The eel genome consortium:
Leiden University, MNHN/CNRS/Paris, AORI/University of Tokyo, Norwegian School of Veterinary Science/Oslo, ZF-screens BV/Leiden

EEL GENOME SYMPOSIUM 2014

new perspectives in evolution, reproduction and ecology

16-17 January 2014

Leiden, The Netherlands

Abstract book

in collaboration with:

Program

Thursday 16 January 2014				
9.00 - 9.30 h	registration and coffee/tea			
Genomics (chair: Ron Dirks)				
Time	Speaker	Affiliation	Title	Abstract
9.30 - 9.40 h	Herman Spaink	Leiden; Netherlands	Opening of the Symposium	n/a
9.40 - 9.50 h	Sylvie Dufour	Paris; France	Introduction	n/a
9.50 - 10.10 h	Christiaan Henkel	Leiden; Netherlands	Towards finished eel genomes	Page 4
10.10 - 10.30 h	Jérémy Pasquier	Rennes; France	Integration of European eel transcriptome into the PHYLOFISH project, a high throughput phylogenomic analysis designed to study teleost gene evolution after whole genome duplication	Page 5
10.30 - 10.50 h	Scott Pavey	Québec City; Canada	The draft genome of the American eel (Anguilla rostrata)	Page 6
10.50 - 11.10 h	Shaadi Pooyaei Mehr	New York; USA	Muscle transcriptome profiling of Kaupichthys hyoproroides	Page 7
11.10 - 11.40 h	coffee/tea break			
Ecology (chair: Caroline Durif)				
11.40 - 12.00 h	Olga Haenen	Lelystad; Netherlands	Eel diseases: an overview	Page 8
12.00 - 12.20 h	Jens Frankowski	Rostock; Germany	Large scale genetic monitoring of Atlantic eels in Northern Germany	Page 9
12.20 - 12.40 h	Tomasz Podgorniak	Bordeaux; France	Selectivity of water dams on glass eel : transcriptomics approach	Page 10
12.40 - 13.00 h	Marti Pujolar	Aarhus; Denmark	Genomic diversity and differentiation in European eel using RAD sequencing-generated single nucleotide polymorphisms	Page 11
13.00 - 14.30 h	lunch break + poster session			
Posters				
	Agnès Bardonnet	Saint-Pée-sur-Nivelle; France	Expression profiles for six sex-specific genes during gonadal development in eels	Page 32
	Anne-Gaëlle Lafont	Paris; France	Characterization of three nuclear and two membrane estradiol receptors in the eel: involvement in experimental maturation process	Page 33
	Gersende Maugars	Paris; France	Pituitary hormone glycoprotein receptors in eel: characterization and evolutionary insights	Page 34
	Marina Morini	València; Spain	Characterization and expression of the Vitellogenin receptor in the European eel	Page 35
	Arjan Palstra	Yerseke; Netherlands	Silver eel broodstock conditioning, artificial reproduction and larval rearing at IMARES	Page 36
	Michaela Mandelli	Bologna; Italy	Effect of photoperiod on endocrine profiles in european eels anguilla anguilla during artificially induced ovarian development	Page 37
Evolution (chair: Finn-Arne Weltzien)				
14.30 - 14.50 h	Sylvie Dufour	Paris; France	Impact of genome duplications on teleost reproductive neuroendocrine systems: the eel model	Page 12
14.50 - 15.10 h	Gersende Maugars	Paris; France	Discovery of duplicated genes for LH receptor in a basal teleost, the European eel (Anguilla anguilla)	Page 13
15.10 - 15.30 h	Jérémy Pasquier	Paris; France	Diversity and evolutionary history of the Kisspeptin system across vertebrates: the benefits from studying unconventional model genomes	Page 14
15.30 - 15.50 h	Steven van Beurden	Utrecht; Netherlands	On the genomics of host-pathogen co-evolution: the viral interleukin-10 story	Page 15
18.30 - 20.30 h	Symposium dinner at the Garden restaurant of Holiday Inn Leiden, Haagse Schouwweg 10, Leiden			

Program (continued)

Friday 17 January 2014				
8.30 -9.00 h	arrival with coffee/tea			
Endocrinology (chair: Juan Asturiano)				
Time	Speaker	Affiliation	Title	Abstract
9.00 - 9.20 h	David Peñaranda	València; Spain	Modulation of testis steroidogenic enzymes gene expression and steroid synthesis by temperature thermal regime during spermatogenesis in European eel	Page 16
9.20 - 9.40 h	Marina Morini	València; Spain	DHP receptors and PLCZ1 expression during the European eel spermatogenesis	Page 17
9.40 - 10.00 h	Anne-Gaëlle Lafont	Paris; France	First evidence of a duplicated leptin/leptin receptor system in a vertebrate species, the eel, and involvement in sexual maturation	Page 18
10.00 - 10.20 h	Finn-Arne Weltzien	Oslo; Norway	The pituitary gland of the European eel reveals massive expression of genes involved in the melanocortin system	Page 19
Toxicology (chair: Guido van den Thillart)				
10.20 - 10.40 h	Ibon Cancio	Plentzia; Spain	Transcriptional profiles of Hg in the liver of European eel (Anguilla anguilla)	Page 20
10.40 - 11.00 h	Tamás Müller	Gödöllő; Hungary	Possible way for extraction of sublethal pollutants from aged european eel	Page 21
11.00 - 11.20 h	coffee/tea break			
11.20 - 11.40 h	Lucia Privitera	Suffolk; UK	Effects of short term metal exposure on glass eel DNA integrity and freshwater adaptation	Page 22
11.40 - 12.00 h	Gordon Cramb	St. Andrews; UK	Myo-inositol biosynthesis and osmoregulation in the eel; potential disruption by organophosphorus xenobiotics	Page 23
12.00 - 12.20 h	Marti Pujolar	Aarhus; Denmark	A transcriptomic approach to assess the effect of environmental pollution in the European eel	Page 24
Physiology (chair: Yoshio Takei)				
12.20 - 12.40 h	Delphine Cottin	Paris; France	Origin and role of Transient Receptor Potential Vanilloid (TRPV) ion channels in vertebrates	Page 25
12.40 - 13.00 h	Allison Churcher	Faro; Portugal	Differential gene expression in the olfactory epithelium of Anguilla anguilla	Page 26
13.00 - 14.00 h	lunch break			
14.00 - 14.20 h	Arjan Palstra	Yerseke; Netherlands	Transcriptomic characterisation of the interactions between metabolism and sexual maturation under exercise in European eel	Page 27
14.20 - 14.40 h	Ron Dirks	Leiden; Netherlands	Molecular markers in pectoral fin to predict hormone-induced maturation of farmed female European eels (Anguilla anguilla)	Page 28
Physiology (continued; chair: Arjan Palstra)				
14.40 - 15.00 h	Yoshio Takei	Tokyo; Japan	The eel: an excellent but enigmatic model for the study of osmoregulation	Page 29
15.00 - 15.20 h	Ohiane Diaz de Cerio	Plentzia; Spain	Transcriptomic responses of Anguilla anguilla elvers fed dietary LPS	Page 30
15.20 - 15.40 h	Erik Burgerhout	Leiden; Netherlands	Biomarkers for broodstock selection of farmed female European eels	Page 31
15.40 - 16.00 h	coffee/tea break			
Panel discussion (chair: Ron Dirks)				
16.00 - 16.45 h	Ron Dirks	Leiden; Netherlands	(1) Symposium paper, (2) funding opportunities toward finished eel genomes, (3) any other business	n/a
16.45 - 17.00 h	Guido van den Thillart	Leiden; Netherlands	Closure of the Symposium	n/a
17.00 - 18.30 h	drinks and snacks			

Oral presentation

Towards finished eel genomes

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The genomes of the European and Japanese eel were sequenced and assembled in 2010–2011, and published in 2012. Since then, these draft genome sequences have enabled a number of studies, for instance on genome-wide gene expression and on the function and evolution of specific genes. However, the genome assemblies are relatively fragmented, preventing high-quality annotation, as well as large-scale comparative genomics studies.

This fragmentation is an inevitable result of the sequencing technology employed. The eel genome projects were made possible by advances in sequencing technology, specifically the advent of relatively affordable, massively parallel short read sequencing on the Illumina platform. At the time, Illumina sequencing was effectively limited to fragments <300 bp. Reconstructing entire eukaryotic genomes (in the case of eels, ~10^9 bp) from such small fragments is challenging, and often fails at local polymorphic or repetitive genome content.

In the two years since the eel genomes were assembled, substantial progress has been made in both sequencing technology and bioinformatics algorithms. I will discuss the prospects of using these technologies in future eel genomics projects, with the ultimate goal of producing fully finished reference genome sequences.

Oral presentation

Integration of European eel transcriptome into the PHYLOFISH project, a high throughput phylogenomic analysis designed to study teleost gene evolution after whole genome duplication

J. Pasquier[1], C. Cabau[2], P. Gouret[3], L. Journot[4], C. Klopp[2], P. Pontarroti[3], J. Postlethwait[5], Y. Guiguen[1], J. Bobe[1]

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Investigation of gene evolution after whole genome duplications is crucial to better understand the mechanisms by which genomes evolved and drive the development and physiology of vertebrates. The PHYLOFISH project will take advantage of the additional whole genome duplication rounds of teleost to address this question. In addition, a special attention has been paid to species presenting a key taxonomic position in the teleost tree of life, including the European eel as a basal teleost.
Using next generation sequencing, this project has already provided original information on the transcript repertoires of 24 fish species, including European eel. The eel RNA libraries have been produced from 10 organs, including ovary, testis, brain and liver, in addition to a leptocephalus larva. The number of sequenced reads per libraries were comprised between 35 million to 83 million and enabled to reconstruct more than 60 thousand contigs. The totality of the generated datasets will be released and accessible through a web browser providing multiple and comparative information on the transcript repertoires of the 24 investigated species.
This evolutionary-relevant sequence dataset has then been used as a basis for the development of a high throughput analysis combining gene phylogenies, synteny information, and expression profiling. As we have investigated a wide range of teleost species, including basal species such as European eel, the results of this project should provide high resolution and genome-wide answers concerning the fate of paralogous genes after genome duplication events in teleosts. The project will provide new insight into the influence of sub- and neo-functionalization process on teleost gene diversity. In addition, and because these gene duplications also have a major impact on the quality of gene annotation in teleosts, the PHYLOFISH project will propose a phylogenetically-supported refinement of teleost gene nomenclature. This will link gene information across many vertebrate species, allowing to bridge functional information from conventional model species to emergent model species.

