Workshop organized by COST ACTION CA22163 "Solving bottlenecks in eel reproduction to support sustainable aquaculture" (EEL SUPPORT), supported by COST (European Cooperation in Science and Technology)

REPORT

October, 7th & 8th 2024
Pula, Croatia

Incorporated in the program of ***EIFAAC International Symposium***

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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Monday, October 7th 2024

1. PLENARY LECTURE

After registration of EEL SUPPORT participants and coffee networking, a plenary lecture “The quest for eel reproduction: a millennial history and current challenges” was given to all participants (120 participants) of the EIFAAC Sympozium by Sylvie Dufour. In this lecture, activities of CA22163 EEL SUPPORT were presented.

Abstract of the plenary lecture

THE QUEST FOR EEL REPRODUCTION: A MILLENNIAL HISTORY AND CURRENT CHALLENGES

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The question of the mystery of the eel reproduction was raised more than two thousand years ago by the Greek philosopher Aristotle, in the books « History of Animals » and « Generation of Animals ». The European eel, *Anguilla anguilla*, has indeed a complex migratory life cycle with the reproduction in a still undiscovered spawning area in the Sargasso Sea and the juvenile growth in continental waters. Furthermore, the European eel is still sexually immature when it leaves the continent at the silver stage, and remains blocked at this prepubertal stage as long as the reproductive migration is prevented, such as in captivity.

First attempts to induce the eel sexual maturation by hormonal treatments were successfully performed by Maurice Fontaine and co-workers at the MNHN, Paris, in the male (1936), and then in the female (1964). Based on these pioneer advances in the European eel, huge research efforts in Japan were dedicated to the Japanese eel, *A. japonica*, leading to the completion of its life cycle in captivity (2010).

As for other eel species, the population of the European eel has dramatically declined in the last decades, and it is classified as a critically endangered species in the red list of the International Union for Conservation of Nature (IUCN). The collapse of the eel population is emblematic of the multiple impacts of anthropogenic global change, such as overfishing, habitat reduction and degradation, migration impairment, pollutants, and climate change.

Eel aquaculture is largely developed in Asia, and at a much lesser extend in Europe. However, in both cases, aquaculture still depends on catches of wild juvenile glass eels, thus being unsustainable and contributing to the pressure on wild eel populations. This is because on the one hand the control of the Japanese eel life cycle has not reached yet a commercial scale, and on the other hand the life cycle of the European eel has not yet been completed in captivity.

Concerning the control of eel sexual maturation, progress has been made in the characterization of the neuroendocrine systems involved in the blockade and stimulation of the gametogenesis, providing the knowledge base for current or new hormonal treatments. However, large research avenues are still open to decipher the role and interactions of the environmental factors of the oceanic reproductive migration and spawning ground, and how they would be involved in the natural activation of eel sexual maturation and reproduction. These factors, potentially as diverse as swimming activity, salinity, temperature, light, hydrostatic pressure, geomagnetism, and social cues, may lead to innovative ecophysiological methods to induce eel sexual maturation, or at least to facilitate and improve the effects of hormonal treatments.

A European Union COST Action «Solving bottlenecks in eel reproduction to support sustainable aquaculture » (EELSUPPORT) has been launched in 2023. This international consortium aims at sharing the state-of-the-art, identifying and filling knowledge gaps, in order to collaboratively design optimal protocols for broodstock conditioning, for induction of sexual maturation, egg and larvae production, and for rearing larvae to the glass eel stage. EEL SUPPORT's ambition is to contribute to closing the European eel lifecycle, in order to support sustainable aquaculture.

Beside aquaculture for consumption, completing the eel life cycle would also provide compulsory knowledge and expertise for potential *ex situ* conservation of the European eel, in the context of the dramatic decline of its population, as currently atempted for other critically endangered migratory European fishes such as the Allis shad or the European sturgeon. Investigating the impact of environmental factors, such as temperature increase or anthropogenic contaminants, on eel broodstock

quality and reproduction, will also provide a relevant knowledge base for European eel conservation and management in the context of the impact of global change on watersheds.

Acknowledgement

This publication is based upon work from COST Action EEL SUPPORT, CA22163, supported by COST (European Cooperation in Science and Technology).

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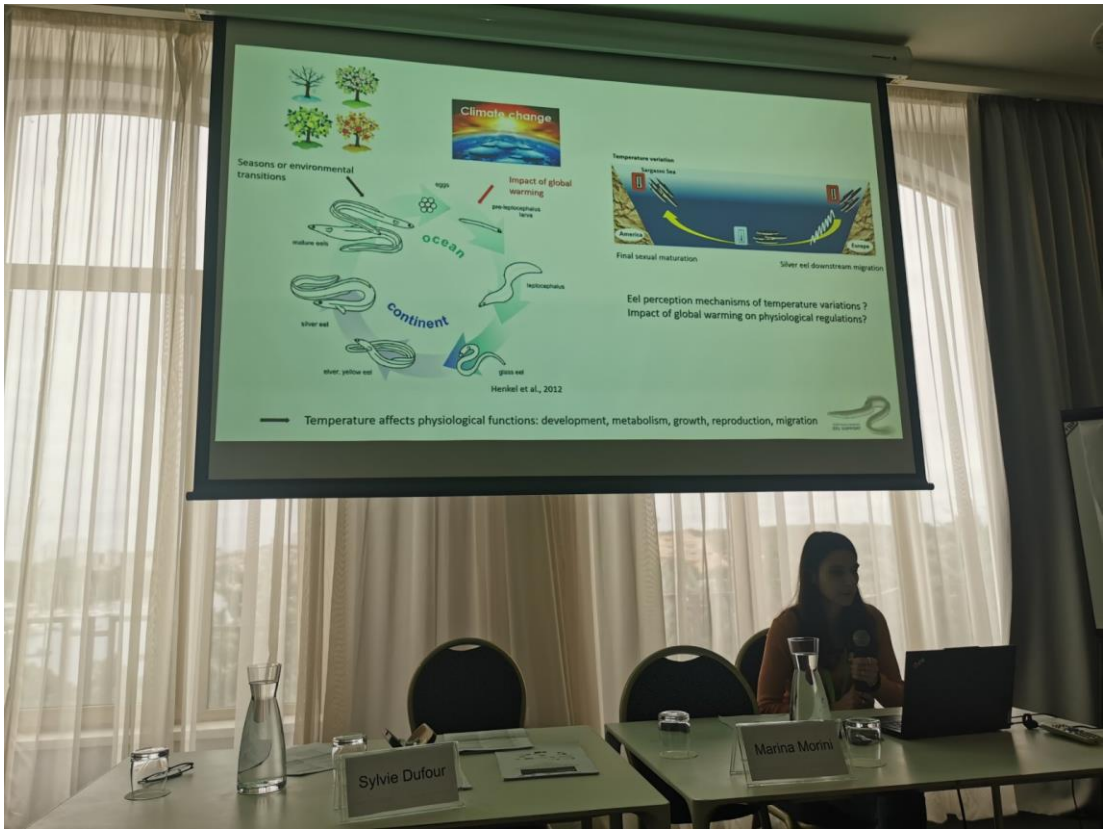
2. WORKSHOP SESSION ORGANIZED BY CA22163 EELSUPPORT

