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RESEARCH ARTICLE

Prepubertal gonad investment modulates thymus function: evidence in a teleost fish

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ABSTRACT

Thymus plasticity following gonadectomy or sex hormone replacement has long since exemplified sex hormone effects on the immune system in mammals and, to a lesser extent, in 'lower vertebrates', including amphibians and fish. Nevertheless, the underlying physiological significances as well as the ontogenetic establishment of this crosstalk remain largely unknown. Here, we used a teleost fish, the European sea bass, *Dicentrarchus labrax*, to investigate: (1) whether the regulation of thymus plasticity relies on resource trade-off with somatic growth and reproductive investment and (2) if the gonad–thymus interaction takes place during gonadal differentiation and development. Because gonadal development and, supposedly, thymus function in sea bass depend on environmental changes associated with the winter season, we evaluated thymus changes (*foxn1* expression, and thymocyte and T cell content) in juvenile *D. labrax* raised for 1 year under either constant or fluctuating photoperiod and temperature. Importantly, in both conditions, intensive gonadal development following sex differentiation coincided with a halt of thymus growth, while somatic growth continued. To the best of our knowledge, this is the first study showing that gonadal development during prepuberty regulates thymus plasticity. This finding may provide an explanation for the initiation of the thymus involution related to ageing in mammals. Comparing fixed and variable environmental conditions, our work also demonstrates that the extent of the effects on the thymus, which are related to reproduction, depend on ecophysiological conditions, rather than being directly related to sexual maturity and sex hormone levels.

KEY WORDS: 11-Ketotestosterone, 17β-Oestradiol, T-lymphocyte, Gonadal development, Life history theory, Thymic epithelial cell

INTRODUCTION

Jawed vertebrates have developed an adaptive immune system relying on B- and T-lymphocytes, which clonally express immunoglobulin-based receptors for antigen recognition (Boehm and Swann, 2014). T cells represent a key component of the adaptive immunity, because they coordinate the immune response by the secretion of a variety of pro- and anti-inflammatory cytokines. In jawed vertebrates, self-tolerant T cells differentiate within a specialized lymphoid organ – the thymus (Boehm and Swann, 2014). The thymus provides an evolutionarily conserved microenvironment for T cell maturation, which shares numerous features across various taxonomic groups, ranging from gene expression to functional anatomy. The morphofunctional specialization of the thymus enables T cell development, with immature T cells migrating through the cortex and the medulla to perform successive proliferation, selection and differentiation steps (Bajoghli et al., 2015; Bajoghli et al., 2019; Barraza et al., 2020; Bowden et al., 2005). This complex sequential process is performed in the thymic microenvironment formed by distinct stromal cells comprising heterogenous populations of thymic epithelial cells (TECs).

The thymus of all gnathostomes is characterised by an extremely high plasticity, compared with other immune organs. Various stimuli, such as stress or pregnancy, along with rising sex- or stress-related hormone levels, may induce transient (or 'acute') thymus atrophy, whereas the thymus undergoes chronic atrophy with ageing (Chaudhry et al., 2016; Hince et al., 2008; Honma and Tamura, 1984; Nakanishi, 1986; Shanley et al., 2009; Torroba and Zapata, 2003; Zapata et al., 1992). As for the latter, which is also referred to thymus involution, thymus organization changes considerably, including: (1) a reduction of the thymic parenchyma formed by the cortex and medulla (the site of thymopoiesis), as well as (2) an increase of thymic perivascular space and adipose tissue (Gruver et al., 2007; Rezzani et al., 2013). The reproduction-associated regulation of thymic function has been well studied in rodents, yet little is known about other vertebrates. Surgical or chemical ovariectomy or castration induce a transient thymus regrowth in vertebrates as varied as humans, rodents, cattle, rabbits, amphibians and lizards (Calder et al., 2011; Gubbels Bupp and Jorgensen, 2018; Hareramadas and Rai, 2006; Hince et al., 2008; Lutton and Callard, 2006; Martin et al., 2008; Moulton, 2018). The injection of sex hormones, such as oestrogens or androgens, on the other hand, triggers drastic thymic atrophy in rodents, associated with a deep qualitative and quantitative modulation of the intrathymic T cell maturation (Calder et al., 2011; Gubbels Bupp and Jorgensen, 2018; Hince et al., 2008; Moulton, 2018). Similar findings have been reported for fish, birds, reptiles and amphibians (Hareramadas and Rai, 2006; Lutton and Callard, 2006; Sufi et al., 1980; Zapata et al., 1992). In vertebrates, the variation of sex hormone levels relates to changes in the reproductive system. Accordingly, in various mammalian species, such as fish, reptiles, amphibians and birds, correlations between massive thymus atrophy and sexual maturation, breeding seasons and gestation have been described. However, the timing of the onset (before or after puberty) and the extent of thymus atrophy (partial or total) appear to be inconsistent between the different species (Cockburn, 1992; Hirakawa et al., 2018; Honma and Tamura, 1984; Kendall, 1980; Lutton and Callard, 2006; Nakanishi, 1986; Peel and Belov, 2017; Steinmann

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et al., 1985; Torroba and Zapata, 2003; Zapata et al., 1992). In humans and rodents, it is commonly accepted that after puberty, when sexual maturity is reached, high plasma sex hormone levels accelerate ageing-related thymus atrophy. Nevertheless, the driving factors triggering the first signs of thymus atrophy remain unknown to date. In fact, in humans, rodents and horses, the ageing of thymus occurs early in development (Aw and Palmer, 2012; Hince et al., 2008). Interestingly, Cockburn (1992) suggested that the timing and the extent of thymus atrophy appear to correlate with the different strategies in life-history across vertebrate classes (Cockburn, 1992). Cockburn (1992) observed that vertebrates that reproduce once (semelparous), such as eel, salmon, and marsupial species undergo a complete thymus involution, which commences before puberty. Although, it is clear that sex hormones represent an important regulator of thymus function in vertebrates, the mechanisms that regulate thymus plasticity remain to be identified (Aw and Palmer, 2012; Chaudhry et al., 2016).

Reproduction and immunity constitute two essential components for population and organism survival. Nevertheless, in the face of variable environmental constraints and limited energetic resources, the life history theory explains that living organisms need to optimize fundamental biological functions, such as growth, maintenance and reproduction. The immune system comprises the defence element of the maintenance program and, like reproduction, represents an energy-consuming element of fitness (Martin et al., 2008; Segner et al., 2017; Wang et al., 2019). This assumption has long since been postulated with respect to various trade-offs between the immune and the reproductive systems (extensively reviewed by Segner et al., 2017 and Martin et al., 2008). Accordingly, the link between energetic resources, metabolism and the immune system constitutes a novel field of research, coined ‘immunometabolism’ (Chapman et al., 2020; Jung et al., 2019). In fact, to support the intensive proliferation step necessary for an efficient adaptive immune response and naïve thymic T cell differentiation, memory T cells and their progenitors must increase their metabolism (Chapman et al., 2020; Jung et al., 2019). As a consequence, immunity and metabolism are tightly orchestrated at the organism level by complex communication networks, involving cytokines, metabolites and hormones (Lercher et al., 2020). Thymus function is tightly linked to the systemic metabolic status. In fact, nutrient supplementation or caloric restriction as well as metabolic disorders, such as diabetes and obesity can all impact thymic T cell maturation and thymus plasticity (Konarzewski and Książek, 2013; Pae et al., 2012; Rezzani et al., 2013). The specific costs of thymus development and maintenance remain, however, less well studied. It must be assumed that thymic T cell maturation and naïve self-tolerant T cell egress require various steps of intensive proliferation that are energetically costly. Indeed, to recognize self, more than 90% of the immature T cells are eliminated by positive selection, which is followed by negative selection in the thymus to discriminate non-self (Klein et al., 2014; Seddon and Yates, 2018).