This work was supported by French National Research Agency grant PHYLOFISH (ANR-10-GENM-017) to JB.

Oral presentation

The draft genome of the American eel (*Anguilla rostrata*).

Scott A. Pavey[1], Jeremy Gaudin[1], Eric Normandeau[1], Louis Latourneau[2], Sébastien Boisvert[1], Jacques Corbeil[1], Céline Audet[3], and Louis Bernatchez[1].

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American eel (*Anguilla rostrata*) reproduce in a single panmictic population in the Sargasso Sea in the Atlantic Ocean. After hatching, they migrate as leptocephali to a large diversity of salt, brackish, and freshwater habitats in Eastern North America. Despite panmixia (Côté *et al.* 2013), there are extreme differences in morphology, growth rate, and sex ratio by rearing habit (Jessop 2010); resulting in the contradiction of a panmictic species that also seems to exhibit phenotypic attributes of locally adaptation. There is tremendous conservation concern, because some of these unique rearing groups are in steep decline (Castonguay *et al.* 1994). Also, due to their complex life history, it is not possible to economically produce the full life cycle in a fish farm, so every fish consumed has a wild origin. We chose a single individual for full genome sequencing. We sequenced 100X coverage with paired-end reads and in addition 2kb and two 4.5kb insert mate pair libraries including one Nextera library. We assembled using the program RAY (Boisvert et al. 2010) with a kmer length of 41bp, and scaffolded with SSPACE (Boetzer *et al.* 2011). The resulting assembly and scaffolding indicated a total genome size of 1.5 Gb, which is in line with estimates by more direct methods. There are 398,895 contigs, the largest of which is 72,008 bp and the contig N50 is 5,818 bp. There are 121,797 scaffolds, the largest of which is 866,215 bp and the scaffold N50 is 74,883 bp. The annotation is in progress with the application MAKER2. So far we have annotated 15,400 protein coding genes.

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- Jessop BM (2010) Geographic effects on American eel (*Anguilla rostrata*) life history characteristics and strategies. Canadian Journal of Fisheries and Aquatic Sciences 67, 326-346.

Oral presentation

Muscle transcriptome profiling of *Kaupichthys hyoproroides*

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A high ecological diversity and a great number of species organized in 20 families define the Anguilliformes [1]. Recent research in eels mainly focuses on the on the critically endangered freshwater eel *Anguilla anguilla* [2] investigating survival risks and stress response due to anthropogenic introduced water pollution (mainly pesticides) [3] or in systematics in general. Little is known about other non-migratory eel species inhabiting coral reefs, and nothing in regards to their genomics. *K. hyoproroides* is an Atlantic circum-tropical species and some authors proposed to place it in synonymy with Indo-Pacific species *Kaupichthys diodontus* [1]. Here we present the first high throughout transcriptome data of this circum-tropical marine living False Moray, *Kaupichthys hyoproroides*. Two individual high quality muscle RNAseq data sets were obtained using Illumina Hi-seq, 80-bp paired end reads. De novo assembly with two main de novo assembly programs has highlighted the assembly statistics in these two datasets. Comparative transcriptomic analyses between these two individuals have led to identification of similarly expressed genes (essential as calibration for future studies). Furthermore, comparisons at the order level with existing transcripotme data and EST's of *A. anguilla* and *A. japonica* has shed light on order shared genes. Besides, these first annotated transcriptomes enable subsequent qualitative and quantitative studies in stress response and local adaptation using *K. hyoproroides* as ecologically important fish for anthropogenic reef destruction.

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2. Coppe A, Pujolar J, Maes G, Larsen P, Hansen M, Bernatchez L, Zane L, Bortoluzzi S: Sequencing, de novo annotation and analysis of the first *Anguilla anguilla* transcriptome: EeelBase opens new perspectives for the study of the critically endangered European eel. BMC genomics 2010, 11(1):635.
3. Peters G, Delventhal H, Klinger H: Physiological and Morphological Effects of Social Stress on the Eel, *Anguilla anguilla* L. In: Fish Diseases. Springer; 1980: 225-227.

Oral presentation

Eel diseases: an overview

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Over the last decades, the European eel (*Anguilla anguilla*), American eel (*A. rostrata*) and the Japanese eel (*A. japonica*) populations have strongly declined. This has led to a hypothesis about global causative factors, as pollution, habitat loss, fisheries, migration barriers, predation by birds, and diseases. In this presentation, an overview will be given on the predominant diseases and parasites of eel.

In the 1980s, the swimbladder parasitic nematode *Anguillicoloides crassus* was introduced in wild European eel. It caused acute and severe swimbladder lesions in eel populations, but this effect became milder in time. In a study on the health of wild spawners (silver eels, *A.anguilla)* originating from the Dutch River Rhine and Lake IJsselmeer it was concluded, that the silver eels had a proper condition, with nonspecific fin hemorrhages, and were frequently infected with *Trypanosoma* spp., *A. crassus*- and/or *Anguillid herpesvirus 1* (AngHV-1), depending on the season. AngHV-1 is the most frequent observed virus in wild eel in the Netherlands. The virus has recently been molecularly characterized in detail by Van Beurden et al. (2012), who also performed a retrospective study on the prevalence of European eel viruses in the Netherlands.

As eel culture fully depends on wild caught glass eels, the health status of silver eels, as a factor influencing the recruitment of glass eels, is of relevance to both wild and cultured eel stocks. Until the 1990s, eel farms used wild, *A. crassus* infected stocking eels for stocking their farms. However, since then, only glass eels, not infected with *A. crassus*, were used as stocking eels. This resulted in *A. crassus* free eel farms. In eel culture, predominantly, *Anguillid herpesvirus 1* (AngHV-1), the IPNV related virus *Eel virus European* (EVE), and *Eel rhabdovirus European X* (EVEX) were found to be related to disease outbreaks, sometimes as double infections, and sometimes combined with a pathogenic bacterium, such as *Vibrio vulnificus*. This bacterium as single pathogen also caused severe disease cases at Dutch eel farms. Characterisation of the *V. vulnificus* isolates resulted into seven new *V. vulnificus* genotypes. Moreover, certain *V. vulnificus* genotypes are zoonotic which recently lead to a zoonotic casus in a Dutch eel farmer.

In conclusion, although no direct relationship can be made between the decline of the eel population and specific pathogens, a number of pathogens have shown potential to severely hamper the health of eel in the wild and under farmed conditions.

Oral presentation

Large scale genetic monitoring of Atlantic eels in Northern Germany

Jens Frankowski[1], Malte Dorow[2], Ralf Bastrop[1]

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In response to the detection of American eels in inland waters of the state Mecklenburg-Vorpommern, located in north-eastern Germany (Frankowski *et al.* 2009), a genetic monitoring program was established using the identification protocol of Frankowski & Bastrop (2010). The method allows the unambiguous species identification of *A. anguilla* and *A. rostrata* as well as the detection of Atlantic eel hybrids by means of nuclear vs. mitochondrial markers. Since 2006 tissue samples were collected in coastal and inland waters from all eel life stages to answer various eel biology and management related questions. Further, since 2006 the sampling of on grown eels used for stocking was integrated in the monitoring program. In the period from 2006 to 2012 over 10.000 identifications were conducted and supplemented by individual data (e.g., length, total mass, silvering stage). Based on this database it was shown that (1) since 2006 the stocked fish solely were European eels, (2) European eels were autochthonous - no natural occurring *A. rostrata* have been recorded, (3) the distribution of *A. rostrata* reflected their anthropogenic introduction, (4) translocated American eels developed into silver eels in freshwater habitats and migrated together with European eels to coastal waters (Reckordt *et al.* 2013, in press), (5) among European eels some individuals exhibited hybrid ancestry (Boëtius 1980).
From the eel management perspective the genetic monitoring helped to prevent a new invasion of A. rostrata into the study region and to understand the expansion of the translocated species.
The observed pattern of interglacial introgression through hybridisation is discussed with respect to the hypothesis of separation of Atlantic eel species during larval migration (Schmidt 1923).

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Oral presentation

Selectivity of water dams on glass eel : transcriptomics approach

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Physical obstacles to upstream migration such as dams are a major impairment of natural colonisation and dispersion of eels. Dams and obstacles also increase the energy demand of fish, even if there is a fish friendly device to allow them to swim across the obstacle. Besides, local accumulation of eels below dams may increase the mortality associated with predation. Obstacle to eel migration may select on different trait of life history. While quantitative impact of dams is subjected to numerous studies, little is known about their intra-specific selectivity. Even the fishway efficiency analyses are scarcely hinting at their potential selective effect (Castro-Santos 2004; Noonan, Grant & Jackson 2012).

The main issue of this study is to pinpoint phenotypic traits that predisposed glass eels to dams successful passage. The approach we adopted is individual-centered and without any a priori hypothesis on traits involved by the putative dams selective pressure. We analyzed the expression of 15000 known eel genes based on previous studies (Coppe et al. 2010; Pujolar et al. 2012). Transcriptome analysis of three main tissues (brain, liver and muscle) from individuals sampled on three successive forebays separated by dams indicate different gene expression profiles in brain between the two upstream forebays. The functional role of the overall set of regulated genes strongly suggests cytoskeletal changes and synaptic plasticity. Their interpretation at higher phenotypic level and further research perspectives are discussed.

