Symposium

Towards reproduction of eel in captivity to support sustainable aquaculture

October 20, 2016 | Wageningen | The Netherlands

eelric
eel reproduction innovation centre

Symposium
'Towards reproduction of eel in captivity to support sustainable aquaculture' (part of the EELRIC workshop)

Silver eel drawing by Marco Graziano

Thursday closed workshop (max 32 persons) Impulse Speakers Corner	
8.30	registration
9.00-10.20	opening and 3 talks
9.00-9.20	Arjan Palstra - Wageningen University & Research (NL) WHY are WE HERE NOW?
9.20-9.40	William Swinkels - DUPAN (NL) DUPAN and EELRIC
9.40-10.00	Mark Lokman - University of Otago (NZ) Tinkering with induced spawning protocols
10.00-10.20	Shigeho Ijiri - Hokkaido University (JP) Molecular mechanism of maturation-inducing steroid synthesis during oocyte maturation in Japanese eel
10.20-10.50	coffee
10.50-12.10	4 talks
10.50-11.10	Luz Perez - Polytechnical University of Valencia (ES) Eel research at UPV-Valencia (Spain)
11.10-11.30	Tamas Muller - Szent Istvan University (HU) Hungarian experiments on artificial propagation of aged European eel
11.30-11.50	Arjan Palstra - Wageningen University & Research (NL) Broodstock conditioning, stimulation of maturation and successful reproduction of European eels in Wageningen
11.50-12.10	Pauline Jehannet- Wageningen University & Research (NL) Ultrasonography to assist with the timing of spawning
12.10-13.20	lunch break
13.20-14.40	4 talks
13.20-13.40	Tagried Kurwie - Mahurangi Technical Institute (NZ) New Zealand eel aquaculture where at?
13.40-14.00	Lasse Marohn- Thünen-Institut (DE) Early life stages in spawning area and lab – Eel research at the Thünen Institute
14.00-14.20	Petra van Dijk - Glasaal Volendam (NL) Artificial reproduction of European eel in Volendam
14.20-14.40	Annalisa Zaccaroni - University of Bologna (IT) Recent advances in eel artificial reproduction in Italy
14.40-15.10	coffee
15.10-16.30	4 talks
15.10-15.30	Jonna Tomkiewicz - Technical University of Denmark (DK) Artificial reproduction of European eel in Denmark
15.30-15.50	Ryusuke Sudo - National Research Institute of Aquaculture (JP) Artificial seedlings production of the Japanese eel
15.50-16.10	Ron Dirks - ZFscreens BV (NL) Toward improved assembly of the European eel genome sequence using Nanopore sequencing technology
16.10-16.30	Arjan Palstra - Wageningen University & Research (NL) Wrap up and group picture
16.30-18.00	drinks
18.30-21.30	**dinner Reehorst**

Dinner Venue:

Hotel and Congrescentrum
the ReeHorst:
Restaurant Valentino
Bennekomseweg 24
6717 LM Ede

Welcome

Dear colleague,

Welcome at the symposium 'Towards reproduction of eel in captivity to support sustainable aquaculture'!

We are proud to offer you a programme with presentations of eel reproduction researchers from Japan, New Zealand, Denmark, Italy, Spain, Germany, Hungary and The Netherlands who will give a progress update of their work. These researchers are potential future partners within the Eel Reproduction Innovation Centre (EELRIC). EELRIC is initiated by the collaboration between Stichting Duurzame Palingsector Nederland (DUPAN) and Wageningen University and Research. EELRIC is a platform for the reproduction of eel in captivity and home for an international consortium of partners sharing experience and collaborating to create breakthroughs (more information provided on the www.eelric.eu website).

We certainly hope that you will enjoy this symposium!

With kind regards,

Dr. Ir. Arjan Palstra (PhD)
Senior researcher Aquaculture (fish physiology)

WAGENINGEN
UNIVERSITY & RESEARCH

Ir. William Swinkels
Treasurer DUPAN and director of Nijvis group

DUPAN
Stichting Duurzame Palingsector Nederland

The launch of

eelric
eel reproduction innovation centre

World-wide, eel populations have decreased strongly in numbers since the 1970s. The existing eel farms still depend on the catches of glass eels in nature which are then raised to market size. Only a restricted number of glass eels is available for aquaculture and societal concern exists about the lack of sustainability. Successful reproduction in captivity could supply aquaculture with glass eels and close the production cycle. This way, sustainability of both eel aquaculture as well as management of the natural populations will be enhanced. Several European research groups work independently on the reproduction of European eel in captivity but progress is still limited. The aim of Eel Reproduction Innovation Centre EELRIC is to function as a platform for the reproduction of eel in captivity and as a home for an international consortium of partners sharing experience and collaborating to force breakthroughs. EELRIC is initiated and owned by the partnership between Stichting Duurzame Palingsector Nederland (DUPAN) and Wageningen University and Research Centre (WUR). The launch of EELRIC represents an essential step towards reproduction of eel in captivity to support sustainable aquaculture.

Team descriptions and abstracts

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The eel reproduction team

Ir William Swinkels
Board member DUPAN
Chair Dutch Association of fish farmers (NEVEVI)
CEO Nijvis Group (eel farms, traders, processors)

Current research on eel reproduction

Eel farms – which produce the majority of eels for consumption - depend on the restricted catches of glass eels in nature which are then raised to market size. Successful reproduction in captivity could supply aquaculture with glass eels and close the production cycle. This would be a new and important solution in the entire range of sustainability measurements, for both eel aquaculture as well as management of the natural populations. Several European research groups work independently on the reproduction of European eel in captivity but progress is still limited. The aim of Eel Reproduction Innovation Centre EELRIC is to function as a platform for the reproduction of eel in captivity and as a home for an international consortium of partners sharing experience and collaborating to force breakthroughs. EELRIC is initiated and owned by the partnership between the foundation of the Dutch eel sector (DUPAN) and Wageningen University & Research (WUR). The launch of EELRIC represents an essential step towards reproduction of eel in captivity to support sustainable aquaculture.

DUPAN is the Dutch foundation of Eel traders (NeVePaling), the Dutch Association of fish farmers (NEVEVI) and the organisation of professional fishermen (CvB). The foundations purpose is to achieve the recovery of the European eel. It undertakes activities that contribute to a sustainable recovery of the eel stocks in the Dutch and European inland waterways. In addition, DUPAN is working on sustainable solutions for the catch and farming of eel, for environmental and animal-friendly processing, and is stimulating scientific research for eels. By doing so the DUPAN Foundation strives towards a sustainable conservation of Dutch eel and the eel sector.

Notes

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Current research on eel reproduction

Much of our eel research has focused on the function of the androgen 11-ketotestosterone. This steroid hormone has wide-ranging effects, most notably in the context of (at least in part) inducing the migratory phenotype and being a major regulator of ovarian lipid physiology – two subject areas that have obvious relevance for the artificial propagation of these fish. Focus on androgen signalling is set to continue, partly as a basic science enquiry, partly to further pursue its value as a tool in induced spawning protocols. In addition, we are increasingly interested in exploring the use of sustained-release implants at the expense of weekly injections as a measure to saving time and reducing handling of broodstock. We further run a project aimed at understanding the ovarian physiology of pre-pubertal fish and their responses to gonad-stimulating drugs – again, a basic science pursuit that is likely to yield some applications downstream.

Tinkering with induced spawning protocols – can sustained-release androgen implants enhance the outcomes of fertility treatment in female eels?