After plenary session, Workshop session organized by COST ACTION CA22163 "Solving bottlenecks in eel reproduction to support sustainable aquaculture" (EELSUPPORT), and supported by COST (European Cooperation in Science and Technology) started. Presentations were open to all, and apart from EELSUPPORT participants, 30-35 EIFAAC Symposium participants were present at the session.

The session was chaired by Marina Morini & Sylvie Dufour

Oral presentations:

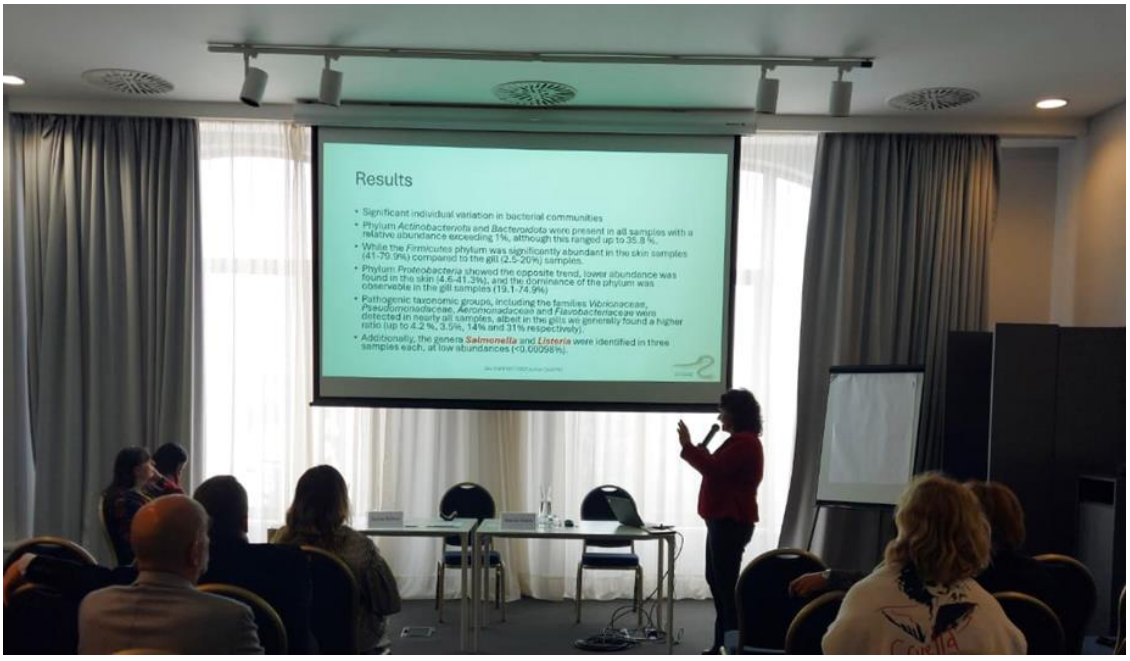
- Marina Morini, Christina A. Bergqvist, Juan F. Asturiano, Dan Larhammar, Sylvie Dufour: LOOKING FOR TRANSIENT RECEPTOR POTENTIAL RECEPTORS IN THE EUROPEAN EEL: AN EVOLUTIONARY APPROACH **(Best oral presentation awarded by EIFAAC)**



- Ana, Gavrilović, Oliver Barić, Tena Radočaj, Ákos Horváth, Tamás Müller, Kinga Katalin Lefler, Réka Enikő Balogh, Irena Vardić, Nevena Kitanović, Damir Kapetanović, Jurica Jug-Dujaković: SCREENING OF EEL POPULATION FOR BROODSTOCK SELECTION

- Michael I. Pedersen, Gorm Rasmussen, Niels Jepsen: IMPACT ASSESSMENT OF EEL STOCKING UPSTREAM VESTBIRK HYDROPOWER STATION, GUDENAA, DENMARK

- Milán Farkas, Balázs Kovács, Irena Vardić Smrzlić, Damir Kapetanović, Tena Radočaj, Oliver Barić, Jurica Jug Dujaković, Ana Gavrilović*: PRELIMINARY ANALYSES OF EEL SKIN AND GILL MICROBIOME FROM THE NERETVA DELTA, CROATIA *(two presenters, oral presentation given by Ana Gavrilović; poster presentation by Balázs Kovács)*



- Dijana Blazhekovikj - Dimovska, Stojmir Stojanovski: STATUS OF EEL POPULATION IN OHRID LAKE