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Oral presentation

Genomic diversity and differentiation in European eel using RAD sequencing-generated single nucleotide polymorphisms

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The application of genomic approaches into ecological and evolutionary studies has been facilitated by recent advances in the speed, cost and accuracy of next-generation sequencing technologies. Using genotyping-by-sequencing approaches (i.e sequencing of a reduced representation of the genome followed by single-nucleotide polymorphism (SNP) discovery) allows the production of data on hundreds of thousands of genome-wide SNPs. One such genotyping-by-sequencing approach is RAD-sequencing that utilizes high-throughput sequencing of restriction-site-associated DNA tags (RADs).

Here we employed a RAD-sequencing approach to (1) assess the population structure in European eel with a genome-wide array of SNPs and (2) evaluate the scope of neutral vs. adaptive differentiation in European eel by identifying potential candidate SNPs responding to environmental selection. A total of 259 European eel (Anguilla anguilla) individuals collected at 8 locations across the geographic distribution of the species, ranging from Iceland to Morocco were RAD sequenced. An average of 9.59 million reads of 90 bp per individual was generated. After trimming sequences to 75 bp and quality filtering, on average, 7.90 million (82.19%) reads per individual were retained. An average of 69.96% of the quality-filtered reads aligned to the European eel draft genome and 4.46% of the reads were discarded due to alternative alignments.

Aligned reads were assembled into 335,343 loci. Using the program Stacks, a total of 453,062 SNPs were identified. Data analysis considering either all 453,062 SNPs, 50,354 SNPs with a minimum allele frequency >0.05 or 41 mitochondrial SNPs showed no significant differences across samples at genetic diversities or allele frequencing, suggestive of panmixia at the genomic level. Finally, evidence of local adaptation was tested by searching for elevated population differentiation using FST-based outlier analyses and by testing for significant associations between allele frequencies and environment variables. Loci showing either significantly greater population differentiation or significant covariance with environmental variables (temperature, latitude and longitude) relative to reference SNP distributions were considered candidate loci for local adaptation.

Oral presentation

Impact of genome duplications on teleost reproductive neuroendocrine systems: the eel model.

Sylvie DUFOUR[1], Gersende MAUGARS[1], Anne-Gaëlle LAFONT[1], Jérémy PASQUIER[1], Delphine COTTIN[1], Marina MORINI[1], Nédia KAMECH[1], Karine ROUSSEAU[1], Ron DIRKS[2], Guido van den THILLART[2]

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Two rounds (1R and 2R) of whole genome duplication have occurred in early vertebrates, with an additional third round (3R) at the basis of the teleost lineage. These duplications provided the genomic basis for multiple morphological and physiological innovations. In particular, the teleost-specific 3R may have contributed to the remarkable diversification of this group, the largest amongst vertebrates. Duplicated genes (paralogs) may have been conserved or lost, according to lineages and species. Conservation of paralogs can be related to the emergence of new functions (neofunctionalization) or to the partition of pre-existing functions (subfunctionalization). As extant representatives of a basal group of teleosts (Elopomorphs), *Anguilla* species provide a powerful model to investigate the origin and early fate of paralogs in teleosts. Based on examples from the brain-pituitary gonadotropic axis, we are investigating the impact of the three rounds of genome duplication on the numbers, evolutionary histories and roles of neuroendocrine paralogs in teleosts. Likely related to its basal position, the eel often possesses the largest number of paralogs amongst teleosts, with some remarkable exceptions such as a single aromatase gene in the eel (Jeng et al., 2012). Phylogeny and synteny analyses reveal that multiple paralogs in the eel may not always originate from the 3R. For instance, while the two eel leptin receptor paralogs likely originated from the teleost-specific 3R (Lafont et al., this meeting), the three eel Kisspeptin receptors would come from the vertebrate 1R and 2R (Pasquier et al., 2012 and this meeting). Concerning glycoprotein hormone receptors, while duplicated eel TSH receptors likely originated from the teleost-specific 3R, duplicated eel LH receptors would rather reflect an ancestral actinopterygian feature (Maugars et al., this meeting). The eel genome (Henkel et al, 2012) has revealed as a potent tool to characterize the presence, origin and roles of paralogs in the neuroendocrine control of eel and teleost reproduction.

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Oral presentation

Discovery of duplicated genes for LH receptor in a basal teleost, the European eel (*Anguilla anguilla*).

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In vertebrates, pituitary gonadotropin follicle stimulating hormone (Fsh) and luteinizing hormone (Lh) via their interaction to the receptors, Fsh receptor (Fshr) and Lh receptor (Lhr), control the reproductive function. In the present study, we report for the first time the characterization of two Lhrs in a vertebrate, the European eel (*Anguilla anguilla*). Three receptors, Fshr, Lhr1 and Lhr2 were identified on the draft genomes of the European eel and Japanese eel (*Anguilla japonica*) (Henkel *et al.*, 2012a, b) and the sequences were confirmed by cDNA isolation from European eel testis or ovary. The *fshr* and the two *lhr* genes found in the eel showed the typical vertebrate intro-exon structure with 10 exons for *fshr* and 11 exons for both *lhr*. To further investigate the phylogeny of the two Lhrs, new orthologous Fshr and Lhr sequences were identified in the genome of representative vertebrates. A single *fshr/lhr-related* gene was found in the cyclostome lamprey. The basal sarcopterygian, coelacanth showed only one *fshr* and one *lhr* gene as in tetrapods (mammals, birds, amphibians). In a non-teleost actinopterygian, the spotted gar, a single *fshr* and two *lhr* genes could be annotated. In teleost species, one *fshr* and either one or two copies of *lhr* gene could be found. Phylogeny analysis showed that the Lh receptors were distributed into three subclades: one clade including only sarcopterygian sequences and two sister clades of actinopterygian sequences in which branched in basal position each eel Lhr and each gar Lhr. These results suggest that eel *lhr* duplicated genes result from actinopterygian specific duplication that occurred before the teleost whole genome duplication (3R). Sequence alignment revealed a great divergence between the two eel Lhrs at the signaltransduction domain and to a lesser extent at the hairpin-like shape ligand-binding domain. Transcripts of the three receptors were found in eel testes and ovaries. In addition differential extra-gonadal expression were observed for the *fshr* and the two *lhrs*, with a high expression in the retina for the *lhr1*, while *fshr* and *lhr2* transcripts showed a strong expression in the anterior brain. A sharp increase of all receptor transcripts was measured in ovary and testis at silver stage compared to yellow stage. In contrast differential regulation of the expression of the three gonadotropin receptors in the BPG axis was observed during experimental sexual maturation.

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b. Henkel CV, Dirks RP, de Wijze DL, Minegishi Y, Aoyama J, et al., 2012. First draft genome sequence of the Japanese eel, Anguilla japonica, Gene, 511(2) : 195-201.

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Oral presentation

Diversity and evolutionary history of the Kisspeptin system across vertebrates: the benefits from studying unconventional model genomes.

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In the recent years, Kisspeptin (Kiss) and its receptor (KissR) arose as new key actors of the Brain-Pituitary-Gonad (BPG) axis in mammals. While only one *Kiss* and one *KissR* have been described in this lineage, the breakthrough of multiple *Kiss* and *KissR* genes in other vertebrate phyla, such as teleosts, stimulated the interest into the origins of their diversity and respective evolutionary histories.

We investigated the presence of new *Kiss* and *KissR* genes in the genomes of various vertebrates species of relevant phylogenetical positions, including a cyclostome (Sea lamprey), a chondrichthyan (ghost shark), a basal sarcopterygian (coelacanth), a non-teleost actinopterygian (spotted gar) and a basal teleost (European eel). We performed phylogenetic and synteny analyses to determine the homologous relationships among the different *Kiss* and *KissR* genes. Those two approaches brought new evidences for a larger diversity of both *Kiss* and *KissR* gene families among vertebrate species. In particular, from the European eel genome, we identified two *Kiss* genes and for the first time in a teleost, three *KissR* genes. The European eel is, so far, the only teleost species presenting a *KissR* ortholog to the mammalian *KissR*. More generally in vertebrates, our findings clearly demonstrate that *Kiss* can be classified in three clades and *KissR* in four clades. This leads us to propose new and phylogenetically supported nomenclatures for both gene families. Our results also suggest that such diversity originated from the early vertebrate genome duplication rounds (1R and 2R). In contrast, no trace of the teleost-specific 3R remains neither in *Kiss* nor in *KissR* gene diversities, even in the European eel. This highlights the complex evolutionary histories of both gene families, involving early duplications followed by independent losses of *Kiss* and *KissR* genes across vertebrate radiation.

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Oral presentation

On the genomics of host-pathogen co-evolution: the viral interleukin-10 story

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For millions of years, viruses have been co-evolving with their respective hosts. During this process, viruses have developed numerous ways to deregulate the host immune response in order to avoid immune surveillance and subsequent elimination from the host. Large DNA viruses invariably encode a raft of genes that specifically target host biological processes. One of the most intriguing strategies is the acquisition of host immunomodulatory genes, which are used against the host during the course of infection. Host and pathogen genomics go hand-in-hand in the study of this important class of viral immune evasion genes.

One of the best examples of such a gene is the pleiotropic cytokine Interleukin-10 (IL-10), which has been acquired independently by several herpesviruses and poxviruses from their respective hosts. The key features of cellular IL-10 relate mainly to its capacity to exert potent immunosuppressive functions through various mechanisms, and these features make this a protein with potential immune evasion functions for viruses. The IL-10 story in relation to the European eel (*Anguilla anguilla*) and the eel herpesvirus *Anguillid herpesvirus 1* will be used to demonstrate the involvement of genomics in the discovery and structural characterization of these genes. The involvement of another twenty cellular and viral IL-10 variants, several of which were discovered during genome sequencing projects, allows important evolutionary conclusions to be drawn. The use of transcriptomics in the functional assessment of these viral cytokine homologs, and their potential as vaccines or therapeutics, will be reviewed on the basis of research on related viral IL-10 variants.

