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Abstract

Water temperature governs physiological functions such as growth, energy allocation, and sex determination in ectothermic species. The European sea bass (*Dicentrarchus labrax*) is a major species in European aquaculture, exhibiting early dimorphic growth favoring females. The species has a polygenic sex determination system that interacts with water temperature to determine an individual's sex, with two periods during development that are sensitive to temperature. The current study investigated the influence of water temperature on energy allocation and sex-biased genes during sex determination and differentiation periods. RNA-Sequencing and qPCR analyses were conducted in two separate experiments, of either constant water temperatures typical of aquaculture conditions or natural seasonal thermal regimes, respectively. We focused on eight key genes associated with energy allocation, growth regulation, and sex determination and differentiation. In Experiment 1, cold and warm temperature treatments favored female and male proportions, respectively. The RNA-seq analysis highlighted sex-dependent energy allocation transcripts, with higher levels of *nucb1* and *pomc1* in future females, and increased levels of *egfra* and *spry1* in future males. In Experiment 2, a warm thermal regime favored females, while a cold regime favored males. qPCR analysis in Experiment 2 revealed that *ghrelin* and *nucb1* were down-regulated by warm temperatures. A significant sex-temperature interaction was observed for *pank1a* with higher and lower expression for males in the cold and warm regimes respectively, compared to females. Notably, *Spry1* displayed increased expression in future males at the all-fins stage and in males undergoing molecular sex differentiation in both experimental conditions, indicating that it provides a novel, robust, and consistent marker for masculinization. Overall, our findings emphasize the complex interplay of genes involved in feeding, energy allocation, growth, and sex determination in response to temperature variations in the European sea bass.

Keywords: Temperature sensitivity, feeding regulation, metabolism, growth, sex determination.

1 Introduction

Water temperature plays a critical role for aquatic ectotherms, influencing their physiology, behavior, life history, as well as growth and development (Abram et al., 2017; Birnie-Gauvin et al., 2021; De Jong et al., 2023; Kua et al., 2020; Neuheimer et al., 2018). Abiotic factors affect metabolic rates of ectothermic animals, and among them teleost fishes have evolved a remarkable array of phenotypic and genotypic strategies to thrive in environments with fluctuating thermal conditions (Clarke, 2003; Xia et al., 2023). Indeed, multiple metabolic processes such as appetite, feeding behavior, digestion, and enzyme activity, are directly influenced by temperature in teleosts (Volkoff and Rønnestad, 2020).

Warmer temperatures generally increase metabolic demands, affecting growth rates and energy allocation patterns. Temperature can also have size-dependent effects on growth. Juveniles and adults of the same species may exhibit different responses; consequently, energy allocation patterns vary with temperature and size, affecting fitness and survival (Gillooly et al., 2001; Lindmark et al., 2022). Interestingly, environmental conditions can also affect the sexual fate of the gonad during early development (Geffroy and Douhard, 2019). In some fish species, the process of sex determination exhibits an intricate interplay between genetic and environmental factors, with temperature playing a pivotal role in shaping the gonadal fate (Geffroy and Wedekind, 2020). The phenotypic sex can be modulated by multiple genes in some ectothermic species, and this polygenic sex determination system can be affected by temperature (Miyoshi et al., 2020; Vandeputte et al., 2007), as in the European sea bass (*Dicentrarchus labrax*) (Geffroy et al., 2021a). This teleost species is important for European mariculture but is also a model for research in genetics and sex determination (Vandeputte and Piferrer, 2018).

In European sea bass, high temperatures during early life can masculinize populations by modulating promoter methylation of sex-specific genes such as sox3 and sox9a (Geffroy et al., 2021a; Navarro-Martín et al., 2011). Conversely, exposure to cold temperatures (<17 °C) from the early larval phase to metamorphosis (55-66 days post-hatch – dph) triggers ovary development (Navarro-Martín et al., 2009; Pavlidis et al., 2000; Sfakianakis et al., 2013; Vandeputte and Piferrer, 2019). However, recent studies showed that late exposure (after 80 dph) to a relatively warm temperature (>21°C) enhanced females production (Clota et al., 2024; Vandeputte et al., 2020), while prolonged exposure (up to 244 dph) to a relatively low temperature (16 °C) biased the sex ratios towards males (Vandeputte et al., 2020). This demonstrated the existence of two distinct thermosensitive periods in European seabass, as the same temperature treatment applied at different times results in opposite effects on the sex ratio, and thus possibly distinct mechanisms involving methylation and modulation of growth. Indeed, the mechanisms governing sex determination are also tightly linked to growth in European sea bass, females are larger than males very early in the development, adding another layer of complexity in our understanding of sex determination in the species (Díaz et al., 2013; Faggion et al., 2021). Therefore, it is difficult to distinguish causes from consequences: does sex influence differential growth rate or does early growth rate influence sex determination?

A recent study highlighted a tendency for future females to display more energy content in their body (joules·mg^{-1}) than future males (Geffroy et al., 2021a). It has thus been hypothesized that, compared to males, future females accumulate more energy during the sex determination period, with associated up-regulation of molecular pathways for lipid and glucose metabolism (Geffroy, 2022). Appetite regulation and energy allocation, are mechanisms dependent on molecular factors, such as peptide-hormones or transcription factors, that respond to nutrients

and modulate complex molecular pathways operating either centrally (brain) or peripherally (e.g. liver) (Challet, 2019; Kubrak et al., 2022). Yet, the molecular pathways associated with energy allocation that can be modulated by temperature during the sex determination period are almost unexplored in teleost fishes.

In the above-mentioned study (Geffroy et al., 2021a), molecular factors like epidermal growth factor receptor a (*egfra*) and sprouty 1 (*spry1*) were up-regulated early in the development of future phenotypic males. In mammals, EGFR is involved in multiple functions related to glucose and amino acid metabolism, lipogenesis during cell proliferation, growth, and differentiation processes (Orofiamma et al., 2022). SPRY1, is a negative regulator of receptor tyrosine kinase, and has been studied in mammals for its role in controlling adipogenesis (Mandl et al., 2019) and angiogenesis (Sabatel et al., 2010). Interestingly, a recent study in mice showed that *spry1* was up-regulated by *dmrt1* (doublesex- and mab-3-related transcription factor 1) (Zhang et al., 2023), a key gene in testis formation and masculinization in mammals and fishes (Ogita et al., 2020). Previous studies, on juvenile and adult teleosts, demonstrated that orexigenic factors like *ghrelin*, as well as anorexigenic factors like pro-opiomelanocortin (*pomc*), are modulated by temperature in *Salmo salar L.*, *Carassius auratus,* and zebrafish (*Danio rerio*) (Chen et al., 2019; Hevrøy et al., 2012; Woods et al., 2014). *Pomc* participates in the domestication process of the mandarin fish (*Siniperca chuatsi*) (Lu et al., 2023), and is involved in stress signaling, mediating cortisol synthesis (Cerdá-Reverter et al., 2011). More recently, it was demonstrated that growth and stress signaling acting through *pomc* are related with sex, with female tilapia (*Oreochromis niloticus*) displaying higher levels of *pomc*, and its expression is mediated by estrogen response elements in the promoter region of the gene (Wan et al., 2021). Furthermore, *pomc* deficiency in zebrafish increases growth and promotes sex reversal (Shi et al., 2020; Yang et al., 2023).

Other key regulators of energy that have an impact on sex determination in other species are AMP-activated protein kinase (*ampk*) and the mechanistic target of rapamycin (*mtor*) transcription factors (Geffroy, 2022). They both act as cellular sensors of energy homeostasis (Hardie, 2014; Wullschleger et al., 2006), regulating glucosensing capacity, protein and lipid metabolism in rainbow trout (*Oncorhynchus mykiss*) (Otero-Rodiño et al., 2019; Polakof et al., 2011), or modulating key peptides like Ghrelin in rats and mice (Martins et al., 2012; Yin et al., 2015). Nucleobindin protein 1 (*nucb1*) that encodes for the nesfatin-1 like peptide (NLP), is an anorexigenic peptide that stimulates insulin secretion (Ramesh et al., 2015; Sundarrajan et al., 2016). The pantothenate pathway is also crucial regarding energy storage (Geffroy, 2022). Indeed, malonyl Coenzyme A (CoA), a final metabolite of the pantothenate pathway, is a critical substance that regulates other metabolic pathways involved in cholesterol production and lipogenesis. The pantothenate kinase (*pank*) gene encodes a catalytic enzyme that phosphorylates pantothenate (vitamin B5). Paralog genes share the same function in mammals but with different sub-cellular distribution, regulation and expression (Zizioli et al., 2016). In the zebrafish, pank1a is predicted to localize in both the cytosol and the nucleus (https://zfin.org/ZDB-GENE-040426-2846#summary). Interestingly, lipid metabolism mediated by *pank1a* cause sex reversal of genetic female to phenotypic male in the medaka (*Oryzias latipes*)(Sakae et al., 2020).

Understanding the complex interrelationships among water temperature, energy allocation and sex determination is crucial for predicting and managing thermal requirements for natural populations but also for aquaculture production. The aim of the present study was to investigate the molecular pathways that are modulated by water temperature and that can be involved in sex determination of the European sea bass. To this end, we first re-analyzed the transcriptomic data from Geffroy and collaborators (2021a) to depict the expression pattern of genes involved

in energy allocation at two stables temperatures (21°C vs 16°C) applied between early larval phase to metamorphosis. Then, we performed a specific experiment with two distinct seasonal thermal regimes that follow natural temperatures, to investigate the expression patterns of the above-mentioned genes.

2 Materials & methods

2.1 Ethical statement on Animal for research

For both experiments, fish rearing was performed at the Ifremer Plateforme Expérimentale d'Aquaculture (Palavas-les-Flots, France), accredited to use and breed laboratory animals (n° C341926). Experiments were performed in accordance with relevant guidelines and regulations provided by the ethic committee (no 36) of the French Ministry of Higher Education, Research and Innovation and the experiment received the following agreement number: APAFIS#19676-2019021915002143 for Experiment 1 and APAFIS #34987-202201251229160 6 for Experiment 2. All procedures involving animals were also performed following ARRIVE guidelines (https://arriveguidelines.org/) and the EU Directive 2010/63/EU for animal experiments.