- Devrim Memiş, Gökhan Tunçelli, Süleyman Öztürk: AQUACULTURE OPPORTUNITIES FOR THE CONSERVATION OF EUROPEAN EELS IN TURKEY: CHALLENGES AND POTENTIAL SOLUTIONS

- Jurica Jug-Dujaković, Ana Gavrilović: THE IMPORTANCE OF SYSTEM DESIGN ON THE EARLY LARVAL REARING OF EEL

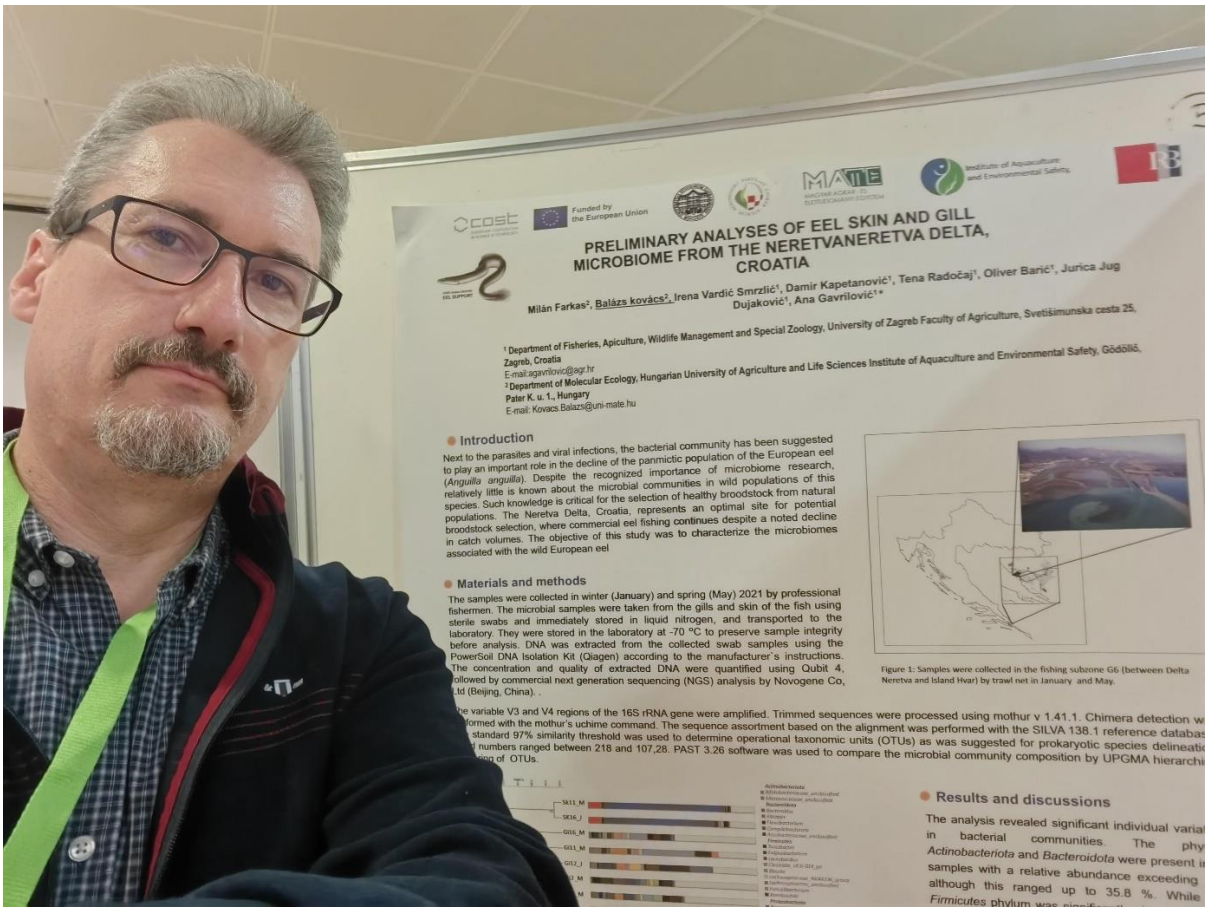
Poster presentation followed the discussion at coffee break.

3. POSTERS

Dijana Blazhekovikj - Dimovska, Stojmir Stojanovski: THE POTENTIAL OF USING MEDICINAL PLANTS FOR TREATING THE PARASITES IN FISH

Milán Farkas, Balázs kovács*, Irena Vardić Smrzlić, Damir Kapetanović, Tena Radočaj, Oliver Barić, Jurica Jug Dujaković, Ana Gavrilović: PRELIMINARY ANALYSES OF EEL SKIN AND GILL MICROBIOME FROM THE NERETVANERETVA DELTA, CROATIA

Tena Radočaj, Oliver Barić, Irena Vardić Smrzlić, Damir Kapetanivić, Jurica Jug-Dujaković, Ana Gavrilović: INFESTATION OF EUROPEAN EEL (*Anguilla anguilla*) WITH *Acanthocephalus* sp. IN THE NERETVA DELTA



Abstracts of oral and poster presentations

Notice: Notice: All abstracts will be published as a separate chapter in the Proceedings of the International EIFAAC Symposium that will be issued as FAO occasional publication. Visual identity of COST and EELSUPPORT will be added to the Chapter.

Oral presentation (Best oral presentation awarded by EIFAAC)

LOOKING FOR TRANSIENT RECEPTOR POTENTIAL RECEPTORS IN THE EUROPEAN EEL: AN EVOLUTIONARY APPROACH

Marina Morini[*1,2], Christina A. Bergqvist[3], Juan F. Asturiano [2], Dan Larhammar [3], Sylvie Dufour [1]

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The Transient Receptor Potential (TRP) channels constitute a superfamily of multifunctional membrane proteins, involved in many sensory and physiological functions. Currently, the TRP superfamily members have been classified into nine TRP families in metazoans, including TRPV and TRPM families. In 2021 the Nobel prize was attributed to the discovery in mammals of TRPV1 and TRPM8 sensitive to hot and cold temperatures, respectively. Few data are yet available in other vertebrates and metazoans. This study aimed to investigate the number, origin, and evolution of TRPV and TRPM families among metazoans, with a special focus on the impact of whole genome duplications (WGD), gene-specific duplications, and gene losses, in teleosts and in particular in the European eel (Anguilla anguilla).