Lokman P.M.[1], Matsubara H.[2], Divers S.L.[1], Damsteegt E.L.[1], Di Biase A.[3], Setiawan A.N.[4], Wylie M.J.[1], Thomson-Laing G.[1], Mordenti O.[3], Todo T.[5], Ijiri S.[5], Adachi S.[5]

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The steroid hormone 11-ketotestosterone (11KT) is a surprising and unexpected regulator of a suite of biological events in both male and female eels. Much of our focus has been on females so as to by-pass male-specific, spermatogenesis-associated 11KT synthesis. Accordingly, we have reported extensively on the remarkable experimental effects of 11KT in females, including the widespread phenotypic changes of yellow eels towards a silver eel-like phenotype: remodeling of the head, increased eye size, blackening of the pectoral fins and a suite of changes to the size and function of internal organs akin those observed during the silvering transformation. We also found some effects on behavior.

In recent years, a role of 11KT in ovarian physiology of anguillids has become increasingly evident; *in vivo* treatment of yellow eels with 11KT increases expression of the Fsh receptor and results in increased size of the ovary, accompanied by increased oocyte diameters. Significantly, these findings could be reproduced *in vitro* and coincided with increased deposition of lipids into small oocytes (~ 0.1 mm) if very low density lipoproteins were supplemented to the media. The accumulation of lipids is, at least in part, dependent upon 11KT-mediated increased ovarian expression of the enzyme lipoprotein lipase – however, lipoprotein receptor(s) are likely to also be involved.

We have applied the findings from these basic science studies to experimentally induce oogenesis in females of 3 temperate eel species and 11KT has consistently delivered encouraging outcomes, depending on the dose administered. Notably, treatment of silver eels with 11KT prior to or during 'classical' pituitary homogenate-induced artificial maturation decreases the experimental duration and accelerates lipid accumulation in the oocyte. By itself, 11KT dramatically increases oocyte size and lipid content, but vitellogenin/yolk protein accumulation has not been observed. We are currently investigating the effects of steroid hormones on expression of the gonadotropin receptors and the estrogen and androgen receptors in the ovary and

liver of silver females to investigate whether observed 11KT effects can be attributed to cascades other than those leading to enhanced Fsh signaling and lipid accumulation. High throughput approaches (next-generation sequencing) may prove especially suitable to identify 11KT-mediated effects which may help direct future tinkering with induced spawning protocols of freshwater eels – indeed, reliance on implant-based approaches may prove rather beneficial in terms of consistency of effects and animal survival (reduced handling, cheaper drugs of uniform quality, repeatability, etc), especially if implants are cheap and can be administered without major trauma to the fish.

A key question that remains to be addressed is whether egg quality is affected by 11KT – enhanced egg quality could contribute notably to larval survival and accordingly, would strengthen the rationale to include 11KT (pre)treatment in pituitary homogenate-based fertility treatments of female eels. This issue is under investigation.

Notes

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Molecular mechanism of maturation-inducing steroid synthesis during oocyte maturation in japanese eel

Shigeho Ijiri* and Shinji Adachi
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The Japanese eel, *Anguilla japonica*, is one of the most important species of the aquaculture industry of East Asia. However, the supply of glass eels from wild catch declined drastically at present, and it is important that a reliable supply of larvae is established. Cultivated eels do not undergo vitellogenic growth under aquarium conditions unless animals are given repeated injections of gonadotropic preparations such as salmon pituitary extract (SPE). By this regular SPE injections, most eels could not complete oocyte maturation. Recently, Dr. Hirohiko Kagawa found that high-dose SPE injection could induce oocyte maturation and succeeded to obtain fertilizable eel eggs. This study was aimed to reveal the molecular mechanism underlying induction of oocyte maturation by the high-dose SPE injection.

Eels received weekly injections of SPE at 30 mg/kg body weight. After completion of vitellogenesis, the SPE dose was increased 10 x to induce oocyte maturation. During induced oocyte maturation up to ovulation, ovaries were biopsied and sera were collected. Serum concentrations of 17α,20β-dihydroxy-4-pregnen-3-one (DHP), which has been identified as a maturation-inducing steroid of the Japanese eel, were measured by time-resolved fluoroimmunoassay. Changes in mRNA levels of *cyp17a1* which converts progesterone to androstenedione, *cyp17a2* which converts progesterone to 17OHP, and *hsd17b12L* which converts 17OHP to DHP in the ovaries were measured by quantitative PCR (Figure 1).

Serum DHP concentrations were significantly increased after oocyte maturation. After high-dose SPE injection, *cyp17a1* expression decreased to undetectable levels. However, mRNA levels of *cyp17a2* and *hsd17b12L* increased gradually as the maturation proceeded until completion of ovulation. This study demonstrated at the first time that eels can produce DHP and suggested that DHP production is controlled mainly by 17OHP production due to a rapid drop in *cyp17a1* expression and not upregulation of *cyp17a2* and *hsd17b12L* mRNA expression.

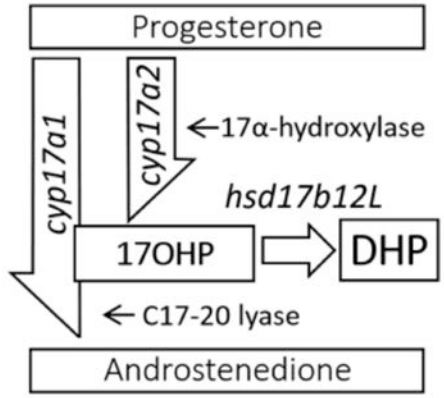

Figure 1. Molecular control of DHP synthesis.

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Current research on eel reproduction

Our group of research[1], leaded by Juan F. Asturiano[2] has been working on European eel reproduction since 1998 (recent PhD Thesis[3]). We have been involved in research projects funded by our Regional Government, by the Spanish Government, and by the European Union. Our research focuses the European eel reproductive physiology, the endocrine control of its gametogenesis and aspects related with the spermatology of this species (including sperm quality, sperm cryopreservation and studies on the effect of different ions on the sperm motility). In the last years we have been studying the combined effect of temperature and different hormonal treatments on the sex development, and the expression of key genes related with reproduction, the role of fatty acids during the gametogenesis, and the participation of several receptors, enzymes, etc. Nowadays we are studying the effect of temperature pre-treatments, before starting the hormonal treatment, on the male eel reproduction. Also, recently we started a collaboration with a Spanish biotechnology company to test the use of recombinant European eel FSH and LH on the maturation of both males and females. We collaborate with several European and Asian groups.