2.2 Experiment 1: Effects of exposure to two constant temperatures on energy homeostasis-related genes

We used the transcriptomic data generated from our previous study (Geffroy et al., 2021a) that compared European sea bass kept at constant low (16°C) and high temperature (21°C) during the sex determination period (0 to 60 dph). Briefly, fish were held in triplicate tanks at each water temperature. Since fish growth was stimulated at the higher temperature, sea bass development differed greatly between treatments (Figure 1). To enable data comparison, the first sampling was performed at the same sum of degree-days, a procedure previously used to standardize measurement of growth in fishes (Chezik et al., 2014). Hence, 29 fish from the low temperature (LT) treatment and 39 from the high temperature (HT) treatment were sampled at 78 and 53 dph corresponding at ~550 Degree Days base 10°C ($DD_{10°C}$). Then, 42 juveniles were collected at 124 dph (LT, n = 21) and 117 dph (HT, n = 21), when they reached the exact same length and weight ~ 1100 Degree Days (Table 1). The RNA-seq was performed on the whole body of the 68 individuals at 550 $DD_{10°C}$ and in the gonads of the 42 juveniles, to focus on genes related to sex determination and sex differentiation.

In Geffroy et al (2021a), the genetic sex tendency (GST) of individual larvae and juveniles, before phenotypic sex is determined, was predicted based on the genotype of 2030 European sea bass from the same cohort using the Thermofisher DlabChip European sea bass array of 57k Single Nucleotide Polymorphism (SNP) markers (Griot et al., 2021). The result of the SNP-based model allowed us to classify fish with positive values of GST as being more likely to develop as females in a neutral environment and those with negative values as being more likely to develop as males. Here, the RNA-seq data were re-analyzed to identify genes related to feeding, metabolism and growth, which would correlate with the GST and/or temperature regime.

2.3 Experiment 2: Effect of two fluctuating water thermal regimes on energy homeostasis-related genes

The aim of this experiment was to compare the effect of simulated natural regimes of water temperature on the transcript abundance of genes previously identified as being key for metabolism and growth during the sex determination period in sea bass.

2.4 *Experimental design and larval sampling*

The three populations of fish used were produced by artificial fertilization of females and males from wild-caught broodstock of the Atlantic (7♀x30♂), East Mediterranean (12♀x30♂) and West Mediterranean (14♀x30♂) *Dicentrarchus labrax* at the Ifremer experimental platform in Palavas-les-Flots, France. After artificial fertilization, the embryos were incubated for 20 days post-hatching at 13°C, and then fish were mixed in equal proportions and disposed into quadruplicate 110 L RAS tanks at a density of 33 larvae per liter (Figure 1). Quadruplicate tanks were interconnected with a recirculating biofilter and UV sterilization. The water temperature was increased at 1°C/week ratio until the environmental temperature for Atlantic (cold regime), or East Mediterranean (warm regime) was reached (Figure 1). Photoperiod was adjusted weekly to average for the natural thermal regime according to the Copernicus database. Water temperature was regulated with electric heater/cooler units. From 10 days post hatching (dph) until 144 $DD_{10°C}$, larvae were fed with Cryoplankton Large (Planktonic AS, Norway), the nauplius from *Semibalanus balanoides*, then Artemia nauplii until 300 $DD_{10\ °C}$, and changed thereafter to a commercial sea bass diet (Pro Start and Pro Wean, BioMar, Nersac, France).

At the all-fins stage (~480 $DD_{10°C}$), 18 fishes per temperature treatment were collected (6 per tank, note that one tank was left undisturbed) and whole bodies were measured and weighted (Table 1) before being placed in 2 ml tubes and quickly frozen in liquid nitrogen. At the juvenile stage (~1300 $DD_{10°C}$), 21 fishes per temperature regime were collected (7 per tank) and quickly euthanized with an overdose of benzocaine (150 mg/L). The liver and gonads were dissected, deposited in 2 ml tubes and quickly frozen in liquid nitrogen. All the samples were stored at -80°C until processing.

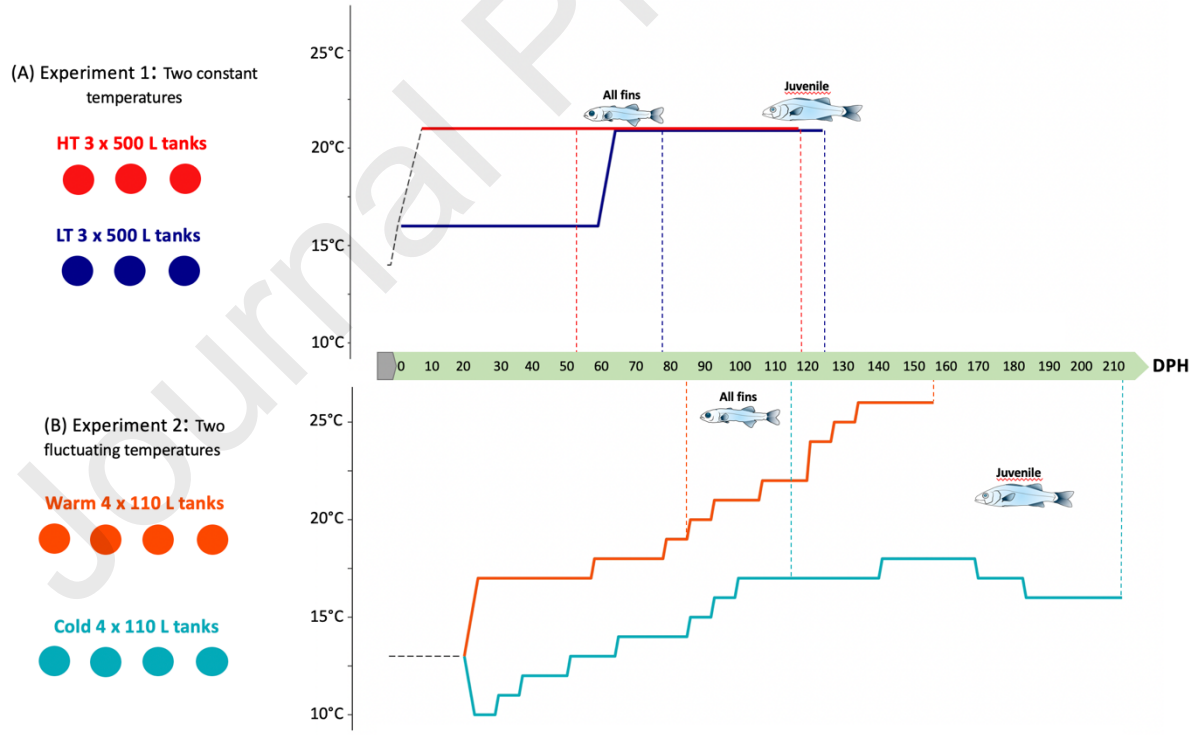

Figure 1. Experimental Design for Experiment 1 (Upper Figure): This experiment aims to evaluate the transcriptomic profile of European sea bass larvae under typical aquaculture conditions at two constant temperatures (16 and 21°C) until the end of the all-fins stage. Experimental design for Experiment 2 (Lower Figure): This experiment focuses on assessing the impact of simulating two natural thermal regimes (Atlantic and East Mediterranean Sea) on

gene expression by qPCR during the sex determination and differentiation process of European sea bass. In both experiments, dashed blue lines indicate the sampling times for individuals kept at low temperatures, while dashed red lines indicate the sampling times for individuals kept at high temperatures. Additional details can be found in the Material and Methods section.

2.5 *RNA extraction and reverse transcription*

The all-fins stage period coincides with rapid primordial germ cell proliferation (Roblin & Brusle, 1983), but the gonad is still too tiny for being accurately sampled. However, previous studies showed that whole larvae (instead of gonads) can be used to assess gene expression of sex-related genes, as this approach was previously performed and validated in comparable studies on the subject (Blázquez et al., 2009, 2008). Still, for having distinct parts of the body that included the brain and the liver, the head was separated from the trunk for all these samples. The total RNA of 72 samples (36 trunks and 36 heads) from 480 $DD_{10°C}$, and 126 samples from ~1300 $DD_{10°C}$ were extracted following the TRIzol reagent (Invitrogen) standard protocol. Nucleic Acid (ng/uL) concentration was measured with the NanoDrop® ND-1000 V3300 Spectrophotometer (Nanodrop Technology Inc., Wilmington, DE, USA). The RNA quality was checked on an Agilent bioanalyzer (Agilent) using electrophoretic trace method. The RNA integrity number was between 8.5 and 10 for all samples. 1 mg of total RNA was used as positive template for reverse transcription and two negative controls were included, no retro-transcriptase enzyme (-RT) and ultra-pure water instead of RNA template (-C). Considering the amount of the tissues, two different kits for reverse transcriptions was used. For the all-fins stage, the M-MLV reverse transcriptase kit (PROMEGA were used to get cDNA following the manufacturer instructions. For the ~1300 $DD_{10°C}$ samples, considering the small amount of tissue for liver and gonads, the cDNA was synthesized using the qScript cDNA SuperMix (Quantabio, QUIGEN, Beverly, MA, USA) following manufacturer's instructions. In both cases Oligo(dT) adaptor primer was used for the reverse transcription reaction. The cDNAs from all the samples were diluted 40-fold in nuclease free water for the quantitative PCR (qPCR) reactions.

2.6 Quantitative PCR (qPCR)

All primers that were not published before were designed with the Primer3Plus tool, ensuring that one of the forward or the reverse primer hybridizes on the exon-exon junction to avoid genomic amplification (Table S1 and S2). The reaction mix (1.5 µl) was dispensed with the Echo® 525 liquid handling system (Labcyte Inc., San Jose, CA, USA) into a 384-well reaction plate as follows: 0.75 µL of SensiFAST™ SYBR® No-ROX Kit (Bioline, London, UK), 0.4 or 0.6 µM of final concentration for each primer, approximately 0.625 ng of cDNA, and ultra-pure water to complete the volume. Triplicates reactions were assessed for each standard point, duplicates for the target and reference genes, negative controls were included for each gene (-RT, -C and ultra-pure water instead of cDNA template). Primer specificity was assessed with the melting curve to confirm the amplification of one product. The qPCR products were sequenced by Sanger method to confirm the identity of the amplicons. The housekeeping genes Ribosomal protein L13 (*l13*), eukaryotic translation elongation factor 1 alpha (*eef1α*) and beta-actin (*β-actin*) are all recommended for qPCR and have been successfully used previously for European sea bass (Sadoul et al., 2018). We used sry-related HMG box3 (*sox3*) the evolutionary precursor of sex determining region Y (*sry*) in mammals (Herpin and Schartl, 2015), to know the molecular sex of the fish at the all-fin stage, as we previously found that it significantly correlates with the genetic sex tendency (more transcripts in futures males). At the juvenile stage, we used gonadal soma derived factor (*gsdf*) and gonadal aromatase (*cyp19a1a*) as sex

markers in the gonads, as we previously found that they discriminate sex well at this stage (Goikoetxea et al., 2022).