Oral presentation

Modulation of testis steroidogenic enzymes gene expression and steroid synthesis by temperature thermal regime during spermatogenesis in European eel

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The influence of temperature on maturation process has been evidenced (Sato et al., 2006), but little is known about the role on the European eel (*A. anguilla*) maturation process (Pérez et al., 2011). The temperature effect was evaluated on testis steroidogenic enzymes gene expression and steroid synthesis. Three hundred males were hormonally-induced with weekly doses of hCG under three thermal regimes (T10: 10 °C first 6 weeks, 15 °C next 3 weeks and 20 °C last 6 weeks; T15: 15 °C first 6 weeks and 20 °C last 9 weeks; and T20: constant 20 ºC). Eight fish per treatment were weekly sacrificed. Sperm was collected and volume, density and motility parameters were estimated. Blood samples were extracted to determine testosterone (T), estradiol (E2), 11-ketotestosterone (11KT) and 17,20βP plasma levels (Fig.1). Total RNA was extracted from testicular fragments and retro-transcripted in cDNA. Primers were designed and used to develop specific qPCR to quantify the expression level of some genes encoding some key steroidogenic enzymes (*P450ssc, P450c17, cyp19a1, 11β-HSD, cyp21, 20β-HSD*; Fig. 1).

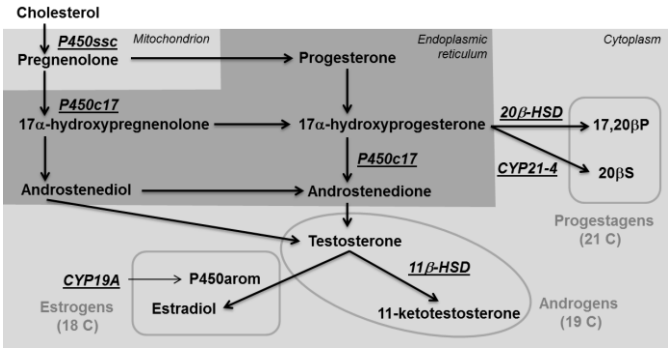

Figure. 1. Main steroid biosynthetic pathways in the fish gonad. Studied enzymes are underlined.

The process of testis maturation, as well as the sperm quantity and quality, were affected by water temperature. T20 induced the best sperm results (Gallego et al., 2012). Gene expression evaluation probed that this temperature effect was caused through the modulation of the expression of steroidogenic enzymes. T20 and T15-treated males showed earlier peaks of steroidogenic enzymes gene expression, while T10 males showed delayed peaks, which was translated on different timings of steroids production. I.e.: P450scc and P450c17, involved in the path of T synthesis, maintained high expression at low temperatures coinciding with high T levels. However, the gene expression of steroidogenic enzymes involved into the T conversion to E2 and progestagens synthesis were delayed at lower temperatures. A relative good correlation was found between 11KT plasma levels and *11β-HSD* gene expression, but the E_2 plasma level increased before than the *cyp19a1* gene expression peaked. The sea water acclimation was enough to increase E_2 plasma level, independently of water temperature.
These results suggest that could be a differential gene regulation depending on the environment temperature, which might be part of the regulatory system used by the European eel males during the reproductive migration to spawning area in the West Atlantic.

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Oral presentation

DHP receptors and PLCZ1 expression during the European eel spermatogenesis

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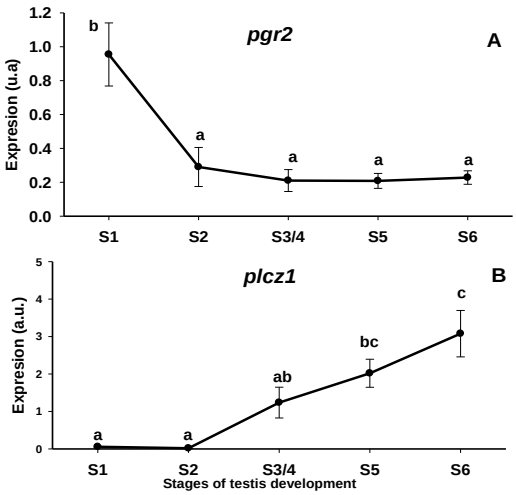
In some teleost including Japanese eel, it has been suggested that DHP regulates sperm maturation. It seems that DHP action is mediated through an increase in the seminal plasma pH, which in turn increases the cAMP content in sperm, thereby allowing the acquisition of sperm motility (Miura & Miura, 2011). However, DHP has been also proposed as an essential factor for the initiation of testicular meiosis in Japanese eel (Miura & Miura, 2011). We studied the expression of two nuclear DHP receptors, *pgr1, pgr2* through spermatogenesis.
In mammal sperm, a sperm specific phospholipase C, the PLCZ1 (phospholipase C zeta 1) enters into the egg and triggers the egg activation after fertilization, by inducing the Ca^{2+} release from intracellular stores. Fish could have a similar mechanism, as sperm extracts from tilapia induced release or oscillations in Ca^{2+} in mouse and sea urchin eggs (Coward et al., 2003). *plcz1* gene expression has been observed in medaka testis, but not in testis from 2 fugu species. We studied, by first time in teleosts, the evolution of *plcz1* expression through spermatogenesis.
Male eels were induced to mature with weekly injections of hCG. 5-8 males were sacrifized weekly, and the testis stage of development (S1-S6) was determined by histology and representative samples were selected for gene expression analyses. Also, samples from 3 immature males and females were collected for screening study. Total RNA was extracted, and specific qPCR were developed to quantify gene expression.



We studied the expression of the two nuclear DHP receptors present in eel testis. Both receptors were highly expressed, but different expression profiles were observed: *pgr1* expression was stable during testis development, while *pgr2* expression was the highest at S1 (when the only germ cell type was spermatogonia), what agree with the proposed role for DHP on inducing spermatogonia to enter in meiosis (Miura et al., 2011) (fig. 1A). Final maturation may be mediated through one or more undetermined membrane bound DHP receptors.
Anguilla anguilla plcz1 gene showed 73-76% sequence conservation with *plcz1* from other fish species (medaka, tilapia, 2 fugu species). Screening revealed high *plcz1* expression in testis, but very low in ovary. Through male sex maturation, testis *plcz1* gene expression was very low in stages 1 and 2, increasing from S3+4 (spermatids) to S6 (full spermiation), when it was 75-fold higher than at S1 (figure 1B). That suggests that testis *plcz1* mRNA synthesis starts after the onset of spermiogenesis, like it was shown in some birds and mammal species (Yoneda et al., 2006). By first time, *plcz1* gene expression has been analyzed through fish spermatogenesis, suggesting that the same sperm-oocyte activation mechanism observed in mammals could be operating in teleost fish.

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Oral presentation

First evidence of a duplicated leptin/leptin receptor system in a vertebrate species, the eel, and involvement in sexual maturation

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Leptin is a recently discovered metabolic hormone also involved in the control of reproduction in vertebrates. In tetrapods, one gene has been evidenced for coding the leptin and one gene for the leptin receptor. So far in teleosts, up to two leptin genes have been identified but only one receptor. Duplicated genes in teleosts may result from the whole genome duplication event (3R) that occurred specifically in this group. The analysis of the European and Japanese eel genomes led us to characterize two leptin genes and, for the first time in vertebrate species, two leptin receptor genes. Based on this new discovery, and the increasing number of available vertebrate genomes, we performed phylogenetic analyses of both leptin and its receptor families. We further analyzed the leptin receptor evolution history by performing synteny analysis in the genomes of various vertebrate species, including the European and Japanese eels. Our analyses suggest that the two leptin and the two leptin genes characterized in the eel are the result of the 3R, specific to the teleost lineage. As the eel is so far the unique example of duplicated leptin receptor in teleosts, the duplicated copy of this gene may have been lost early in the teleost lineage, after the elopomophe emergence. We developed specific qPCRs to investigate the differential tissue distribution of the two leptins and the two leptin receptors in the eel. Only one of the two leptin genes was expressed in the adipose tissue, which constitutes the leptin site of production in mammals, while the other one was expressed in the liver, site of leptin production in most of the teleost species. The four genes of the leptin system, hormones and receptors, are expressed in the organs of the brain-pituitary-axis (BPG). We analyzed their regulation in the BPG axis and in the liver during experimental maturation. This study provides the first evidence of a conserved duplicated leptin/leptin receptor system in vertebrates and of a potential involvement of these duplicated peptides and receptors in the eel sexual maturation.

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Oral presentation

The pituitary gland of the European eel reveals massive expression of genes involved in the melanocortin system

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Hormones secreted from the pituitary gland regulate important processes such as development, growth and metabolism, reproduction, water balance, and body pigmentation. Synthesis and secretion of pituitary hormones are regulated by different factors from the hypothalamus, but also through feedback mechanisms from peripheral organs, and from the pituitary itself. In the European eel extensive attention has been directed towards understanding the different components of the brain-pituitary-gonad axis, but little is known about the regulation of upstream processes in the pituitary gland. In order to gain a broader mechanistic understanding of the eel pituitary gland, we have performed RNA-seq transcriptome profiling of the pituitary of prepubertal female silver eels. RNA-seq reads generated on the Illumina platform were mapped to the recently assembled European eel genome. The most abundant transcript in the eel pituitary codes for pro-opiomelanocortin, the precursor for hormones of the melanocortin system. Several genes putatively involved in downstream processing of pro-opiomelanocortin were manually annotated, and were found to be highly expressed, both by RNA-seq and by qPCR. The melanocortin system, which affects skin color, energy homeostasis and in other teleosts interacts with the reproductive system, has so far received limited attention in eels. However, since up to one third of the silver eel pituitary's mRNA pool encodes pro-opiomelanocortin, our results indicate that control of the melanocortin system is a major function of the eel pituitary.