Although in vertebrates the thymus is particularly sensitive to hormonal signalling from the reproductive system, the physiological requirements governing thymus growth, atrophy and maintenance as well as the ontogenetic establishment of this crosstalk remain to be experimentally investigated. We, therefore, aimed at examining whether: (1) changes in thymus plasticity are governed by both energy trade-off and environmental changes such as reproduction and growth and (2) reproductive differentiation, development and the associated rise of plasmatic sex hormones are correlated with thymus atrophy. In European sea bass *Dicentrarchus labrax* the reproductive development and associated endocrine changes as well as the thymus development and its oestrogenic regulation have been well described (Blázquez et al., 2008; Carrillo et al., 2009; Paiola et al., 2017, 2018; Romano et al., 2011). Despite some similarities in the oestrogenic regulation of thymus function with mammals, the effects of gonadal development and sexual maturation are mostly unknown (O’Neill, 1989; Paiola et al., 2017, 2018). We therefore investigated the kinetic changes of various thymic parameters in juvenile sea bass in relation to endogenous (reproductive axis, growth and energetic resources) and exogenous (photoperiod and temperature) factors that regulate the physiology of ectothermic organisms, including reproduction and immunity. In *D. labrax*, the seasonal reproductive investment initiates with the entry into the winter season, during which temperatures grow colder and the photoperiod is continuously shortening. Hence, in this study, sexually undifferentiated juvenile sea bass were raised – and analysed monthly – over 1 year under either fixed or variable photoperiod and temperature, the latter following the natural seasonal variations.

MATERIALS AND METHODS

Animals

Fingerling European sea bass *Dicentrarchus labrax* (Linnaeus 1758) at 90 days post hatch (dph) were obtained from the hatchery L’écloserie marine de Gravelines (Gravelines, France) in May 2018 and randomly allotted to two experimental setups. The two cohorts of fish were raised under these conditions over 12 months until May 2019, comprising both an entire summer and an entire winter season. 110 animals under fixed photoperiod and temperature were kept in 100 litre glass aquaria in a semi-static renewal system in climate chambers. This condition consisted of a constant temperature (19°C) and photoperiod (14 h:10 h light:dark) with weekly changes of 70% of the tanks filled with 100 litres of aerated artificial seawater (35‰, Tetra marine sea salt, Melle, Germany). The number of tanks was adjusted to seven tanks throughout the experiment to maintain a maximum fish density of 5 g l^{-1}. The water quality (nitrate/nitrite) of the semi-static condition was maintained using external filters (Pro 4+250 and Ecco Pro 200; Eheim GmbH & Co. KG, Deizisau, Germany) and a skimmer (skim 2.0 17 watts, Aquarium System, Roubaix, France). 130 animals were kept to the experimental setup referred to as ‘natural’ in two 1800 litre tanks inside the facilities of Aquacaux, a sea farm at Octeville-sur-Mer (France). This condition consisted of a continuous flow of filtered and aerated marine seawater supplied directly from the sea to the tanks at environmental temperatures and a photoperiod that followed the natural conditions present in the Normandy region of northern France. The animals of both conditions were fed daily *ad libitum* with Turbot label rouge fish pellets (Le Gouessant, Lamballe, France). All fish were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 2010/63/EU). Fish mortality occurred only during the first month of acclimation. It was not significantly different between the two experimental setups. The experimental protocol was approved by the French Ministry of Higher Education, Research and Innovation (agreement number: A7601606).

Sampling

A total of 15–17 fish from each experimental setup were randomly sampled at monthly intervals. At each sampling, the environmental parameters, including photoperiod and temperature were recorded. The fish were anesthetized with tricaine methanesulfonate (MS 222;

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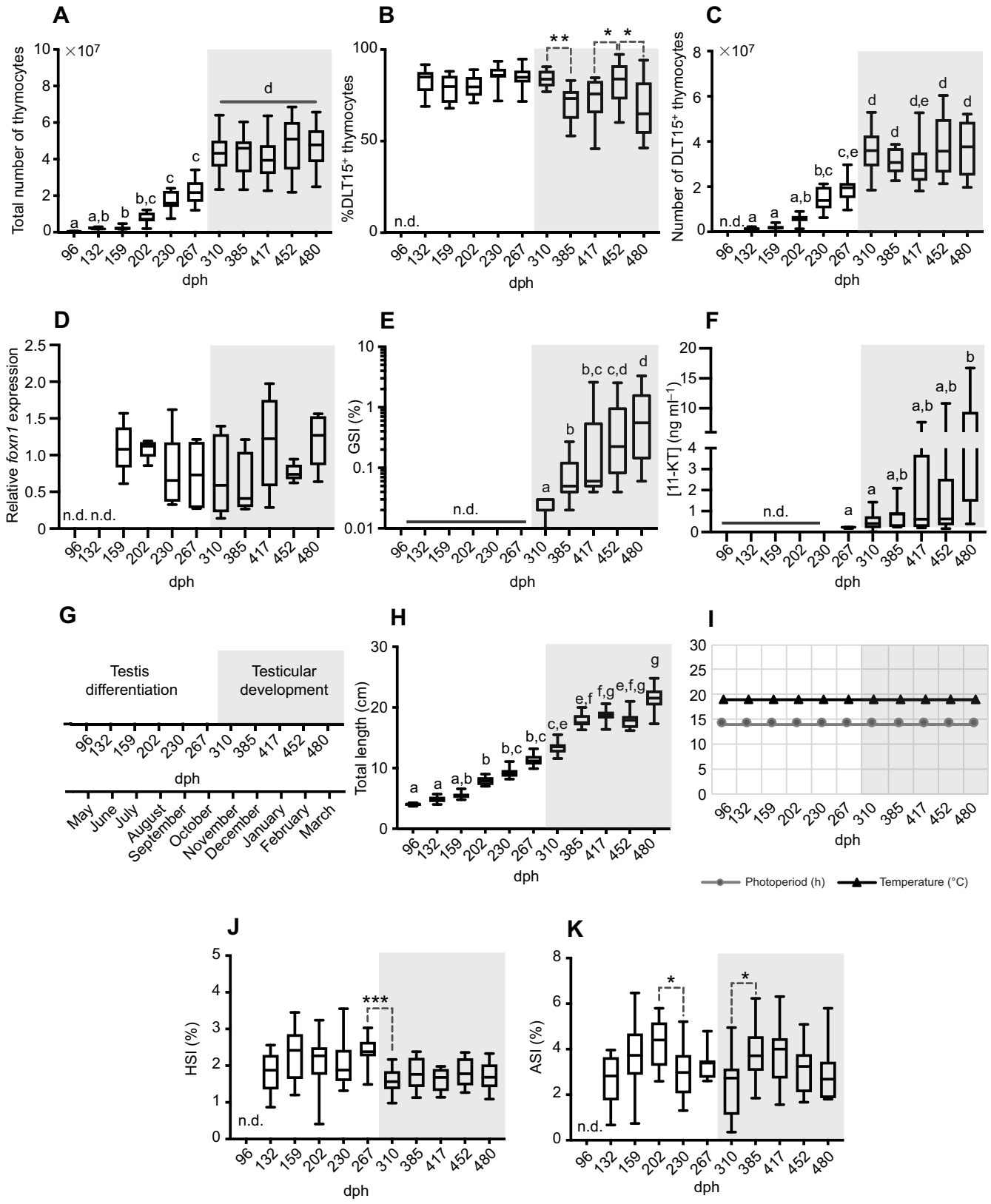

Fig. 1. Thymic changes in relation to the reproductive differentiation and development in male juvenile *Dicentrarchus labrax* over 1 year under fixed temperature and photoperiod. (A) Total number of thymocytes. (B) Proportion of DLT15$^+$ thymocytes (detected by a pan-T cell antibody specific for sea bass) relative to the total number of thymocytes. (C) Number DLT15$^+$ thymocytes. (D) Relative expression of *foxn1* [marker for thymic epithelial cells (TECs)]. (E) Gonadosomatic index (GSI). (F) Plasma 11-ketotestosterone (11-KT) concentration. (G) Testicular growth and increased plasma [11-KT] characterize the period of testicular development, i.e. the phase with more reproductive investment (highlighted in grey). (H) Total length. (I) Constant temperature (19°C) and photoperiod (14 h:10 h light:dark) on a monthly scale. (J,K) Somatic indices, including hepatosomatic (HSI) and adiposomatic (ASI) indices. Statistical differences between sampling are shown in A–K as lowercase letters or asterisks (*P<0.05, ***P<0.0001). n.d., not determined; dph, days post hatch.