The qPCR reactions were performed in a LightCycler 480 (Roche) with a denaturation step at 95°C for 3 min, 40 cycles of amplification (95°C, 10 s), hybridization (60°C, 10 s) and elongation (72°C, 10 s). All the data were analyzed with the standard curve method. Relative levels of gene transcription were obtained using the following equation (E_{ref}^(Ct_{ref}))/ E_{target}^(Ct_{target}) with the cycle threshold of target gene (Ct_{target}) normalized by the geometric mean of three housekeeping genes (Ct_{ref} : *l13*, *β-actin* and *eef1a*). E_{ref} represent the mean efficiency of the standard curve for the three housekeeping genes and E_{target} the efficiency of the target gene. At the juvenile stage, one individual (from the cold regime) did not amplify, 2 gonads presented very high and unusual values of housekeeping genes (one from the cold and one from the warm regime): those 3 samples were thus removed for the statistical analysis.

2.7 *Statistical analysis*

Before any analysis of gene expression, we tested all housekeeping genes relative to temperature or sex with t-test to ensure that there was no difference linked to the treatment. Linear models were used to test for correlation between the molecular sex (*cyp19a1a, gsdf*, and *sox3*) and the genes of interest, adding the thermal regime (cold and warm) as a co-factor. Since the number of fish sexed at the juvenile stage was low (n=37) we used a Pearson's Chi-squared test with Yates' continuity correction to test the effect of temperature on sex ratio. For each tissue (head, trunk, liver or gonads), differences between groups (molecular sex or thermal regime) were assessed by t-test or ANOVA (when temperature and sex were tested simultaneously), plots include mean and standard error (se).

3 Results

3.1 *Experiment 1: constant temperatures, 16 vs 21°C*

By re-analyzing the RNA-Seq data at 550 $DD_{10°C}$, we detected 2 new transcripts that correlate with the molecular sex: *nucb1* and *pomc1*, with more transcripts in futures females (Figure 2). As previously identified in Geffroy et al. 2021a, *egfra* and *spry1* presented more transcripts in future males than in future females (Figure 2). Regarding temperature treatment, we identified 5 genes with significant differences in transcript abundance, namely *ampk1, ghrelin, mtor*, *pank1a* and *spry1* (Figure 2).

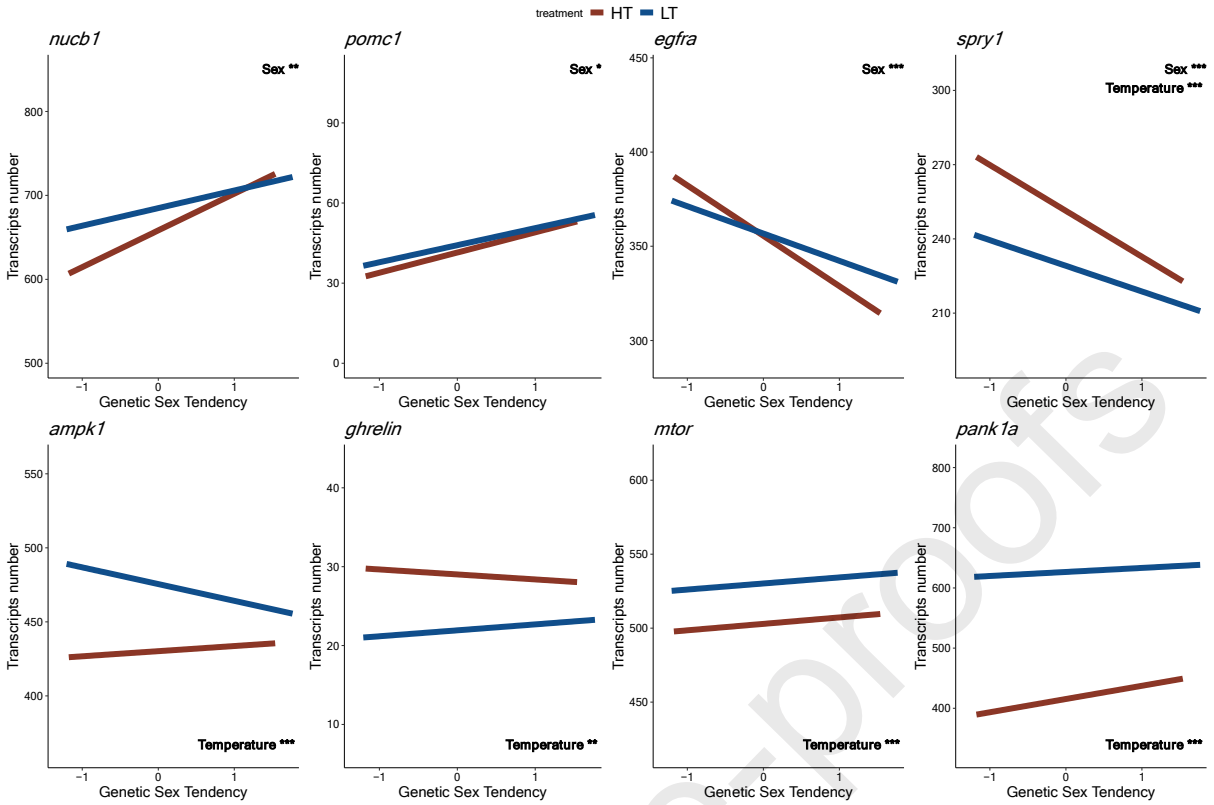

Figure 2. Comparative linear correlation of transcript number with the genetic sex tendency (GSD) at low temperature (LT, 16°C, blue line) and high temperature (HT, 21°C, red line) treatments at the all-fins stage. Abbreviations: *** = p-value < 0.001; ** = p-value < 0.01; * = p-value < 0.05; AMP-activated protein kinase 1 (*ampk1*); epidermal growth factor receptor a (*egfra*); mechanistic target of rapamycin (*mtor*); nucleobindin protein 1 (*nucb1*); pantothenate kinase 1a (*pank1a*); pro-opiomelanocortin 1 (*pomc1*); sprouty 1 (*spry1*).

When the RNA-seq data from gonads at 1100 $DD_{10°C}$ were re-analyzed, *spry1, mtor, ampk1*, and *pank1a* were also differentially expressed between future females and males (Figure 3).

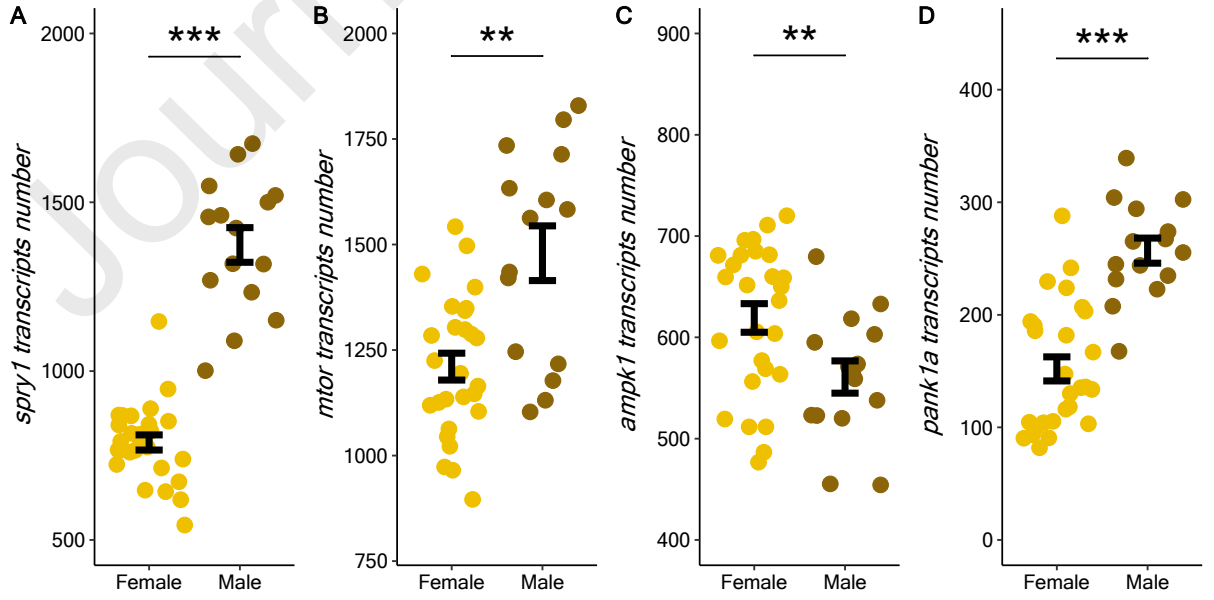

Figure 3. Number of RNA-Seq transcripts in the gonads of phenotypic females (yellow dots) and phenotypic males (brown dots) at the juvenile stage. The number of transcripts for A) sprouty 1 (*spry1*); B) mechanistic target of rapamycin (*mtor*); C) AMP-activated protein kinase 1 (*ampk1*) and D) pantothenate kinase 1a (*pank1a*) are represented. Each bar represents the mean ± se. Abbreviations: *** = p-value < 0.001; ** = p-value < 0.01.

3.2 Experiment 2: fluctuating cold and warm temperatures

At the all-fins stage, we detected a strong and negative correlation between *sox3* and the weight of fish, confirming that *sox3* is an early sex marker in European sea bass (Figure 4A). The levels of transcripts of *cyp19a1a* and *gsdf* also allowed differentiation of females from males at the juvenile stage (Figure 4B). Note that two individuals with intermediate values, from the warm thermal regime, were removed to ensure a good discrimination between sexes. Based on this discrimination, we detected that temperature significantly affected sex ratio (Chi-squared p-value < 0.01), with more males produced in the cold treatment (19 *vs* 2) and more females in the warm treatment (10 *vs* 6). In addition, the females were about twice the weight of males (9.3 ± 0.8 g *vs* 5.3 ± 0.4 g; t-test p-value < 0.001), and females were also significantly longer than males (9.1 ± 0.25 cm *vs* 7.4 ± 0.2 cm; t-test p-value < 0.001).