Running projects

- Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources. The need to harmonize and standardize evolving methodologies, and improve transfer from academia to industry (*AQUAGAMETE*). (COST Action FA1205; http://aquagamete.webs.upv.es/). J.F. Asturiano (Chair).
- European eel as a model to study the temperatura as a modulator of sexual maturation in teleosts. Potential application in aquaculture. (*REPRO-TEMP;* AGL2013-41646-R). Spanish Ministry of Economy and Competitiveness.
- Improved production strategies for endangered freshwater species (*IMPRESS*) H2020. Marie Sklodowska-Curie Actions. European Training Network. (Grant agreement nº: 642893; http://www.impress-itn.eu/)

1. http://acuiculturaenvalencia.blogspot.com.es/ (use the "translate" to read it in your own language)
2. https://www.researchgate.net/profile/Juan_Asturiano
3. http://hdl.handle.net/10251/3063; http://hdl.handle.net/10251/34625; http://hdl.handle.net/10251/48490; http://hdl.handle.net/10251/49988; http://hdl.handle.net/10251/68513

Eel research at UPV Valencia (Spain)

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Our region has a special relation with eels, as they are part of the local gastronomy and fisheries at the Albufera lagoon, and because the biggest Spanish eel farm is located at only 25 km from Valencia. This contribution summarizes the European eel research from the Grupo de Acuicultura y Biodiversidad (UPV, Valencia) from 1998. Our group has developed three main lines of research:

1) Spermatology and sperm physiology

We have tested many different maturation-inducing treatments[1-3]; investigated the dynamics and duration of the spermiation and the eel sperm production; checked and standardized[4] the use of techniques for the sperm quality assessment (sperm motility by CASA software, spermatozoa morphometry by ASMA[5], or sperm viability using different staining techniques and flow cytometry); and worked in short and long-term sperm preservation techniques, including a cryopreservation protocol[6,7]. We also studied the ionic composition of the European eel seminal plasma in relation to the sperm quality, in order to develop a good sperm extender for this species[8]. Based on this previous knowledge, we were able to produce the first eel cryolarvae[9] in collaboration with DTU team in the frame of the PRO-EEL project (www.pro-eel.eu).
Regarding sperm physiology, we have studied the role of different ions on the sperm motility initiation in the European eel. From a practical point of view, we demonstrated that the presence in the seminal plasma of Na^+ and K^+, but not Ca^{2+}, is necessary to maintain the sperm ability for further activation[10-12]. Seminal plasma protein profile[13] showed an increase in the levels of 20–60 kDa proteins in sperm samples with enhanced motility, and some of the major proteins were identified and correlated with sperm quality.

2) Teleost female gametogenesis

We started working on female European eel artificial maturation[14], with bad results in terms of egg quality. Later, we combined hormonal and thermal treatments[15] and observed interesting differences in FSH and LH expression, as well as in the steroidogenic process, in relation to thermal regimes. In a thermal regime starting with low temperatures, FSH expression levels were higher than at 20 °C, and more similar to the naturally maturing fish species. Later we tested the effect of three thermal regimes (combined with CPE) and observed that low temperatures facilitated early ovarian development but high temperatures accelerated the vitellogenesis progression[16]. For the first time, a down-regulation of ovarian *cyp19a1* caused by high temperatures in CPE-treated eels was observed. Finally, in a later experiment it was possible to obtain

good egg quality, as well as some larvae, mainly from one thermal treatment were low temperatures were used[17].

3) Control of reproduction of the European eel

We first observed that male eel treatment with hCG decreased the FSH gene expression, while LH expression and DHP plasma profiles agreed with a role in the final testis maturation[18]. The effect of thermal regimes on male eel artificial maturation was also studied: both their effect on sperm production and quality[3], and their effect on the steroid plasma levels and the gene expression of steroidogenic enzymes[19]. At 10 °C spermatogenesis stopped at A spermatogonia stage, 15 °C were necessary to induce a shift in the steroidogenesis from androgen to estrogen and progestin synthesis, and 20 °C were necessary to reach the spermiation.

We have also studied the expression of several receptors (GnRH[20], estrogen[21], progestin[22], aromatase and androgen[23] receptors) in relation to sex maturation, and the fatty acid mobilization in the eel body from muscle to liver and testis through the spermatogenesis[24] as well as its role on the sperm quality[25-27].

One problem of those studies was that it was almost impossible to differentiate the effects of the temperature from those derived from the hormonal treatment. Then, we started a series of studies to evaluate the effect of the environmental treatments (temperature and salinity) without adding any external hormone, on the brain-pituitary-gonad axis of male European eels. Transcriptomic analyses of this data are in course.

We also started a collaboration with a local biotechnology company (Rara Avis Biotec, S.L.), which produces specific recombinant FSH and LH of European eel for our research. We did the first trials with males[28], demonstrating that the half-life of these recombinant gonadotropins is long enough to induce *in vivo* effects, being able of inducing full testis development and spermiation using immature males. However, further experiments combining these recombinant hormones are required to improve the hormonal treatments.

1) 10.1111/j.1095-8649.2000.tb02227.x; 2) 10.1111/j.1365-2109.2005.01366.x; 3) 10.1016/j.aquaculture.2012.04.041; 4) 10.1016/j.theriogenology.2013.01.019; 5) 10.1016/j.theriogenology.2005.08.008; 6) 10.1007/s10695-005-1553-x;
7) 10.1016/j.cryobiol.2009.06.001; 8) 10.1111/j.1439-0531.2008.01206.x; 9) 10.1111/rda.12706; 10) 10.1016/j.cbpa.2015.10.009; 11) 10.1016/j.cbpa.2016.04.008; 12) 10.1016/j.cbpa.2016.09.024; 13) 10.1016/j.cbpa.2016.06.025; 14) Pérez et al., Cybium 2008;15) 10.1016/ygcen.2011.08.009; 16) 10.1016/j.aquaculture.2014.07.020; 17) Vílchez et al. 10th ISRPF; 18) 10.1016/j.ygcen.2009.08.006; 19) 10.1016/j.cbpa.2016.03.012; 20) 10.1016/j.mce.2013.01.025; 21) 10.1016/j.cbpa.2016.08.020; 22) Nuclear and membrane progestin receptors in the European eel: characterization and expression in vivo through spermatogenesis. Morini et al. (submitted); 23) 10.1111/rda.12321; 24) 10.1016/j.aquaculture.2014.03.045; 25) 10.1016/j.aquaculture.2014.10.016; 26) 10.1016/j.cbpa.2014.11.022; 27) 10.1016/j.cbpa.2015.01.015; 28) Specific recombinant gonadotropins induce spermatogenesis and spermiation for the first time in a teleost fish, the European eel (*Anguilla anguilla*). Peñaranda et al. (submitted).

Notes

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Current research on eel reproduction

Szent István University has been dealing with artificial induction of sexual maturation and propagation of European eel by using conservation biology value of the relatively old eel population. The aims are:1) to prepare the broodstock for artificial propagation by using short-term medical and long-term detoxification treatments and different salinity conditions; 2) to develop new technologies in egg management and larvae rearing.
Last year we have produced larvae and we were able to keep them alive up to eight days. During collaboration with Japanese researchers (Hokkaido University, Tokyo University of Agriculture), hybridisation experiments between Japanese eel and European eel were carried out by using European eel sperm samples, hybrid larvae were reared up to 14 days. At this workshop we would like to join the Eel Reproduction Innovation Centre EELRIC with the aim to function as a platform for the reproduction of eel in captivity and as a home for an international consortium of partners sharing experience and collaborating to create breakthroughs.

Running projects
- Artificial reproduction of European eel; Foundation for the Development of Animal Husbandry Sciences (Hungarian Fundation)
- Captive breeding of European eel; Mohamed bin Zayed Species Conservation Fund (no: 162512761)

Hungarian experiments on artificial propagation of aged European eel (maturation in different salinity, investigations on the extraction of sublethal pollutants, sperm cryopreservation)

Müller T., Horváth Á., Boltizár O., Várkonyi L., Bernáth G., Urbányi B., Horváth L.
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The European eel in Lake Balaton (Hungary) forms a unique stock. Eels hail from colonization in the period of 1961-1991, thus, this aged, landlocked population is unable to migrate. Over this long period of time, persistent pollutants can build up in fat stores, which can be harmful during the artificial induction of sexual maturation and propagation of over minimum 25-year old eels (in 2016). The nearly 100% infection rate by *Anguillicoloidescrassus* is another aggravating factor for succcesful maturation. According to our hypothesis, if the parasite and supposed pollutants could be eliminated in vivo from the aged eels, and the oogenesis whould be successfully completed. For in vivo detoxification, humic acids and zeolites were used. Short-term medical treatment and long-term detoxification treatments assisted to eliminate toxic agents or at least a part of them in vivo, and improved the reproductive fitness (fulvic acid in the peat influences the steroidogenesis). Consequently the pretreated old females ovulated, indicating successful oocyte maturation. Fertilisation tests were successful and hatched larvae survived for up to 8 days at 17 °C from one captive spawning.

