EEL SUPPORT
ANNUAL CONFERENCE

6st June 2024
Demre, Antalya
Türkiye

EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

Funded by
the European Union

CONTENTS

Değerli katılımcılar,

Antalya T.C. Tarim ve Orman Bakanlığı Akdeniz Su Ürünleri Araştırma, Üretme Ve Eğitim Enstitüsü Müdürlüğü'nde düzenlenecek olan CA22163 nolu "Solving bottlenecks in eel reproduction to support sustainable aquaculture (EELSUPPORT)" adlı COST aksiyonu çerçevesinde sizleri aramızda görmekten mutluluk duyuyoruz. Bu konferansın amacı, dünya çapında 1970'lerden bu yana azalan yılan balığı popülasyonlarının akuakültür yoluyla sürdürülebilir üretimini sağlamak ve doğal popülasyonu balıkçılık baskısından kurtararak doğal popülasyonun yönetimine katkıda bulunabilmektir.

COST Aksiyonu EEL SUPPORT, en son teknolojiyi üye ülkeler arasında paylaşmak, bilgi boşluklarını belirlemek, bu boşlukları doldurmak için işbirlikçi stratejiler geliştirmek ve bu bilgiyi sentezlemek ve gözden geçirmek için mevcut ağ oluşturma araçlarını kullanacaktır.

Bu konferansta keyifli ve faydalı bir konaklama geçirmenizi diliyor, Antalya'nın hoş ortamından ve sıcak yazından keyif alacağınızı umuyoruz.

Süleyman Öztürk, Gökhan Tunçelli ve Devrim Memiş (Yerel Organizatörler)
Caroline DURIF (EELSUPPORT Aksiyon Başkan Yardımcısı)
Arjan Palstra (EELSUPPORT Aksiyon Başkanı)

Dear participiants,

We are happy to see you among us within the framework of the COST action named "Solving bottlenecks in eel reproduction to support sustainable aquaculture (EELSUPPORT)", numbered CA22163, which will be held at the Mediterranean Fisheries Research, Production and Training Institute Directorate of the Ministry of Agriculture and Forestry, Antalya, Republic of Türkiye. The aim of this conference is to ensure the sustainable production of eel populations, which have been decreasing worldwide since the 1970s, through aquaculture and to contribute to the management of the natural population by saving the natural population from fishing pressure.

COST Action EEL SUPPORT will use existing networking tools to share cutting-edge technology among member countries, identify knowledge gaps, develop collaborative strategies to fill these gaps, and synthesize and review this knowledge.

We wish you a pleasant and useful stay at this conference and hope you will enjoy the pleasant environment and warm summer of Antalya.

Süleyman Öztürk, Gökhan Tunçelli and Devrim Memiş (Local Organizers)
Caroline DURIF (EELSUPPORT Action Vice-Chair)
Arjan Palstra (EELSUPPORT Action Chair)

Annual conference of EELSUPPORT project will be held at the Mediterranean Fisheries Research, Production and Training Institute Directorate which is located in Demre, Antalya, Türkiye

Address
Akdeniz Su Ürünleri Araştırma, Üretme ve Eğitim Enstitüsü Müdürlüğü.
Beymelek Mahallesi, 07570, Demre Kampüsü, Finike Karayolu 6 km
Demre / Antalya
Türkiye

Phone

+90 242 872 14 00

Web site
https://arastirma.tarimorman.gov.tr/akdenizsuurunleri

SCIENTIFIC PROGRAMME
WEDNESSDAY, June 5 2024

9.00-9.10 **Welcome**
- Suleyman Ozturk

9.10-10.30 **MC Meeting**
- Arjan Palstra

10.30-10.45 **Coffee break**

10.45-12.00 **MC Meeting**
- Arjan Palstra

12.00-13.30 **Lunch break**

13.30-18.00 **Parallel WG Meetings incl coffee break:**
- WG1 meeting Jonna Tomkiewiecz, Justas Dainys
- WG2 meeting Sylvie Dufour, Marina Morini
- WG3 meeting Laura Gentile
- WG4 meeting Annalisa Zaccaroni

19.00-22.00 dinner

THURSDAY, June 6 2024

8.30-9.00 **Registration of attendance**

9.00-10.30 **Invited speakers (chairs Marina Morini and Annalisa Zaccaroni)**
- Shigeho Ijiri *History of the development of artificial maturation of the Japanese Eel*
- Ryusuke Sudo *Recent progress in artificial seedlings production of the Japanese eel*
- Jonna Tomkiewicz *Closing the cycle of the European eel*

10.30-11.00 **Coffee-break**

11.00-12.30 **Invited speakers**
- Sylvie Dufour *Endocrinology of the European eel*
- Reinhold Hanel *The Sargasso Sea hypothesis: myths and facts*
- Caroline Durif *A brief history of European eel management*

12.30-14.00 **Lunch break**

14.00-15.00 **Research presentations (chairs Sandra Ramos and Salima Aroua)**

- Laura Gentile *Advancements in artificial reproduction: Insights from the University of Bologna*
- Neil Duncan *Comparison of hormonally induced oogenesis between flathead grey mullet (Mugil cephalus) and European eel (Anguilla anguilla)*
- Herman Spaink *Shaping eel microbiomes for aquaculutral applications*