Fish, as ectothermic organisms, are sensitive to temperature changes related to seasons or environmental transitions. They are particularly vulnerable to the increase in watershed or ocean temperature due to global warming. Temperature may affect all aspects of fish life cycle and physiological functions, such as development, metabolism, growth, reproduction, migration, etc...

The European eel species is in the red list of the International Union for Conservation of Nature (IUCN) as critically endangered. Eel overfishing, together with other anthropogenic factors such as pollution, river dams, diseases, and global climate change, have indeed caused a drastic reduction of the eel populations during the last decades. Eels have a complex lifecycle and encounter high variations in temperature and salinity during their continental and transoceanic migrations. For instance, decreasing temperature is reported as one of the environmental factors triggering the silver eel downstream migration. It has been also suggested that final sexual maturation in the spawning ground would be related to an increase in water temperature. The eel's perception mechanisms of temperature variations at these key stages and the physiological responses are still unknown, as well as the potential impact of global warming on these regulations. The eel, as a representative of elopomorphs, an early diverging group of teleosts, the largest group of vertebrates, presents also the interest of a key phylogenetical position for the evolutionary studies.

We performed gene searches, phylogenetic and syntenic analyses across metazoans, and revealed larger number and lineage specific diversity of TRPV and TRPM genes than previously assumed. Four TRPV types (A, B, C and D) and four TRPM types (alpha, beta, beta-like and gamma) were identified in non-vertebrate metazoans. In vertebrates, local gene duplications and vertebrate WGD (1R/2R) led to the expansion of the TRPM and TRPV families with up to nine TRPV types (TRPV1 to 9) resulting from TRPVC and D, and up to eight TRPM types (TRPM1 to 8) resulting from TRPMalpha and beta.

The additional teleost WGD (3R), as well as species-specific local gene duplications and gene losses, led to diverse repertoires of TRPM and TRPV across teleost species. The European eel possesses ten TRPM resulting from the conservation of two 3R-paralogs (a and b) of TRPM1, TRPM4 and TRPM7

while of only a single 3R-paralog of TRPM2, TRPM3, TRPM5 and TRPM6. This species possesses three TRPV corresponding to the conservation of a single 3R-paralog of TRPV1, TRPV4 and TRPV5.

This study proposes a phylogenetically based classification of TRPM and TRPV types across metazoans, and provides necessary tools for predicting and naming TRPM and TRPV types and paralogs according to lineages and species, as well as for relevant inter-specific comparative investigations. These data open new research avenues for the comprehensive studies of the multiple TRPM and TRPV and their potential involvement in sensing and responses to environmental changes.

Acknowledgement

This publication is based upon work from COST Action EEL SUPPORT, CA22163, supported by COST (European Cooperation in Science and Technology), Seal of Excellence MSCA Post-doctoral Fellowship from Sorbonne University, MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana (THINKINAZUL/2021/012) to SEASPERM, Swedish Research Council, Swedish Brain Foundation.

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Oral presentation

SCREENING OF EEL POPULATION FOR BROODSTOCK SELECTION

Ana, Gavrilović*[1], Oliver Barić [1], Tena Radočaj [1], Ákos Horváth [2], Tamás Müller [2], Kinga Katalin Lefler [2], Réka Enikő Balogh [2], Irena Vardić [3], Nevena Kitanović [3], Damir Kapetanović [3], Jurica Jug-Dujaković [4]

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The basis for colecting and selecting the broodstock of eel, as well as other types of fish, is the good condition and health of the individuals. In addition, the presence of numerous toxic chemicals, including pesticides and heavy metals, in the body of fish are known to cause reproductive disorders (Delbes et al., 2022). For the success of the broodstock conditioning, it is also necessary to know the stage of gonadal development (gametogenesis) in the natural environment so that the conditioning can be carried out as efficiently as possible. Since the commercial fishing of European eel in the Neretva delta in Croatia represents a long-standing tradition that has been maintained to this day, despite the decline in catches, this area represents a potential place for the selection of eel broodstock for artificial spawning.

Given that the eel population of the Neretva delta is a potential location for collecting the broodstock, the aim of this work was to examine the health status, the concentration of pesticides in the meat, as well as to determine the stage of gonadal development.

Asymptomatic eels were sampled from the catches of commercial fishermen from January to November 2021. Of the 94 eels collected during this period, 32 specimens from January and May were subjected to virological analyzes. Three different organs (liver, spleen and kidney) were isolated and stored in 70% ethanol. Detection of the most frequently reported eel viruses, AngHV-1 and EVEX, was performed by PCR. All 94 collected eels were processed for routine histology of the gonads to determine maturation stage, endoparasitological analysis, analysis of pesticide concentration and mercury (Hg) in the meat.

A total of 14 out of 32 fish were positive on AngHV-1, while all samples were negative on EVEX. The prevalence of most widespread genus of intestinal fish parasites, the thorny headed worms *Acanthocephalus* sp., was 56.5%, while the prevalence of swim bladder parasite *Anguillicoloides crassus* was 32%. The concentration of Hg varied from 0,9 to 0,17 mg/kg. Out of 14 examined pesticedes, endosulphane alfa and p, p – DDE were above the detection limit. Several different oocyte maturation stages described by Lokman and Young (1998) were identified.

In addition to the importance of using broodstock from virus-free areas and virological examinations of eel from the areas of unknown health status, these results highlight the importance of broodstock health assessment and biosecurity measures, including quarantine and preventive bathing, to ensure higher survival and better larval quality. Detoxification protocols should be considered in case of accumulation of endocrine disruptors.