The whole process of sexual maturation may be induced not only in saline but also in freshwater conditions by treating female eels with carp pituitary extract. One to seven days before the induced ovulation females were adapted to artificial seawater. The ovulated eggs were fertilised by active males indicating that seawater is not an obligatory environmental factor for stimulating gametogenesis and for artificial maturation in the European eel. Euryhaline eels are able to adapt to a wide range of salinities due to its catadromous life strategy involving maturation but there is a relatively short timeperiod when this genus shows different character – stenohaline propertises – (egg fertilisation, embryogenesis, early leptocephalus larvae stage). The freshwater rearing of maturation period has been proven it. Theoreticaly it would be better to determine the energy free ion background condition for maturation period. Osmoregulation is the active process by which an eels maintains its level of water content so we suppose that ioncomform (osmocomform) condition the eel use minimal energy to maintain its homeostasis and turn more energy for reproduction processes.

Sperm cryopreservation would allow preserving the best-quality sperm samples and making the synchronization of spermiation and ovulation unnecessary. Apart from this, it has been found suitable for the preservation of genetic resources of endangered species as well for general breeding purposes in cultured species. Eggs from Japanese eel females (*Anguilla japonica,* in Hokkaido University and Tokyo University of Agriculture) were artificially fertilised with native sperm of Japanese eel males and cryopreserved sperm from European eel (extender was modified Tanaka solution and methanol as cryoprotectant). There were no statistical differences ($p<0.05$) among the measured parameters such as fertilization, hatch and survival after 10 days post-hatch rates due to large individual differences. The malformation rate of larvae compared to the hatching rate was higher in cryopreserved groups (42.8 – 100%) than in the control (fertilization with native sperm: 16 – 49.9%, $p=0.069$) indicating that the methodology needs further refinement. Genetic analysis (PCR-RFLP) proved that the malformation was not caused by haploidity because the investigated randomly collected hybrid samples clearly showed the presence of both species specific, parental alleles.

Notes

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Job van Mil (MSc intern)

Current research on eel reproduction

Wageningen Livestock Research collaborates with DUPAN in a long-term research trajectory that aims to reproduce European eel in captivity. Currently, in a first phase project of this trajectory with Leon Heinsbroek, the Aquaculture and Fisheries group and DUPAN, and in collaboration with the Dutch Ministry of Economic Affairs, the aims are: 1) Optimal conditioning of the broodstock eels by feminisation, optimal feeding and simulated migration; 2) Subsequent artificial reproduction in order to optimise and newly develop larval rearing systems and diets. This year we have produced larvae from wild and farmed eels and we were able to keep them alive up to eight days. In a second phase we aim to upgrade our efforts to routine larval production and optimal larval feeding and growth by making use of state-of-the-art (genomic) tools and expanding our internal, national and international collaborations. At this workshop we will launch the Eel Reproduction Innovation Centre EELRIC with the aim to function as a platform for the reproduction of eel in captivity and as a home for an international consortium of partners sharing experience and collaborating to create breakthroughs.

Running projects

- Dutch Eel; Dutch Ministry of Economic Affairs (BO-20-010-114) and DUPAN
- Launching the eel reproduction innovation centre EELRIC; TKI-A&F (SMP16026)
- Innovative eel reproduction research and international collaboration; Dutch Ministry of Economic Affairs (KB-21-001-001)

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Research

Wageningen Eel Reproduction Experts (WERE) collaborates with DUPAN and Wageningen Livestock Research (WLR) in a long-term research trajectory that aims to reproduce European eel in captivity. Currently, in a first phase project of this trajectory funded by DUPAN and the Dutch Ministry of Economic Affairs, the aims are: 1) Optimal sourcing and conditioning of the broodstock eels by feminisation, optimal feeding and/or simulated migration; 2) Subsequent artificial reproduction in order to develop and optimise larval rearing systems and diets. This year we have produced larvae from wild and farmed eels which remained alive up to eight days. In a second phase we aim to upgrade our efforts to routine larval production and optimal larval feeding and growth.

Running projects

- Dutch Eel; Dutch Ministry of Economic Affairs (BO-20-010-114) and DUPAN

Broodstock conditioning, stimulation of maturation and successful reproduction of European eels in Wageningen

Palstra A.P.

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In nature, European eels (*Anguilla anguilla*) sexually mature during and/or after the ~6000 km semelparous reproductive migration from their freshwater habitats to the spawning grounds in the Sargasso Sea. In captivity, eels have been stimulated to mature with the purpose to artificially reproduce by injecting gonadotropins, either through hypophysation or, more recently, with recombinant FSH and LH. As the use of wild migrant silver eels as broodstock for artificial reproduction trials has become difficult due to management protection measures, conditioning methods should be developed to use farmed eels as broodstock. One such method was developed for Japanese eels and concerns the feminisation of glass eels through feeding estradiol-17β for 5-6 months which accelerates the previtellogenic oocyte development. Recently, we showed that early sexual maturation of farmed European eel can be enhanced by a simulated migration under mimicked photothermal conditions (Mes et al., 2016). With this method to make farmed eels silver we have developed another important tool for the conditioning of farmed broodstock eels.

In collaboration with ZFscreens BV (Leiden, the Netherlands), we have also combined both methods (Böhm et al., 2016): 1) For the first time European eels were feminised; 2) Feminised eels were then subjected to simulated migration to assess the effects on maturation, and 3) Feminised migrant eels were subsequently stimulated to fully mature by hypophysation. Correlations between the individual maturation responses to simulated migration and to hormonal injections were analysed to identify potential predictors for the selection of farmed broodstock. Results showed that the feminisation procedure for European eels had been successful. All eels were stimulated in their early maturation by simulated migration as indicated by an increase of the eye index. Fourteen out of the eighteen feminised migrants could be fully matured by hypophysation after 11-17 weekly injections. The eye indices after simulated migration correlated positively with the weight increase after injection 11 as a result of the hydration response of the oocytes (0.9-13.6%) and indicating the speed of the maturation response. The eels that ovulated were those that had the higher eye indices after simulated migration. Therefore we can conclude that simulated migration can be applied for both conditioning and selection of feminised broodstock eels.

In the currently running project, facilities have been expanded from a large swim-gutter to experimental freshwater and seawater RAS set-ups and a larval hatchery system. The aims are: 1) Optimal conditioning of the broodstock eels by feminisation, optimal feeding and simulated migration; 2) Subsequent artificial reproduction in order to optimise and newly develop larval rearing systems and diets. Simulated migration has been applied to condition batches of farmed and wild eels. These eels were then matured by hypophysation and ultrasonography was explored for a potential role to assist with timing of spawning (Jéhannet et al., this abstract book). Four batches of larvae were produced from wild and farmed eels thus far and they were kept alive up to eight days post hatching. Early development was studied as well as hatching capacity, active behaviour and occurence of morphological abnormalities. Now, together with Glasaal Volendam, further development up to the moment of first exogenous feeding is studied. Also a new batch of feminised eels has been produced. Together with a batch of farmed eels they are now being fed with a newly developed broodstock diet with the purpose to use them in near future reproduction trials.