15.00-15.30 **Coffee-break**

15.30-16.30 **Research presentations**

- Suleyman Ozturk *European eel (Anguilla anguilla) aquaculture and investigation of early larval rearing possibilities*
- Francesca Bertolini *Exploiting blood transcriptomics as non-lethal tools in european eel research and monitoring*
- Erik Burgerhout *Epigenetics in freshwater eels: lessons from other species*

16.30-18.00 **Posters and drinks**

19.00-22.00 **Dinner**

POSTERS

P1 **THREATS TO THE PRESENCE OF EUROPEAN EEL (*Anguilla anguilla*) in GÖKÇEADA STREAMS**
ÖZCAN GAYGUSUZ, GÖKHAN TUNÇELLİ, ÇİĞDEM GÜRSOY GAYGUSUZ, İDİL CAN TUNÇELLİ, SEDAT OZAN GÜREŞEN, DEVRİM MEMİŞ*

P2 **REAL-TIME OPTIMIZATION IN RECIRCULATING AQUACULTURE SYSTEMS FOR EEL PRODUCTION**
ALAA JAMAL

P3 **EUROPEAN EEL (*Anguilla anguilla*) IN LAKE OHRID, NORTH MACEDONIA: CONSERVATION STATUS, CHALLENGES AND PERSPECTIVES**
ALEKSANDAR TRAJCHOVSKI, ALEKSANDAR CVETKOVIKJ

ABSTRACTS:
ORAL PRESENTATIONS

HISTORY OF THE DEVELOPMENT OF ARTIFICIAL MATURATION OF THE JAPANESE EEL

SHIGEHO IJIRI[1], HIDEKI TANAKA[2], SHINJI ADACHI[1]
[1]3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan. Faculty of Fisheries Science, Hokkaido University. [2]468-3 Uragami, Nachikatsuura, Wakayama 649-5145, Japan. Aquaculture Research Institute, Kindai University.

The Japanese eel, Anguilla japonica, is the most important species in the freshwater aquaculture industry of Japan. To address the rapid decline of glass eel catch, a research project for artificial maturation of the eel was commenced in the late 1960s. The first larvae and yellow eel production were achieved in 1974 and 2003, respectively.

In 1964, Fontaine et al. succeeded in producing eggs in European eel by carp pituitary injections. With the help of this knowledge, our group produced eggs in Japanese eel by injections of salmon pituitary homogenates (SPH), then succeeded in producing the first eel larvae. Although SPH injections were enough effective to induce vitellogenic growth, oocyte maturation, and ovulation were hard to induce by the SPH injection alone. In 1985, we identified DHP as a maturation-inducing steroid for the first time in vertebrates from Amago salmon. Thanks to this finding, oocyte maturation and ovulation were easily induced by DHP injection to post-vitellogenic eels induced by SPH injections.

After the mid-1990s, it became not easy to collect silver eels from the wild. Because most Japanese eels became male in the captive condition, we faced difficulty in obtaining mother eels for artificial maturation. In this situation, Tachiki et al. established the method to produce female eels by feeding E2 to glass eels for five months in 1993. Not only the feminizing effect, but the E2 treatment also accelerated primary oocyte growth. In the accident, we also found that low-temperature treatment to the eels in the third year accelerated oil droplet accumulation, in which the eels became ready for proceeding sexual maturation.

The methods in preparing mother eels and for the artificial maturation have not been changed basically until now, except for using recombinant gonadotropins, instead of SPH. Until the late 1990s, we constantly produced fertilizable eggs and were enabled to test rearing experiments using artificially produced eel larvae.

In this paper, we reflect on these processes including the endocrine control underlying the controlled maturation in the Japanese eel.

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

NOTES

RECENT PROGRESS IN ARTIFICIAL SEEDLINGS PRODUCTION OF THE JAPANESE EEL

RYUSUKE SUDO, TAKASHI YATABE, MASATAKA SATOMI, KENTARO HIGUCHI, RYUTARO TAKASAKI, KOHEI NAKAMURA, YUKINORI KAZETO.
Fisheries Technology Institute, Minamiizu Field Station, Japan Fisheries Research and Education Agency, Minamiizu, Kamo, Shizuoka 415-0156, Japan

Anguillid eels are one of the most important and popular food fish that are usually eaten as "kabayaki" (grilled eel with soy sauce), which is a traditional dish in Japan, and they are a highly valued aquaculture species in East Asian countries. However, supply of seedings for the present eel culture industry depends completely on the capture of naturally recruiting glass eels in estuaries whose abundance has decreased markedly due to overfishing, aquatic habitat loss or degradation, or other factors such as ocean-atmosphere environmental changes. For conservation of anguillid eel populations, one effective way is to establish mass production techniques for hatching and rearing seedlings.
Our group (Japan Fisheries Research and Education Agency) has succeeded in obtaining artificially matured gametes using hormonal treatment of Japanese eel adults. Viable leptocephali and glass eels have then been successfully produced by the development of appropriate rearing techniques. In 2010, our group achieved the completion of the Japanese eel life cycle for the first time under laboratory conditions using healthy larvae (F2 generation) obtained from artificially propagated male and female brood stock (F1 generation). However, large-scale commercial production of seedlings for aquaculture has not been practical to date.
Recently though, techniques for artificial seedlings production of Japanese eels have progressed. New protocols for maturation techniques using recombinant gonadotropins have been established. A new diet that is mainly composed of hen egg yolk, milk protein and fish protein hydrolysate was developed. In addition, a new scaled-up rearing tank was developed to provide growth and survival rates that are equivalent to those of small experimental tanks. In this presentation, we introduce recent advances in these techniques.