Acknowledgement

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Oral presentation

IMPACT ASSESSMENT OF EEL STOCKING UPSTREAM VESTBIRK HYDROPOWER STATION, GUDENÅEN, DENMARK

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To meet the EU's recovery plan for eels, about 1.5 million on-grown eels (2-5 g) are stocked into freshwater areas in Denmark annually. Knowledge about the effect of stocking is insufficient. The purpose of this study has therefore been to shed light on the effect of stocking eel in the upper part of river Gudenå. In the experimental area, the natural immigration of elvers is limited and a fish trap at Vestbirk Hydropower can retain fish that migrate downstream from the upstream rearing area.

In 2001 and 2002, a total of 78,633 eels were stocked with an invisible CW (Coded Wire) tag. The fish were released in two size groups of 2.8 grams and 9.5 grams respectively. The 2001 release was in the main stem of the river and in 2002 it was in tributaries. After 6 years, the first eel with a CW-tag was recaptured in the fish trap. In the years from 2007 until 2020, 712 tagged eels were recaptured. The gender distribution of the recaptured eels was 27% males at length 33-42 cm (62-155 g) and 73% were females at length 45 to 90 cm (174-940 g).

The catch efficiency of the trap was estimated to be 37% of all eels migrating from the release area and the total migration was thus estimated to be 1,924 silver eels. This corresponds to a total survival of 2.45% of the 78,633 eels that were released. The survival of the "large" eels averaged 2.51% and the small eels 2.38%. The small 2.8-gram eels have roughly the same survival in 2001 (2.33%) as in 2002 (2.43%), but the survival of the large eels is almost double as high in 2001 (3.11%) compared to 2002 (1.87 %), which indicates higher survival when large eels are released into the Gudenå's main stem contrary to release into the tributaries (P< 0,001).

The average annual growth in length in the two size groups were similar between 2.9 and 3.4 cm/year for small versus large eel respectively and were not significantly different (P= 0,1542). The average annual growth in length were significantly faster for females compared to males (P= 0.007).

The gross yield per released eel is positive for both size groups, but if the yield is calculated as net yield, i.e. minus the released biomass, the net yield is positive for the small, released eels (3.3 and 3.4 g per stocked eel) but negative for the large, released eels (-1.6 and -4.5 gram per released eel).

The total survival and thus the yield were less than what was documented in similar studies e.g. marine Fjord areas, which possibly can be attributed to slower growth and higher age and the presence of predators, cormorants, and otters, in the river Gudenå.

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Oral presentation

PRELIMINARY ANALYSES OF EEL SKIN AND GILL MICROBIOME FROM THE NERETVANERETVA DELTA, CROATIA

Milán Farkas[1], Balázs kovács*[1], Oliver Barić[2], Irena Vardić Smrzlić[3], Damir Kapetanović[3], Tena Radočaj[2], Jurica Jug Dujaković[4], Ana Gavrilović*[2]

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Next to the parasites and viral infections, the bacterial community has been suggested to play an important role in the decline of the panmictic population of the European eel (*Anguilla anguilla*). Despite

the recognized importance of microbiome research, relatively little is known about the microbial communities in wild populations of this species. Such knowledge is critical for the selection of healthy broodstock from natural populations. The Neretva Delta, Croatia, represents an optimal site for potential broodstock selection, where commercial eel fishing continues despite a noted decline in catch volumes. The objective of this study was to characterize the microbiomes associated with the wild European eel.

The samples were collected in winter (January) and spring (May) 2021 by professional fishermen. The microbial samples were taken from the gills and skin of the fish using sterile swabs and immediately stored in liquid nitrogen, and transported to the laboratory. They were stored in the laboratory at -70 °C to preserve sample integrity before analysis. DNA was extracted from the collected swab samples using the PowerSoil DNA Isolation Kit (Qiagen) according to the manufacturer`s instructions. The concentration and quality of extracted DNA were quantified using Qubit 4, followed by commercial next generation sequencing (NGS) analysis by Novogene Co, Ltd (Beijing, China). A total of 14 samples underwent metagenomic amplicon sequencing (Illumina), targeting the V3-V4 region of the 16S rDNA gene to accurately determine the composition of the bacterial communities in the selected samples.

The analysis revealed significant individual variation in bacterial communities. The phylum *Actinobacteriota* and *Bacteroidota* were present in all samples with a relative abundance exceeding 1%, although this ranged up to 35.8 %. While the *Firmicutes* phylum was significantly abundant in the skin samples (41-79.9%) compared to the gill (2.5-20%) samples. The phylum *Proteobacteria* showed the opposite trend, lower abundance was found in the skin (4.6-41.3%), and the dominance of the phylum was observable in the gill samples (19.1-74.9%). Pathogenic taxonomic groups, including the families *Vibrionaceae*, *Pseudomonadaceae*, *Aeromonadaceae* and *Flavobacteriaceae* were detected in nearly all samples, albeit in the gills we generally found a higher ratio (up to 4.2 %, 3.5%, 14% and 31% respectively). Additionally, the genera Salmonella and Listeria were identified in three samples each, at low abundances (<0.00098%).

The presented results contribute to filling knowledge gaps in microbiome research of eel and point out that individual microbiome differences are significant in natural populations. Moreover, the detection of pathogenic bacteria underscores the necessity of health assessments and stringent biosecurity measures, including quarantine and molecular/microbial testing, for eel producers to enhance survival rate and quality.

Acknowledgement

This study is based upon work from COST Action CA22163 “Solving bottlenecks in eel reproduction to support sustainable aquaculture” (EELSUPPORT), supported by COST (European Cooperation in Science and Technology). Part of the activities was financed within the Project "Fisherman- Scientific Network of the City of Ploče" performed under Measure I.3. "Partnership between scientists and fishermen for the period 2020-2022., while one part has been supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2021, (TKP2021-NVA-22), the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences.