Sigma-Aldrich) and, before being killed with an overdose of MS 222, blood was sampled from the caudal vein with a heparinised syringe. Biometric measurements, including total body mass and length, mass of the liver, gonads and visceral adipose tissue were recorded for each dissection. One of the two thymic lobules was used to isolate thymocytes for flow cytometric analyses, whereas the second lobe served for gene expression analyses. The plasma was snap frozen and stored at −80°C after elimination of the red blood cells by a centrifugation at 3000 ***g*** and 4°C for 10 min. Gender was determined by gonad histology using Hematoxylin–Eosin–Saffron staining. In the fixed photoperiod and temperature setup, the onset of puberty in male fish corresponding to 'precocious males' was identified when the fish released sperm after gentle pressure on the abdomen and when spermatozoids in the gonad sections became abundant (see representative histological section of the testes in Fig. 2G,H). The mass of the body, liver, gonads and visceral adipose tissue were used to establish the hepatosomatic (HSI), the gonadosomatic (GSI) and the adiposomatic (ASI) indices in % =organ mass (g)/body mass (g)×100.

Thymocyte isolation

After dissection, one of the thymic lobes was gently passed through a 100 µm cell strainer adding 10 ml of Leibovitz medium L15 without $NaHCO_3$ and L-glutamine (PAN BIOTECH, Aidenbach, Germany) adjusted with NaCl to 360 mOsm kg^{-1}. The cells were centrifuged at 400 ***g*** and 4°C for 10 min. The supernatant was discarded and the pellet resuspended and homogenised in isosmotic L15. To determine the total number of thymocytes, the cell suspension was incubated with propidium iodide (PI) at 50 µg ml^{-1} for 10 min in the darkness. Subsequently, thymocytes were counted by flow cytometry in 25 µl cell suspension using the gates selecting all PI-negative thymocyte populations and thus excluding the debris as well as necrotic/apoptotic cells. Thymocyte viability was higher than 98% at each sampling. All flow cytometric measurements were carried out with a NovoCytes™ (ACEA Biosciences Inc., San Diego, CA, USA) and analysed by NovoExpress® software (ACEA Biosciences Inc.).

Thymocyte immunostaining

The proportion of T cells amongst all thymocytes was assessed by immunostaining using a pan-T cell specific monoclonal antibody (Scapigliati et al., 1995). The protocol was adapted for use with 96-well plates with a V-shaped bottom (Nunc, Thermo Fisher Scientific). Briefly, after centrifugation at 400 ***g*** and 4°C for 5 min and elimination of the supernatant, 100,000 thymocytes were incubated with 15 µl DLT15 for 1 h on ice. Subsequently, the cells were washed twice with isosmotic phosphate-buffered saline (PBS) and incubated on ice with 0.05 µg secondary anti-mouse m-IgGκ BP-FITC antibody (Santa Cruz Biotechnology, Dallas, TX, USA) for 30 min in the darkness. The cells were washed again three times and the proportion of $DLT15^+$ thymocytes was assessed by flow cytometry. This parameter corresponds to the proportion of thymocytes with a high fluorescence intensity at λ=530 nm relative to the thymocytes of the same individual incubated without primary antibody. Based on the total thymocytes counts (PI^- cells) and the $DLT15^+$ cell proportions measured by immunostaining, the number of $DLT15^+$ cells could be estimated for each age.

Gene expression

Relative expression of forkhead-box n1 (*foxn1*), which is a major TEC marker was analysed relative to the expression of two reference genes: elongation factor α 1 (*ef1a*) and 40S ribosomal protein S30 (*fau*) in a single thymic lobe beginning at 155 and 159 dph for natural or fixed photoperiod and temperature setups, respectively as previously described (Paiola et al., 2018). *Ef1a* and *fau* were found to be stably expressed upon oestrogen treatment or reproductive cycle in sea bass in natural conditions (Paiola et al., 2018; Pinto et al., 2018). 155 and 159 dph were the ages when thymus growth provided enough thymocytes to use only one lobe for the immunostaining and the other one was available for gene expression. For each sampling, a minimum of five animals was analysed. After dissection, the thymic lobes were immersed in RNAlater® (Sigma-Aldrich) and stored at −20°C until RNA extraction. Following removal of the tissue from RNAlater® and embedding within TRI Reagent® (Sigma-Aldrich), thymic lobules were homogenised twice using Precellys® lysis kits (CK14; 1.4 mm ceramic, zirconium oxide beads in 2 ml standard tubes; Bertin instruments, Montigny-le-Bretonneux, France) for 10 s at 5000 rpm and subsequently centrifuged at 12,000 ***g*** for 15 min at 4°C. RNA was extracted with minor changes to the supplier's instructions. To improve RNA recovery and to help to visualise the pellet, the nucleic acid precipitation with isopropanol was done overnight at −80°C with 0.05 mg ml^{-1} of Glycoblue (Ambion, Austin, TX, USA) and 0.5 mol l^{-1} ammonium acetate. Potential DNA contamination was removed by digestion with the TURBO DNA-free Kit (Invitrogen-Ambion, Carlsbad, CA, USA) according to the supplier's instructions. RNA quality and quantity were assessed with a Nanodrop One (Thermo Fisher Scientific).

Reverse transcription and real-time RT-PCR was accomplished as previously detailed (Paiola et al., 2018). Briefly, quantitative RT-PCR was performed with two technical replicates per sample in 384-microwell plates loaded with 1 µl of diluted cDNA (1:20) and 2 µl of PCR mix (enzyme, dNTP, primers and LightCycler® 480 SYBR Green I Master mix from Roche Molecular Diagnostics, Pleasanton, CA, USA) using an Acoustic Automated Liquid Handler (Echo® 525, Labcyte™, San Jose, CA, USA). The qPCR conditions for the LightCycler® 480 II (Roche) were as follows: initial incubation at 95°C for 10 min followed by 45 cycles at 95°C for 10 s, 60°C for 10 s and 72°C for 10 s. Cycle thresholds (Ct) were determined with LightCycler 1.5 480. The PCR efficiency for each primer was assessed using serial dilutions of cDNA pooled from all samples. The relative expression ratio of *foxn1* was measured using the geometric mean of the two housekeeping genes and the efficiency of each primer pair based on the formula of Pfaffl (2001). Throughout the manuscript, the normalized expression of *foxn1* is referred as 'gene expression'.

Plasma sex hormone levels

17β-oestradiol (E2) and 11-ketotestosterone (11-KT) were measured in duplicate for each fish as previously described (Pinto et al., 2016). The E2 and 11-KT were analysed by radioimmunoassay using specific antiserum against E2 and 11-KT (Guerreiro et al., 2002; Kime and Manning, 1982) and dextran-coated charcoal to separate free hormones. For each sampling from 310 and 264 dph in fixed or natural photoperiod and temperature, respectively, six samples were analysed per sampling event and per group when possible (precocious and non-precocious males). At 237 and 267 dph, only three samples could be analysed because of the size of the fish and the low quantity of serum that could be obtained.