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

NOTES

CLOSING THE CYCLE OF THE EUROPEAN EEL

JONNA TOMKIEWICZ, SEBASTIAN N. POLITIS, SUNE RIIS SØRENSEN, PARASKEVAS KOUMPIADIS
Section for Marine Living Resources, National Institute of Aquatic Resources, Technical University of Denmark, Denmark. snpo@aqua.dtu.dk

Closing the European eel (*Anguilla anguilla*) life cycle in captivity for a self-sustained and sustainable eel aquaculture requires efficient hatchery techniques and technology. With this goal in mind, EU-supported research initiatives set out in the beginning of this century to develop procedures for captive reproduction and larval culture aiming at glass eel production. These efforts have led to two decades of successful achievements through dedicated international and national efforts. Research initially focused on development of assisted reproduction protocols applying hormone therapy, and broodstock feeds thereby enabling production of high-quality gametes and viable offspring (Tomkiewicz et al., 2019, Jéhannet et al., 2024). Recent years research, building on these results, have gained terrain in the establishment of larval culture technology and techniques as well as formulation of feeds for ongrowing larvae sustaining their development into the leptocephalus stage (Politis et al., 2022; Benini et al., 2023; Bandara et al., 2024).

While new knowledge, products and methods have incrementally extended longevity, variable reproduction success, offspring quality, and high mortality in the early larval stages challenge upscaling of larval culture and the completion of the larval phase. These challenges have urged researchers to revisit procedures with the aim to enhance quality and survival through different stages. This presentation will overview progress in European eel offspring production and larval culture and discuss bottlenecks and impediments that need attention spanning from reproduction through the larval culture to reach metamorphosis and the glass eel stage in sufficient numbers. Among other, we look at the still limited insights in larval demands to ambient culture conditions and stage specific nutritional requirements as well as the efficiency required for a commercial closed-cycle hatchery production of European eel.

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

Bandara KA, Politis SN, Sørensen SR, Benini E, Tomkiewicz J, Vadstein O, 2024. Effect of Food Amounts on Larval Performance, Bacteriome and Molecular Immunologic Development during First-Feeding Culture of European Eel. Microorganisms, 12(2), 355.

Benini E, Bandara KA, Politis SN, Engrola S, Nielsen A, Conceiçao LEC, Santos A, Sørensen SR, Tomkiewicz J, 2023. Exploring first-feeding diets for European eel larval culture: insights at morphological, nutritional, and molecular levels. PLoS One 18, e0283680.

Jéhannet P, Heinsbroek LTN, Swinkels W, Palstra AP, 2024 Recent insights into egg quality and larval vitality of the European eel (*Anguilla anguilla*). General and Comparative Endocrinology, 354, 114531.

Politis, SN, Sørensen SR, Conceicao L, Santos A, Benini E, Bandara K, Sganga D, Branco J, Tomkiewicz J. European eel larviculture: First establishment of feeding Leptocephalus culture. https://aquaeas.org/Program/PaperDetail/39870

Tomkiewicz J, Politis S, Sørensen S, Butts I, Kottmann J, 2019. European eel – an integrated approach to establish eel hatchery technology in Denmark. In: Eels Biology, Monitoring, Management, Culture and Exploitation: Proceedings of the First International Eel Science Symposium, 2019. 5m Publishing, Sheffield, UK.

NOTES

REPRODUCTIVE ENDOCRINOLOGY OF THE EUROPEAN EEL

Sylvie DUFOUR,

Biology of Aquatic Organisms and Ecosystems (BOREA), National Museum of Natural History (MNHN), National Center for Scientific Research (CNRS), Sorbonne University, 75231 Paris Cedex 05, France

The mystery of the life cycle and reproduction of the European eel, Anguilla anguilla, arouse curiosity since a long time, going back to the Greek philosopher Aristoteles. The first attempts to induce eel sexual maturation by hormonal treatments were performed by the French physiologist, Maurice Fontaine, who successfully obtained mature European male and female eels. These pioneering advances set up the basis of current treatments for inducing maturation in other eel species such as the Japanese eel. They also demonstrated that the blockade of sexual maturation in the silver eel resulted from a deficient production of pituitary gonadotropins. Subsequent endocrine studies aimed at characterizing the actors of the brain-pituitary-gonad axis in the European eel. Neuroendocrine investigations revealed a dual brain control of pituitary gonadotropins in the eel, with a classical stimulatory effect by the neuropeptide gonadotropin-releasing hormone (GnRH), and a striking inhibitory effect by the neuromediator dopamine. Other brain neuropeptides such as kisspeptins, gonadotropin inhibitory hormone (GnIH), neurokinin B, were also shown to control eel pituitary gonadotropins, but all having inhibitory effects. Gonad sexual steroids exert a major positive feedback on GnRH and LH, likely related to the induction of LH ovulatory / spermiation peak. Gonad peptide hormone, activin, has a remarkable stimulatory effect on FSH while a negative one on LH. Investigations also address the potential interactions between the gonadotropic and other neuroendocrine axes in the eel. Growth hormone (GH) potentiates the stimulatory effect of estradiol on the induction of vitellogenin synthesis by the liver, and insulin-like growth factor (IgF1) directly stimulates pituitary LH synthesis, indicating multiple positive interactions of the somatotropic axis on eel reproduction. Concerning the corticotropic axis, cortisol exerts various positive effects such as the stimulation of LH expression and the mobilization of metabolic and mineral stores necessary for gametogenesis and migration, while it also has an inhibitory effect on vitellogenin production by the liver. A large set of investigations aims at characterizing in the eel the paralogous genes for each hormone and receptor of interest, as a result of the teleost specific genome duplication. Thus, the discovery and expression of a thyrotropin receptor paralog in the eel ovary, allows to infer a potential direct role of thyrotropin in the control of reproduction. Finally, important lines of investigation by various European research laboratories concern the effects on the eel gonadotropic axis of migration-related factors, such as swimming activity as well as environmental parameters including salinity, temperature, light, hydrostatic pressure, magnetic field, and their variations. Altogether, these investigations contribute to raising basic knowledge on