Oral presentation

Transcriptional profiles of Hg in the liver of European eel (Anguilla anguilla).

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Many enigmas surround different aspects of freshwater eel biology and life cycle. In the same way different hypothesis about why eels are disappearing from European continental waters have been proposed. One such proposal defends that poor fat accumulation of eels, due to pollution in continental waters, might be stopping eels from reproductive migration to the Sargasso Sea. Thus, habitat deterioration could be blamed for the decline in the catchment potential of the European rivers. In this context, and with the aim to study the mode of action of environmentally relevant chemicals in eels, the multi tissue transcriptome was sequenced (454 Titanium Roche) in order to design a high density custom oligonucleotide microarray (eArray, Agilent). To validate this tool a laboratory experiment was carried to analyse the gene trasncrition profiles related to chemical compounds released from pulp and paper mills; 100 µg/L mercury and 150 µg/L β-sitosterol (only Hg data is presented here). 20 yellow European eel (Anguilla anguilla) elvers (11.7±5.35 g) obtained from a local farm (Acuivas SL, Usurbil, Gipuzkoa) were exposed for 3, 6 and 9 days. Pyrosequencing allowed the design and construction of a 60K microarray platform containing 3923 gene signatures identified through BlastN analysis and 7212 sequences annotated through BlastX coupled to Blast2Go analysis. Additional 226 sequences were incorporated from NCBI databases and 3551 from the information available in EeelBase in 2011. Two probes were generated per sequence and they were spotted twice in the array.

Hepatic gene expression profiling of the exposed eels indicated that Hg significantly down-regulated (LIMMA, adj. $p<0.005$) only gene signatures related to selenoprotein W-1 (SeW), something typically described in mammals exposed to methyl-mercury. Increasing the adj. p value to <0.05, 116 genes were significantly regulated (38 down-regulated and 79 up-regulated). Among them, we found additional selenoproteins such as ROS metabolism related genes; glutathione peroxidases (gpx1 & gpx4b) and thioredoxine which were up-regulated. In addition, complement system genes (C3 & C4b) were also up-regulated. Studying enriched Go pathways ($p<0.005$) and in relation with lipid homeostasis we observed that the following pathways were enriched after exposure to Hg: fatty acids degradation and metabolism of arachidonic acid, linoleic acid, ether lipids, alpha linolenic acid, and glycerophospholipids. In addition, among the top 10 significantly enriched KEGG pathways, p53 signalling, apoptosis, and MAPK signalling were present, suggesting possible effects on cell cycle regulation.

In summary, transcriptome pyrosequencing and subsequently designed microarray provided the molecular tools to successfully study the gene transcription profiles of toxic chemical compounds such as Hg in European eel tissues. In addition to study the molecular modes of action of specific chemical compounds, the developed gene expression microarray will be useful in active monitoring of the quality of freshwater environments using caged sentinel eels.

This study was funded by Basque Government (SAIOTEK S-PE09UN32; Consolidated Research Groups IT810-13) and UPV/EHU (UFI 11/37). Technical and human support provided by SGIker (UPV/EHU) is acknowledged.

Oral presentation

Possible way for extraction of sublethal pollutants from aged european eel

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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 and there is no possible emigration and immigration of eels from and to the lake. Whereas this aged, landlocked population is unable to migrate, therefore physiological parameters of sexual maturity greatly differ from other fresh water populations which have seaside connections. 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 21 years old eel. The nearly 100% infection rate by Anguillicoloides crassus is another aggravating factor for maturation experiments. According to our hypothesis, if the parasite and supposed pollutants could be eliminate in vivo from the aged eels, the oogenesis should be successfully completed. For in vivo detoxification of the alive organims humic acids and zeolites were used. Short-term medical treatment (Mebendazole, Levamisole) and long-term detoxification treatments (peat as alive organims the humic acids - Sphagnum sp. + Zeolite) assisted to eliminate toxic agents or at least a part of them in vivo, and improved the reproductive fitness, consequently all of our old females ovulated indicating successful oocyte formation.

Figure. Egg stripping

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Oral presentation

Effects of short term metal exposure on glass eel DNA integrity and freshwater adaptation.

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The European eel (*Anguilla anguilla*) recruitment population has been declining rapidly since the 1980's and despite limited management and restoration programmes, there has been little improvement in natural recruitment. In response to this decline, the EU "Eel Recovery" regulation, (Council Regulation 1100/2007) was implemented in 2007. To satisfy the EU Eel Recovery Plan, member states must develop Eel Management Plans. The major factors regulating eel populations are still uncertain but pollution has been suggested to have a detrimental effect on eel health and potentially on their reproductive success and survival.

The present study examined the impact of short term metal exposure on the DNA integrity and the subsequent freshwater adaptation of glass eels during their transition from the marine to the freshwater environment. Glass eels approaching the UK coast in the Bristol Channel were obtained from commercial fishermen and exposed for 2 weeks in the laboratory to environmental levels of common metals (Cu, Zn, Pb, Cr) present in the Bristol Channel. The concentrations were representative of historic and current levels in the coastal zone environment. After exposure, the eels were either sampled for Comet assay analysis or transferred to freshwater (freshwater challenge). The results from this study suggest that even short term exposure to metals can affect glass eel condition and are discussed in relation to the conservation and management of the European eel population.

Keywords: European eel, glass eel, metals, osmoregulation, comet assay.

Oral presentation

Myo-inositol biosynthesis and osmoregulation in the eel; potential disruption by organophosphorus xenobiotics

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The European eel (*Anguilla anguilla*) exhibits a remarkable genetic plasticity that is essential for its successful acclimation to the marine environment during the migration back to the Sargasso Sea to breed. Although electrolytes are the major osmolytes present within cells and extracellular fluids, organic metabolites, including sugars and amino acid, are also osmotically active components of the cell cytosol. Consequently changes in the concentration of organic metabolites can contribute to the process of cell volume regulation resulting from perturbations in plasma osmolality. A number of organic metabolites are known to play important roles in cellular osmoregulation in marine "osmoconformers" such as elasmobranchs, however there have been few studies into potential roles for these organic osmolytes in "hypo-osmotic regulators" such as seawater (SW) teleosts. Myo-inositol is a major osmolyte that plays an important role in cyto-protection in many organisms exposed to a variety of environmental stresses, including increases in environmental salinity. In the eel (1), and other euryhaline teleosts like tilapia (2) myo-inositol has been shown to accumulate in various tissues as fish acclimate to SW or hypersaline environments. Inositol can accumulate in cells either by increasing the activity of the sodium-linked myo-inositol transporter (SMIT1) or by enhanced *de novo* synthesis. Myo-inositol phosphate (MIP) synthase and inositol monophosphatase (IMPA) are the two enzymes required for the *de novo* synthesis of inositol from glucose 6-phosphate. SW-acclimation is accompanied by tissue-specific increases in expression of mRNA and protein for only one of four IMPA isoforms (IMPAs1.1, 1.2, 1.3 and 2) found in the eel. Increased expression of IMPA1.1 is especially prevalent in epithelial tissues directly exposed to the aquatic environment, such as the gill, oesophagus, skin and fin. In contrast, levels of tissue expression of SMIT1 and MIP synthase mRNA exhibited no significant differences between FW- and SW-acclimated fish. These results suggest that increases in IMPA1.1 expression are primarily responsible for the enhanced levels of intracellular inositol detected in tissues from SW-acclimated fish and that neither MIP synthase nor SMIT1 appear be salinity-sensitive genes in the eel. We hypothesise that enhanced IMPA1.1 expression and inositol accumulation are necessary for cellular adaptation to increases in plasma osmolality in SW-adapted fish and the osmolyte is especially important for the protection of body surface epithelial/epidermal cells from the severely dehydrating effects of the SW. Recent studies *in vitro* have indicated that a number of structurally unrelated organophosphate pesticides are disruptors of IMPA enzymatic activity and therefore could potentially affect the regulation of intracellular inositol concentrations in fish inhabiting heavily polluted environments. Consequentially, it is possible that exposure of fish to certain pesticides in the FW environment may adversely affect the osmoregulatory role of the myo-inositol biosynthesis pathway in migratory fish and thus compromise the survival and/or fecundity of fish returning to SW to breed.

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Oral presentation

A transcriptomic approach to assess the effect of environmental pollution in the European eel

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Together with overfishing, parasite infestation and diseases, environmental pollution has been put forward as one of the main causes to explain the current decline in European eel recruitment. While eels are regarded as fairly resistent species, often living in unproductive waters and polluted habitats, they are prone to bioaccumulation of lipophilic contaminants due to their particular ecology (i.e. a benthic feeding behaviour and a high fat content). Evidence has been presented that chemical compounds such as PCBs, pesticides and heavy metals could have a serious effect on the health of the European eel, particularly affecting migrating adult eels, since it has been hypothesized that mobilization of lipids and lipophilic contaminants to the gonads during the transoceanic migration could result in a low quality of spawners. In order to study the effect of pollutants on eels, we first developed a transcriptomic platform for global gene expression profiling in European eel that consisted of about 15,000 annotated genes. This array was then applied to detect differentially expressed genes between polluted sites. The study was replicated in low and high polluted sites in Belgium and Italy using adult silver eels that were individually measured for 36 PCBs, several organochlorine pesticides and brominated flame retardants and nine heavy metals. A parallel response was observed in both Italian and Belgian individuals that consisted in the upregulation of detoxification genes including genes that take part in phase I of the xenobiotic metabolism, i.e. CYP3A, phase II, i.e. glutathione-5-transferase, and, oxidative stress, i.e. glutathione peroxidase. In addition, key genes in the mitochondrial respiratory chain were downregulated in the highly polluted sites, pointing to a low energetic status of the individuals. Although we did not measure metabolism directly, the suggested lowered metabolic rate and condition observed in pre-migrating eels in our study points to a poor quality of spawners that could potentially impair migration and reproduction in the Sargasso Sea, and ultimately, contribute to a lowered spawning stock.

