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Short- and long-term effects on growth and expression patterns in response to incubation temperatures in Senegalese sole

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ABSTRACT

In this study, the short- and long-term effects of embryo incubation temperatures (16, 18 and 20 °C) on development and growth of the flatfish Senegalese sole (*Solea senegalensis*) was determined by investigating the expression patterns of the epigenetic regulators DNA methyltransferases (dnmt) and histone 3 (H3) and genes belonging to the retinoic acid (RA), insulin-like growth factors (IGFs) and hypothalamic-pituitary-thyroid (HPT) axes. Results indicated that egg incubation temperature affected embryo development, but not survival, and incubation at 16 °C significantly delayed development. Coincident with these effects, levels of muscle-specific *dnmt3aa* transcripts and histone H3 protein levels were significantly different between the 16 and 20 °C groups at hatch. The larvae from eggs incubated at 20 °C relative to the 16 °C group had significantly higher transcript levels of four genes belonging to the HPT axis (*trhr1a*, *tshr*, *thrb* and *dio2*), four genes of the RA axis (*aldh1a2*, *cyp26a1*, *rara2*, *rarg*), *igfbp1* and the glycolytic enzyme *gapdh2*. Taken together the data suggest that higher egg incubation temperatures enhance energy production, which accelerates cell proliferation and larval development and that hatching is a key moment for the regulation of epigenetic mechanisms. Long-term effects of egg and larval incubation temperatures were revealed by a higher mRNA abundance of the thyroid-related genes *tgb* and *tpo* and the RA degrading enzyme *cyp26a1* in pre- and metamorphic larvae when they were incubated at 20 °C as embryos and may be related to the earlier initiation of metamorphosis in the pelagic larval stages. Evaluation of growth in pelagic larvae and juveniles after weaning (one trial from 42 to 119 and another from 164 to 247 days post-hatch using a longitudinal approach) revealed that juveniles from embryos incubated at 20 °C had a higher growth rate. All these data demonstrate that the thermal regime during embryogenesis modulated mechanisms that regulate larval plasticity and caused imprinting evident in juvenile sole as persistent changes in key endocrinological pathways and growth performance.

1. Introduction

Environmental temperature is a major factor that governs fish development and growth imposing severe changes in metabolism, physiology, behavior and morphology (Pittman et al., 2013). This notable response to environmental temperature is linked to the absence of thermal homeostasis (they are poikilotherms) and the specific evolutionary consequences for development are relatively poorly explored. Normally, early life stages develop faster at higher temperatures due to the modifications induced in molecular and metabolic responses (Campos et al., 2013c; Camus and Koutsikopoulos, 1984; Das et al., 2006; Martell et al., 2006; Politis et al., 2018; Radonic et al., 2005; Thépot and Jerry, 2015). However, when the environmental temperature is outside of the thermal tolerance range, survival rates decrease and the incidence of malformations substantially increases (Das et al., 2006; Little et al., 2013). In addition to the rapid cellular and metabolic responses to temperature, thermal regimes also modulate embryo and larval phenotype and have long-term effects (also known as developmental or transgenerational plasticity). This epigenetic programing of early life stages has been reported to strongly influence metabolic rates and acclimation capacity, sex determination and muscle development in juvenile fish (Pittman et al., 2013; Schnurr et al., 2014, 2011). This

Abbreviations: CDH, cumulative degree-hours; dnmt, DNA methyltransferases; dph, days post-hatch; EST, expressed sequence tag; H3, histone 3; HPT, Hypothalamic-pituitary-thyroid; IGFs, insulin growth factors; RA, retinoic acid

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may be accomplished by DNA methylation, histone modifications or chromatin remodeling (Kim and Kaang, 2017).

DNA methylation is controlled by the DNA methyltransferases Dnmt1 and 3 that are involved respectively in maintenance and de novo DNA methylation and regulate chromatin state transitions (Goll and Bestor, 2005). Moreover, chromatin structure and remodeling is highly dependent on post-translational modifications of histone proteins (mainly methylation and acetylation) that drive stable changes in gene expression patterns and result in different animal phenotypes (Kim et al., 2009; Kim and Kaang, 2017). The histone family has been highly conserved during evolution and there is a high level of redundancy in the genome (Cheung et al., 2000; Maehara et al., 2015; Okada et al., 2005; Ren and van Nocker, 2016). Histone H3 is an important target for epigenetic modifications that affect chromatin structure and remodeling processes. H3 isoforms include the replication dependent or canonical H3, the replication independent or replacement histones (H3.3) that are uncoupled from DNA replication and expressed throughout the cell cycle and the tissue- and centromere-specific forms (Akiyama et al., 2011; Ren and van Nocker, 2016).

One well studied reprogramming effect of temperature during early development of fish occurs in muscle and leads to modified fibre composition and growth patterns in adults (Johnston, 2006). A long-lasting influence of rearing temperature on muscle structure and somatic growth related the modifications induced in hypertrophy and hyperplasia of muscle fibres has been reported for several fish species (Alami-Durante et al., 2007; Albokhadaim et al., 2007; Johnston et al., 2009, 2003, 2000a; López-Albors et al., 2008; Macqueen et al., 2008; Steinbacher et al., 2011). However, temperature during early development can also influence other traits such as appetite and feeding behavior in juvenile fish (Albokhadaim et al., 2007) and sex differentiation, which causes skewed population ratios of sex in some species (Chen et al., 2014; Luckenbach et al., 2009; Navarro-Martín et al., 2011; Piferrer and Guiguen, 2008; Wen et al., 2014). Recently, the role of thermal imprinting during early development was shown in sea bream and was associated with a modified bone response to a cold challenge in juveniles, which was associated with modifications in the response of the thyroid, IGF-GH and cortisol axes (Mateus et al., 2017a). Additionally, in the sea bream and sea bass adult stress responsiveness was also significantly modified when eggs and larval fish were reared under different thermal regimes (Fokos et al., 2017; Mateus et al., 2017b). All these data indicate that the temperature regime during fish development modifies their developmental trajectory. To understand how early temperature regimes in hatchery stages affect juveniles and aquaculture productivity more studies are urgently needed.