Statistical analysis

All statistical analyses were conducted using Prism (version 7.0a, GraphPad Software Inc., USA). Data were visualized as box-and-whisker plots, indicating the median, the 25th and 75th percentiles

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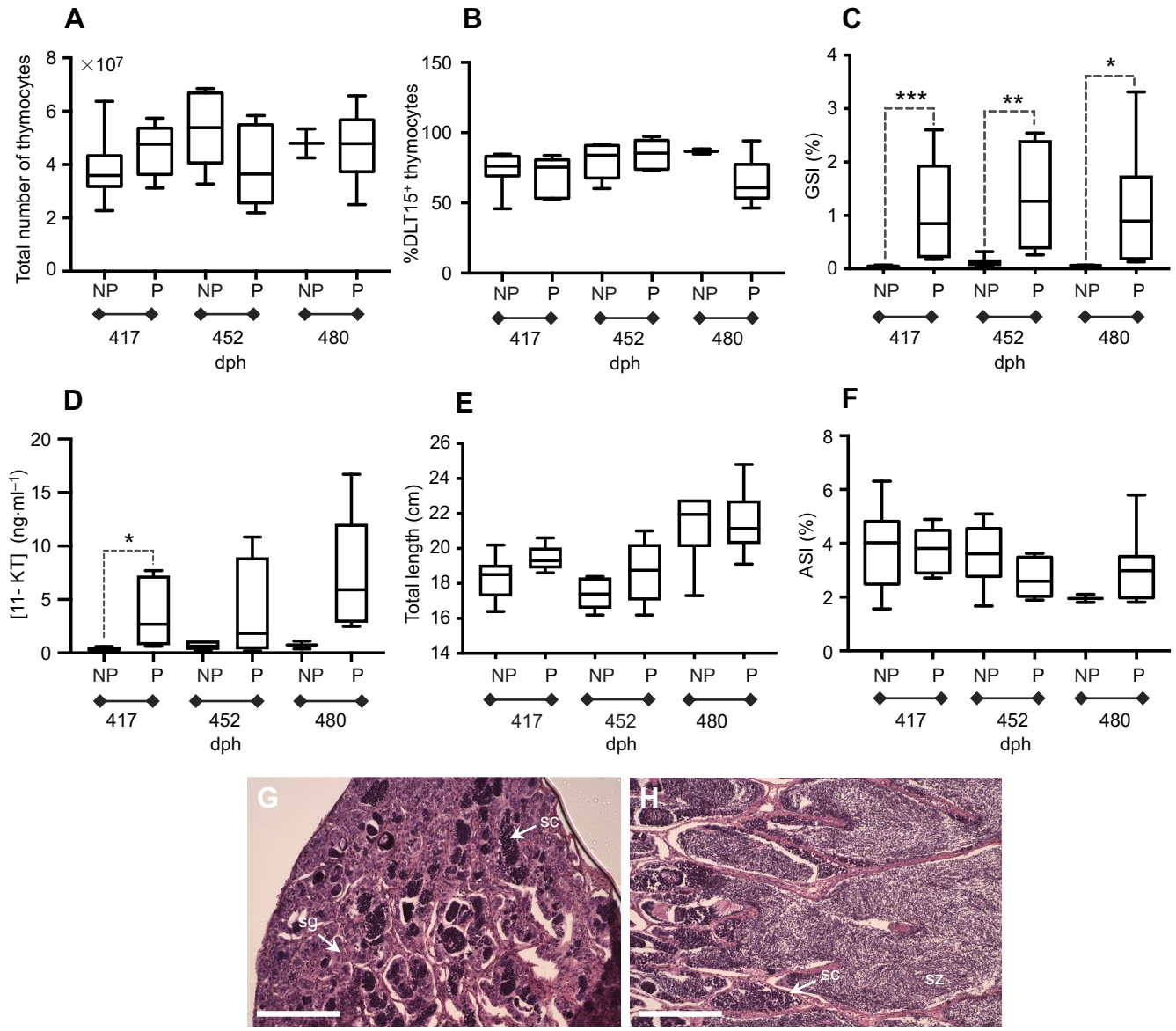

Fig. 2. Relationship between sexual maturation, thymic changes, somatic growth and energetic resources in male juvenile *D. labrax* raised under fixed temperature and photoperiod for 1 year. (A) Total number of thymocytes. (B) Proportion of DLT15$^+$ thymocytes (detected by a pan-T cell antibody specific for sea bass) relative to the number of thymocytes. (C) Gonadosomatic index (GSI). (D) Plasma level of 11-ketotestosterone (11-KT). (E) Total length. (F) Adiposomatic index (ASI). At 417, 452 and 480 dph, the sampled males were subdivided in two groups: non-precocious (NP) and precocious males (P), i.e. sexually immature and pubescent fish, respectively. Asterisks indicate statistical differences between precocious and non-precocious males for each sampled group (*P<0.05, **P<0.001, ***P<0.0001). Histological sections showing the testis of a non-precocious (G) and a precocious (H) male at 352 dph. sg, spermatogonia; sc, spermatocyte; sz, spermatozoa. Scale bars: 150 µm.

as well as the minimum and maximum values. Prior to these analyses, outliers were eliminated using the ROUT outlier test (Q=1%). The datasets were checked for normality using the Shapiro–Wilk test and for equal variances using the Brown–Forsythe test. Because normal distribution and homoscedasticity could not be confirmed in all groups, the non-parametric Kruskal–Wallis test (H-test) was used. The mean of each group was compared using the two-stage step-up method of Benjamini, Krieger and Yekutieli (*post hoc* test), which corrects for multiple comparisons by controlling the false discovery rate (FDR). The results were considered significant at an α-level of 5% (P<0.05). For comparison between precocious and non-precocious fish, the non-parametric Mann–Whitney U-test was also used.

RESULTS

Thymus changes in relation to gonad development under fixed environmental factors

The number of thymocytes was measured for each age and condition, and was used as an estimate of thymus size. In fact, thymocytes represent the principal thymic cell type, which outnumbers TECs (i.e. the stromal cells) as well as thymic B-cells and dendritic cells by two to three orders of magnitude (Hirakawa et al., 2018; Klein et al., 2014; Nagakubo et al., 2017). As a proxy for the evaluation of the thymus activity, i.e. thymic T cell differentiation or thymopoiesis, we measured the proportion and the number of DLT15$^+$ cells among the thymocytes as well as the relative expression of *foxn1*. DLT15 is a monoclonal antibody specific for pan-T cells of sea bass, staining T cells in early steps of

differentiation as well as mature T cells (dos Santos et al., 2000; Scapigliati et al., 1995). FOXN1 is a major transcription factor and a cell marker for TEC differentiation and function in vertebrates that can serve as an indicator of thymus activity, because it regulates thymopoiesis, thymus atrophy and regeneration (Bajoghli et al., 2009; Dumont-Lagacé et al., 2020).

The definition of puberty is a source of confusion. In teleosts, as in mammals, puberty can be defined as the final developmental stage of the reproductive system when fertility and secondary sex characteristics appear. In fish and mammals, puberty initiates when first functional gametes are spawned and ends when adult reproductive capacity is reached and physical changes are completed (Bell, 2018; Felip et al., 2006; Liu et al., 2019; Okuzawa, 2002). As an intermediate between juvenile and pubertal stages, the peripubertal or prepubertal stage characterizes the beginning of plasma sex-hormone rise and gonadal development after birth in mammals. This stage ends when the individual becomes capable of reproducing sexually, i.e. the onset of puberty (Bell, 2018; Bellvé et al., 1977; Okuzawa, 2002; Papadaki et al., 2005; Peters, 1969). In sea bass, prepubertal stage(s) corresponds to the year(s) when the brain–pituitary–gonad axis is incompletely activated, i.e. brain, pituitary and sex hormones rise without achievement of gonad maturation and spawning (Okuzawa, 2002; Papadaki et al., 2005). In sea bass, puberty has been poorly described. In Mediterranean aquaculture, puberty is generally reached at 2 and 3 years of age for males and females, respectively (Carrillo et al., 2009; Felip et al., 2006). In aquaculture culture conditions, as in the present work, an important fraction of the males can be 'precocious', becoming pubescent at 1 year of age (Felip et al., 2006). The fertility of pubescent males, which were precocious and non-precocious males, is similar to each other and lower than in 'adult' fish of more than 3 years of age (Felip et al., 2006). The end of puberty is difficult to define because the fecundity apparently increases with the size and the age of the fish and because there are no obvious secondary sex characteristics in this species (Mayer et al., 1990).