Acknowledgements

Earlier described research was supported by a grant from the Dutch Ministry of Economic Affairs and the European Fisheries Fund: "Innovative reproduction of European eel" in the context of the Dutch Operational Programme "Perspectief voor een duurzame visserij" (Application #4610010911889 to R.P. Dirks), and KB-21-001-001 project "Eel reproduction" to A.P. Palstra funded by the Dutch Ministry of Economic Affairs. Later described research was supported by a grant from the Dutch Ministry of Economic Affairs and DUPAN: "Dutch Eel" (BO-20-010-114).

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Böhm, T., Graziano, M., Blom, E., Brittijn, S.A., Dirks, R.P.H., Palstra, A.P. (2016) Simulated migration of feminised eels to stimulate and predict the sexual maturation response. Annual conference COST action FITFISH (www.fitfish.eu), April 22 2016, Belgrade, Serbia.

Ultrasonography to assist with timing of spawning

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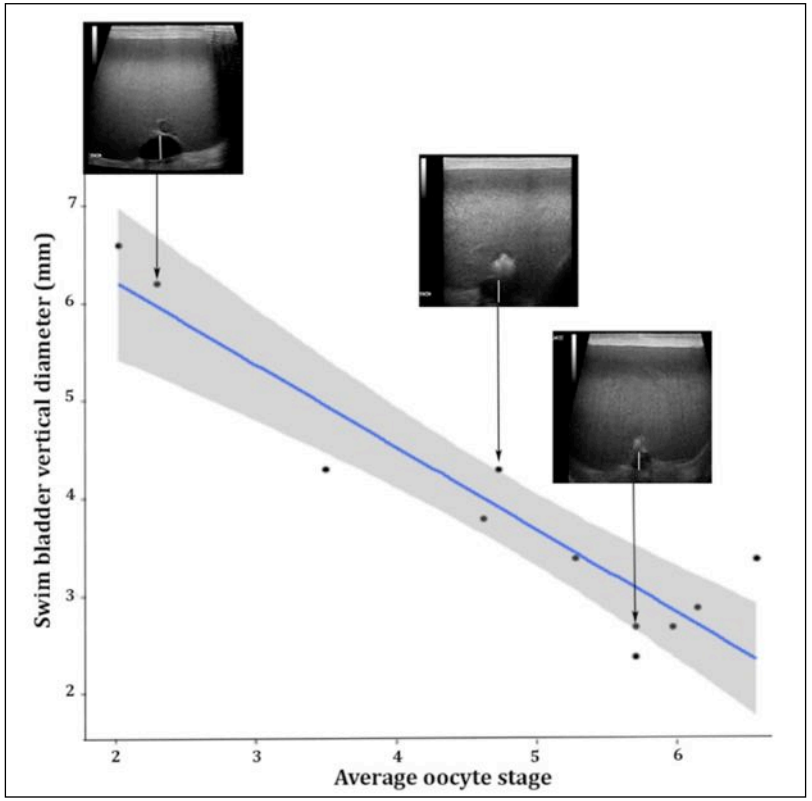
Ovulation in the European eel is induced by injection of DHP (17α,20β-dihydroxy-4-pregnen-3-one). The timing of injection is mainly based on the developmental stage of the oocytes. The oocyte stage is determined by biopsy or by external indicators of the oocyte hydration responses: body weight index (BWI) and body girth index (BGI). However, in European eel, BWI and BGI are inaccurate indicators because the individual hydration response is highly variable. Consequently, biopsies are taken to know the developmental stage of the oocytes. However, this method is invasive and could lead to infection since oocytes are sampled by using a needle or by inserting a cannula through the cloaca in the oviduct. The aim of this study was to find indicators of final oocyte maturation non-invasively by applying ultrasonography. BWI and BGI measurements, oocyte biopsy and ultrasonography were first performed on farmed female eels to identify ultrasound parameters, which indicate oocyte hydration. For this, correlation analysis between ultrasound parameters, average oocyte stage and average oocyte diameter were performed to determine the parameter of highest accuracy. Ultrasound parameters that were determined were body wall thickness, cloaca opening, grey scale median (GSM) of gonads and liver, and diameters (vertical and horizontal) and surface area of liver, gonads and swim bladder. Selected parameters were subsequently validated for wild silver female eels. Data of farmed and wild eels were then combined for correlation analyses and principal component analyses (PCA). Gonad and liver parameters were not correlated with the average oocyte stage. Body wall thickness was negatively correlated to the average oocyte stage ($y=-0.018x+0.4069$, $p=0.049$ and $R^2=0.293$) but variability was quite high. The swim bladder vertical diameter (Sbv) was negatively correlated with the average oocyte stage (farmed eels: $y=-0.0841x+0.7899$, $p=3.09e-5$ and $R^2= 0.8673$, $n=11$; and incl. wild eels: $y=-0.0795x+0.7831$, $p=6.27e-6$ and $R^2=0.8026$, $n=15$) and with the oocyte diameter (farmed eels: $y=-0.0012x+1.4287$, $p=0.0108$ and $R^2=0.5323$, $n=11$; and incl. wild eels: $y=-0.0009x+1.1957$, $p=0.0141$ and $R^2=0.3816$, $n=15$). Swim bladder area (Sba) was also negatively correlated to the average oocyte stage but less strict (farmed eels: $y =-9.0093x+76.313$, $p=0.002$ and $R^2= 0.6665$; and incl. wild eels: $y=-8.1846x+75.557$, $p=0.003$, $R^2=0.4959$). Sba and the average oocyte diameter were significantly correlated for farmed eels ($y=0.0115x-5.001$, $p=0.0422$ and $R^2=0.3833$) but not significantly when including the wild eels ($p=0.080$, $R^2=0.217$, $n=15$). Females with an average oocyte stage between 4.5 and 7.0 and an average oocyte diameter higher than 800 µm, had Sbv values ranging between 2.4 and 4.3 mm and Sba values between 11 and 52 mm². PCA revealed that Sbv, Sba, average oocyte diameter and average oocyte stage contributed mostly to PC1 that explained 50% of the variance. For PC2, BWI and BGI contribution explained an additional 24% of the total variance. In conclusion, results show that during the final stages of oocyte maturation, the swim bladder becomes smaller. This is most probably due to the pressure that is caused by the increasing gonad due to the oocyte hydration response. The correlation between Sbv and oocyte stage is more strict than for BWI or BGI which makes Sbv not only a parameter that can be determined non-invasively but also one that is more accurate in indicating the oocyte developmental stage. Therefore, ultrasonography, and in particular the ultrasound imaging of the swim bladder, represents a useful tool to assist with the timing of spawning as induced by injection of DHP.

Fig. 1: Swimbladder vertical diameter (Sbv) decreased during final oocyte maturation of farmed eels. Linear regressions between Sbv and the average oocyte stage/diameter with ultrasound images of Sbv (indicated by the white lines).



Notes

New Zealand eel aquaculture where at?

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New Zealand has mainly two species of eels the shortfin *Anguilla australis* and *A. dieffenbachii* which is endemic to New Zealand. A third species *A. reinhardtii* was naturally introduced to New Zealand waters and are often found in eel catches.

This presentation is to describe the attempts of farming the eel in New Zealand and the national management plan to conserve the species especially Longfin because it is believed to be in decline. Historical account of our attempts at closing the lifecycle of New Zealand shortfin eel *A. australis* is discussed. In addition to producing thousands of eggs on demand, nearly all year round and keeping the larvae alive for few days we managed to get the broodstock to re-feed after the starvation period. Ideas for future work will also be discussed.