Senegalese sole (*Solea senegalensis*) is an eurytherm flatfish that has optimal survival and growth rates over a wide thermal range (from 13 to 28 °C) in the wild (Vinagre et al., 2006). It is an increasingly popular aquaculture species in the Mediterranean due to its high commercial value and its prolonged reproductive season (in spring and autumn) since spawning can occur over a broad thermal range, 13 and 23 °C, although fecundity is highest between 15 and 21 °C (Anguís and Cañavate, 2005). This extremely wide thermal range for spawning means that embryonic and larvae development may occur under highly divergent environmental conditions with consequences for physiological traits. Campos et al. (2013c) demonstrated that larval growth, muscle phenotype and the expression pattern of myogenesis-related genes were different in post larval sole of larvae reared at 15 to 21 °C until hatch. Moreover, Campos et al. (2013b) demonstrated that metamorphic larvae reared at 15 °C had increased methylation of the *myog* promoter and its expression was lower in the skeletal muscle when compared to larvae grown at higher temperatures (18 and 21 °C). However, lower temperatures during the pelagic stage resulted in reduced larval survival at settlement but not at 100 dph (Campos et al., 2013a).

In addition to the effect of epigenetics on growth Blanco-Vives et al. (2011), demonstrated that daily thermocycles oscillating between 19 and 22 °C from hatch to 97 dph had a strong impact on the timing of gonad differentiation and sex ratios in sole populations. Moreover, incubation temperature was a key regulator of bone development and the incidence of skeletal deformities in sole juveniles (Dionísio et al., 2012). All these data demonstrate that in early developmental stages temperature can program key production traits in early developmental stages of sole and this makes the species an interesting model for understanding epigenetic mechanism behind thermally induced phenotypic plasticity and how this may impact on aquaculture performance. In this context, the present study aimed to: i) quantify the expression of DNA methyltransferases and histone H3 during embryogenesis and establish their regulation by temperature; ii) quantify the short- and long-term responses of genes modulating growth and metamorphosis, eg. HPT, GH-IGF and RA axes, in response to incubation temperatures; and iii) evaluate the long-term effects of incubation temperatures on somatic growth and metamorphosis. The knowledge generated in this study will provide new data of interest for the aquaculture industry since it will reveal how manipulation of temperature during embryonic development may benefit somatic growth in juveniles and adults.

2. Materials and methods

2.1. Fish trials

2.1.1. Embryo incubation trial and larval rearing

All procedures were authorized by the Bioethics and Animal Welfare Committee of IFAPA and given the registration number 06–11–15-337 by the National authorities for regulation of animal care and experimentation.

Fertilized eggs for Senegalese sole were obtained from CUPIMAR (San Fernando, Cadiz, Spain). Broodstock was fed daily with polychaeta, mussels and squid (~1% biomass). Eggs were collected early in the morning (9:00 a.m.) and transferred to a 1000 mL measuring cylinder to separate buoyant (viable) from non-buoyant (non-viable) eggs. The number of viable eggs in each fraction was estimated using a volumetric method (1100 eggs mL^{-1}). Water temperature and salinity in the broodstock tank (20 animals; ratio 2 M:1F) during spawning were 18 °C and 32 ppt, respectively. Fertilized embryos were collected and randomly distributed between nine cylindro-conical tanks (500 L) at a density of 140 eggs L^{-1} in an open seawater circuit supplied with gently aerated seawater. The temperatures selected to evaluate the effects of thermal reprogramming were based on the thermal range, 16–20 °C, tolerated for sole reproduction and used in the hatchery stage by the Mediterranean aquaculture industry. When embryos were at the beginning of gastrula (50% epiboly), the water temperature was shift progressively from 18 °C to the target incubation temperatures, 16 °C, 18 °C and 20 °C. The temperature change occurred over 1 h by mixing water at 20 °C (well water) and 13 °C (cooled using a water cooler carrier) and treatments were carried in triplicate tanks. During the experiment the temperature was continuously recorded with temperature data loggers (HOBO PENDANTs Onset Computer Corporation, Massachusetts) located in each of the experimental tanks. The average temperatures for each treatment group were 15.5 ± 0.9 ºC, 18.0 ± 0.3 ºC and 20.3 ± 0.2 ºC (Suppl. file 1). The embryos exposed to the 18 °C and 16 °C treatments were maintained at these water temperatures for 42 h and 52 h, respectively to ensure the change in thermal regime occurred at the same developmental stage in all temperature treatment groups. Thereafter, the water temperature in all experimental tanks was increased in approximately 1 h to the temperature normally used by industry (~20 °C) (Fig. 1).

To monitor embryo development, tanks were sampled every hour or every two hours after temperature treatments were initiated. The developmental stage of the embryos (10–20 embryos per sampling point) was recorded using a Nikon SMZ800 dissecting microscope connected to a Leica DC320 camera. At each sampling time point a pool of