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Oral presentation

STATUS OF EEL POPULATION IN OHRID LAKE

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Lake Ohrid, known as one of the oldest lakes globally, has the distinction of being the deepest lake in the Balkans region, with a maximum depth of 288 m and a mean depth of 155 m. Even with its exceptionally high level of endemism (approximately 1,200 native species, at least 212 endemics; almost half of the 21 native fish species are endemic), the presence of non-endemic species is still considerable in Lake Ohrid. The lake likely stands out as the only one in the world that primarily receives water from many surface and sub-lacustrine springs. The River Crn Drim, part of the Adriatic drainage basin, is the only outlet for the lake. The non-endemic category consists of species that are mobile or migratory, like the European eel. Over the past 60 years, the natural migration routes for eels in Macedonia have been blocked due to the construction of dams for hydroelectric power stations on the River Crn Drim. The eels are obstructed by the dams as they try to leave Lake Ohrid and reach the sea, where they naturally spawn. The issue is somewhat addressed by importing and introducing young eels into Lake Ohrid.

In the past 7-8 years, juvenile eel has not been introduced into Lake Ohrid, The visible decrease in the population of this fish species is noticeable every year. The State Statistics Office has reported the following results in the catch of eel (in kg) in recent years: 2020 – 200 kg; 2021 – 280 kg; 2022 – 293 kg and 2023 – 282 kg.

In the past few decades, the focus on eel diseases has been dominated by the nematode *Anguillicola crassus*. The Japanese eel (*Anguilla japonica*) is the native host, and the parasite is prevalent in both open waters and fish farms in Japan, but it causes minimal damage since it is almost non-pathogenic for the Japanese eel. The introduction of the parasite in Europe occurred in the early 1980s through the import of infested eels from East and Southeast Asia. The spread of *Anguillicola crassus* quickly reaches many European countries, affecting open waters and fish farms and resulting in significant losses.

Anguillicola crassus in Lake Ohrid was determined by Stojanovski et al. (2002) to be present in nearly half (39.71%) of the eel population in the lake, likely introduced through the import of young eels for stocking. Stojanovski et al. (2010) discovered that *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) and *Pseudodactylogyrus bini* (Kikuchi, 1929), monogenean trematodes, were present in 51.31% of the eel examined in Lake Ohrid. The presence of gyrodactylid species in Lake Ohrid was last investigated in 2018 (within the Bilateral Macedonian - Austrian Scientific - Research Project 2018-2020) by Blazhekovikj - Dimovska and Stojanovski during their eel sampling, but no parasites in eel were found***Acknowledgement***

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Acknowledgment

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Oral presentation

AQUACULTURE OPPORTUNİTİES FOR THE CONSERVATİON OF EUROPEAN EELS İN TURKEY: CHALLENGES AND POTENTİAL SOLUTİONS

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European eels are distributed from the northern coasts of the Atlantic Ocean to the Baltic and Mediterranean Seas. They can migrate as far as Asia and South America, but they do not breed in those regions. The European eel (*Anguilla anguilla*) is the only species of the Anguillidae family that inhabits the Mediterranean, Aegean Sea, and Black Sea. These migratory species spend most of their lives in rivers (from 6 to 20 years) and generally move to the sea from April to November to spawn at ages of 6 to 9 years or older. During this transition, the color of the eels turns silver in about 3-4 months. When they reach 60-80 cm in length, they achieve sexual maturity in their silver form. The stages of an eel's life are as follows: Leptocephalus (pelagic larval eel), glass eel (transparent small eel), elver (small juvenile eel), yellow eel (juvenile eel residing in continental waters), and silver eel (sexually maturing eel) (EIFAC/ICES, 2001).

Eels can grow to a length of 50-150 cm, rarely up to 200 cm, and can weigh 4-6 kg, living up to 85 years. Male eels typically do not exceed 51 cm in length. Eels spawn in their natural environment in the western Atlantic Ocean, at a depth of 1000 m, in waters with a minimum temperature of 7 ℃ and salinity not less than 35.5‰. Spawning occurs from early spring to late summer. Larvae, which are 7-15 mm long, live at depths of 200-300 m. By the end of the third month, at water temperatures of 20 ℃, they reach a length of 25 mm and reside at 25-50 m depths. When the larvae reach 50-55 mm in length by June of their second summer, they migrate to the central parts of the Atlantic Ocean. By the third summer, they grow to 60-88 mm and reach the European coast. At this point, they finally take the shape of a small eel. Eels that arrive in rivers feed on worms, crustaceans, insect larvae, mollusks, fish, frogs, fish eggs, and other creatures (Slanstenenko, 1955).

Glass eels migrate upriver, crossing all kinds of obstacles. In river environments, eels face challenges in reaching maturity and preparing for spawning migration to the Atlantic Ocean. Many studies on eel stomach contents show that eels are carnivorous, feeding on fish as well as benthic invertebrates (Yalçın Özdilek & Solak, 2007). Eels may remain in the yellow eel stage, characterized by golden pigmentation, for up to 14 years and reach a length of 60 to 80 cm. After this stage, they move back to the sea, often crossing wet grasslands in search of an appropriate river or lake, particularly at night (Akşıray, 1987).

Eel fisheries and capture-based eel aquaculture are unique among fisheries due to the extremely migratory nature of the eel's life cycle (Ottolenghi et al., 2004). *Anguilla anguilla* are found in marine, brackish, and freshwater environments worldwide, where they are consistently caught due to their accessibility and market demand. In 1998, the International Council for the Exploration of the Sea (ICES) declared that the eel spawning stock was beyond safe biological limits (EIFAC/ICES, 2002).

Today, according to the official website of the General Directorate of Fisheries and Aquaculture of the Ministry of Agriculture and Forestry, European eels (*Anguilla anguilla*), whose exports are subject to a quota under the "Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)," have an export quota for 2024 set at 100 tons per year.