At fixed photoperiod and temperature, the total number of thymocytes in the thymus constantly increased to reach a plateau at 310 dph (Fig. 1A; *H*-test, $H_{10,157}$=135.1, P<0.0001; *post hoc* test, P<0.05). In contrast to the total number of thymocytes, the proportion of DLT15$^+$ thymocytes (Fig. 1B) remained constant from 96 to 310 dph, decreased significantly thereafter at 385 dph and significantly increased again at 452 dph (*H*-test, $H_{9,135}$=41.34, P<0.0001; *post hoc* test, P<0.05). Correspondingly, the total number of DLT15$^+$ thymocytes (Fig. 1C) significantly increased until 310 dph and then remained stable (*H*-test, $H_{9,128}$=106.8, P<0.0001; *post hoc* test, P<0.05). The expression of *foxn1* did not change significantly over the experimental period (Fig. 1D; *H*-test, $H_{8,128}$=12.57, P=0.1277; *post hoc* test, P<0.05). These results indicate a constant cell ratio between TECs and thymocytes because they are the major components of the thymus microenvironment.

In sea bass, the thymic rudiment and thymus regionalization in the cortex and medulla appear, respectively, at 25–27 dph and 50 dph, with a constant water temperature of 15–17°C (Picchietti et al., 2015; Romano et al., 2011). At 180 dph, thymus organisation is similar to the adult thymus, indicating that the thymus is functionally mature at this age and thus that the observed thymus changes are not related to thymus ontogenesis.

Considering the sex differentiation, sea bass becomes fixed after a labile period between 66 and 250 dph, depending on the size and the gender of the fish (Blázquez and Saillant, 2018; Piferrer et al., 2005). Accordingly, the gender of the fish could be determined without ambiguity by histological analysis from 267 dph onwards, indicating that gonadal differentiation was accomplished by that time. Under fixed experimental conditions, the juveniles developed a clearly male-biased sex ratio (88% males on average throughout the experiment) leading to the sampling of two female per sampling on average. Because the low and inconsistent number of females per time point did not allow for statistical evaluation (e.g. no females were sampled at 417 dph), these individuals were excluded from further analyses. In *D. labrax*, gonadal sex determination is under polygenetic as well as environmental control (Blázquez and Saillant, 2018; Piferrer et al., 2005). This explains the development of different and highly biased sex ratios in our two experimental conditions, i.e. fixed or natural. The GSI could only be calculated from 310 dph onwards, when it was possible to weigh the gonads for the first time (Fig. 1E). In the following ages, a period of high testicle growth/development commenced (Fig. 1G), characterised by a constant and significant increase of the GSI from 310 dph until the end of the experiment at 480 dph (*H*-test, $H_{4,69}$=40.39, P<0.0001; *post hoc* test, P<0.05). The plasma level of 11-KT started increasing from 267 dph to 480 dph in two phases (Fig. 1F; $H_{5,42}$=16.62, P=0.0053). In fact, at 310 and 385 dph, [11-KT] tended to increase in comparison to levels at 267 dph, although without significant statistical differences, which could be due to the low number of samples that could be analysed at 267 dph (n=3; P=0.2763, 267 versus 310 dph and P=0.0696, 267 *versus* 385 dph). Thereafter, [11-KT] significantly increased at 480 dph (P=0.0053, 310 versus 480 dph). During the entire experiment from 96 dph to 480 dph, the total body length of the fish significantly increased (*H*-test, $H_{10,161}$=153.7, P<0.0001; *post hoc* test, P<0.05) indicating constant growth, but with a reduction of growth at 417 and 452 dph (Fig. 1H). The HSI significantly decreased from 310 dph onwards (Fig. 1J; *H*-test, $H_{9,145}$=31.21, P<0.0001; *post hoc* test, P<0.05). This decrease was observed together with the increase in GSI, the plateau in the number of both thymocytes and DLT15$^+$ thymocytes as well as the fluctuating proportions of DLT15$^+$ thymocytes until the end of the observation period and is highlighted as 'testicular development' in grey in Fig. 1G. Eventually, the ASI showed few significant changes over the entire experimental period (Fig. 1K), with two phases of slight increase, from 132 to 202 dph as well as from 310 to 385 dph, each followed by decreases of the ASI. This was, however, supported only by one significant decrease at 230 dph and one significant increase at 385 dph (*H*-test, $H_{9,145}$=30.6, P=0.0003; *post hoc* test, P<0.05).

In the fixed photoperiod and temperature setup, a significant proportion of pubescent animals (i.e. precocious males, because male sea bass puberty normally occurs in the second reproductive season) was identified at each sampling: 33% at 417 dph, 42% at 452 dph and 86% at 480 dph (see representative histological sections of the testes in Fig. 2G,H). Only one precocious fish was sampled at 385 dph and, therefore, was not included in the analyses. The total number of thymocytes, the proportion of DLT15$^+$ thymocytes (Fig. 2A,B) as well as their number (Fig. S1A) did not differ between the precocious and non-precocious males. Correspondingly, *foxn1* expression was not significantly different between the two groups of males (Fig. S1B). The precocious fish, however, had a significantly higher GSI compared with sexually immature males (*U*-test, P=0.0008 at 417 dph, P=0.0013 at 452 dph and P=0.0220 at 480 dph). Considering the 11-KT levels, precocious fish had a significantly higher level of 11KT at 417 dph in comparison to the non-precocious fish (*U*-test, P=0.0159 Fig. 2D) and no statistical significance obtained for the other time points. Precocity also had no effect on total length, HSI or ASI (Fig. 1E and Fig. S1).

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Thymic changes in relation to gonad development under varying temperature and photoperiod

In fish growing under natural photoperiod and temperature, the total number of thymocytes significantly and constantly increased to reach an approximately constant level between 204 dph and 431 dph (Fig. 3A; *H*-test, $H_{11,167}$=130.6, P<0.0001; *post hoc* test, P<0.05). Subsequently, the number of thymocytes further increased significantly at 459 dph and 487 dph (P=0.0252 and P=0.0047, respectively, versus 431 dph). The proportion of DLT15$^+$ thymocytes was stable over the first months from 96 dph to 264 dph and significantly decreased thereafter (Fig. 3B; *H*-test, $H_{10,150}$=105.8, P<0.0001). A slight, but significant decrease occurred at 313 dph when compared with the preceding month (*post hoc* test, P=0.0031 for 313 dph versus 264 dph) followed by a larger decrease at 350 dph (P=0.0002, 313 dph versus 350 dph), after which the proportion of DLT15$^+$ thymocytes tended to increase again at 400 dph, 431 dph and 459 dph in comparison with 350 dph (P=0.0720, P=0.1594 and P=0.0725, respectively). This increase became statistically significant at 487 dph compared with the lowest value at 350 dph (P=0.0108) but remained at a significantly lower level than that of the first 6 months, which corresponded to the summer period. Accordingly, the DLT15$^+$ thymocyte number increased until 204 dph (*H*-test, $H_{10,105.9}$=105.8, P<0.0001) and remained statistically unchanged until a significant decrease at 350 dph (P=0.0053, 313 dph versus 350 dph; Fig. 3C). At 400 and 431 dph, the number of DLT15$^+$ thymocytes tended to increase (P=0.2179, 350 dph versus 400 dph and P=0.2864, 400 dph versus 431 dph) to become significantly different at 459 dph (P=0.0048, 431 dph versus 459 dph). In agreement with the decrease of thymic T cell content and, therefore, the ratio change between TECs and thymocytes, *foxn1* expression (Fig. 3D, *H*-test, $H_{9,58}$=8.771, P<0.0001) was significantly increased at 313 dph (P=0.0201, 264 dph versus 313 dph) to reach a plateau at 350 dph (P=0.0001, 264 dph versus 350 dph). Subsequently, *foxn1* expression significantly decreased at 459 dph (P=0.0001, 431 dph versus 459 dph) to reach the basal level.