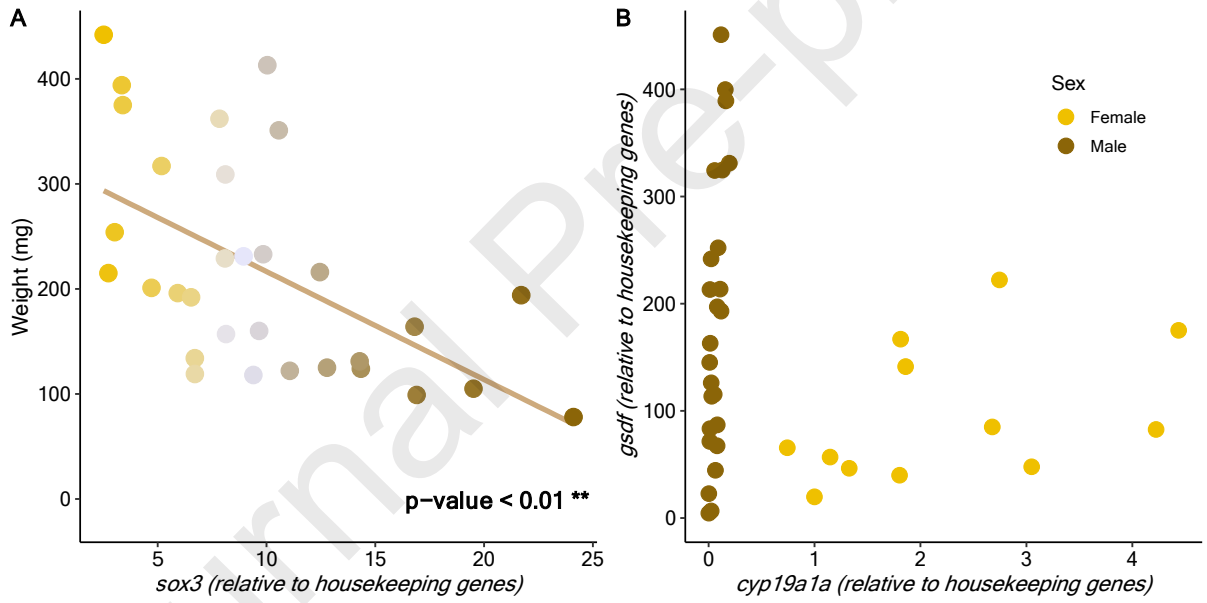

Figure 4A) Linear correlation between *sox3* and body weight (mg) at all-fins stage; B) Relative expression of *gsdf* and *cyp19a1a* in the gonads at juvenile stage of fish from the Experiment 2. Phenotypic females (yellow dots) are discriminated from phenotypic males (brown dots) based on gene expression. Abbreviations: ** = p-value < 0.01; sry-related HMG box3 (*sox3*); gonadal aromatase (*cyp19a1a*).

3.3 Differentially expressed genes related to sex

At the all-fins stage, of the 8 genes investigated by qPCR only *spry1* (p-value = 0.01) and *mtor* (p-value < 0.001) presented a significant and positive correlation with *sox3* abundance in the trunk, indicating that future males have relatively more transcript abundance of both genes than future females (Figure 5). Interestingly, this pattern was conserved for *spry1* at the juvenile stages with more transcripts in male gonads and liver (t-test, p-value < 0.05) compared to females (Figure 5).

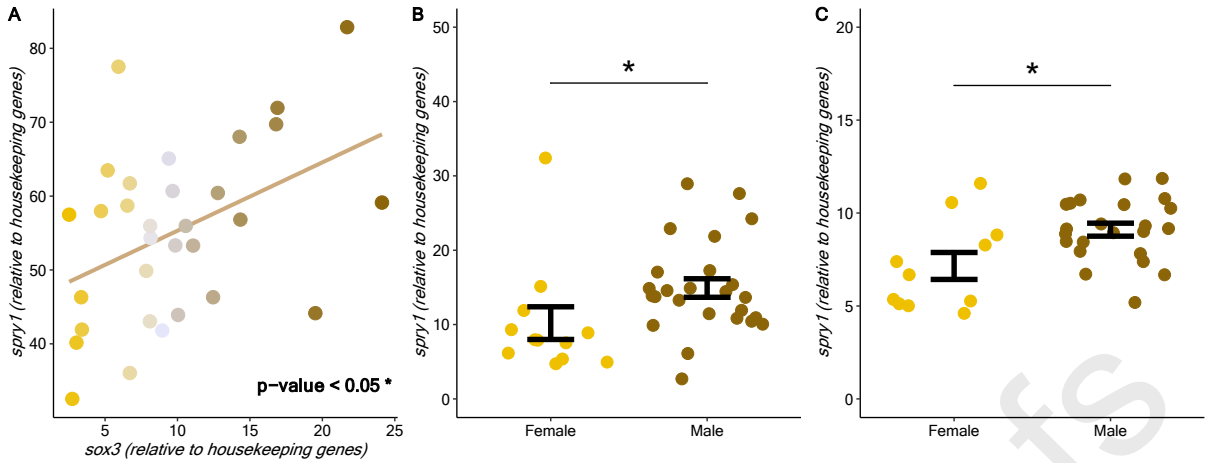

Figure 5A) Linear correlation between the relative expression of *spry1* and *sox3* at the all-fins stage. B) Gene expression of *spry1* in the gonads and the liver (C) of phenotypic females (yellow dots) and phenotypic males (brown dots) at juvenile stage. Note that the sex is based on gene expression assessed in the Figure 4. Each bar represents the mean ± se. Abbreviations: * = p-value < 0.05; sprouty 1 (*spry1*).

3.4 Differentially expressed genes related to fluctuating temperature

At the all-fins stage, only *nucb1* (ANOVA, p-value < 0.001) and *ghrelin* (ANOVA, p-value < 0.05) were differentially expressed in the trunk as a function of temperature, with more transcripts of both genes in cold compared to warm regime. This pattern was conserved in the head for *nucb1* (ANOVA, p-value < 0.01) (Figure 6A). At the juvenile stage, the same result was detected for *nucb1* in gonad (ANOVA, p-value < 0.01) and liver (ANOVA, p-value < 0.001) (Figure 6B). Gene expression of *mtor* in the liver was also higher in the cold compared to the warm regime (ANOVA , p-value < 0.01). There were no significant differences according to temperature for the other 6 genes tested.

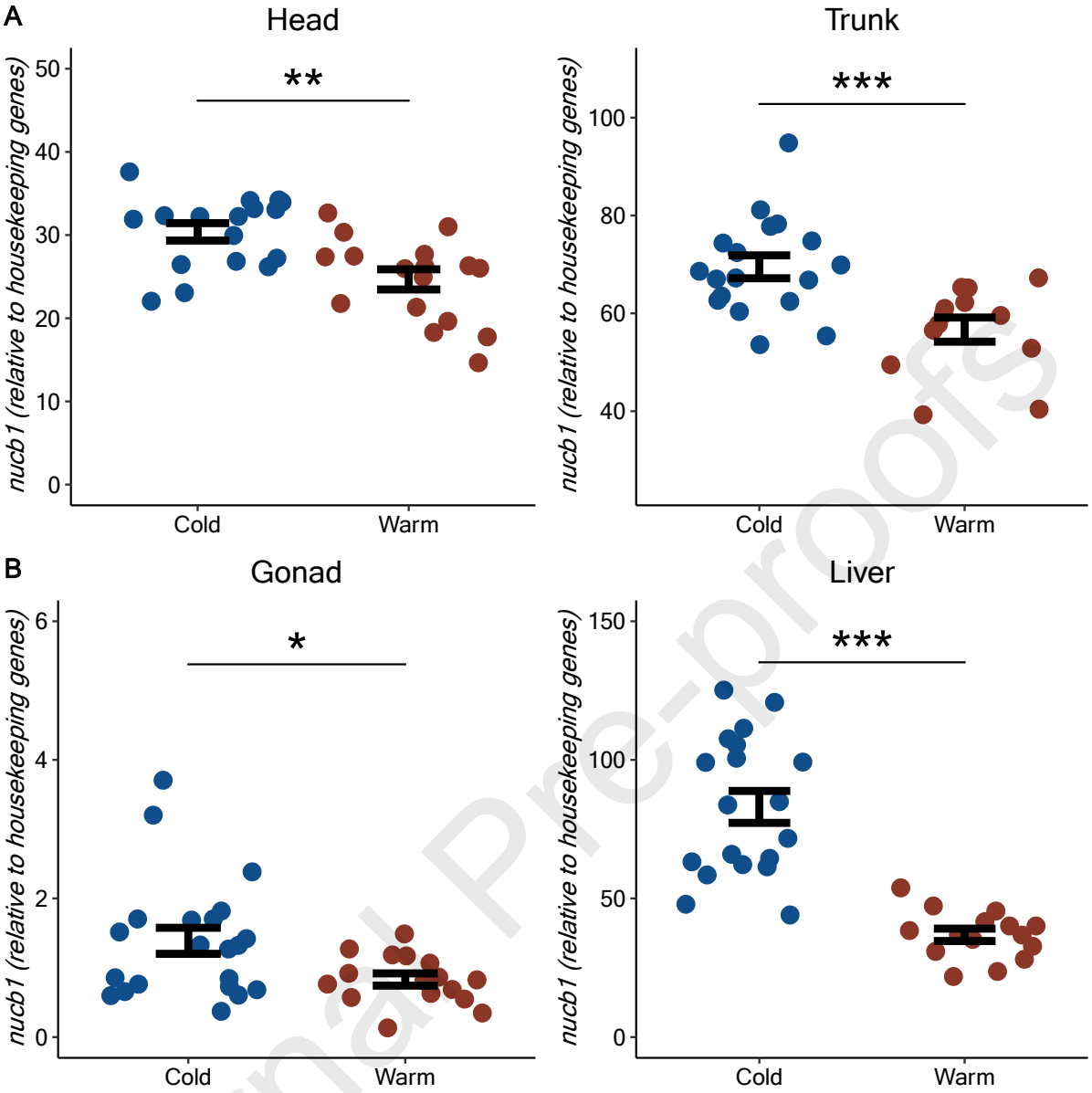

Figure 6A) Relative expression of *nucb1* as a function of water temperature in the head and trunk of European sea bass larvae at the all-fins stage, and B) in the gonads and liver of fish at juvenile stage from the Experiment 2. Cold regime (blue dots) and warm regime (red dots). Each bar represents the mean ± se. Abbreviations: *** = p-value < 0.001; ** = p-value < 0.01; * = p-value < 0.05; nucleobindin 1 (*nucb1*).

3.5 *Interaction between sex and temperature*

At the all-fins stage, only *pank1a* in the trunk presented a significant interaction between sex (*sox3*) and temperature regime (p-value < 0.05; Figure 7A). In the cold thermal regime, *pank1a* was increased in future males, while it decreased in the warm thermal regime. The exact same pattern was found in the liver of juveniles (p-value < 0.05, Figure 7B).