At present, the dramatic decline in eel stocks indicates that eels are under pressure, either from fishing activities or environmental degradation. It is necessary to update the reasons for the decrease in European eel stocks under current conditions and prepare an action plan to assess and protect the existing stocks in Turkey. More research projects are needed to monitor these fish, re-determine their existing

habitats, and explore opportunities for breeding them under controlled conditions. In this study, important eel habitats in the Mediterranean region will be investigated, and potential opportunities for aquaculture will be explored.

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Oral presentation

THE IMPORTANCE OF SYSTEM DESIGN ON THE EARLY LARVAL REARING OF EEL

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Until now, within the framework of our Project, the focus in eel larvae breeding was concentrated on food and feeding. Despite its importance, the design of larval rearing systems was completely outside our focus. This article highlights the correlation between larval feeding and behavior, as well as relevant environmental factors and the design of larval rearing systems.

Given the similar situation in fish species whose early-stage breeding has been established and is successfully taking place, the development of the breeding system took place in parallel with the development of larval nutrition and feeding strategy. Feeding with live food requires a completely different approach to the design of the drain and supply of fresh water to the tanks, unlike feeding with inert food. System and tank designs for the laboratory research have their specificities and were never fully applied in commercial production.

Even discussions on some relevant environmental factors of the rearing of eel larvae (as temperature) were insufficient. During the early development of the larvae, a temperature of

18ºC is recommended for optimal utilization of the yolk, but at this temperature it has been recorded that the larvae become immunologically weakened and very sensitive to pathogens. Slow decrease in temperature during yolk-sac larval stage was not suggested, but according to latest meeting with Japanese experts, temperature of 24ºC was mentioned as optimal for larval stage during initial feeding. Negative phototaxis was used in the feeding strategy to scare the larvae and impel them to move to the bottom where slurry-type feed was spread. Light at the surface and feeding at the bottom of the tank determine the specific design of the tank with a drain at the bottom but considering the speed of decomposition of small food particles, an additional drain can be applied. The method of water intake and the speed and manner of water movement, especially due to the applied feeding method and the desired movement of the larvae during feeding, are of key importance. The shape of the larval tank as well as its volume will also determine the movement of the water. Even though in European eel the expression of digestive enzymes was detected just after hatching, the introduction of probiotics into larval tanks is being considered. The bacterial load would strongly influence the water quality, and the design of the tank would have to be adjusted if another way of introducing probiotics was not applied. The colour and transparency of the wall and bottom of the larval tank was changed several times in most protocols for different species. In research laboratory conditions, transparent tank walls are suitable, but in commercial systems they are usually inefficient and are not used.

Successful research results in early larval rearing of various fish species include effective rearing system design that correlates with other relevant rearing factors, and eel larval rearing is certainly no exception. This is why this type of bioengineering must be included in the research.

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Poster presentation

INFESTATION OF EUROPEAN EEL (*Anguilla anguilla*) WITH *Acanthocephalus sp.* IN THE NERETVA DELTA

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One of the most widespread genus of intestinal fish parasites in Europe is Acanthocephalus. These are obligate pathogenic worms that cause considerable damage to the intestinal wall. Most of them infest different fish, but for some species the European eel (*Anguilla anguilla*) is preferred as a final host. *A. anguilla* is a catadromous species assessed as critically endangered. Some of the causes of endangerment

are habitat loss, pollutants and diseases. The aim of the study was to determine the prevalence of acanthocephalans in digestive system of *A. anguilla* in the Neretva Delta. Eel sampling was carried out from January to November 2021, at the main confluence of the Neretva River and 94 eels were collected. Clinical examination revealed the presence of parasites in the digestive system. The parasites found were counted for each individual fish, morphologically examined and stored in 96% ethanol for molecular analysis. Molecular analysis of the parasites in the digestive system confirmed the morphological determination, i.e. that they are thorny headed worms *Acanthocephalus* sp. The prevalence in the total sample was 56.5%. In further research, more detailed analysis of the relationship between the fish size and the degree of infestation and water quality parameters should be performed.

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Poster presentation

THE POTENTIAL OF USING MEDICINAL PLANTS FOR TREATING THE PARASITES IN FISH

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The growth of aquaculture in recent years has made it a viable source of food supply, as long as it is conducted in a way that is environmentally friendly, socially responsible, and takes into account food safety and animal welfare.

The number of infectious disease outbreaks, specifically parasitic diseases, has increased in aquaculture, affecting the fish population and causing significant economic losses. Conventional treatments like

antibiotics, anti-parasitics, and chemical compounds have been used by fish farmers to prevent and control fish diseases. Numerous studies have demonstrated the accumulation of these chemical residues in fish tissues, enhanced resistance to pathogens, and a negative effect on aquatic organisms' environment. Chemical compounds are difficult to control in open waters where aquaculture is conducted.

Nowadays, alternative methods are being developed by scientists and fish technologists, with many plant-derived compounds from selected plant species becoming increasingly promising supplements, because of their effectiveness, safety, environmental protection, and lower drug resistance. A significant number of compounds derived from plant extracts can be used to prevent and control fish parasites in aquaculture, particularly against protozoans, myxozoans, and monogeneans.

Essential oils are secondary metabolites of medicinal plants that possess biological compounds such as terpenes, terpenoids, alkaloids, flavonoids, saponins, coumarins, and phenolics. Preventing and controlling diseases in aquaculture systems can be achieved by using them as an effective treatment. Using essential oils in fish diets can also have an effect on their growth, immunity, and resistance to infectious diseases.

In recent years, the use of plant-derived compounds and the potential to discover new formulas containing essential oils, plant extracts, and their bioactive compounds have been continuously increasing, in relation to phytotherapy. Serious research and further examinations are required to determine the appropriate concentration for the administration and to demonstrate the effectiveness of these plant-derived compounds and their pharmacological activities in controlling fish parasitic diseases.