The gonads could be identified without ambiguity by histological analysis as either testis or ovaries from 237 dph onwards, indicating that the fish were sexually differentiated at this age. These conditions, which followed the natural variations (Fig. 3I), resulted in a clear female-biased sex ratio of the population (90% females in average across the experiment). The limited number of differentiated males was, therefore, excluded from further analyses. From 237 dph onwards, the mass of the gonad could be reported and the GSI calculated (Fig. 3E). The GSI slowly, but significantly increased until 400 dph (*H*-test, $H_{7,106}$=60.8, P<0.0001; *post hoc* test, P<0.05). The plasma [E2] significantly changed with a peak at 431 dph (Fig. 3F; *H*-test, $H_{7,44}$=12.63, P=0.0816; *post hoc* test, P=0.0016 400 dph versus 431 dph, P=0.058, 431 dph versus 459 dph and P=0.0022, 431 dph versus 487 dph). Concurrently, the hepatosomatic index, which is known to increase with plasma E2 in sea bass (Felip et al., 2001; Mañanós et al., 1994), increased significantly from 237 dph to 350 dph (Fig. 3J; *H*-test, $H_{8,119}$=59.01, P<0.0001), with 264 dph and 313 dph being significantly different from the preceding months, respectively (*post hoc* test, P=0.0302, 237 dph versus 264 dph and P=0.0293 313 dph versus 264 dph). This increase was followed by a sharp decrease at 400 dph (P<0.0001 in comparison with 350 dph). At 431 dph, the HSI increased, but without this difference being significant (P=0.1591, 400 dph versus 431 dph, P=0.2439 431 dph versus 459 dph) and subsequently stabilised at about the initial levels of 204 dph and 237 dph. The poor correlation between the changes in E2 and the HSI probably resulted from the low plasma sample size (n=3) that could be measured at 237 dph. During the entire observation period, the total length significantly increased (Fig. 3H; *H*-test, $H_{11,169}$=149.9, P<0.0001; *post hoc* test, P<0.05), indicating constant growth. Nevertheless, growth was much slower over the last half a year as compared to the first 6 months. The ASI significantly increased from 130 dph until 264 dph (*H*-test, $H_{10,153}$=58.97, P<0.0001; *post hoc* test, P<0.05), whereupon it underwent slight ups and downs, which, however, where not statistically significant, as both the quartile and the minimum to maximum ranges were quite scattered. As highlighted in grey (Fig. 3), the changes in the thymic parameters as well as the changes in the HSI and E2 level fell into the period of ovarian growth and the concurrent decrease in temperature and length of day from September until March.

DISCUSSION

Thymic changes are related to prepubertal gonadal growth

Measurements of thymocyte number, the proportion and total number of DLT15-positive thymic cells (i.e. immature and mature T cells) as well as the relative *foxn1* expression (TEC marker) were used as a proxy for functional and volume changes of the thymus. Essentially, our results show that in juvenile sea bass, the thymus initially grows along with somatic growth. Thymus growth appears to be halted when intensive testicular and ovarian growth and development begins with the prepubertal stage. In females in natural conditions, the thymus even showed a decrease of T cell content, indicative of thymus atrophy during ovarian development. Interestingly, in females, the pause in thymus growth and the decrease of T cell content coincided with an increase in the relative expression of *foxn1*. This suggests that thymic involution during reproductive investment is caused by a depletion of the thymocytes, rather than by a loss of TECs, i.e. the thymic microenvironment, as recently described in mice during pregnancy (Dumont-Lagacé et al., 2020). Under natural conditions, thymus growth restarted at the end of gonadal development. Consequently, this study shows that thymus atrophy in female sea bass occurs with the first gonadal development during proliferation of primary oocytes in prepuberty (Papadaki et al., 2005). In contrast, in female zebrafish, thymus atrophy occurs in puberty with no apparent reduction of thymus growth in prepuberty (Kernen et al., 2020). In medaka, the thymus growth is apparently reduced by puberty and the rise of sex hormones. Accordingly, thymus size dimorphism occurs after puberty and disappears with reproductive senescence (Ghoneum and Egami, 1982; Liu et al., 2019). In mice, the number of thymic cells and the associated T cell egress from the thymus peak 1 month after birth and decrease subsequently with prepubertal stage (Bell, 2018; Domínguez-Gerpe and Rey-Méndez, 2003; Gray et al., 2006; Gui et al., 2012). Accordingly, from 1 to 3 months of age, the thymocyte number decreases in both female and male mice, this decrease being more pronounced in males (Gui et al., 2012). Similarly, in horses, thymus atrophy begins at 6 months of age during the prepubertal stage (Aw and Palmer, 2012; Hess and Roser, 2004). The human thymus involutes rapidly after 1 year of age (the youngest age so far investigated; Steinmann et al., 1985), which indicates that in humans, minipuberty and the associated gonadal growth and transient rise of sex hormones can trigger thymus involution during the first 6 months of life (Bell, 2018; Lanciotti et al., 2018; Moreira-Filho et al., 2018). Sex differentiation in vertebrates starts with the hypothalamic production of gonadotropin-releasing hormone, which triggers the secretion of gonadotropins from the pituitary gland. The gonadotropins

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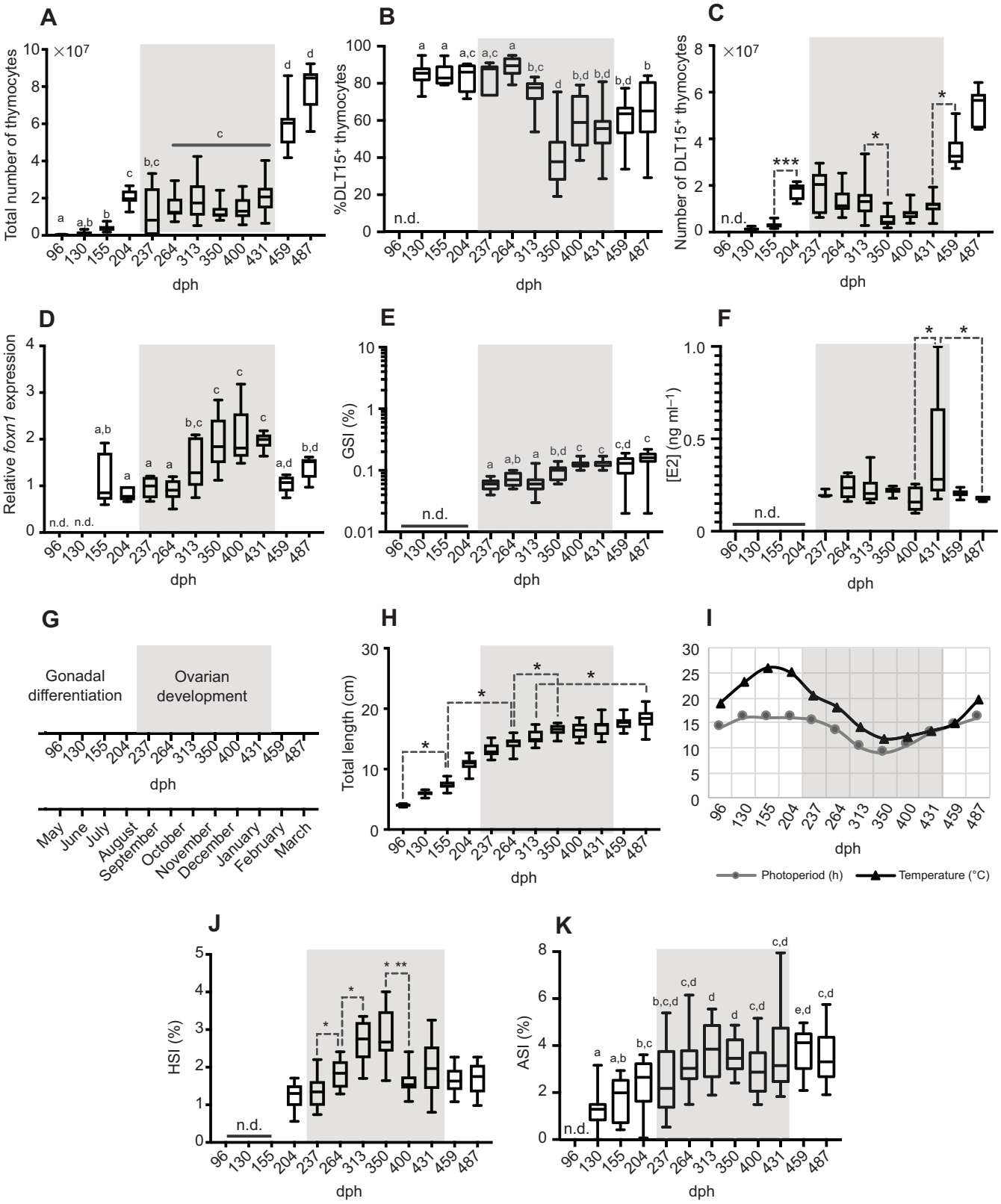