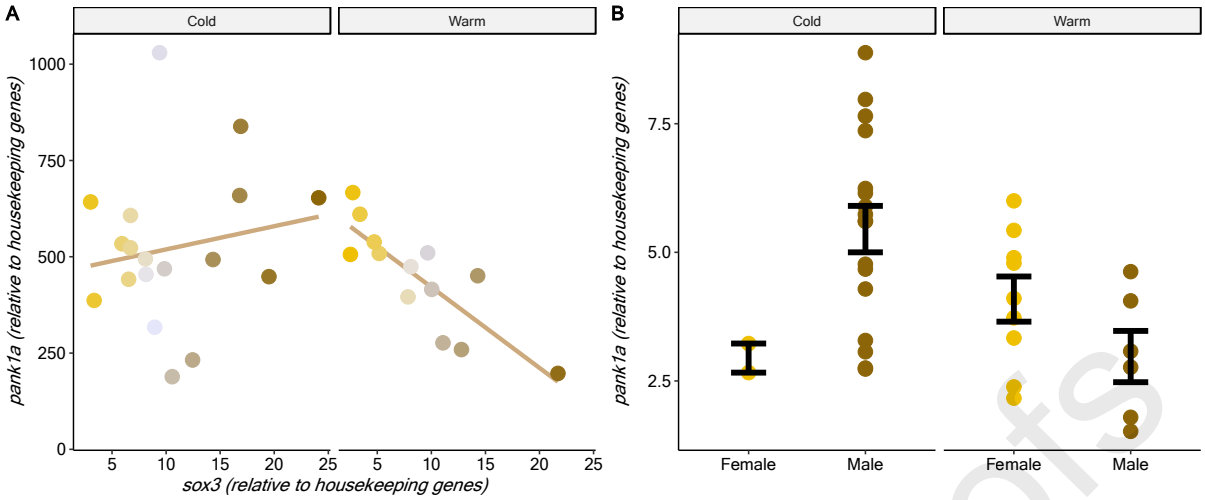

Figure 7A) Linear correlation between the relative expression of *pank1a* and *sox3* of fish at the all-fins stage at the two temperature in the Experiment 2. B) Gene expression of *pank1a* in the liver (B) of phenotypic females (yellow dots) and males (brown dots) at juvenile stage. Each bar represents the mean ± se. In both A and B) there is a significant interaction between Sex and Temperature. Sry-related HMG box3 (*sox3*); pantothenate kinase 1a (*pank1a*).

4 Discussion

The present study employed two methodologies—RNA-seq and qPCR—in conjunction with two experimental setups to elucidate the impact of water temperature on sex-specific gene expression related to energy allocation in the European sea bass. These genes are associated with anorexigenic and orexigenic functions, as well as transcription factors regulating energy homeostasis, glucose and lipid metabolism during the sex determination and differentiation periods.

In the European sea bass, sex-dimorphic growth in favor of females becomes apparent during larval development, and significantly separates males from females as soon as 103 dph, corresponding to the end of the sex determination period (Faggion et al., 2021). We demonstrated that at the all-fins stage (84-117 dph, ~28.7 mm, ~480 $DD_{10°C}$), *sox3* transcript abundance, measured by qPCR in the trunk, is up-regulated in lighter individuals (*i.e.* likely future males), confirming the previous transcriptomic study showing that *sox3* is an early marker of masculinization in the European sea bass (Geffroy et al. 2021a). Molecular sex differentiation at the juvenile stage (157-212 dph, ~82.0 mm, ~1300 $DD_{10°C}$) was determined by quantifying the RNA relative abundance of *cyp19a1a* and *gsdf* in the gonads, allowing us to sex individuals successfully, as previously reported for this species (Vandeputte et al., 2020). At this stage too, the females were significantly heavier and longer than males.

Interestingly, for fish collected at the juvenile stage in Experiment 2, the warm treatment favored feminization (10 females *vs* 6 males) whereas the cold treatment favored masculinization (19 males *vs* 2 females). We have previously demonstrated two distinct thermosensitive periods in European sea bass development, whereby temperature increase between 80 and 100 dph favors feminization but prolonged exposure (up to 244 days post-hatching) to cold water promotes masculinization (Clota et al., 2024; Vandeputte et al., 2020). In the present experiment 2 that follows natural thermal regimes, the temperature of the warm

regime was relatively low (< 18°C) before 78 dph and then increased, reaching 22°C at 107 dph. According to our previous work (Clota et al., 2024; Vandeputte et al., 2020); this temperature pattern is the best to favor female production (early cold + late warm, Figure 8). Whereas prolonged exposure to low temperatures in the cold regime treatment (< 17°C before 140 dph) is expected to favor masculinization (Figure 8). Since there was no exposure to warm temperatures early in development in either treatment in Experiment 2, differential methylation might not be at play, an effect previously observed (Geffroy et al., 2021a). It could rather be an effect of temperature on growth rate, where the warm regime promotes energy accumulation and ultimately favors female differentiation, while the cold regime negatively affects energy storage favoring male differentiation.

At the molecular level, the RNA-Seq re-analysis of fish kept at constant warm or cold temperatures (Experiment 1) revealed energy allocation transcripts that are dependent upon sex but independent of rearing temperature during the sex determination period. We found higher levels of *nucb1* and *pomc1* in future females compared to males; previous studies reported that nesfatin-1-like peptide (NLP), the mature peptide of *nucb1*, stimulates POMC synthesis in murine cell culture (Nasri and Unniappan, 2021) suggesting a link between these two genes. In the Mozambique tilapia, *Oreochromis mossambicus, pomc1* is upregulated in females' brain compared to males early in development, and *in vitro* analysis demonstrated that β-estradiol significantly enhances its expression (Wan et al. 2021), concordant with our results. However, since *pomc1*$^{-/-}$ mutants of zebrafish (*Danio rerio)* exhibit higher growth and enhanced appetite (Shi et al., 2020; Wan et al., 2021; Yang et al., 2023), we would have expected lower levels of *pomc1* in females than males, as reported in *Pogona vitticeps* after male to female sex-reversion (Deveson et al., 2017). Levels of *ampk1* in the trunk were unaffected by the sex tendency of fish, but upregulated in ovaries undergoing molecular sex differentiation.

On the other hand, differential expression of *egfra* and *mtor* in future males (in trunk) and testis undergoing molecular sex differentiation respectively, suggests a sex dimorphic role for energy allocation during male sex determination. Even though it is hard to elucidate the *ampk1*/*egfra/mtor* roles because of their multifunctionality during early development (Lee, 2015; Orofiamma et al., 2022), sex dimorphic patterns for mTORC1 and AMPK was previously reported in nematodes (Robles et al., 2021) and EGFR is involved in the control of glucose uptake and glycolysis (Orofiamma et al., 2022). Overall, the genetic background appeared to affect *egfra*, *nucb1,* and *pomc1* in a sex dependent manner, independently of environmental effects, while *ampk1, ghrelin, mtor*, and *pank1a* were influenced by the temperature and, later, differentially expressed according to sex in the gonad (except for *ghrelin*). Only *spry1* was actually influenced by both temperature (more in the early warm treatment) and genetic sex tendency (more in future males) in Experiment 1, and differential expression level was maintained in testis undergoing sex differentiation. These last genes (*ampk1, mtor, pank1,* and *spry1*) are, therefore, the most interesting to study to understand the role they play during the second thermal sensitivity window, where exposure to warm temperatures (i.e. after 80 dph) promotes feminization (Figure 8), probably by enhancing growth.

To gain further understanding of energy allocation patterns in the gonads during sex determination and sex differentiation, we focused on the 8 genes detected in Experiment 1. The qPCR results obtained in a totally different experimental design (Experiment 2) provided specific insights into the effect of late increased temperatures on sex. First, *ghrelin* and *nucb1* were always down-regulated by warm temperatures, independently of the sex of individuals, while only *nucb1* was positively correlated with female genetic sex tendency in Experiment 1. This highlights that late exposure to warm temperatures might not affect the phenotypic sex of

individuals through modulated expression of *nucb1*. Second, the qPCR results of Experiment 2 confirmed the RNA-seq results from the Experiment 1 for *spry1*, indicating a significant increase in *spry1* relative expression at the all-fins stage for future males, but also in the gonads and livers of males undergoing molecular sex differentiation at the juvenile stage. While the link between *spry1* function and sex differentiation in teleost is currently unknown, in mammals, *dmrt1* positively regulates the expression of *spry1* (Zhang et al., 2023). Other reported functions for *spry1* are related to reduced accumulation of body fat and adipogenesis (Mandl et al., 2019; Urs et al., 2012); regulation of early gonadal white adipose tissue growth (Yang et al., 2021); epithelial morphogenesis (Koledova et al., 2016); and inhibition of angiogenesis (Sabatel et al., 2010). All these processes are key for early gonadal development (Devlin and Nagahama, 2002; Sharma et al., 2023). Regarding the localization, SPRY1 is predicted to be found in the cytosol and is expressed in multiple developing organs during embryogenesis, including the brain and the pronephros in the zebrafish (Komisarczuk et al. 2008). The fact that *spry1* was upregulated by warm temperature in Experiment 1 only indicates that it is a real masculinizing factor that does not respond to the second thermal window (i.e. is not up-regulated by cold temperature in Experiment 2). This is essential for our understanding of how energy regulation by temperature can differentially affect sex and suggests that *spry1* acts by decreasing the growth potential of individuals.

Third, enhanced expression of *mtor* was detected in individuals of Experiment 2 undergoing male sex determination at the all-fin stage. Then, higher gene expression of *mtor* was detected in the liver of juveniles in the cold regime, which produced more males. Interestingly, the cold temperature favoring females in Experiment 1 led to enhanced expression of *mtor* in the whole body, independently of the genetic background of fish. Together, these results highlight that cold temperatures lead to increased expression of *mtor* and suggest that fish respond differently in terms of sex differentiation, according to the moment when the increased temperature was applied. In this framework, temperature would first affect *mtor* gene expression and energy balance and then the phenotypic sex.