Oral presentation

Origin and role of Transient Receptor Potential Vanilloid (TRPV) ion channels in vertebrates

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The Transient Receptor Potential (TRP) multigene superfamily encodes membrane proteins that function as ion channels. The TRP family is subdivided into seven subfamily: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin), TRPA (Ankyrin) and TRPN (NOMPC-like). These ion channels are widely expressed in many different tissues and are activated in response to various chemical and physical stimuli. Among them, the TRPV channels are known to play a critical role in thermosensation in many homeothermic animals, such as mammals. Several TRPV members have been recently characterized in ectothermic vertebrates. However, whether these receptors are also physiologically important in environmental detection for ectothermic vertebrates still remains unknown.

Here, we proposed to study the origin and evolution of TRPV channels among vertebrates and to investigate their physiological function in ectothermic vertebrates using a biological model of high phylogenetic and ecological interest, the European eel (*Anguilla anguilla*). The analysis of eel genome revealed the existence of three TRPV genes (TRPV1, TRPV4, TRPV6) while six members are currently characterized in mammals (TRPV1 to TRPV6). These TRPV genes were then cloned and their expression was quantified using real-time quantitative PCR in various tissues (heart, brain, gills, skin, liver, gonad…). Quantitative PCR analyses revealed differences among TRPV genes in their tissue distribution. Indeed, while the expression of TRPV1 and TRPV4 was detected in several tissues (brain, skin, eye, spleen…), TRPV6 was mostly expressed in gills. These preliminary results therefore suggest that these receptors may play different functions in environmental detection of ectothermic vertebrates. A perspective to this study would be to investigate the regulation of these genes in response to variations of environmental factors (ex: temperature, salinity or pressure) related to European eel life cycle. This work may help to establish the functional role of each receptor.

Keywords: Transient Receptor Potential Vanilloid, qPCR, vertebrate, teleostean, evolution

Oral presentation

Differential gene expression in the olfactory epithelium of *Anguilla anguilla*

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Several aspects of eel biology suggest that olfaction plays an important role at key life stages. While a few studies have addressed the role of olfaction in eels, the molecular components of the olfactory system have not yet been characterized. Our first goal was to identify receptors and other genes involved in olfactory signal transduction in eels. To address this goal, we sequenced and assembled the olfactory epithelial transcriptome (OE) of *Anguilla anguilla* using RNA-Seq. Our assembly and characterization of the transcriptome revealed that eels express a diversity of genes in the OE including a wide array of receptors that are known to be involved in vertebrate olfaction (odorant receptors, type 1 and 2 vomeronasal receptors and trace amine associated receptors). Our comparison of the *A. anguilla* OE transcriptome to the proteome of zebrafish revealed that 89% of the zebrafish proteins have significant alignments (E-value ≤ 0.001) in the OE assembly suggesting that majority of the *A. anguilla* protein coding genes are expressed in the OE. The second goal was to identify genes that may have stage specific functions. For this, we looked at the patterns of differential gene expression in the OEs of freshwater, seawater and sexually mature males. Using this approach, we identified a number of transcripts that are differentially expressed at these three stages. Interestingly, though many genes were expressed at lower levels in sexually mature males, we identified several chemosensory receptors that are expressed at higher levels in this group. This result suggests that these receptors have roles in pheromone communication and reproduction in eels.

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Oral presentation

Transcriptomic characterisation of the interactions between metabolism and sexual maturation under exercise in European eel

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The interplay between metabolism and sexual maturation is a complex physiological process involving the coordinated action of different organs and different levels of regulation with the objective of producing high-quality gametes in a timely fashion. Because these processes are inevitably interlinked in a migrating fish, swimming during spawning migration should be viewed as an integral part of normal reproductive development for long-distance migrants.
Experimental swimming trials with female eels in fresh water revealed lipid deposition and oocyte growth (reviewed by Palstra and van den Thillart [1]) which was also apparent for migrating female silver eels in the River Rhine [2]. Simulation of oceanic migration in seawater resulted in suppressed gonadotropin expression and vitellogenesis in females, while in contrast continued sexual maturation was observed in silver males [1]. The transcriptomic approach has been instrumental in studies on rainbow trout. A salmonid cDNA microarray platform was used to demonstrate a down-regulation of the transcriptomic response in the ovary after long-term swimming [3]. Deep RNA sequencing revealed increased transcriptional activity in skeletal muscle and specifically an up-regulation of genes involved in muscle growth and developmental processes in white muscle after long-term swimming [4].
A swimming-induced ovarian developmental suppression at the start of vitellogenesis during long-term reproductive migration may be a strategy to avoid increased drag resistance due to oocyte growth and to prevent precocious muscle atrophy [5]. What we have shown for rainbow trout and European eel is that the onset of vitellogenesis is a clear suppression point and an example of phenotypic plasticity during long-distance reproductive migrations. It thus appears that the physiological processes occurring in the muscle and ovary are conflicting. When there is a need to migrate, energetic processes in the muscle that provide fuel for contraction and for muscle growth are up-regulated and those in the ovary are down-regulated (migration phenotype). When there is a need to start vitellogenesis, the situation in muscle and ovary is reversed (maturation phenotype).
Now that the genomic resources for eel have become available, further investigations are made possible on the transcriptomic characterisation of the interactions between metabolism and sexual maturation as experienced during reproductive migration in European eel. Transcriptomic analyses will be instrumental in demonstrating the mechanisms behind swimming-suppressed vitellogenesis in females and the swimming-induced spermatogenesis in males. This will raise information on natural ways to induce sexual maturation and increase gamete quality, on using swimming exercise in protocols for broodstock conditioning (*see also other abstract*).

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Oral presentation

Molecular markers in pectoral fin to predict hormone-induced maturation of farmed female European eels (*Anguilla anguilla*)

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The European eel (*A. anguilla*) is an intriguing organism that has been extensively studied for many decades. The natural population is severely threatened by known and unknown factors, such as overfishing, parasites, migration barriers and/or climate change, and is now listed as a critically endangered species on the IUCN Red List. Sustainable aquaculture may relieve the pressure on wild stocks and thereby contribute to restoration of the natural population; however, one has not yet succeeded in closing the complete life cycle of European eels in captivity. Artificial maturation of female European eels can be achieved via a lengthy, laborious and expensive procedure, including weekly injections with pituitary extracts over a period of 3 to 6 months. The success rate is highly variable, probably depending on the quality and initial maturation status of the brood stock animals. A method for early selection of responders to the hormone treatment would prevent the unnecessary and lengthy treatment of non-responding eels. Sexual maturation of European eels is accompanied by morphological changes of the pectoral fin. Therefore, we examined whether pectoral fin samples could be used to monitor the response of female eels to the hormone treatment. Farmed silver eels were subjected to the standard protocol of weekly injections with pituitary extracts. Representative groups were sampled at 0, 4, 12 and 13-18 weeks of hormone treatment. Four responders and three non-responders were identified based on the gonado-somatic index and their pectoral fin transcriptomes at the start and the end of the trial were mapped using Illumina RNAseq technology. Highly stringent selection resulted in 23 up-regulated and 21 down-regulated maturation marker genes that could be placed into a small number of categories. The molecular pectoral fin markers can be used to monitored hormone induced sexual maturation of female European eels. In addition, these markers provide important new insight into several fundamental processes in eel biology.

Oral presentation

The eel: an excellent but enigmatic model for the study of osmoregulation

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The eel is a strongly euryhaline teleost that can survive a direct transfer from fresh water (FW) to seawater (SW) or vice versa, and it can tolerate distilled water to concentrated SW. Thus, the eel has been used for the study of osmoregulation for a century in Europe (A. anguilla), U.S.A. (A. rostrata), and Japan (A. japonica). Because of limited value for food resources in Europe and U.S.A., however, the number of researchers and laboratories working on osmoregulation of this interesting fish is decreasing compared with more commercial fish such as salmonids and tilapia. Meanwhile, the grilled eel is one of the most popular and luxury foods in Japan, and a recent abrupt decline in glass eel catch urged the government to invest for the research on eel to restore its population. Our laboratory has a long history of research on osmoregulation in eels since late 1960s, and we still succeed this tradition. We are particularly interested in the hormonal regulation of SW adaptation using eel as a model fish. The technique for analyses ranges from comparative genomics to organismal physiology. These studies identified a number of sodium-extruding and hypotensive hormones, which are diversified in teleost fish and shown to play critical roles in SW acclimation. These hormones include cardiac natriuretic peptides (ANP, BNP and VNP), guanylins (guanylin, uroguanylin and renoguanylin), adrenomedullins (AM1-5), and others. Thanks to the completion of the eel genome project led by Ron Dirks and others, we recently started transcriptome analyses in eels to identify key genes for SW acclimation, not only those of hormones but also osmotic-responsive transcription factors and transporters etc. In this presentation, we will introduce our recent work on hormonal regulation of SW acclimation in eels with emphases on the unique characteristics of the eel compared with other typical teleosts. These characteristics can be the clue to enable this mysterious species to prosper to date.

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Oral presentation

Transcriptomic responses of Anguilla anguilla elvers fed dietary LPS

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Dietary immunostimulants are considered as an effective tool for enhancing the immune status of cultured organisms and improving their general welfare. Immunostimulants are chemical substances which activate transiently elements of the immune response and may potentially render animals more resistant to infectious diseases. They also reduce the risk of disease outbreaks, if administrated prior to situations known to result in stress and impaired general performance (e.g. handling, change of temperature and environment, weaning to inert diets) or prior to expected increase in exposure to pathogenic microorganisms and parasites. In this study, we have evaluated the effect of dietary LPS derived from Pantoea agglomerans (LPSp, SOMACY SL100, MACROPHI Inc., Japan) in Anguilla anguilla elvers considering the general condition, growth and immune status of the animals.