reproductive physiology in teleosts and to opening new ways for inducing experimental maturation in the eel.

NOTES

THE SARGASSO SEA HYPOTHESIS: MYTHS AND FACTS

REINHOLD HANEL
Thünen Institute of Fisheries Ecology, Herwigstrasse 31, 27572 Bremerhaven, Germany

European eel stock assessment is still limited by significant data gaps for the different life stages. While the management target of the Eel Regulation issued by the European Union in 2007 is an increase in silver eel escapement, scientific advice provided by the International Council of the Exploration of the Sea (ICES) is based on glass eel recruitment series as reference point. Information about potential changes in larval abundances in the spawning area were largely missing, after scientific investigations specifically targeting eels in the Sargasso Sea ended in the late 1980s. The unique oceanic life history of spawning offshore followed by a long larval stage has also made it difficult to understand the causes of recent anguillid eel population declines. Conflicting hypotheses highlight either excessive mortalities during the eel's continental life stages or climate change aspects affecting the leptocephalus larvae. However, in order to disentangle potential causes of recent population decline and more rapidly observe changes in the development of the stock, larval surveys in the spawning area can play a significant role.
After five consecutive oceanographic surveys from 2011 to 2023 to study the European eel spawning area, the benefits and pitfalls of a larval eel assessment in the central Sargasso Sea as well as changes in larval eel abundance over time are presented. The results so far give some indication that recruitment of glass eels at the continental coasts has decreased stronger than the larval densities in the spawning area.
With regard to silver eel behavior in the open sea, we were able to document that spawning still takes place across about 2000 km in the Sargasso Sea despite the sharp decline in the number of spawners. We documented a very stable pattern of diel vertical movements with amplitudes of about 300 meters and maximum diving depths of over 1200 meters.
However, the larval ecology of eels in the Sargasso Sea still largely remains a mystery. Despite some efforts to better understand the feeding ecology of the larvae, there are still two conflicting hypotheses found in the scientific literature, describing eel larvae either as active predators of gelatinous zooplankton or as scavengers on marine snow.

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

NOTES

A BRIEF HISTORY OF EUROPEAN EEL MANAGEMENT

CAROLINE DURIF, JAN DAG POHLMANN, CEDRIC BRIAND, HILAIRE DROUINEAU

The European eel is widely spread between north Africa and Norway. The species, during its multiple life-stages is present in the sea where it spawns, but also far up into freshwater systems, sometimes over 1000 km upstream. Eel may grow in coastal marine waters, estuaries, inland lakes and rivers. Yet, all European eel belong to the same genetic population creating a colossal challenge for stock assessors and managers. Life-history traits such as age and size at maturation or sex ratio vary between the north and southern part of the population and therefore complicating the stock-recruitment relationship.
Threats also vary depending on habitat and geography. Almost all life-stages are targeted by the fishery but differently depending on the country. Glass eel are fished mainly in the center of the distribution, yellow eel in the south and silver eel mainly in the north. Different fishing gear are used which compromises the comparison of catches and landings between eel fisheries. As is the case with all migratory fishes, hydropower also consitutes a major threat to eel. It affects both upstream and downstream migrating individuals and contributes to habitat loss.
The Working Group on Eel has since the 1970s been working toward a common assessment to provide advice to managers on both the national and international level. Today, WGEEL includes 29 countries and over 100 members. It is a prime example for transboundary management of a fisheries resource.

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

NOTES

ADVANCEMENTS IN ARTIFICIAL REPRODUCTION: INSIGHTS FROM THE UNIVERSITY OF BOLOGNA

LAURA GENTILE[1], ANTONIO CASALINI[1], PIETRO EMMANUELE[1], RICCARDO BRUSA[1], OLIVIERO MORDENTI[1].
[1]*Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy*