Fig. 3. Thymic changes in relation to the reproductive differentiation and development in female juvenile *D. labrax* over 1 year under natural environmental conditions. (A) Total number of thymocytes. (B) Proportion of DLT15$^+$ thymocytes (detected by a pan-T cell monoclonal antibody specific for sea bass) relative to the total number of thymocytes. (C) Number DLT15$^+$ thymocytes. (D) Relative expression of *foxn1* (marker for TECs). (E) Gonadosomatic index (GSI). (F) Plasma 17β-estradiol (E2) concentration. (G) Ovarian growth and increased plasma [E2] characterize the period of ovarian development, i.e. the phase with more reproductive investment (highlighted in grey). (H) Total length. (I) Natural temperature and photoperiod variability. (J,K) Somatic indices, including hepatosomatic (HSI) and adiposomatic (ASI) indices. A–E show statistical differences between time points (P<0.05) as lowercase letters; asterisks designate statistical differences between time points (*P<0.05, ***P<0.0001). n.d., not determined.

stimulate the gonadal production of oestradiol, which is necessary for ovarian development, but is also considered necessary for the testicular differentiation in numerous teleost species (Guiguen et al., 2010). In sea bass, aromatase expression and activity increased significantly in both males and females during gonadal differentiation (Blázquez et al., 2008). Subsequently, the oestrogen levels and gonadal aromatase activity decreased towards basal levels in the case of testicle formation, whereas high oestradiol levels and gonadal aromatase activity persisted and increased, respectively, during ovarian differentiation (Blázquez et al., 2008; Guiguen et al., 2010). In male sea bass, the sex differentiation is followed by a first period of spermatogenesis, which is characterised by a rise of both plasma and gonadal androgen (11-KT) levels (Papadaki et al., 2005). Consequently, our results suggest that prepubertal gonadal development and the associated rise in [11-KT] in males and [E2] in females negatively impacted thymus activity in sea bass, as they do in mammals after puberty (Gui et al., 2012; Hince et al., 2008). In mammals, oestrogen- and testosterone-mediated thymus atrophy results from a direct effect on TECs (Olsen et al., 2001; Staples et al., 1999). In European sea bass, TECs express oestrogen receptors and, *in vivo*, oestrogen modulates TEC-related gene markers similarly to what can be observed in mammals (Dragin et al., 2016; Paiola et al., 2017, 2018). Therefore, our study corroborates the hypothesis that E2 and 11-KT are evolutionarily conserved mediators linking the hypothalamic–pituitary–gonadal (HPG) axis to the thymus (Paiola et al., 2018; Segner et al., 2017).

Thymus plasticity and energetic trade-off with reproduction

Growth and reproduction represent competing, energy-consuming fitness traits, directing limited resources from somatic growth to reproduction (Hill and Elias, 2018; Taranger et al., 2010). In the present study, the coinciding reductions of somatic growth with mid and late gonadal development (i.e. 385 and 350 dph) in both fixed and natural conditions, therefore, are in line with the life history theory. Importantly, the reduction of thymic growth occurred together with early gonadal growth and, thus, preceded the reduction of somatic growth, i.e. 310 and 204 dph. This observation suggests that the reproduction-related changes in the thymus result from an energy trade-off with the reproductive system, as hypothesised previously (Aw and Palmer, 2012; Chaudhry et al., 2016; Cockburn, 1992; Quaglino et al., 2014). This hypothesis is further supported by a more pronounced thymus atrophy during gonadal growth in fish of the prepubertal stage following the natural changes of temperature and photoperiod, in spite of lower GSI and low sex hormone levels in comparison to the pubescent fish raised under fixed conditions. Gender differences may well account for the differences in thymus atrophy between the two conditions. In mice and humans as well as two other teleost species, the rate of thymus atrophy is, however, higher in males (Domínguez-Gerpe and Rey-Méndez, 2003; Ghoneum and Egami, 1982; Gui et al., 2012; Tamura et al., 1981). The differences between the two experimental conditions are, therefore, likely to result from effects of natural photoperiod and temperature cycles corresponding to the winter season, during which European sea bass naturally invest in gametogenesis. Under constant temperature and photoperiod, the thymus of precocious males did not differ significantly from non-precocious males despite the development of functional gonads and higher sex hormone levels, i.e. a higher amount of energy invested into spermatogenesis in comparison to the non-precocious males. In this artificial condition without major environmental changes, the investment in gonadal development and maturation did not drastically affect thymus plasticity. Hence, this situation radically differs from previous observations made in different ectotherms, including sea bass, kept under natural conditions (Hince et al., 2008; Honma and Tamura, 1984; Zapata et al., 1992; M. Paiola et al. unpublished observations). In response to lower temperatures, sea bass – like other teleost species – decrease their metabolism, their swimming activity and lower their food intake (Chen et al., 2019; Pastoreaud, 1991; Pérez-Ruzafa and Concepción, 2015). We observed that food consumption clearly decreased during the winter months, in spite of the fish receiving as much food as they could eat. Thus, the major reproductive investment, as characterized by the rise of sex hormones, is made when sea bass face a restricted caloric intake in winter conditions. For this reason, sea bass under natural conditions mobilise reserves from their visceral adipose tissue and negatively regulate their thymus function (M. P. et al., unpublished observations). Accordingly, in fish and mammals, reproductive investment has been associated with an important mobilization of visceral fat (McElroy and Wade, 1987; Sundararaj et al., 1982). In fact, in teleosts, as well as in mammals, reproduction depends considerably on energy storage (Hill and Elias, 2018; Taranger et al., 2010). Under naturally varying photoperiod and temperature, adipose tissue mobilization was not observed along with thymus atrophy in the present study, probably because of the modest reproductive investment. The absence of food limitation together with constant temperature probably stimulated both immune and reproductive systems, as shown by the mitigation of the trade-offs between reproduction and thymus, as well as the high proportion (86%) of the 1-year old juveniles that were at the pubertal stage by the end of the experiment. Likewise, high-fat diet increased thymocyte count and induced precocious puberty in rodents (Sánchez-Garrido et al., 2013; Trottier et al., 2012; Ullah et al., 2019). In lizards, reproduction and immunity were both stimulated when food was abundant and the physiological trade-off between reproduction and immune system was alleviated (French et al., 2007; Ruiz et al., 2010, 2011). Taken together, these observations provide evidence for reproduction-related thymus atrophy resulting from energy trade-offs between thymus and gonadal development, involving the HPG axis. The process is mediated by direct effects of gonadal hormones on the thymus (Hareramadas and Rai, 2006; Paiola et al., 2017, 2018, 2019; Staples et al., 1999), but because the degree of thymus atrophy did not correlate with the plasma sex hormone levels in *D. labrax*, other cues related to the metabolic state probably regulate the process and link it with the hormonal status.

