hematopoietic tissue, remarkably severe in the kidney glomeruli. The causative agent is a hexagonal DNA virus (80 nm), tolerant up to 42°C that induces cytopathic effect in Japanese eel endothelial cell

(JEEC) 7 days after inoculation. Interestingly, experimental challenges with virus showed a high resistance to re-challenge. Moreover, the treatment of fish under non-feeding conditions at 35°C has been shown as an useful measure to control the disease. More recent work focused on the design and trials with double stranded RNA poly immunization of mature fish.

In Canada, no eel viruses were detected in glass eels sampled for pathogen inspections prior to transfer from Atlantic Canada to Lake Ontario between 2006-2010.

Discussion

Infectious eel diseases are a threat to wild and farmed eel. Eel farming is currently dependent on capture of wild glass eels, enabling potential introduction of pathogens to eel farms, which are often closed recirculation systems. Lately, in the Netherlands, farmed yellow eels are restocked into the wild to restore wild eel populations, without prior health check (unlike the transfers noted above in Canada). This represents a risk for wild eels, as EVE infected farmed eel could infect EVE-naïve wild eel populations, resulting in unknown long-term consequences for the wild population.

Which are the most important diseases of eel?

It was agreed by the participants that we should make a distinction between diseases of wild and farmed eels. In the Netherlands, AngHV1 in combination with stress and bacteria are the most important disease factors affecting farmed eels, but it is noteworthy that AngHV1 with *Trypanosoma* sp. and *A. crassus* are often found in wild silver eel. In Germany, elvers are very sensitive to picornavirus and birnavirus and when they enter fresh water, they are more sensitive to

AngHV1 and different rhabdoviruses. AngHV1 is the main problem in German eel culture. The prevalence of *A. crassus* has decreased in the mainland of Western Europe. In the UK *A. crassus* is most important, although this parasite only masked the eel decline. In Japan, VECNE is most important in farmed eels. In South Korea, most important are viral diseases where inactivated vaccines have been used (Joh et al., 2011). In Canada there is a concern over infections of *A. crassus*, which are moving up the Atlantic coast from the US and are currently found in Cape Breton, Nova Scotia.

Which of these diseases may have affected the recruitment of the eel?

Infectious diseases in general should be considered as possible serious factors in the decline of the wild and cultured eel stocks. In the Netherlands, the combination of AngHV1, *Trypanosoma* sp., *A. crassus* and stress in migrating silver eel might induce disease during spawning migration.

Which would be sensible prevention measures?

Health assessment of glass eels, similar to Canadian practice, should be conducted in the European area also. Disease preventive measures need to be formulated and assessed, resulting in strict and detailed international legal measures. In Germany, pilot bath immunisation at farm level against AngHV1 and birnavirus with inactivated virus showed to be successful, with a loss reduction from 90% to 10% in elvers. Farmed eels should be tested for the most important diseases before restocking into the wild, and quarantine and testing should be used when importing eels from other zones.

Which research topics are important for the future on eel diseases?

Some topics were identified:

- Spread of eel pathogens over Europe: a survey.
- Immunity and viruses in relation to spawning - integral approach.
- Influence of contamination and parasites to eel reproduction.
- The immune system during development from glass eel (elvers) to silver eel - how eels handle viruses, bacteria and parasites?
- Presence of AngHV1 in the wild - where do eels get infected in Europe, and what happens when they are in seawater (by use of swim tunnel experiments).
- Molecular characterization and taxonomic analyses of historical eel virus isolates, and development of molecular diagnostic assays.
- Development of a sensitive and accurate molecular assay for the detection of larval infection by the swim bladder nematode *A. crassus*.
- Influence of contamination and parasites to eel migration (by use of swim tunnel experiments and/or tracking systems).
- Prophylaxis and immunoprophylaxis in eel culture (vaccination).

The workshop recommended that eel diseases will be proposed in new international research calls to further protect the eel populations worldwide through knowledge and resulting practices.

Acknowledgements

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References and additional material

Abstracts of the Workshop and Hand-outs

The Abstracts and Hand-outs are available at the EAFP website (www.eafp.org/split-workshops/). For Abstracts of the Workshop, the speaker is underlined.

Bergmann SM, Wonnenmann H, Dauber M and Fichtner D (2011). Virus induced disease in eel (*Anguilla anguilla*) aquaculture and in wild in European waters. Workshop Abstract.

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Haenen O, Roozenburg I, Voorbergen M, van Beurden SJ and Engelsma MY (2011). Eel disease problems in cultured and wild eels since 25 years. Workshop Abstract.

Inouye K, Miwa S, Aoshima H, Oka H and Sorimachi M (2011). A histopathological study on the etiology of intense congestion of the gills of Japanese Eel, *Anguilla japonica*. Workshop Hand-out.

Mladineo I, Konecny R, Schabuss M and Kennedy CR (2011). Composition and richness of intestinal helminth communities in populations of eel, *Anguilla anguilla* - examples from Croatia and Austria. Workshop Abstract.

Muñoz P, Ruiz de Ybañez R, Martínez-Carrasco C, Peñalver J and Mayo-Hernández E (2011). Parasitological status of wild European eels (*Anguilla anguilla* L.) from two ecosystems of the Western Mediterranean. Workshop Abstract.

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Saraiva A (2011). Origin, distribution, and morphology of the genus *Pseudodactylogyrus* Gussev, 1965. Workshop Abstract.

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van Beurden SJ, Galinier R, Haenen OLM and Engelsma MY (2011). Eel rhabdoviruses: How many viruses, how many strains? Workshop Abstract.

Related Conference Presentations

Çolak SÖ, Soylu E, Erdoğan F and Erdoğan M (2011). Metazoan parasite communities of European eel *Anguilla anguilla* L. from Köyceğiz-Dalyan estuarian channel system, Turkey. Oral Presentation O-150. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, Sept. 2011.

Joh SJ, Ahn EH, Choi YJ, Jang H, Lee HJ and Kwon, JH (2011). Bacteria isolated from farm-cultured eels (*Anguilla japonica*) in Korea. Oral Presentation O-152. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, Sept. 2011.

Munro ES, Stewart KJ, Murray WJ and Haenen OLM (2011). Characterization of a virus isolated from farmed European eel elvers (*Anguilla anguilla*) undergoing a mortality event in The Netherlands. Oral Presentation O-140. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, Sept. 2011.

van Beurden SJ, Haenen OLM, Peeters BPH, Rottier PJM and Engelsma MY (2011). Molecular characterization of eel herpesvirus *Anguillid herpesvirus 1*. Oral Presentation O-143. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, Sept. 2011.

Vendramin N, Varvarigos P, Cappelozza E and Bovo G (2011). Co-presence of herpes virus anguillae (HVA) and infectious pancreatic necrosis (IPN) virus in European eels *Anguilla anguilla*: first detection in Greece. Poster Presentation P-220. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, Sept. 2011.