Elvers (1.7 ± 0.2 g) obtained from local fishermen (Delta del Ebro, Spain) were fed at different doses of LPSp (0, 20 and 40 μg LPSp kg BW-1 day-1) for a period of 70 days (4 replicates per dietary group). LPSp was added to the diet (52% protein, 24% fat, 9% ash) by top coating the pellets with the lyophilized powder of LPSp dissolved in 2% fish oil (diets manufactured by ALLERQUA, Denmark). The trial was conducted in 100 L tanks (40 L functional volume) connected to a recirculation unit (IRTAmar®) and water quality was as follows: temperature, 21 ± 0.5 ºC; pH, 7.5 ± 0.1; photoperiod, 12 L:12 D and oxygen at saturation levels. At the end of the trial, fish were measured for growth (BW), size dispersion, histological organization of the intestinal mucosa and the transcriptomic responses of the spleen were evaluated by means of a 60K (15000 gene signatures) custom oligonucleotide microarray (Agilent Technologies Inc.).

No significant differences in BW nor survival were observed among groups regardless of the dose of LPSp (BW: 3.9 ± 0.3 g, survival: 98-99%). Micorarray analysis (LIMMA $F<0.05$; adj. p value < 0.005) resulted in 81 significantly up-regulated genes and only 3 down-regulated genes, in the highest LPSp dose. The lowest dose of LPSp up-regulated 26 genes and down-regulated 50. Both doses resulted in regulation of immune related genes, such as, sensors for detection of viral and bacterial products, innate antiviral response genes, cytokines, adipocytokines, and their regulatory nuclear factors. The pathway enrichment analysis (FatiGO; $p < 0.005$) of up-regulated genes (40 μg LPSp kg BW-1 day-1) indicated among the most significantly regulated KEGG pathways: cytokine-cytokine receptor interaction, Jak-STAT signalling, natural killer cell mediated cytotoxicity, Toll-like, RIG-I-like and NOD-like receptor signalling and Wnt, Notch and mTOR signalling.

In conclusion, LPSp diet highly regulates spleen transcription levels, mainly enhancing the immune response capacity of the immunostimulated elvers.

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Oral presentation

Biomarkers for broodstock selection of farmed female European eels

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In captivity, European eels (*Anguilla anguilla*) can only be reproduced artificially by applying hormonal treatments with pituitary extracts or purified gonadotropins, which takes 4-6 months[1]. However, female eels show a high individual variation in responsiveness to the treatment, ranging from no response at all to the production and release of eggs. The number of non-responders is often higher than 50% and response to treatment is probably related to the initial maturation state of the female [2,3]. In order to increase reproductive success, broodstock needs to be selected for responsive females, either prior to or in the early phase of the treatment. The goal of the present study was to identify markers for broodstock selection.

Farmed silver eels were weekly injected with salmon pituitary extract and sampled at 0, 4, 12 and 18 weeks. When final maturation could be induced within 18 weeks, sampling occurred 1 day after ovulation. Expression of steroidogenic genes in ovarian tissue of responding and non-responding eels was examined using a custom-built microarray based on the European eel genome sequence. In addition, blood plasma levels of sex steroid hormones were measured using ELISA. By using those techniques, we were able to identify candidate biomarkers to distinguish responders from non-responders. Microarray analysis of responders and non-responders showed that 5 out of 17 genes encoding steroidogenic enzymes were significantly up-regulated.

Microarray analysis showed that steroidogenic enzymes involved in the production of 11-KT (11β-HSD2, 17β-HSD3) were highly expressed in responders as compared to non-responders. This increased expression indicates that in responding females pre- or early vitellogenic growth was initiated, which was confirmed by histological analysis. The present data are similar to previously observed results of RNA-seq transcriptome analysis of advancing maturation stages from yellow to silver and spawned females (Minegishi et al., unpubl. data). Sex steroid plasma levels of E2 and T increased significantly during artificial maturation, as also shown in the present study and various other studies [4, 5]. A significant correlation was found between GSI and E2, ΔE2 and ratio E2 (rE2) already after 4 weekly injections. The rE2 levels of responders and non-responders sampled after ovulation or after 18 weekly injections indicate that ca. 80% and 99% of the females responding to the treatment may be selected after 4 and 12 weeks, respectively.

It is concluded that the expression level of steroidogenic enzymes within ovarian tissue may be used as broodstock selection marker. Additionally, based on relative fold increase of E2 blood plasma levels after 4 weekly injections responsive female eels may be selected.

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Poster presentation

Expression profiles for six sex-specific genes during gonadal development in eels

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The timing of sex differentiation varies greatly between teleost species. For instance it can be histologically detected very early as in Nile tilapia (19-21 days post fertilization, dpf), intermediate as in Rainbow trout (63 dpf) or late in juvenile stages as in sea bass (120– 150 dpf). In eel, histological sex differentiation occurs very lately and it is more a question of size, than age. Previous studies showed that first sign of histological sex differentiation occur between 25 and 30 cm (Beullens et al. 1997). In this gonochoristic species, it has been proposed that the undifferentiated gonad could develop into either an intersexual stage (Syrski organ) or directly into ovaries. The Syrski organ could then develop into either an ovary or a testis. In order to discriminate females, males and intersexual eels (all between 29 and 40 cm) at different stage of gonad development, we investigated the expression profile of 6 genes (qPCR) involved at different step of the sex differentiation process. Among these genes, the gene encoding the DM related transcription factor 1 (*dmrt1*) was previously reported to well discriminate male from female gonad at the earliest stages of sexual differentiation in several fish species. The gene encoding the Anti-Müllerian hormone (*amh*) and the gene encoding the gonadal soma derived factor (*gsdf*) were also reported to have a male-biased expression profile during sex differentiation. The expression of two "oocytes-specific" genes: zygote arrest 1 (*zar1*) and zona pellucida glycoprotein 3 (*zp3*), was also studied. In addition, the expression of the Forkhead box (Fox) transcription factors (foxn5) previously reported (from a transcriptomic analysis) to be female-biased, was also investigated. The PCA analysis based on this 6 genes (Fig 1) allowed to well discriminate female from male at different developing stages. In addition, data confirmed our previous results revealing that the Syrski organ could not develop into an ovary Geffroy et al. 2013). Nevertheless, the histological classification of developmental stages was different from the PCA classification (based on gene expression profile) for males and intersexuals eels, revealing the complex process that shapes sexual differentiation in eels.

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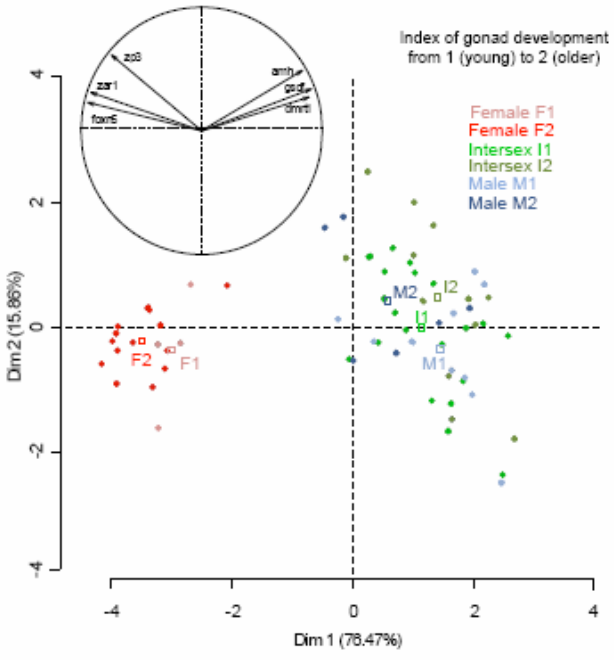

Poster presentation

Characterization of three nuclear and two membrane estradiol receptors in the eel: involvement in experimental maturation process

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Estrogens are sex steroid hormones critically involved in the maturation and reproduction process. In vertebrates, estradiol interacts through two different types of receptors. As classically described for steroids, estradiol binds to nuclear receptors (ER) and acts as a transcription factor. Recently, a less conventional pathway has been evidenced. Estradiol can signal through non-genomic mechanisms by activating membrane G protein-coupled estrogen receptors (GPER). By means of the new genomic data obtained in the European and Japanese eels, we characterized the full set of estradiol receptors in the eel, their involvement in vitellogenesis and experimental maturation, and got more insights into their evolution history in vertebrates.

We identified and characterized three nuclear receptors (ERa, ERb1, ERb2) and two membrane receptors (GPER1, GPER2) in the eel, while in tetrapods only two ER (ERa and ERb) and one GPER are present. Phylogenetic analysis of vertebrate ER genes defined two clades of vertebrate ER, ERa and ERb, and two sub-clades of ERb in teleosts. In the eel, as in most of the teleost species, two ERb are present, resulting from the third whole genome duplication event (3R) that occurred specifically in the teleost lineage. GPER phylogenetic and syntenic analyses suggest that, as for ERb, the two membrane estradiol receptors characterized in the eel resulted from the specific teleost 3R.

Specific qPCR primers were developed for each receptor. The five nuclear and membrane receptors present a large tissue distribution, including the organs of the BPG-liver axis (brain, pituitary, ovary, liver), with the highest expression in the pituitary. In teleosts, this steroid plays a crucial role in the liver vitellogenesis and ovary maturation, but acts also through feed back pathways, at the brain and pituitary levels. We investigated the regulation of the five receptors in the BPG-liver axis, during eel experimental maturation. The nuclear receptor ERa was up regulated in all the studied organs, while the four other receptors presented a differential regulation during the maturation process. We further investigated their regulation *in vitro* in hepatocyte cell culture after estradiol treatment to induce vitellogenesis. Only ERa was regulated and presented an auto up regulation in estradiol-treated hepatocytes. In the same way, we observed an up regulation of ERa in pituitary cell cultures treated with estradiol. In conclusion we characterized the full set of estradiol receptors in the eel. Their differential tissue distribution and regulation may have contributed to the conservation of the duplicated receptors in this species.