The connection between the HPG axis and metabolism is mediated by: (1) a central and peripheral action of metabolic peptide hormones, such as leptin, growth hormone (GH) and insulin-like growth factor-1 (IGF-1), both in mammals and teleosts (Hatef and Unniappan, 2019; Hill and Elias, 2018; Manfredi-Lozano et al., 2018; Shahjahan et al., 2014) and (2) a central sensing of nutrients (glucose, fatty acids and amino acids) in mammals, which is likely to also exist in fish (Conde-Sieira and Soengas, 2017; Hill and Elias, 2018; Soengas et al., 2018). Leptin, GH and IGF-1 positively regulate both the reproduction and the immune system in mammals, including T cell differentiation and thymus plasticity (Hill and Elias, 2018; Naylor and Petri, 2016; Sánchez-Garrido et al., 2013; Savino et al., 2015). In addition to metabolic hormones, T cell proliferation and differentiation depend on cellular metabolic reprogramming, which requires extracellular nutrients (Chapman et al., 2020; Kedia-Mehta and Finlay, 2019). In non-mammalian species, and particularly in fish, knowledge about the effects of leptin, GH and IGF-1 and

nutrient sensing on reproduction and on immune cells is scarce, but some studies suggest that these mechanisms are evolutionarily conserved (Li et al., 2019; Procaccini et al., 2017; Verburg-van Kemenade et al., 2016). Accordingly, French et al. (2011) showed that leptin treatment partially reversed the negative effect of food restriction on follicle growth and cutaneous-wound healing in female lizards. Building upon this knowledge, we speculate that under favourable conditions the (neuro)endocrine system, probably including neuropetides like leptin, either: (1) stimulates the thymus function, thus compensating its downregulation by high sex hormones levels, or (2) fine tunes sex hormone signal transduction in thymic cells. This is corroborated by the observation that leptin injection alleviated the immunosuppressive action of testosterone in birds (Alonso-Alvarez et al., 2007).

Thymus plasticity and environmental factors

Our results provide strong evidence for the environmental parameters, such as temperature and photoperiod, to modulate the thymus plasticity as previously reported in tilapia and brown trout (Álvarez et al., 1998; Attia et al., 2010). In fact, under variable photoperiod and temperature, thymic growth halted whilst the fish invested into gonadal growth. Gametogenesis, however, also coincided with the seasonal decrease of temperature and with shorter photoperiods. Thymus growth recommenced at 459 dph, when temperature and photoperiod increased in spring. Similarly, Honma and Tamura (1984) observed that the thymus of 1-year-old medaka under natural light conditions showed either hyperplasia during the period of the year with the highest day length, or a transient atrophy during the rainy season when the fish were less exposed to light. When they maintained fish with a winter-atrophied thymus under either high temperature or long photoperiod as well as a combination of both, they observed the most pronounced thymic hyperplasia when both elevated temperature and long-day conditions were combined. More recently, Barraza et al. (2020) observed that 15 days of exposure of rainbow trout to extended photoperiod with 16 h of light and 8 h of darkness increased thymus weight and thymocyte number in comparison with photoperiod with 12 h of light and 12 h of darkness. Overall, this demonstrates that fish living in seasonally contrasted environments modulate thymic T cell maturation according to multiple external parameters. Because many animals, including fish, have their period of reproductive investment tightly aligned with changing photoperiod and temperatures (Carrillo et al., 2009; Taranger et al., 2010), our observations in sea bass support a tight interaction between reproductive investment, thymus function and environmental changes. In fact, in connection with their endogenous clocks, organisms tightly synchronize their physiology with the seasonal changes of their environment by sensing the biotic and abiotic factors called zeitgeber or synchronizers (del Pozo et al., 2014). In this context, various hormones including glucocorticoid, melatonin, GH and IGF-1 have been suggested to integrate the seasonal immune and reproductive changes and thus to mediate the energy trade-off between reproduction and immunity (Cowan et al., 2017; Martin et al., 2008). Cortisol, the major glucocorticoid in sea bass, is a powerful inducer of thymus atrophy in vertebrates. The large fluctuation in its levels in the plasma in summer and its generally low level in winter rather excludes an involvement of this stress hormone in the thymus plasticity observed in the present study (Honma and Tamura, 1984; Pascoli et al., 2011; Planas et al., 1990; Savino et al., 2015; Zapata et al., 1992). In European sea bass, the plasma levels of melatonin, GH and IGF-1 are known to be low in winter and high in summer, which suggests that the positive effect of melatonin, GH and IGF-1 on thymus size may be involved in the observed thymic changes (de Celis et al., 2004; Honma and Tamura, 1984; Janković et al., 1994; Ren et al., 2017; Vera and Migaud, 2014). Because secretion of melatonin, GH and IGF-1 is regulated by the temperature and photoperiod changes (Cowan et al., 2017; Vera and Migaud, 2014), these hormones are probably involved in the synchronization of both reproductive and thymus function with environmental changes.

Conclusions

This study on European sea bass shows that gonadal development impacts thymus growth and can trigger first signs of atrophy before puberty and sexual fertility. The latter may be already the case in the first year in sea bass held in aquaculture and is normally completed at 5–6 years of age in wild fish. Our study, therefore, may provide an argument for considering early postnatal gonadal development as a factor that initiates thymus ageing in humans during minipuberty and in mice and horses during prepuberty. Besides being an important developmental window for genital organ development, minipuberty is believed to be an essential period for somatic and cognitive development (Becker and Hesse, 2020; Lanciotti et al., 2018). This suggests that the effects of gonadal growth on the thymus, i.e. halt of thymus growth or even thymus atrophy, could stem from resource reallocation. Early gonadal development in both sea bass and mammals has been associated with a rise of plasma sex hormone concentrations. Our study therefore suggests that prepubertal rises in levels of sex hormones negatively regulate thymus plasticity and mediate this trade-off, albeit the levels being relatively low at that age compared with the pubertal level (Bell, 2018; Papadaki et al., 2005).

Interestingly, in sea bass, the extent of effects of gonadal development and sex hormones on the thymus is modulated by environmental factors including photoperiod and temperature. Under fixed photoperiod and temperature, higher temperatures probably allow the use of ample nutritional energy for reproductive investment, growth and thymus maintenance. This study, therefore, provides experimental evidence that helps to explain the physiological relevance of the evolutionarily conserved thymus atrophy in view of limited resources and energy trade-offs between immunity, growth and reproduction. Hence, it confirms the hypothesis of a link between energetic costs, sex steroid hormones and thymus atrophy (Aw and Palmer, 2012; Chaudhry et al., 2016; Cockburn, 1992; Kernen et al., 2020). This finding has importance beyond fish. Intensive research is being conducted to identify strategies to reverse thymus atrophy related to chemotherapy or ageing, but the underlying physiological function, and the mechanisms controlling thymus plasticity remain largely enigmatic (Aw and Palmer, 2012; Chaudhry et al., 2016). Importantly, among the various therapeutic strategies considered to regenerate the thymus and reverse the associated immunodeficiency, chemical and surgical sex steroid inhibition has been approved as a therapy in case of prostate cancer, for instance (Chaudhry et al., 2016; Kinsella and Dudakov, 2020). In this study, we have shown that under specific ecophysiological conditions, the negative effects of sex hormones on thymus plasticity can be alleviated. Similarly in humans *in utero*, when nutrients are provided by the placenta, the thymus grows and develops during the surge of plasma sex hormones related to the ‘first endocrine puberty’ (Becker and Hesse, 2020; Prabavathy, 2014). Together with recent important scientific progress in the field of the immunometabolism, these results suggest that the metabolic regulation of thymus plasticity at the cell and organism level represents a promising new field of investigation for thymus regeneration.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: M.P., T.M.; Methodology: M.P.; Software: M.P.; Validation: M.P.; Formal analysis: M.P.; Investigation: M.P., C.M., J.H., A.D., P.I.S.P.; Resources: G.S.; Data curation: M.P.; Writing - original draft: M.P.; Writing - review & editing: M.P., T.K., T.M.; Visualization: M.P.; Project administration: T.M.; Funding acquisition: T.M.

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Supplementary information
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