The European eel is listed as "critically endangered" under the IUCN Red List of Threatened Species. One of the strategies to counteract this decline is to improve the aquacultural system, allowing a reduction in wild elvers and juvenile eel harvesting.
Aquaculture production may represent an effective tool to fulfil customer requests and preserve natural stocks providing eels for the food industry and, optimistically, for future restocking projects. Over the last 10 years, the University of Bologna has tried to standardize the artificial reproduction protocol for this species. We have always used wild broodstock from the North Adriatic lagoons. In Italy, extensive cultures are managed in coastal lagoons, the European eels are extensively reared through traditional *Vallicoltura* practices, conducted within the *Valli*—defined sections within a lagoon or enclosed earthen pond.
During the last years, we compared the reproductive performance of females even at different stages of maturity to assess reproductive performance and larval quality. The objective of these studies is therefore to evaluate the reproductive potential of female eels characterized by different stages of silvering, for potential inclusion in artificial reproduction and/or restocking programs. We have used and compared two broodstock from two different areas of Italy. In particular, the Tyrrhenian stock and the Adriatic stock. The spawners from these two areas are morphologically different and the yield in terms of reproductive performance and larval quality is comparable. In Italy, research on European eel reproductive biology, and offspring development competence has progressed, using an integrated, multidisciplinary approach to generate new insights. Parallel to zootechnical studies, we have carried out several studies on physiology to improve the protocol. The technique we use today is a technique that allows the eels to have natural spawning without the need for stripping, which is possible thanks to various measures that we apply throughout the weeks of induction.
Here we will discuss the implications of our results concerning the standardization of a single protocol that we can all use. Furthermore, this additional knowledge on reproduction can contribute to understanding the European eel's natural life cycle, thus supporting management and conservation plans for this critically endangered species.

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

NOTES

COMPARISON OF HORMONALLY INDUCED OOGENESIS BETWEEN FLATHEAD GREY MULLET (*MUGIL CEPHALUS*) AND EUROPEAN EEL (*Anguilla anguilla)*

NEIL DUNCAN[1], SANDRA RAMOS-JÚDEZ[2], GIANCARLO CERRUD[1], PAULINE JÉHANNET[3], ARJAN PALSTRA[3], IGNACIO GIMÉNEZ[4]

[1] IRTA La Rapita, Ctra Poble Nou, 43540 La Rapita, tarragon, Spain - neil.duncan@irta.cat

[2] S2AQUA—Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture, Olhão, Portugal - sandra.ramos@s2aquacolab.pt

[3] Wageningen University & Research Animal Breeding and Genomics, Wageningen Livestock Research, PO Box 338, 6700, AH, Wageningen, The Netherlands - arjan.palstra@wur.nl

[4] RARA AVIS Biotec, C/ Moratín 17, 4º, 46002 Valencia, Spain - igimenez@raraavis-bio.com

The aim of this presentation is to compare oogenesis in flathead grey mullet (*Mugil cephalus*) that was induced using recombinant gonadotropins (rGth) with similar hormone therapies used to induce oogenesis in European eel (*Anguilla anguilla*). Oogenesis in these two species has been hormonally induced from early stages, classified as sexually immature or resting, to the production viable ova or eggs. The induced oogenesis can be broken down into different steps of development. The first step is to initiate vitellogenesis with the transition from perinuclear and cortical alveoli oocytes to an oocyte that has incorporated vitellogenin. The process of vitellogenesis must then be maintained over a period of 6-13 weeks to produce an oocyte ready to enter into oocyte maturation. The final step is to induce oocyte maturation and ovulation. These steps have been treated and successfully induced in *Mugil cephalus*. The initiation of vitellogenesis was induced with low doses (6 to 9 μg kg^{-1}) of *Mugil cephalus* recombinant follicle stimulating hormone (rFsh). The process of vitellogenesis was maintained with a weekly injection of 12 μg kg^{-1} of rFsh. However, the application of low doses (4 to 9 μg kg^{-1}) of *Mugil cephalus* recombinant luteinizing hormone (rLh) appeared to be necessary to complete vitellogenesis to a stage from which oocyte maturation could be induced. Oocyte maturation, ovulation and spontaneous tank spawning was induced with two injections of 30 μg kg^{-1} of rLh (priming and resolving doses) separated by 24 hours. The diameter of the oocyte and position of the germinal vesicle when the priming dose was administered appeared to be related to egg and larval quality. This protocol for *Mugil cephalus* will be compared with protocols used to induce oogenesis in the European eel. A wider variety of hormones have been used for European eels. Steroid implants have been employed to initiate vitellogenesis, pituitary extracts and recombinant gonadotropins to maintain vitellogenesis and for oocyte maturation combinations of pituitary extracts, recombinant gonadotropins and 17α,20β-dihydroxy-4-pregnen-3-one (DHP) have been administered. The principal differences will be exposed to generate discussion on species differences and possible protocol improvements.

The work on *Mugil cephalus* is being supported by the Spanish Ministry of Science, Innovation and Universities, Project RTA2021-126070OR-100 ECOMUGIL awarded to ND. This publication is based upon work from COST Action EEL SUPPORT, CA22163, supported by COST (European Cooperation in Science and Technology).