Finally, we detected a significant interaction between sex and temperature for *pank1a* in both trunk (all-fins stage) and liver (juvenile stage) in Experiment 2. A previous study in medaka showed that *pank1a* was mainly expressed in the liver, and early starvation caused female to male sex reversal by inhibiting the pantothenate metabolic pathway, highlighted by the downregulation of *pank1a* (Sakae et al., 2020). Depletion of lipids then caused ectopic *dmrt1* expression in XX somatic cells, which resulted in genetic females (XX) to develop into a phenotypic males (Sakae et al., 2020). In the present study, in both trunk and liver, the relative expression of *pank1a* was significantly down-regulated in females at cold temperature, while it was up-regulated at warm temperature. The warm condition of Experiment 2 promoted feminization and possibly accumulation of energy in females, results that are in line with Sakae et al. (2020), where males showed lower levels of *pank1a* and, thus, possibly less fatty acid synthesis than females. This is also consistent with the RNA-seq results of Experiment 1, where individuals exposed to low temperatures (favoring feminization) presented about two times more transcripts of *pank1a* compared to individuals exposed to warm temperatures during sex determination. Here again, the temperature appeared to first affect energy balance of fish to then affect their phenotypic sex later, possibly through down-regulation of *dmrt1* as in the medaka. Indeed, based on *pank1a* gene expression, one might hypothesize that early exposure to cold and late exposure to warm would both favor fatty acid synthesis, and thus feminization independently of the genetic sex tendency of individuals. In the cold regime of Experiment 2 only two females were produced and it is more difficult to explain why males displayed higher levels of *pank1a*. One possible explanation could involve the necessity of producing CoA (the

end product of the panthothenate pathway), independently of the genetic sex tendency. In this framework, only those individuals with strong genetic female sex tendency would develop as phenotypic females, through other pathways than those related to energy accumulation. One limitation of such a reasoning - and that applies for all results presented here - is that gene expression does not necessarily reflect what happens at the protein level. For instance, we do not know the initial quantity of pantothenate and it is still possible that females displayed much more than males, so that even though they exhibited lower expression of *pank1a* in the cold condition (and possibly less PANK activity), they still produced more fatty acids than males.

In summary, in this species with polygenic sex determination, several genes with additive effects influence the phenotypic sex (Geffroy et al., 2021a). Among them, *nucb1, pomc1,* and *egfra* appeared to be essential players affecting energy balance from the onset of fish development. The present study also highlighted *spry1* as a novel, robust and consistent marker of masculinization, which can be boosted by warm temperature, thereby reinforcing this sexual state. Lastly, *pank1a* might also contribute to the sexual fate of the individual, but would only be affected by the temperature, not the genetic sex tendency. In this framework, *pank1a* would be differentially affected by the temperature in the first thermal sensitivity period, where cold temperature favor feminization, and the second thermal sensitivity period, where warm temperature favor feminization (Figure 8). Providing new data to understand how temperature modulates energy accumulation in a species for which sex is influenced by the environment is also of interest for other researchers in the field, as it opens new avenues for all species with environmental sex determination.

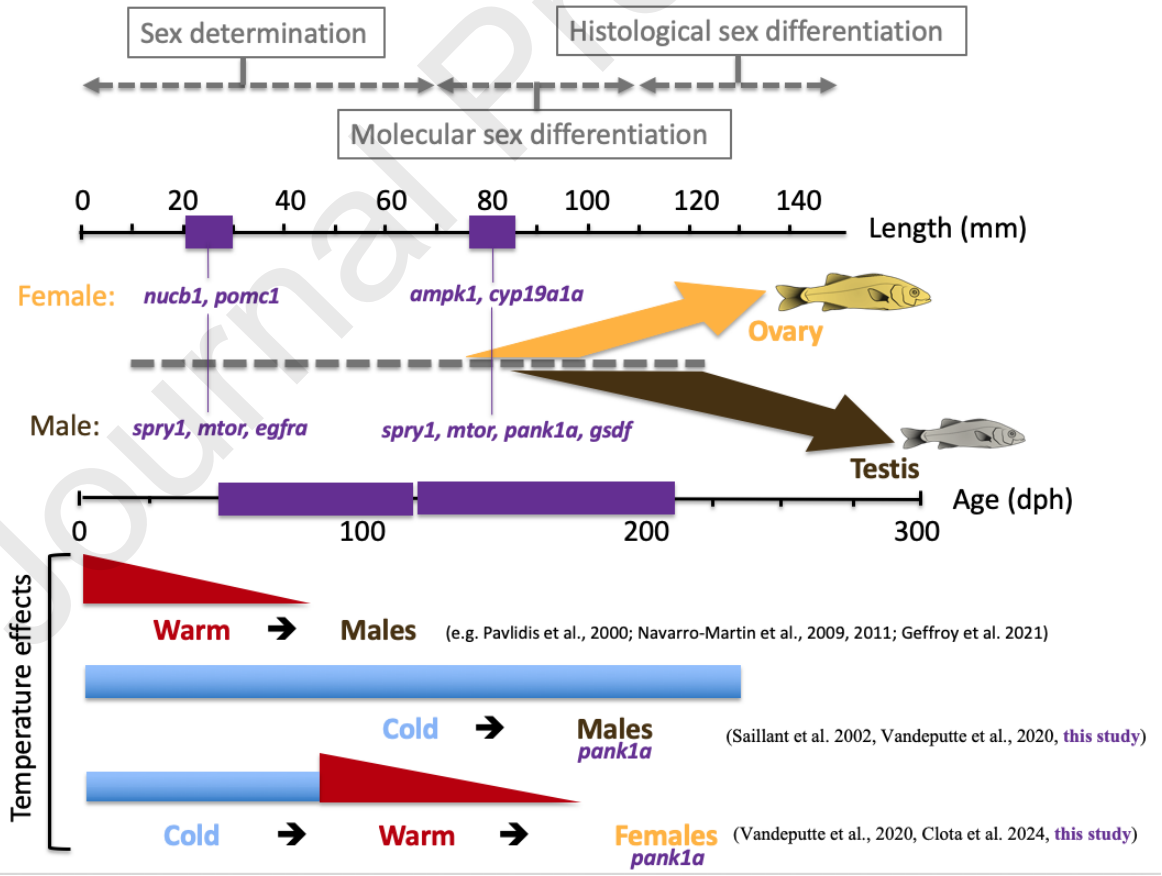

Figure 8. Scheme of the gonadal development of the European Sea bass, modified from (30). The sex determination, molecular sex differentiation and histological sex differentiation are

represented by dashed lines as their duration is influenced by environmental (mainly temperature) and experimental conditions. Molecular sex differentiation refers to the period at which gene expression of sex-related genes (e.g. *cyp19a1a*, *gsdf*) allows to discriminate phenotypic males from phenotypic females, while there are no evidences of histological sex differentiation in the gonad. Purple rectangles indicate the length range (in mm) and age (dph) at which the samples were taken in experiments 1 and 2. The different temperature treatments, having an effect on sex, are represented as well as the identification of the study where those findings were reported. Up-regulation of genes of interests in this study (in purple) are indicated for future females and future males at the timing of sampling. Note that up-regulation of *pank1a* is dependent on the interaction between sex and thermal treatment (see discussion for more details).

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Author contribution

Oswaldo Tovar- Bohórquez: Data curation (equal); Formal analysis (equal); Writing - original draft (equal); Writing - review & editing (equal). **David McKenzie:** Conceptualization (equal); Funding acquisition (equal); Project administration (equal); Writing - review & editing (supporting). **Damien Crestel:** Data curation (equal); Investigation (equal); Writing - review & editing (supporting). **Marc Vandeputte:** Conceptualization (equal); funding acquisition (supporting); Project administration (supporting); Writing - review & editing (supporting). **Benjamin Geffroy:** Conceptualization (supporting); Data curation (equal); Formal analysis (equal); Writing - original draft (equal); Writing - review & editing (equal).

5 Bibliography

Abram, P.K., Boivin, G., Moiroux, J., Brodeur, J., 2017. Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity. Biol. Rev. 92, 1859–1876. https://doi.org/10.1111/brv.12312

Birnie-Gauvin, K., Bordeleau, X., Cooke, S.J., Davidsen, J.G., Eldøy, S.H., Eliason, E.J., Moore, A., Aarestrup, K., 2021. Life-history strategies in salmonids: the role of physiology and its consequences. Biol. Rev. 96, 2304–2320. https://doi.org/10.1111/brv.12753

Blázquez, M., González, A., Papadaki, M., Mylonas, C., Piferrer, F., 2008. Sex-related changes in estrogen receptors and aromatase gene expression and enzymatic activity during early development and sex differentiation in the European sea bass (Dicentrarchus labrax). Gen. Comp. Endocrinol. 158, 95–101. https://doi.org/10.1016/j.ygcen.2008.06.001

Blázquez, M., Navarro-Martín, L., Piferrer, F., 2009. Expression profiles of sex differentiation-related genes during ontogenesis in the European sea bass acclimated to two different temperatures. J. Exp. Zoolog. B Mol. Dev. Evol. 312B, 686–700. https://doi.org/10.1002/jez.b.21286

Cerdá-Reverter, J.M., Agulleiro, M.J., R, R.G., Sánchez, E., Ceinos, R., Rotllant, J., 2011. Fish melanocortin system. Spec. Issue Melanocortins 660, 53–60. https://doi.org/10.1016/j.ejphar.2010.10.108

Challet, E., 2019. The circadian regulation of food intake. Nat. Rev. Endocrinol. 15, 393–405. https://doi.org/10.1038/s41574-019-0210-x

Chen, T., Wong, M.K.H., Chan, B.C.B., Wong, A.O.L., 2019. Mechanisms for Temperature Modulation of Feeding in Goldfish and Implications on Seasonal Changes in Feeding Behavior and Food Intake. Front. Endocrinol. 10, 133. https://doi.org/10.3389/fendo.2019.00133

Chezik, K.A., Lester, N.P., Venturelli, P.A., 2014. Fish growth and degree-days I: selecting a base temperature for a within-population study. Can. J. Fish. Aquat. Sci. 71, 47–55. https://doi.org/10.1139/cjfas-2013-0295

Clarke, A., 2003. Costs and consequences of evolutionary temperature adaptation. Trends Ecol. Evol. 18, 573–581. https://doi.org/10.1016/j.tree.2003.08.007

Clota, F., Goikoetxea, A., Vergnet, A., Blanc, M.-O., Lallement, S., Ruelle, F., Leitwein, M., Allal, F., Geffroy, B., Vandeputte, M., 2024. Post-larval exposure to warm temperature enhances female ratio, while starvation and photoperiod do not: The case of European seabass, Dicentrarchus labrax. Aquaculture 740758. https://doi.org/10.1016/j.aquaculture.2024.740758

De Jong, M.J., Alton, L.A., White, C.R., O'Bryan, M.K., Chapple, D.G., Wong, B.B.M., 2023. Long-term effects of incubation temperature on growth and thermal physiology in a small ectotherm. Philos. Trans. R. Soc. B Biol. Sci. 378, 20220137. https://doi.org/10.1098/rstb.2022.0137