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This second part of the workshop was chaired by Ana Gravilovic and Sylvie Dufour, and addressed the objectives of three EEL SUPPORT Working groups.

WG1 "Broodstock conditioning"

Following the presentations given the day before by Ana Gavrilovic and by Dijana Blazhekovikj-Dimovska, on health problems faced by wild eels, the discussion addressed possible broodstock management protocols to be applied before experimental maturation: they include health control (for bacteria, viruses, parasites,..), anti-pathogen treatments, quarantine. The importance of establishing standardized protocols shared by EELSUPPORT partners was highlighted.

The question of assessment / restoration of eel habitat quality was also debated. ICES recommendation is against restocking, the participants agreed with. At a minimum, habitat quality should be considered in any restoking program.

The question of the recommendation by ICES, as presented by Reinhold Hanel, to ban any European eel fisheries, as a rationale measure for conservation of this critically endangered species, raised an animated discussion. Several participants highlighted the fact that maintaining a limited and regulated small fishery in various countries, may significantly contribute to the conservation of eel populations and their habitats. It allows indeed: - maintaining eel fishery survey by governmental authorities, thus preventing the growth of illegal fisheries, - raising relevant information on eel populations and habitats by traditional eel fishermen, - promoting actions to preserve / restore eel habitat quality.

The participants also emphasized the paradox of eel conservation under the increasing pressure of eel predators (specially birds and mammals) which are themselves under protective measures.

The question of water contaminants and their impact on broodstock quality and capacity to mature and reproduce, as highlighted by Sylvie Dufour during her conference was also debated. The requirement by ICES of a specific threshold value of water contaminants for eels would require extensive experimentations on wild eels, thus impacting eel populations, while it does not seem relevant in the context of a precautionary approach for eel conservation. Current data already obtained on impaired experimental maturation and reproduction of broodstocks from specific rivers should be considered, as well as data on other fish species.

WG2 "Induced maturation and reproduction"

The relationships between COST action EELSUPPORT objectives of sustainable eel aquaculture and the conservation of endangered European eel raised a large discussion addressing several points.

- Following up on the above discussion, it was highlighted that induced maturation and reproduction brings relevant information on broodstock quality that should be considered by conservationists in the survey of eel habitat quality and eel population management.

- Everybody agreed that the reproduction of eel in aquaculture and sustainable production of glass eels would contribute to eel conservation by preventing capture of wild glass eels, but should not aim at releasing cultured eels in the wild. This shared opinion solves the question of the potential impacts of cultured eels on wild eel populations such as reduction of genetic diversity, disease propagation etc…It was emphasized that this "theoretical" question is anyway still far from applying in the case of the European eel. Even for the Japanese eel, the life cycle of which has already been completed in captivity,

the annual experimental production of glass eels (currently only 10000/ year) is very far for the need of million glass eels for raising them in aquaculture farms.

The discussion also addressed innovative perspectives for European eel reproduction such as oocyte cryopreservation and xenotransplantation, a method developed by Akos Horvath and partners on various fish species including the eel. The first attempts using phylogenetically distant host fish, were not conclusive; during the discussion it was suggested to consider the possibility of transplanting European eel gametes in the Japanese eel.

WG 3" Eel hatchery" was discussing three different topics:

The first one was in connection with the presentation given the day before by Jurica Jug Dujakovic on the larval rearing system design. Until now, within the framework of our Project, the focus in eel larvae breeding was concentrated on food and feeding. Despite its importance, the design of larval rearing systems was outside our main focus.

Successful research results in early larval rearing of various fish species include effective rearing system design that correlates with other relevant rearing factors, and eel larval rearing is certainly no exception. This is why we should include this type of bioengineering in research and discussions. Our experience with different design and our discussion with our colleagues that are not included in our project, especially Japanese colleagues should include this topic. Given the similar situation in fish species whose early-stage breeding has been established and is successfully taking place, the development of the breeding system took place in parallel with the development of larval nutrition and feeding strategy.

Feeding with live food requires a completely different approach to the design of the drain and supply of fresh water to the tanks, unlike feeding with inert food. System and tank designs for laboratory research have their specificities and were never fully applied in commercial production. This correlation between larval feeding and behavior, as well as relevant environmental factors and the design of larval rearing systems. was spread.

Negative phototaxis were used in the feeding strategy to scare the larvae and impel them to move to the bottom where slurry-type feed was spread. Light at the surface and feeding at the bottom of the tank determines the specific design of the tank with a drain at the bottom but considering the speed of decomposition of small food particles, an additional drain can be applied. The method of water intake and the speed and manner of water movement, especially due to the applied feeding method and the desired movement of the larvae during feeding, are of key importance. The shape of the larval tank as well as its volume will also determine the movement of the water.

The color and transparency of the wall and bottom of the larval tank was changed several times in most protocols for different species. In research laboratory conditions, transparent tank walls are suitable, but in commercial systems they are usually inefficient and are not used.

The second one was about reconsidering successes and failures of other research who were successfully dealing with establishing larval rearing protocols for other fish species. Discussions so far on even some relevant factors of the eel larval rearing environment (such as temperature) should perhaps have been more extensive. During the early development of eel larvae, a temperature of 18ºC is recommended for optimal utilization of the yolk, but at this temperature it has been recorded that the larvae become immunologically weakened and very sensitive to pathogens. Slow decrease in temperature during yolk-sac larval stage was not considered, but according to latest meeting with Japanese experts, temperature

of 24ºC was mentioned as optimal for larval stage during initial feeding. Such an increase in temperature in the early larval stage of sea bass and yellow perch has proven to be very effective.

The third mentioned the possible introduction of probiotic that was discussed at our last meeting. Even though in European eel the expression of digestive enzymes was detected just after hatching, the introduction of probiotics into larval tanks is being considered. The bacterial load would strongly influence the water quality, and the design of the tank would have to be adjusted if another way of introducing probiotics was not applied.

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