NOTES

SHAPING EEL MICROBIOMES FOR AQUACULTURAL APPLICATIONS

HERMAN P. SPAINK
Einsteinweg 55 2333 CC Leiden, The Netherlands, Institute of Biology, Leiden University

The gut contains a highly complex community of microbes (the microbiome) that is pivotal for the health of all animals. Our project aims at identifying key microbial community functions that are important for stimulation of development and growth and protection against infectious disease in eel aquaculture. This will show whether early life colonization of eel larvae has long term effects.
For this purpose, we want to develop probiotics that are needed for stimulating early development of cultured larvae of the European eel. For this we will apply state of the art microbial culturomics and modelling of microbial communities obtained from eel in nature. We will study selected communities by novel in vivo robotic test methods for microbial functionalities. For this, zebrafish larval high throughput test systems for growth, development and immunity have now been developed in my laboratory.
In order to investigate what microbial species are suitable for these applications we set out to investigate what are common bacterial sources that occur in adult eel and glass eel. We therefore studied the microbial diversity of the gut of adult eels obtained from two eel culturing laboratories in the Netherlands. The gut of the eel was divided in three parts: front-, middle- and hind-gut and the microbiome diversities were accessed by 16S amplicon sequencing. The results show that the microbiome of all parts of the adult gut are highly complex with many hundreds of microbial species at the genus level. The overlap of the microbial diversity in the two eel samples is limited and identifies a common group of microbes that we have tried to culture. However, only a very limited number of microbes could be cultured in standard media. Subsequently, we analyzed the diversity of microbes from surface-sterilized glass eels obtained at two locations in The Netherlands. These larvae contained a surprising complexity of microbes that was very different from the gut of the adult eels. In this presentation at the COST meeting, I will present a comparative analysis of the identified microbial diversity and the research plans for the near future.

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

NOTES

EUROPEAN EEL (*Anguilla anguilla*) AQUACULTURE AND INVESTIGATION OF EARLY LARVAL REARING POSSIBILITIES

SÜLEYMAN ÖZTÜRK[1]*, İSA AYDIN[1], SONER SEZEN[1], ADEM KURTOĞLU[1], BURAK EVREN İNANAN[1], AHMET MEFUT[1], HÜSEYİN SEVGİLİ[3], MAHİR KANYILMAZ[3], SALİH KOCAKAYA[1]

[1]*Mediterranean Fisheries Research, Production and Training Institute, Antalya, Türkiye*

[2]*Aksaray University, Aksaray, Türkiye*

[3]*Isparta University of Applied Sciences, Isparta, Türkiye*

[4]*General Directorate of Agricultural Research and Policy, Ankara, Türkiye*

**Corresponding Author: suleyman.ozturk@tarimorman.gov.tr*

In this study, 15 females and 77 males silvery European eel (*Anguilla anguilla*) broodstocks were used. Approximately 19 kg of female broodstock candidate eels were obtained from Bafa Lake in Aydın Province and brought to the Mediterranean Fisheries Research, Production and Training Institute trial unit on 15.11.2024. The average weight of the fish was determined as 1280 g (min. 808 g and max. 1619 g). The fish were tagged with the CTW tag and placed in 2-ton circular tanks. Hormone application started on 22.11.2023 for gamete recruitment from broodstock fish. Female broodstock were injected with Carp pituitary hormone at a rate of 20 mg/kg weekly. Silver colored male broodstocks were placed in 3 tanks with a volume of 2 tons. HCG hormone was applied to male broodstock at the rate of 1 IU/g weekly from the abdominal area. Weight gain in broodstock fish occurred in the 13th week. However, since sperm could not be obtained from male broodstock, mating could not be done. In the 15th week, 4 female broodstocks have reached maturity suitable for production. A mixture of 40 mg/kg carp pituitary hormone, 40 mg/kg GnRH hormone and 5 mlt/kg metpyramide was injected into developing females from 8 different points in the abdominal region. However, after the fertilization process of the sperm and eggs obtained after the hormone injection, no fertilized eggs could be obtained. Other females that gained weight were moved to a separate tank with a volume of 500 L. The water temperature has been increased to 20 °C. Four male broodstock from which sperm were collected were placed in the same tank. DHP hormone at a rate of 2 mg/kg was dissolved in 96% ethanol and injected to the female from 8 different points in the abdomen. The female started to release eggs 18 hours after the DHP hormone was injected. However, the eggs fertilization was not successed. Thereupon, an attempt was made to collect eggs and sperm by stripping. First, 3 ml of sperm was taken from 4 male broodstocks. 100% physiological saline was added to the collected sperm and stored at +4 C. Then, the eggs were mixed and sperm was added to the eggs. Sea water was added 5 minutes after sperm was added. However, fertilized eggs could not be obtained with this method. In this study, the studies carried out for eel culture since 2017 will be explained.

Key words: *European Eel, hormone treatments, gamete quality, fertilization.*

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

NOTES

EXPLOITING BLOOD TRANSCRIPTOMICS AS NON-LETHAL TOOLS IN EUROPEAN EEL RESEARCH AND MONITORING

FRANCESCA BERTOLINI[1,2], AMAYA ZARATIEGUI PEDROSA[1], MEHIS ROHTLA[3], JONNA TOMKIEWICZ[1], CAROLINE DURIF[4]