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Poster presentation

Pituitary hormone glycoprotein receptors in eel: characterization and evolutionary insights.

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Glycoprotein hormones, Fsh, Lh and Tsh are composed of a common alpha subunit and of a beta subunit that confers the specific biological activity. Fsh, Lh and Tsh act on target tissues via their interaction to specific G protein-coupled receptors that show the peculiarity of a long extracellular binding domain. Until recently, it was considered that only one Lh receptor (Lhr), one Fsh receptor (Fshr) and one Tsh receptor (Tshr) have been conserved through the vertebrate lineage. We investigated the Fshr, Lhr and Tshr in a basal teleost, the European eel (*Anguilla anguilla*). From the draft genome of European eel (Henkel et al., 2012), one single copy of *fshr* and two copies of *lhr* (*lhr1* and *lhr2*) and *tshr* (*tshra* and *tshrb*) genes were identified. Partial or full-length cDNA of the five receptors were successfully isolated. The glycoprotein receptor repertoire was identified in other genomes of representative vertebrates. In gnatosthomes, the receptors were distributed though three distinct clades: Tshr, Fshr and Lhcgr/Lhr. It appears that only one single copy of *fshr* gene has been conserved in gnatosthome lineage including teleost species, suggesting that there was no impact of the teleost specific whole genome duplication (3R). For the Lhcgr/Lhr, one gene was found in sarcopterygian and chondrichtyan genomes. In contrast duplicated *lhr* (cf other abstract of Maugars et al., this symposium) were found in some actinopterygii including the eel and a non-teleost specie, the spotted gar. This suggests that the two *lhr* genes result from a duplication event that occurred in actinopterygii before the emergence of teleosts and that there was no further impact of the 3R on the *lhr* gene number in teleosts. One *tshr* gene was identified in sarcopterygians, chondrichthyans and a non-teleost actinopterygian (spotted gar), while duplicated *tshr* genes were found in some teleost genomes including the eel. These results suggest that the duplicated *tshr* gene may derive from the teleost specific 3R. Syntenic comparison will be further investigated to confirm this putative 3R origin. Sequence comparison revealed divergences at both hormone-binding domain and transduction domain between both duplicated Tshr and Lhr. *Fshr* and duplicated *lhr* transcripts were mainly found in the ovary and testis but also in extra-gonadal tissue. *Tshra* but not *tshrb* was expressed in thyroid follicles while *tshrb* transcript was distributed in pituitary and gonads suggesting only extra-thyroid functions for this receptor. The structural and functional divergences observed may have acted as selective forces in the maintenance of the duplicated *lhrs* and *tshrs*.

Henkel CV, Burgerhout E, de Wijze DL, Dirks RP, Minegishi Y, et al. (2012). Primitive Duplicate Hox Clusters in the European Eel's Genome. PLoS ONE 7(2): e32231.

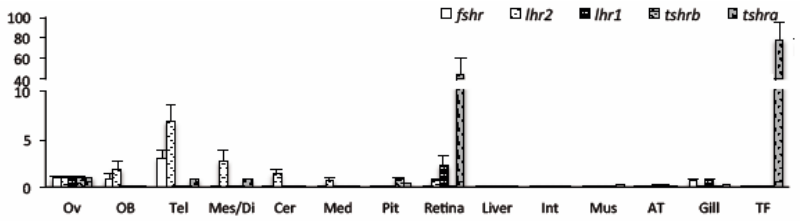

Figure. Tissue distribution of fshr, lhr1, lhr2, tshra and tshrb in European eel. Transcript abundance was assayed by qPCR in ovary (Ov), olfactory bulbs (OB), telencephalon (Tel), di/ mes-encephalon (Di/Mes), corpus cerebellum (Cb), and medulla oblongata (Med), pituitary (Pit), retina, liver, intestine (Int), muscle (Mus), adipose tissue (AT), gill and thyroid follicles (TF). Means ± S.E.M are represented (n=8 eels).

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Poster presentation

Characterization and expression of the Vitellogenin receptor in the European eel

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Teleost eggs contain a substantial yolk mass, which serves as a protein- and lipid-rich source of nutrients for embryonic development and larval growth. Also, egg hydration in pelagic eggs depends on the release of free aminoacids from hydrolysis of yolk proteins. A large portion of the yolk mass is derived from vitellogenin (VTG) which is synthesized by the liver, released to the blood circulation, and incorporated into the oocytes via endocytosis mediated by Vtg receptor (Vtgr), to form the yolk granules. The Vtgr belongs to the low-density lipoprotein receptor superfamily. The receptor-mediated uptake of VTG is crucial for oocyte growth in egg-laying animals because they mediate a key step in oocyte development.
To deep in the study of vitellogenesis in the European eel, we focused on the Vtgr gene, by studying its tissue distribution and expression throughout vitellogenesis induced by hormonal treatment (1) at different temperatures.
First, the search in European and Japanese genomes (2,3) revealed in each species 4 genes coding for LDLR superfamily members. Phylogenetic analysis of LDLR superfamily revealed a single European eel Vtgr, orthologous to other vertebrate Vtgr. The three other genes belong to other clades of the LDLR superfamily. Specific qPCR was developed to assay European eel Vtgr transcript.

Tissue screening was studied in 8 female silver eels. The ovary was the predominant site of expression of Vgtr mRNA. Vtgr mRNA was also expressed in the brain, pituitary, gill, fat and heart. We also investigated its potential regulation in the ovary between yellow and silver stages as well as during experimental maturation at different temperatures. Previous experiments had demonstrated that immature eel ovaries were not able to incorporate Vtg (4). Interestingly, Vtgr mRNA was already expressed at the previtellogenic stage in yellow eel, and its expression does not increase at silvering, nor throughout experimental maturation at different temperatures. However, we previously demonstrated that high temperatures caused a fast vitellogenesis progression (5), which does not seems mediated through increased levels of Vtgr expression, as Vtgr expression profiles were similar between thermal regimes. This study reveals that the Vtgr expression may not be a limiting step for the uptake of Vtg by the oocyte in the European eel.

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Poster presentation

Silver eel broodstock conditioning, artificial reproduction and larval rearing at IMARES

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Experimental scale laboratory studies are required to examine the physiological effects of single cues on metabolism, sexual maturation and the interactions between them in eels. Even if it would be possible to assess such effects in eels during their oceanic migration, for example by applying telemetry, they would still be a product of several actors of the internal and external environment acting at the same time (e.g. exercise, starvation, photothermal conditions, salinity and osmoregulation, depth and pressure, navigation cues and pheromones). When the individual contributions have been assessed, combinations of cues and their interacting effects can be examined and applied for broodstock conditioning.
At IMARES in Yerseke we have extensive aquaculture facilities that provide experimental opportunities for the simulation of migration including four large swim-tunnels (in collaboration with G. van den Thillart, Leiden University) and a large swim-gutter with motor and propellor able to reach speeds above 1 m/s (Fig. 1). We have direct access to seawater and four climate rooms for temperature controlled swimming experiments. Six semi-industrial RAS units with each connected tanks in triplicate can provide optimal water quality, especially important for the induction of natural spawning when large amounts of eggs and sperm are released that threaten the water quality at a crucial moment.
In the currently running project Innovative Reproduction of European Eel (VIP/EFF grant from the Dutch Ministry of Economic Affairs and the European Fisheries Funds, together with partners NewCatch and Glasaal Volendam), IMARES is mimicking natural conditions of fresh water and seawater migration to investigate its use for broodstock conditioning. High-throughput transcriptomic analyses will be instrumental in assessing the effects on the reproductive axis. Subsequently, broodstock will be reproduced by hormonal stimulation and alternative strategies. Produced larvae will be used for optimalisation of rearing and feeding conditions.

Figure 1 *Experimental swimming facilities at IMARES (Yerseke, The Netherlands) include (A) two small (each 8 l) and four large (each 127 l, in collaboration with G. van den Thillart, Leiden University) Blazka-type swim tunnels with Loligo oxygen electrodes to perform respirometry, and (B) a 4x2x1 m swim gutter driven by a motor with propellor reaching speeds over 1 m/s. (C) IMARES in Yerseke is situated next to the Oosterschelde where we have direct access to seawater of 32 ppt for (re)circulation. (D) The newly built research facilities with climate rooms and laboratories will be ready for use in February 2014.*

Poster presentation

Effect of photoperiod on endocrine profiles in european eels *anguilla anguilla* during artificially induced ovarian development

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Artificial reproduction in eels is very challenging due to their peculiar life cycle. No information is yet available on the influence of photoperiod on the sexual development of wild silver eels in captivity. Previous studies have demonstrated the photoperiod to influence sexual maturation of eels showing that dark conditions lead to a better reproductive performance in terms of a greater gonadal maturation and a higher egg production and spawning record.

This study aimed to investigate the physiological mechanisms leading to different outcomes in light or dark conditions on the gonadal steroidogenesis in wild female European silver eels during pituitary extract treatment.

The variation of the body weight index and gonadosomatic index suggested a positive effect in dark conditions. Sexual steroids (androgens and estrogens) showed a significant difference in plasmatic concentration in the animals kept in the dark suggesting a better wellness reproductive situation in this particular housing condition.

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Leiden, The Netherlands

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Genome paper Anguilla anguilla (2012): http://dx.doi.org/10.1371/journal.pone.0032231
Genome paper Anguilla japonica (2012): http://dx.doi.org/10.1016/j.gene.2012.09.064
Website Anguilla genomes: http://www.zfgenomics.org/sub/eel

Items for the panel discussion of Friday 17 January (16.00 h) can be written down below

Please hand in this page during the lunch break of Friday 17 January (13.00 - 14.00 h)

or send items via e-mail to: dirks@zfscreens.com

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