Deveson, I.W., Holleley, C.E., Blackburn, J., Marshall Graves, J.A., Mattick, J.S., Waters, P.D., Georges, A., 2017. Differential intron retention in Jumonji chromatin modifier genes is implicated in reptile temperature-dependent sex determination. Sci. Adv. 3, e1700731. https://doi.org/10.1126/sciadv.1700731

Devlin, R.H., Nagahama, Y., 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Sex Determ. Sex Differ. Fish 208, 191–364. https://doi.org/10.1016/S0044-8486(02)00057-1

Díaz, N., Ribas, L., Piferrer, F., 2013. The relationship between growth and sex differentiation in the European sea bass (Dicentrarchus labrax). Aquaculture 408–409, 191–202. https://doi.org/10.1016/j.aquaculture.2013.05.032

Faggion, S., Vandeputte, M., Vergnet, A., Clota, F., Blanc, M.-O., Sanchez, P., Ruelle, F., Allal, F., 2021. Sex dimorphism in European sea bass (Dicentrarchus labrax L.): New insights into sex-related growth patterns during very early life stages. PLOS ONE 16, e0239791. https://doi.org/10.1371/journal.pone.0239791

Geffroy, B., 2022. Energy as the cornerstone of environmentally driven sex allocation. Trends Endocrinol. Metab. 33, 670–679. https://doi.org/10.1016/j.tem.2022.07.002

Geffroy, B., Besson, M., Sánchez-Baizán, N., Clota, F., Goikoetxea, A., Sadoul, B., Ruelle, F., Blanc, M.-O., Parrinello, H., Hermet, S., Blondeau-Bidet, E., Pratlong, M., Piferrer, F., Vandeputte, M., Allal, F., 2021a. Unraveling the genotype by environment interaction in a thermosensitive fish with a polygenic sex determination system. Proc. Natl. Acad. Sci. 118, e2112660118. https://doi.org/10.1073/pnas.2112660118

Geffroy, B., Douhard, M., 2019. The Adaptive Sex in Stressful Environments. Trends Ecol. Evol. 34, 628–640. https://doi.org/10.1016/j.tree.2019.02.012

Geffroy, B., Gesto, M., Clota, F., Aerts, J., Darias, M.J., Blanc, M.-O., Ruelle, F., Allal, F., Vandeputte, M., 2021b. Parental selection for growth and early-life low stocking density increase the female-to-male ratio in European sea bass. Sci. Rep. 11, 13620. https://doi.org/10.1038/s41598-021-93116-9

Geffroy, B., Wedekind, C., 2020. Effects of global warming on sex ratios in fishes. J. Fish Biol. 97, 596–606. https://doi.org/10.1111/jfb.14429

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of Size and Temperature on Metabolic Rate. Science 293, 2248–2251. https://doi.org/10.1126/science.1061967

Goikoetxea, A., Servili, A., Houdelet, C., Mouchel, O., Hermet, S., Clota, F., Aerts, J., Fernandino, J.I., Allal, F., Vandeputte, M., Blondeau-Bidet, E., Geffroy, B., 2022. Natural cortisol production is not linked to the sexual fate of European sea bass. Fish Physiol. Biochem. 48, 1117–1135. https://doi.org/10.1007/s10695-022-01104-1

Griot, R., Allal, F., Phocas, F., Brard-Fudulea, S., Morvezen, R., Bestin, A., Haffray, P., François, Y., Morin, T., Poncet, C., Vergnet, A., Cariou, S., Brunier, J., Bruant, J.-S., Peyrou, B., Gagnaire, P.-A., Vandeputte, M., 2021. Genome-wide association studies for resistance to viral nervous necrosis in three populations of European sea bass (Dicentrarchus labrax) using a novel 57k SNP array DlabChip. Aquaculture 530, 735930. https://doi.org/10.1016/j.aquaculture.2020.735930

Hardie, D.G., 2014. AMP-Activated Protein Kinase: Maintaining Energy Homeostasis at the Cellular and Whole-Body Levels. Annu. Rev. Nutr. 34, 31–55. https://doi.org/10.1146/annurev-nutr-071812-161148

Herpin, A., Schartl, M., 2015. Plasticity of gene-regulatory networks controlling sex determination: of masters, slaves, usual suspects, newcomers, and usurpators. EMBO Rep. 16, 1260–1274. https://doi.org/10.15252/embr.201540667

Hevrøy, E.M., Waagbø, R., Torstensen, B.E., Takle, H., Stubhaug, I., Jørgensen, S.M., Torgersen, T., Tvenning, L., Susort, S., Breck, O., Hansen, T., 2012. Ghrelin is involved in voluntary anorexia in Atlantic salmon raised at elevated sea temperatures. Gen. Comp. Endocrinol. 175, 118–134. https://doi.org/10.1016/j.ygcen.2011.10.007

Honeycutt, J.L., Deck, C.A., Miller, S.C., Severance, M.E., Atkins, E.B., Luckenbach, J.A., Buckel,

J.A., Daniels, H.V., Rice, J.A., Borski, R.J., Godwin, J., 2019. Warmer waters masculinize wild populations of a fish with temperature-dependent sex determination. Sci. Rep. 9, 6527. https://doi.org/10.1038/s41598-019-42944-x

Koledova, Z., Zhang, X., Streuli, C., Clarke, R.B., Klein, O.D., Werb, Z., Lu, P., 2016. SPRY1 regulates mammary epithelial morphogenesis by modulating EGFR-dependent stromal paracrine signaling and ECM remodeling. Proc. Natl. Acad. Sci. 113. https://doi.org/10.1073/pnas.1611532113

Komisarczuk, A.Z., Topp, S., Stigloher, C., Kapsimali, M., Bally-Cuif, L., Becker, T.S., 2008. Enhancer detection and developmental expression of zebrafish sprouty1, a member of the fgf8 synexpression group. Dev. Dynamics. 237, 2594-2603. https://doi.org/10.1002/dvdy.21689

Kua, Z.X., Hamilton, I.M., McLaughlin, A.L., Brodnik, R.M., Keitzer, S.C., Gilliland, J., Hoskins, E.A., Ludsin, S.A., 2020. Water warming increases aggression in a tropical fish. Sci. Rep. 10, 20107. https://doi.org/10.1038/s41598-020-76780-1

Kubrak, O., Koyama, T., Ahrentløv, N., Jensen, L., Malita, A., Naseem, M.T., Lassen, M., Nagy, S., Texada, M.J., Halberg, K.V., Rewitz, K., 2022. The gut hormone Allatostatin C/Somatostatin regulates food intake and metabolic homeostasis under nutrient stress. Nat. Commun. 13, 692. https://doi.org/10.1038/s41467-022-28268-x

Lee, D.Y., 2015. Roles of mTOR Signaling in Brain Development. Exp. Neurobiol. 24, 177–185. https://doi.org/10.5607/en.2015.24.3.177

Lindmark, M., Ohlberger, J., Gårdmark, A., 2022. Optimum growth temperature declines with body size within fish species. Glob. Change Biol. 28, 2259–2271. https://doi.org/10.1111/gcb.16067

Lu, H.-L., Li, L., Miao, Y.-L., Liang, H., Zou, J.-M., You, J.-J., Liang, X.-F., He, S., 2023. Effects and regulatory pathway of proopinmelanocortin on feeding habit domestication in mandarin fish. Gene 878, 147581. https://doi.org/10.1016/j.gene.2023.147581

Mandl, M., Wagner, S.A., Hatzmann, F.M., Mitterberger-Vogt, M.C., Zwierzina, M.E., Mattesich, M., Zwerschke, W., 2019. Sprouty1 is a weight-loss target gene in human adipose stem/progenitor cells that is mandatory for the initiation of adipogenesis. Cell Death Dis. 10, 411. https://doi.org/10.1038/s41419-019-1657-3

Martins, L., Fernández-Mallo, D., Novelle, M.G., Vázquez, M.J., Tena-Sempere, M., Nogueiras, R., López, M., Diéguez, C., 2012. Hypothalamic mTOR Signaling Mediates the Orexigenic Action of Ghrelin. PLOS ONE 7, e46923. https://doi.org/10.1371/journal.pone.0046923

Miyoshi, K., Hattori, R.S., Strüssmann, C.A., Yokota, M., Yamamoto, Y., 2020. Phenotypic/genotypic sex mismatches and temperature-dependent sex determination in a wild population of an Old World atherinid, the cobaltcap silverside *Hypoatherina tsurugae*. Mol. Ecol. 29, 2349–2358. https://doi.org/10.1111/mec.15490

Nasri, A., Unniappan, S., 2021. Nucleobindin-derived nesfatin-1 and nesfatin-1-like peptide stimulate pro-opiomelanocortin synthesis in murine AtT-20 corticotrophs through the cAMP/PKA/CREB signaling pathway. Mol. Cell. Endocrinol. 536, 111401. https://doi.org/10.1016/j.mce.2021.111401

Navarro-Martín, L., Blázquez, M., Viñas, J., Joly, S., Piferrer, F., 2009. Balancing the effects of rearing at low temperature during early development on sex ratios, growth and maturation in the European sea bass (Dicentrarchus labrax).: Limitations and opportunities for the production of highly female-biased stocks. Aquaculture 296, 347–358. https://doi.org/10.1016/j.aquaculture.2009.07.022

Navarro-Martín, L., Viñas, J., Ribas, L., Díaz, N., Gutiérrez, A., Di Croce, L., Piferrer, F., 2011. DNA Methylation of the Gonadal Aromatase (cyp19a) Promoter Is Involved in Temperature-Dependent Sex

Ratio Shifts in the European Sea Bass. PLOS Genet. 7. https://doi.org/10.1371/journal.pgen.1002447

Neuheimer, A.B., MacKenzie, B.R., Payne, M.R., 2018. Temperature-dependent adaptation allows fish to meet their food across their species' range. Sci. Adv. 4, eaar4349. https://doi.org/10.1126/sciadv.aar4349

Ogita, Y., Mawaribuchi, S., Nakasako, K., Tamura, K., Matsuda, M., Katsumura, T., Oota, H., Watanabe, G., Yoneda, S., Takamatsu, N., Ito, M., 2020. Parallel Evolution of Two dmrt1-Derived Genes, dmy and dm-W, for Vertebrate Sex Determination. iScience 23, 100757. https://doi.org/10.1016/j.isci.2019.100757

Orofiamma, L.A., Vural, D., Antonescu, C.N., 2022. Control of cell metabolism by the epidermal growth factor receptor. Biochim. Biophys. Acta BBA - Mol. Cell Res. 1869, 119359. https://doi.org/10.1016/j.bbamcr.2022.119359