1. National Institute of Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark

2. Division of Animal Sciences, Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

2. Institute of Marine Research, Austevoll Research Station, Storebø, Norway

3. Estonian Marine Institute, University of Tartu, Tartu, Estonia

Non-lethal methods for analyzing fish are of relevance, enabling sustainable practices without sacrificing the stock. In fish, blood transcriptomics is still underutilized, although the few studies available on a wide range of non-mammal vertebrates show how it can be informative for a wide range of assessments. Therefore, blood transcriptomics may offer insights into fish physiology, health and behaviour. In this study, we have investigated the ability of blood transcriptomics to detect salinity habitat history in the European eels. Traditionally, this is determined by examining fish otoliths, a process that requires sacrificing the fish. This work takes advantage of two different datasets. 1) The first consists of a whole blood transcriptomic dataset of 60 eels sampled in different places in Norway, where salinity history based on otolith data was available: these eels were identified as seawater residents, freshwater residents and shifters (i.e. proven shift between the two salinity areas). 2) the second dataset collected whole blood transcriptomic data derived from a longitudinal study of 20 samples, where eels were collected in freshwater and raised in salt water for 4 weeks. For these two datasets, we have combined blood transcriptomics and machine learning to test the ability of transcriptomic blood-based analysis to predict salinity habitat history guided by their otolith-based classification. For both transcriptomic datasets, Differential Gene Expression was performed to reduce to 10-50 most discriminant genes among the approximately 24,000 genes mapped in the European eel genome. Then, several machine learning algorithms were tested and Random forest was selected as the most stable. The combination of the different approaches identified a suitable number of genes (up to 10) whose level of expression was able to correctly classify the 100% of seawater and freshwater residents, and 97% of the shifters. This approach is promising for the replacement or reduction of other lethal analyses in eel research aquaculture, for example for the prediction of the performance of male and female broodstock.

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

NOTES

EPIGENETICS IN FRESHWATER EELS: LESSONS FROM OTHER SPECIES.

ERIK BURGERHOUT
Nofima AS, The Norwegian Institute of Food, Fisheries and Aquaculture Research, Muninbakken 9-13, 9019, Tromsø, Norway

Over the last decades it has become increasingly evident that the environment encountered during critical developmental windows influences the later phenotype, likely through changes in the epigenetic machinery. Especially, an environment that may lead to suboptimal utilization of nutrition during early ontogeny – e.g., one that facilitates fast growth, such as high incubation temperature – appears to alter the life-long programs. For example, in Atlantic salmon, embryonic temperature between fertilization and the 'eyed-stage' appears to leave a 'signature' affecting muscle cellularity that was still visible ca. 3 years later, corresponding with differences in growth at sea. Additionally, incubation temperature during this relatively short-term period is associated with deformities, and it was recently found that it also impacted the immune response later in life. Similar results have been observed in various other teleost species. While there have been several studies on pursuing the optimal rearing conditions in eels, in depth knowledge on the downstream impacts of the environment during early ontogeny is of critical importance.

Tightly intertwined with the above are the potential intergenerational (parent-offspring) effects of the environment. The maternal deposition of bioavailable nutrition into the eggs during gametogenesis is vital to secure proper development of the offspring. Broodstock nutrition and age, and the timing of induction of sexual maturation appear to have an impact on this process. Whereas for most aquaculture species reproduction can be controlled by the manipulation of environmental factors, such as photoperiod and temperature, eels are still being subjected to long-term hormonal treatments to mature. Stressors from these unnatural practices can be expected to have deleterious consequences on offspring quality, likely through disruptions in the process of steroidogenesis and gametogenesis.

Although working with limited information from the natural conditions, knowledge provided by other fish species suggests that to improve eel reproduction from the induction of maturation and embryogenesis, as well as later life's performance, health and welfare the impact of environmental conditions should not be neglected.

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

NOTES

GENOME SEQUENCE OF THE EUROPEAN EEL

RON DIRKS, HANS JANSEN
Future Genomics Technologies, Sylviusweg 74, 2333 BE Leiden, The Netherlands

The first draft genome sequence of the European eel was published in 2012. Although it was still quite fragmented (186,000 contigs), it contributed to multiple scientific publications, including tens of differential gene expression studies. With the commercial introduction of nanopore sequencing in 2014, it became possible to generate much longer sequence reads. This resulted in a significantly improved European eel genome sequence (2,366 scaffolds), one of the first published vertebrate genomes that was solely based on nanopore reads. Finally, a combination of nanopore sequencing and Hi-C was used to generate a chromosome-level assembly of the European eel genome, which has now already contributed to settling a long-standing debate about patterns of early fish evolution. Ten years after the initial launch of nanopore sequencing, the quality of nanopore long reads has reached a level at which Illumina sequencing is no longer necessary for polishing of a nanopore-based genome sequence assembly. Moreover, nanopore sequencing can be used to routinely map DNA methylation and to sequence full-length mRNAs. In conclusion, nanopore sequencing has completely revolutionized the way in which large genome sequences can be *de novo* assembled and annotated.

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

NOTES

ABSTRACTS:
POSTER PRESENTATIONS

THREATS TO THE PRESENCE OF EUROPEAN EEL (*Anguilla anguilla*) in GÖKÇEADA STREAMS

ÖZCAN GAYGUSUZ[1], GÖKHAN TUNÇELLİ[2],
ÇİĞDEM GÜRSOY GAYGUSUZ[3], İDİL CAN TUNÇELLİ[4], SEDAT OZAN GÜREŞEN[5],
DEVRİM MEMİŞ*[2]

[1] *Istanbul University, Faculty of Aquatic Sciences, Department of Marine and Freshwater Resources Management, Istanbul, Türkiye*

[2] *Istanbul University, Faculty of Aquatic Sciences, Department of Aquaculture and Fish Diseases, Istanbul, Türkiye*