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Current research on eel reproduction

We are running a three-year project on artificial eel reproduction that aims to re-establish the production of first feeding eel larvae and investigates the maternal transfer of lipophilic contaminants in eels. The project is funded by the Innovation Fund of the German Federal Ministry of Food and Agriculture an also includes a cooperation with the University of Hamburg where we aim to optimize culture techniques for potential planktonic feed organisms for eel larvae. This project is part of a long-term research approach on European eel at the Thünen Institute of Fisheries Ecology. Our major focus is to contribute to a better understanding of the eel population decline and to develop evaluation criteria for different management strategies. In this context we also conduct regular larvae surveys in the Sargasso Sea, assess the spawner quality throughout German eel habitats and are responsible for the eel data collection framenwork in Germany. Moreover we carry out migration studies and recently started swimming experiments in our newly developed hyperbaric swim tunnels.

Early life stages in spawning area and lab – Eel research at the Thünen Institute

Marohn L., Hanel R.
Thünen Institute of Fisheries Ecology, Hamburg, Germany

In 2014 a joint project on artificial eel production was implemented at the Thünen Institute of Fisheries Ecology in Hamburg, Germany, financed by the Innovation Fund of the German Federal Ministry of Food and Agriculture. Together with the Thünen Institute as the project coordinator, the University of Hamburg and two companies, the BlueBioTech GmbH (microalgae producer) and the Kunststoff-Spranger GmbH (aquaculture engineering) are involved as project partners. The main objective of this three-year research project is to re-establish the production of first feeding eel larvae in Germany on a regular basis and, as a second work package, to optimize culture techniques for potential planktonic feed organisms or particles for eel larvae.

This project is one major pillar in a very broad and long-term research approach on European eel at the Thünen Institute of Fisheries Ecology. Major focus is to contribute to a better understanding of the eel population decline and to develop evaluation criteria for different management strategies. With a combination of field studies in the spawning area of the Atlantic eels in the Sargasso Sea and experimental research on the effects of spawner quality on embryonic and early larval development we want to learn more about the eel's vulnerability to factors that might significantly affect survival, such as chemical pollution.

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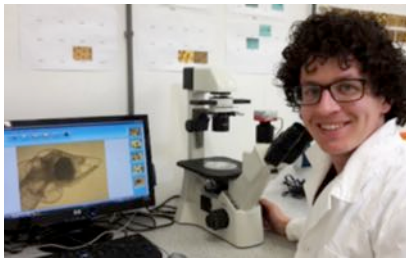

Rick Leemans
Researcher hatchery technology

Current research on eel reproduction

Glasaal Volendam BV is a private company aiming to produce glasseel at a commercial scale. We are funded by shareholders and subsidies. Our facilities in Volendam are specially build for European eel reproduction. This means that we are currently doing full-time applied research.
At this point we have a regular larvae production and we are starting larvae feeding experiments. We work with farmed eels and with feminized animals, both are producing larvae.
Other fields of study include broodstock-feeding trials and development of larval rearing systems.

Notes

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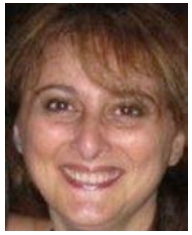

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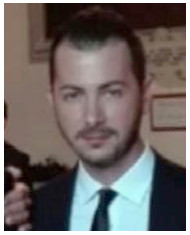

Dr. Antonio Casalini

Current research on eel reproduction

FEEL-EEL Research Group started its activity with European eel in captivity since 2010, in collaboration with Emilia Romagna Region and, recently, with the Department of Zoology, University of Otago, Dunedin, New Zealand. The first research focused on reproductive capacity of wild eel populations from Northern Adriatic Sea. The second step in the research was to obtain spontaneous reproduction in captivity of the European eel by using a new closed recirculating aquaculture system provided with spawning and incubation chambers. The influence of water-flow rates on the spawning, fecundity and egg quality was also investigated. In an additional study we compared the fertility of eggs between artificially matured female silver eels that spawned spontaneously and those that spawned by manual stripping. The effects of the two methods of spawning on ovulation and fertilization rate were also investigated. Applied protocols allows us to obtain up to 200000 larvae/female, which survive up to 21 days. Future research will focus on the identification of the ideal larval diet, to get to complete weaning of larvae.

Running projects

- FEEL-EEL, Emilia Romagna Region: "Setting of induced reproduction for European eel (*Anguilla anguilla*) and feeding test for larval survival rate"

Recent advances in eel artificial reproduction in Italy

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This contribution summarizes results obtained in eel artificial reproduction by Bologna University from 2013-2015. First step was to study the response variability of female silver eels in terms of gonad development and eggs production to a standardized gonadotropic treatment (Carp pituitary extracts—CPE), and to relate this variability to population characteristics. For this purpose, different morphological (Silver index—SI) and hormonal (17b-estradiol—E2, testosterone—T) parameters and lipid content were studied in two wild female populations (Comacchio—CM and Marano-Grado—MG lagoons) during artificial reproduction. The two population proved to differ in terms of morphological, biochemical and hormonal parameters and as response to hormonal induction. As to zootechnical performances, while MG eels released spontaneously into the water, the CMs were stripped in order to check ovulation. The MG eels were statistically the most productive with 40.1 ± 6.33 % BW of eggs released. Furthermore, CM females ovulated mainly between the 19th and 22nd week (77.8% spawned eels) instead in the MG's ovulation goes from the 24th to the 28th week (100% spawned eels). These results seem to indicate that bigger dimensions, higher K, and larger lipid content (Comacchio eels) could fasten gonadic maturation without positively influencing reproductive performance of animals, both in term of quantity and quality of produced eggs. Smaller females with a highest SI (Marano-Grado eels) presented a more regular gonadic development, leading the animals to spontaneous spawning.

The second step in the research was to obtain spontaneous reproduction in captivity of the European eel by using a new closed recirculating aquaculture system provided with spawning and incubation chambers. The influence of water-flow rates on the spawning, fecundity and egg quality was also investigated. The results showed that the designed closed-loop system made it possible to carry out a more spontaneous reproduction for more than 80% of the females that underwent standardized gonadotropic treatment and favored the automatic and complete transfer of the eggs to the hatchery. The results also point out that high or low water current conditions in the tank do not hinder the mating and the emission of gametes by the breeders, but the High-Flow rate in the two incubation chambers showed unsuitable hydrodynamic conditions for embryonic development resulting in a constant loss of viable eggs which reached a mortality of 100% among females with the highest incubation density.

In an additional study we compared the fertility of eggs between artificially matured female silver eels that spawned spontaneously and those that spawned by manual stripping. The effects of the two methods of spawning on ovulation and fertilization rate were also investigated. Wild female European eels were carp pituitary extract-injected to undergo sexual maturation and ovulation; a final injection of 17,20b-dihydroxy-4-pregnen-3-one (DHP) was administered. After the DHP-injection, nine eels were transferred to a new closed recirculating aquaculture system, where they were housed with spermiating males (sex ratio 4/1) to allow spontaneous spawning (SPT-group); the remaining nine eels were transferred to a 250 L tank and ovulation was checked at four-hourly intervals by manual stripping (STR-group). The number of eggs per female in the SPT-group was significantly greater than that in the STR-group. Furthermore, fertilization rates in the SPT-group were notably higher than those observed in the STR-group. Significantly, the best performances were obtained among eels in which at least 50% of oocytes were fully transparent at the time DHP was administered. We conclude that the fertility of eggs from spontaneously spawning eels is superior to that of eggs acquired by strip-spawning and artificial fertilization.