Otero-Rodiño, C., Rocha, A., Sánchez, E., Álvarez-Otero, R., Soengas, J.L., Cerdá-Reverter, J.M., 2019. Sensing Glucose in the Central Melanocortin Circuits of Rainbow Trout: A Morphological Study. Front. Endocrinol. 10, 254. https://doi.org/10.3389/fendo.2019.00254

Pavlidis, M., Koumoundouros, G., Sterioti, A., Somarakis, S., Divanach, P., Kentouri, M., 2000. Evidence of temperature-dependent sex determination in the European sea bass (Dicentrarchus labrax L.). J. Exp. Zool. 287, 225–232. https://doi.org/10.1002/1097-010X(20000801)287:3<225::AID-JEZ4>3.0.CO;2-D

Polakof, S., Panserat, S., Craig, P.M., Martyres, D.J., Plagnes-Juan, E., Savari, S., Aris-Brosou, S., Moon, T.W., 2011. The Metabolic Consequences of Hepatic AMP-Kinase Phosphorylation in Rainbow Trout. PLOS ONE 6, e20228. https://doi.org/10.1371/journal.pone.0020228

Ramesh, N., Mohan, H., Unniappan, S., 2015. Nucleobindin-1 encodes a nesfatin-1-like peptide that stimulates insulin secretion. Gen. Comp. Endocrinol. 216, 182–189. https://doi.org/10.1016/j.ygcen.2015.04.011

Robles, P., Turner, A., Zuco, G., Adams, S., Paganopolou, P., Winton, M., Hill, B., Kache, V., Bateson, C., Pires-daSilva, A., 2021. Parental energy-sensing pathways control intergenerational offspring sex determination in the nematode Auanema freiburgensis. BMC Biol. 19, 102. https://doi.org/10.1186/s12915-021-01032-1

Sabatel, C., Cornet, A.M., Tabruyn, S.P., Malvaux, L., Castermans, K., Martial, J.A., Struman, I., 2010. Sprouty1, a new target of the angiostatic agent 16K prolactin, negatively regulates angiogenesis. Mol. Cancer 9, 231. https://doi.org/10.1186/1476-4598-9-231

Sadoul, B., Alfonso, S., Bessa, E., Bouchareb, A., Blondeau-Bidet, E., Clair, P., Chatain, B., Bégout, M.L., Geffroy, B., 2018. Enhanced brain expression of genes related to cell proliferation and neural differentiation is associated with cortisol receptor expression in fishes. Gen. Comp. Endocrinol. 267, 76–81. https://doi.org/10.1016/j.ygcen.2018.06.001

Sakae, Y., Oikawa, A., Sugiura, Y., Mita, M., Nakamura, S., Nishimura, T., Suematsu, M., Tanaka, M., 2020. Starvation causes female-to-male sex reversal through lipid metabolism in the teleost fish, medaka (*Olyzias latipes*). Biol. Open 9, bio050054. https://doi.org/10.1242/bio.050054

Sfakianakis, D., Papadakis, I., Papadaki, M., Sigelaki, I., Mylonas, C., 2013. Influence of rearing temperature during early life on sex differentiation, haemal lordosis and subsequent growth during the whole production cycle in European sea bass Dicentrarchus labrax. AQUACULTURE 412, 179–185. https://doi.org/10.1016/j.aquaculture.2013.07.033

Sharma, P., Purohit, S., Kothiyal, S., Bhattacharya, I., 2023. Germ cell development in teleost gonads.

Aquac. Fish. https://doi.org/10.1016/j.aaf.2023.07.002

Shi, C., Lu, Y., Zhai, G., Huang, J., Shang, G., Lou, Q., Li, D., Jin, X., He, J., Du, Z., Gui, J., Yin, Z., 2020. Hyperandrogenism in POMCa-deficient zebrafish enhances somatic growth without increasing adiposity. J. Mol. Cell Biol. 12, 291–304. https://doi.org/10.1093/jmcb/mjz053

Sundarrajan, L., Blanco, A.M., Bertucci, J.I., Ramesh, N., Canosa, L.F., Unniappan, S., 2016. Nesfatin-1-Like Peptide Encoded in Nucleobindin-1 in Goldfish is a Novel Anorexigen Modulated by Sex Steroids, Macronutrients and Daily Rhythm. Sci. Rep. 6, 28377. https://doi.org/10.1038/srep28377

Urs, S., Henderson, T., Le, P., Rosen, C.J., Liaw, L., 2012. Tissue-specific expression of Sprouty1 in mice protects against high-fat diet-induced fat accumulation, bone loss and metabolic dysfunction. Br. J. Nutr. 108, 1025–1033. https://doi.org/10.1017/S0007114511006209

Vandeputte, M., Clota, F., Sadoul, B., Blanc, M., Blondeau-Bidet, E., Bégout, M., Cousin, X., Geffroy, B., 2020. Low temperature has opposite effects on sex determination in a marine fish at the larval/postlarval and juvenile stages. Ecol. Evol. 10, 13825–13835. https://doi.org/10.1002/ece3.6972

Vandeputte, M., Dupont-Nivet, M., Chavanne, H., Chatain, B., 2007. A Polygenic Hypothesis for Sex Determination in the European Sea Bass *Dicentrarchus labrax*. Genetics 176, 1049–1057. https://doi.org/10.1534/genetics.107.072140

Vandeputte, M., Piferrer, F., 2019. Genetic and Environmental Components of Sex Determination in the European Sea Bass, SEX CONTROL IN AQUACULTURE, VOLS I AND II. https://doi.org/10.1002/9781119127291

Vandeputte, M., Piferrer, F., 2018. Genetic and Environmental Components of Sex Determination in the European Sea Bass, in: Wang, H.-P., Piferrer, F., Chen, S.-L., Shen, Z.-G. (Eds.), Sex Control in Aquaculture. John Wiley & Sons, Ltd, Chichester, UK, pp. 305–325. https://doi.org/10.1002/9781119127291.ch14

Volkoff, H., Rønnestad, I., 2020. Effects of temperature on feeding and digestive processes in fish. Temperature 7, 307–320. https://doi.org/10.1080/23328940.2020.1765950

Wan, Z.Y., Lin, V.C.L., Hua, Y.G., 2021. Pomc Plays an Important Role in Sexual Size Dimorphism in Tilapia. Mar. Biotechnol. 23, 201–214. https://doi.org/10.1007/s10126-020-10015-2

Woods, I.G., Schoppik, D., Shi, V.J., Zimmerman, S., Coleman, H.A., Greenwood, J., Soucy, E.R., Schier, A.F., 2014. Neuropeptidergic Signaling Partitions Arousal Behaviors in Zebrafish. J. Neurosci. 34, 3142–3160. https://doi.org/10.1523/JNEUROSCI.3529-13.2014

Wullschleger, S., Loewith, R., Hall, M.N., 2006. TOR Signaling in Growth and Metabolism. Cell 124, 471–484. https://doi.org/10.1016/j.cell.2006.01.016

Xia, J., Deng, C., Zheng, X., Huang, Y., Elvidge, C.K., Fu, S., 2023. Differential effects of parental and developmental temperatures on larval thermal adaptation in oviparous and viviparous model fish species. J. Therm. Biol. 117, 103695. https://doi.org/10.1016/j.jtherbio.2023.103695

Yang, X., Pande, S., Koza, R.A., Friesel, R., 2021. Sprouty1 regulates gonadal white adipose tissue growth through a PDGFRα/β-Akt pathway. Adipocyte 10, 574–586. https://doi.org/10.1080/21623945.2021.1987634

Yang, Z., Wong, J., Wang, L., Sun, F., Yue, G.H., 2023. pomc knockout increases growth in zebrafish. Aquaculture 574, 739707. https://doi.org/10.1016/j.aquaculture.2023.739707

Yin, Y., Li, Z., Gao, L., Li, Y., Zhao, J., Zhang, W., 2015. AMPK-dependent modulation of hepatic

lipid metabolism by nesfatin-1. Mol. Cell. Endocrinol. 417, 20–26. https://doi.org/10.1016/j.mce.2015.09.006

Zhang, -Meng-Fei, Wan, -Shi-Cheng, Chen, -Wen-Bo, Yang, -Dong-Hui, Liu, -Wen-Qing, Li, -Ba-Lun, Aierken, -Aili, Du, -Xiao-Min, Li, -Yun-Xiang, Wu, -Wen-Ping, Yang, -Xin-Chun, Wei, -Yu-Dong, Li, -Na, Peng, -Sha, Li, -Xue-Ling, Li, -Guang-Peng, Hua, -Jin-Lian, 2023. - Transcription factor *Dmrt1* triggers the SPRY1-NF-κB pathway to maintain testicular immune homeostasis and male fertility. - Zool. Res.

Zizioli, D., Tiso, N., Guglielmi, A., Saraceno, C., Busolin, G., Giuliani, R., Khatri, D., Monti, E., Borsani, G., Argenton, F., Finazzi, D., 2016. Knock-down of pantothenate kinase 2 severely affects the development of the nervous and vascular system in zebrafish, providing new insights into PKAN disease. Neurobiol. Dis. 85, 35–48. https://doi.org/10.1016/j.nbd.2015.10.010

Abbreviation list

ampk - AMP-activated protein kinase

CoA - Malonyl Coenzyme A

cyp19a1a - Gonadal aromatase

dmrt1 - Doublesex- and mab-3-related transcription factor 1

eef1α - Eukaryotic translation elongation factor 1 alpha

egfra - Epidermal growth factor receptor a

gsdf - Gonadal soma derived factor

l13 - Ribosomal protein L13

mtor - Mechanistic target of rapamycin

NLP - Nesfatin-1 like peptide

nucb1 - Nucleobindin protein 1

pank1a - Pantothenate kinase 1a

pomc1 - Pro-opiomelanocortin 1

SNP - Single Nucleotide Polymorphism

sox3 - Sry-related HMG box3

spry1 - Sprouty 1

Sry - Sex determining region Y

β-actin - Beta-actin

Highlights

- Energy allocation genes are involved in determining phenotypic sex of some animals.
- Temperature differentially modulates *ampk1, ghrelin, mtor,* and *nucb1*.
- Temperature and sex interaction occurs for the expression of *spry1* and *pank1a*.
- *Ampk1*, *mtor*, *pank1*, and *spry1* are involved in the female differentiation
- *Spry1* and *sox3* are early markers of masculinization in the European sea bass.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Geffroy reports financial support was provided by European Maritime and Fisheries Fund. McKenzie reports financial support was provided by French National Research Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.