[3] *Trakya University, Keşan Vocational School, Keşan, Edirne*

[4] *Istanbul University, Faculty of Aquatic Sciences, Department of Fisheries and Seafood Processing Technology, Istanbul, Türkiye*

[5] *Istanbul University, Faculty of Aquatic Sciences, Gökçeada Marine Research Unit, Çanakkale, Türkiye*

**Corresponding Author e-mail: mdevrim*@istanbul.edu.tr

Eels, whose stocks tend to decrease worldwide, are among the endangered fish in Europe. The main reasons for the decline in eel stocks are overfishing, habitat loss, pollution, and various hydraulic obstacles that adults and juveniles encounter while migrating. The European eel (*Anguilla anguilla*) exists in some sea-connected rivers, lagoons, and lakes in Türkiye. Commercial fishing is carried out among inland water fish that have economic value. In accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) export is within the scope of quota. The export quota for eel has been determined as 100 tons in 2024 by the Ministry of Agriculture and Forestry of Türkiye. Eels, which are known to live on Gökçeada, the largest island located in the north-west of Türkiye, are very rare today. There are five dam lakes, one salt lake and many streams connected to the sea in Gökçeada. Two native species (*Anguilla anguilla, Petroleuciscus borysthenicus*) and six invasive fish species (*Atherina boyeri, Carassius auratus, Carassius gibelio, Cyprinus carpio, Gambusia holbrooki, Pseudorasbora parva*) live in these inland water systems. In this study, possible reasons for the decline in eel stocks in Gökçeada were evaluated.

Key words: *Threats, Gökçeada, European Eel, critically endangered species, natural stocks.*

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

NOTES

REAL-TIME OPTIMIZATION IN RECIRCULATING AQUACULTURE SYSTEMS FOR EEL PRODUCTION

ALAA JAMAL
Rishon LeZion, Israel, Agricultural Research Organization – Volcani Institute

With the burgeoning aquaculture industry playing a pivotal role in global food production, the spotlight is increasingly on land-based recirculating aquaculture systems (RAS) due to their efficiency in water usage, stability in conditions, separation from contaminants, and effective waste management practices. Optimization of RAS protocols is essential for maximizing growth outcomes, particularly in the context of eel production, where there is a growing demand for this high-value species. One the other hand, recruitment of eels to the European coast has declined severely in the last decades, and it is estimated that current recruitment is 1–9% of the recruitment observed in the 1970s. The sharp decline is an effect of a serious eel stock decline probably related to high fishing efforts, loss of habitats, parasitic infections, and possible accumulation of contaminants. Therefore, RAS with design and process optimization can provide a solution to the decreasing eel production. Eel farming presents unique challenges, requiring precise control over several variables such as water temperatures and stocking densities to ensure optimal growth and profitability. However, traditional management approaches often lack specificity and fail to integrate comprehensive process descriptions, hindering the attainment of desired outcomes. To address these challenges, this study proposes a novel decision support system (DSS) tailored for real-time optimization in eel production within RAS environments. By integrating a simulation-optimization framework, this approach aims to deliver precise decisions regarding factors. Through holistic simulation models that encompass both fish growth dynamics and water quality management, this system provides a comprehensive understanding of the complex interplay between biological and environmental factors in RAS. Enhanced accuracy is achieved through a closed-loop system that incorporates real-time observations of key parameters, ensuring adaptive decision-making. An optimization algorithm is then utilized to determine optimal strategies for over forecast periods, enabling informed decisions on a daily basis.

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

NOTES

EUROPEAN EEL (*ANGUILLA ANGUILLA)* IN LAKE OHRID, NORTH MACEDONIA: CONSERVATION STATUS, CHALLENGES AND PERSPECTIVES

ALEKSANDAR TRAJCHOVSKI
aleksandar.trajchovski@fvm.ukim.edu.mk
Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine
ALEKSANDAR CVETKOVIKJ
acvetkovic@fvm.ukim.edu.mk
Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine

The European eel population in Ohrid Lake faced significant challenges starting in the 1970s due to the construction of two hydroelectric plants on the Crn Drim River, "Globocica" (1963) and "Spilje" (1969). The economic impracticality of constructing fish pathways due to considerable height differences rendered the dams' barriers to the natural migration of eels. Consequently, the solution has been to stock the lake with eels and capture adult eels before they reach the dams. Statistical data indicates a decline in the eel population following the construction of the hydroelectric power plants. For instance, preceding the construction of "Spilje" between 1955 and 1964, the annual eel stock was approximately 9 tons. However, post-construction, from 1985 to 1997, a discernible reduction occurred, with a yearly catch of only 5.5 tons. This downward trend persisted from 2012 to 2019, with a mere 1.8 tons captured annually and no data from 2020-2024, coinciding with diminished and sporadic stocking activities since 2011, observed in 2016 and 2019. Additionally, concerns regarding the eel health status have surfaced, including reports of the parasite *Anguillicoloides crassus*. Challenges persist with securing funds for eel offspring supply from the government. Furthermore, as a critically endangered species, importing the European eel in North Macedonia, a non-EU member, is very difficult. This complicates the efforts to address the eel's migration issues in Lake Ohrid. Key strategies to mitigate these challenges include implementing fish lifts, exploring alternative stocking solutions, and addressing administrative barriers hindering conservation efforts.
Keywords: European eel, fish migration, Lake Ohrid

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

NOTES