Finally, we determined the effects of dark and light conditions on the E2, testosterone and thyroid hormones levels and on the gene expression levels (vitellogenin 1, vitellogenin 2, and estradiol receptor one) in European eels during ovarian development induced by increasing doses of carp pituitary extracts (CPEs). The subjects were divided into 2 groups: 14-hour light:10-hour dark (Light-group) and 24-hour darkness (Dark-group) and underwent our standard CPE induction protocol. Vitellogenin and estradiol receptor expression levels did not show significant differences between the two housing conditions whereas in both groups vitellogenin mRNA increased starting from first CPE injection. Testosterone and 17-beta estradiol plasma levels were significantly greater in the Dark-group compared with the Light-group starting from the ninth and the 13th week, respectively. These results suggest that darkness could be a useful variable for standardizing gonadal maturation in eels kept in captivity.

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European eel hatchery technology: Recent results on breeding outcome and culture of early life stages

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The recent collaborative research project, PRO-EEL, funded by the EU FP7 program and coordinated by DTU, was successful in establishing methodologies for a stable production of viable offspring of European eel. Furthermore, this research succeeded in enhancing survival of larvae throughout the yolk-sac stage in recirculation aquaculture systems. This work continues in present Danish innovation project, EEL-HATCH, with the aim to improve the development of breeding and hatchery technologies for this species.

Here, our aim is to expand the knowledge base on nutritional and reproductive physiology as well as to move this new knowledge into application development for broodstock feeds and hormonal treatments for high-quality offspring. Another aim is to identify larval biophysical requirements and suited feed for ongrowing in larval culture through basic research that leads to application and technology development. In this presentation, our recent progress in European eel reproductive performance and larval culture technology will be overviewed. Overall, we hope in EEL-HATCH to come one-step-closer towards closing the life cycle for the European eel in captivity.

Notes

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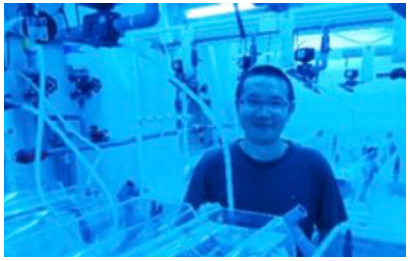
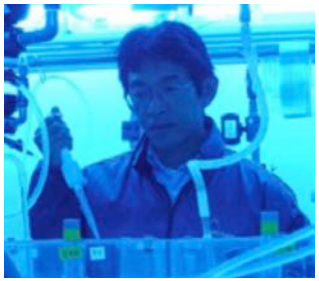


Basic Technology group in Nansei Main Station



Hideki Tanaka (PhD)

Kazuharu Nomura (PhD)

Mission

National Research Institute of Aquaculture has been established to help produce safe and high quality fish and shellfish as food, through promoting the fish/shellfish farming industry. To this end the institute conducts research and development on various aspects of aquaculture technology, which includes production of juvenile Japanese eels, breeding of other important fish/shellfish species in captivity, economical fish feeds based on nutritional physiology, diagnosis, prevention, and treatment of fish/shellfish diseases, and sustainable farming system for aquatic animals. In Research Center for Self-Sustained Eel Culture, we conduct research and development of artificial induction of maturation, good quality feed, breeding and larval rearing of Japanese eel, aiming to established a mass production technology of eel seedlings for self-sustained eel culture.

Running projects

- Development sustainable aquaculture technology independent of wild fishery resource; Agriculture, Forestry and Fisheries Reserch Council, Ministry of Agriculture, Forestry and Fisheries of Japan.
- The project of mass production of Japanese eel; Fisheries Agency.
- The genetic breeding of Japanese eel; National Agriculture and Food Research Organization.

Artificial seedlings production of the Japanese eel

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The Japanese eel (*Anguilla japonica*) is one of the most important freshwater aquaculture fish species in East Asian countries. Seeds for the present eel culture depend completely on the capture of wild glass eels in estuaries whose abundance has decreased markedly due to overfishing, environmental destruction, or other factors such as ocean conditions. This situation leads to unstable supplies and rising glass eel prices which are serious problems in the eel culture industry. In order to solve these problems for stable eel culture management, it is absolutely imperative to establish techniques for artificial production of glass eel seedlings for aquaculture to reduce the demand of wild glass eels.

These techniques have been improved markedly over the last few decades (Fig. 1). Our group (National Research Institute of Aquaculture, Japan Fisheries Research and Education Agency) has succeeded in obtaining artificially matured gametes by using hormonal treatment. Then, viable leptocehali and glass eels have been successfully produced by the development of appropriate rearing techniques. In 2010, our group achieved the completion of the life cycle of the Japanese eel for the first time under artificial conditions using healthy larvae (F2 generation) obtained from artificially propagated male and female (F1 generation). However, the mass production of glass eels has not yet been succeeded. Thus, further improvements are necessary to produce glass eels with high survival rate and growth rate commercially in a sufficient number to meet the demand of aquaculture industries.

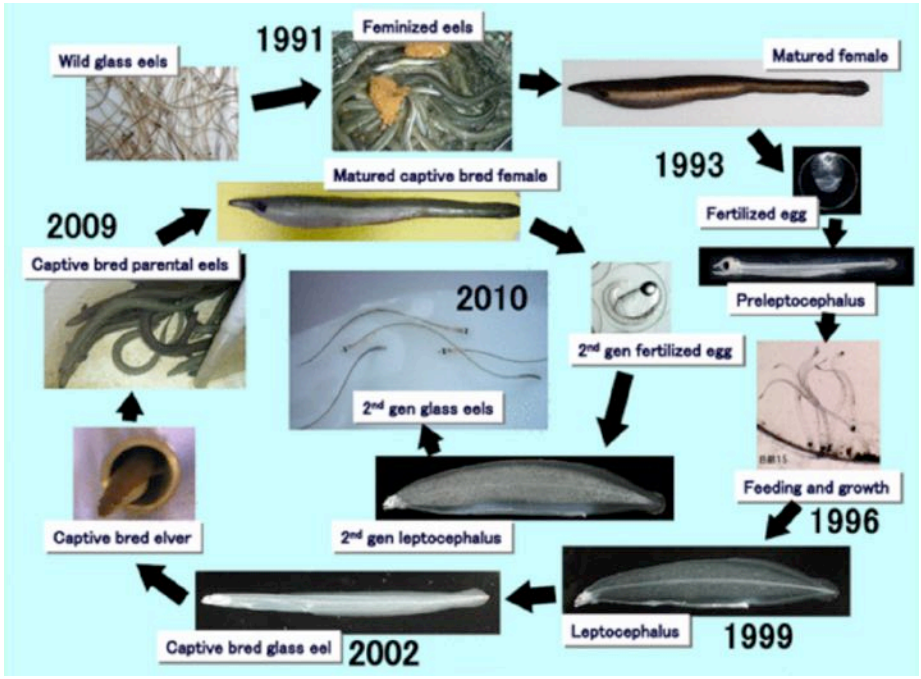

Fig.1
Progressin of research on eels until the attainment of full-life culture. (Tanaka 2015)

Under the current conditions, the start of metamorphosis is delayed. For example, some eels could not start metamorphosis even after they reached a total length of 60 mm or more, which is beyond the size at which metamorphosis occurs in the wild. The delay of metamorphosis prolong the larval stage, which directly affects the rearing costs. To solve this problem, we need to understand the mechanisms of metamorphosis of eels as the first step.

Thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3), are known to induce metamorphosis in many fishes, such as the Japanese flounder, grouper and tilapia. To reveal the role of thyroid function during metamorphosis from leptocephalus to glass eel in the Japanese eel, we measured the whole-body concentrations of THs and thyroid hormone stimulating hormone β-subunit (TSHβ) mRNA expression levels in five stages of artificially hatched eels (leptocephalus, early-metamorphosis, late-metamorphosis, glass eel, and elver). During metamorphosis, both T4 and T3 levels were significantly increased, whereas a small peak of TSHβ mRNA level was observed at the early-metamorphosis stage (Fig. 2). Similarly, TSHβ mRNA levels

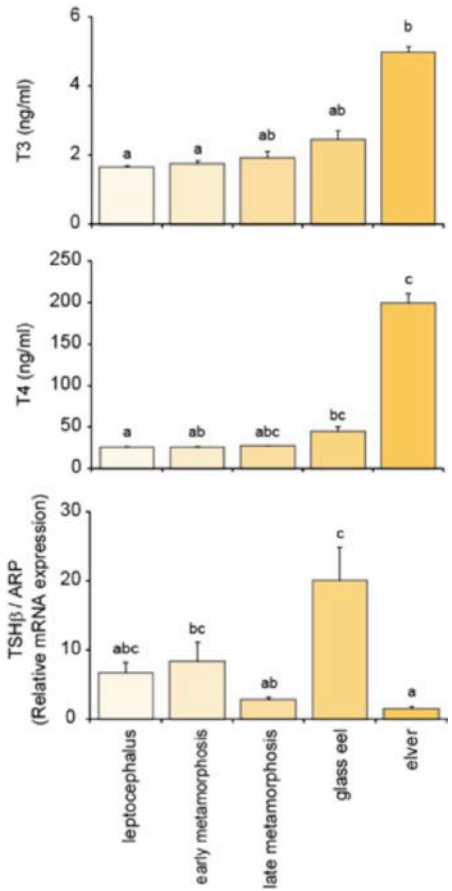

Fig.2 Changes in the levels of T4, T3 and TSHβ mRNA expression during metamorphosis.

were highest in the glass eel stage, and then decreased markedly in the elver stage. In contrast to TSHβ mRNA expression, thyroid hormones (both T4 and T3) increased further from the glass eel to elver stages. In addition, T4 administration to pre-metamorphosis eel larvae shortened the gut length, which is one of the features of metamorphosis. These results indicated that thyroid function in the Japanese eel was active both during and after metamorphosis.

For further understanding of metamorphosis of eels, RNA-seq of head and whole body of eel larvae during metamorphosis was performed. Approximately 373 million reads were generated and assembled 320,321 transcripts. The results of comprehensive analysis of RNA-seq data showed that many genes were drastically changed during the early to late metamorphosis stages. From gene ontology analysis, it is revealed that the expression of genes for transcriptional elongation, regulation of myofibril size and glycolytic process was greatly changed. In near future, more detailed analysis is needed to clarify the mechanisms of metamorphosis of anugillid eels.

* Tanaka H. (2015) Progression in artificial seedlings production of Japanese eel *Anguilla japonica*. Fish Sci 81:11-19

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Current activities

ZF-screens' main activities were related to (1) artificial reproduction of aquacultured fish, including European eel, (2) pharmaceutical and toxicity screens in zebrafish larvae and (3) *de novo* genome assembly and transcriptome profiling via Next generation sequencing. Recently, we have stopped our aquaculture-related activities and shifted our main focus toward third generation sequencing. The European eel is still our favourite model species and the Eel genome version 2.0 project is a perfect platform to test the full potential of the disruptive new nanopore sequencing technology. Our ambitious goal is to assemble a reference quality eel genome, ideally to the level of individual chromosomes. We have already obtained several-fold coverage of MinION long-read data and are beginning to recognize the great advantage compared with Illumina short-read sequencing.

Running projects

- IMPRESS: MSCA-ITN network for the conservation and management of endangered freshwater fish (www.impress-itn.eu)
- ParaFishControl: "Improving our understanding of fish-parasite interactions" (www.parafishcontrol.eu)

Toward improved assembly of the European eel genome sequence using Nanopore sequencing technology

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The eel is one of the oldest known fish species and has been around for at least a hundred million years, i.e. long before most modern fish species appeared. Sadly, three out of nineteen known eel species (European, American and Japanese eel) are currently endangered species. The eel is not only of great ecological, cultural, culinary and commercial value, it has also been a source of inspiration for various scientific discoveries. For instance, the first fluorescent protein ever discovered in a vertebrate animal was recently found in Japanese eel, which has already resulted in a revolutionary new medical test for certain human diseases. [Kumagai et al (2013) A bilirubin-inducible fluorescent protein from eel muscle. Cell 153, p. 1602-1611].

Not so long ago, mapping the complete genome of a vertebrate organism was a very lengthy and expensive process. E.g., it took about fourteen years to sequence the human genome using first generation technology (Sanger sequencing) at a price of 3 billion (!) Dollars. That all changed in 2006 when second generation sequencing (e.g. Illumina) was introduced. In 2010, Leiden University and ZF-screens took the initiative to unravel the genetic code of the European and Japanese eel. To this end, a mini-consortium was built that also included a Norwegian and Japanese university and the National Museum of Natural History in Paris. Within the financial and technical limits of that time we generated the first usable draft genome sequences of the European and Japanese eel and made them available to the scientific community via two publications in 2012. Since then this new source of information has already proven its value, and contributed to more than 20 scientific publications. Nonetheless, the draft genome sequence of the European eel is still highly fragmented. The code is divided over nearly 200,000 small pieces (scaffolds), whereas ideally we would like to have a complete genome sequence consisting of 38 intact chromosomes; however, five years ago mapping a complete genome would still have cost millions of Euros.

Whereas first and second generation sequencing already had a huge impact on multiple disciplines of biological, medical and pharmaceutical sciences, third generation sequencing (e.g. Pacific Biosciences, Oxford Nanopore) is expected to completely change the "omics" landscape again. Since 2005, a company named Oxford Nanopore Technologies (ONT) has been developing a revolutionary new technology that allows the direct analysis of very long DNA fragments by threading the DNA strand through tiny protein pores. Massive parallel production of these long sequence reads is now

within reach and applications that were not even possible a few years ago are now available or will be available soon. Examples include direct sequencing of complete messenger RNAs, direct sequencing of very long DNA molecules (>100 kb), direct mapping of DNA modifications from unprocessed DNA molecules, rapid *de novo* assembly of complex vertebrate genomes, *de novo* assembly of plant or amphibian genomes with sizes of tens or even hundreds of gigabasepairs. The anticipated output of the new, small and cheap nanopore sequencing device, the PromethION, is in the range of terabases per day, whereas its smaller cousin, the MinION, can be taken into the field for rapid streaming applications, such as diagnostics of diseases. It is already clear that this technology will have a major impact on the current shift toward third generation sequencing.

In 2014, ZF-screens joined the early access program of the first available nanopore sequencers and is now reaching a stage where it can use these devices to generate a significantly improved version of the European eel genome. Construction and analysis of the complete eel genome will again be performed in collaboration with and with support from mini-consortium partners, which now also includes the DUPAN foundation. The improved eel genome sequence is expected to be of significant value to research in the field of (among others) artificial reproduction, behavior, migration, population dynamics and evolution.

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