Efeito a longo termo da temperatura do cultivo larvar no crescimento e fibras musculares de corvinas, *Argyrosomus regius*

Long-term effect of larval rearing water temperature on meagre, *Argyrosomus regius*, growth and muscle cellularity

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ABSTRACT

Water temperature can affect growth and fish development. In this study, two different water temperatures differing in 1.5 ºC were tested during meagre larval ontogeny. Once the 30 days period was over, fish were transferred to larger tanks, all at natural water temperature, where they were reared until they were 12 months old. Fish growth and muscle cellularity were analysed at 1, 2, 4, 6 and 12 months old. Results showed that 1 month old meagre under higher temperature had higher length and dry weight (11 mg *versus* 7 mg). Differences were also found for muscle fibre density, where 1 month old larvae from the control treatment showed higher fibre density (13493 mm^2 *versus* 5689 mm^2) and higher fibre recruitment. On the other hand, larvae from the high temperature treatment (HT) showed higher frequency of fibres with an area between 100 and 200 μm^2. Once all fish were kept under the same temperature, the differences in fish length and weight faded. The turnover point seemed to be at 4 months old when mean fibre area was higher in the control (704 μm^2 *versus* 540 μm^2). Fibre recruitment decreased throughout fish life cycle whereas fibre hypertrophy did not show a high variation until adulthood. This study shows that the use of different water temperatures during the larval phase can induce changes in fish weight and length as well as

affect muscle cellularity, namely fibre density and fibre recruitment. However, these differences were mitigated after fish were placed under the same water temperature.

Keywords: meagre, growth, muscle cellularity, fibre hypertrophy, fibre recruitment, temperature.

RESUMO
A temperatura de cultivo desempenha um papel fundamental no desenvolvimento dos peixes. Neste estudo foram testadas duas temperaturas durante a ontogenia larvar da corvina. Após este período, os alevins foram transferidos para tanques maiores, todos à temperatura ambiente, onde permaneceram até atingirem os 12 meses de idade. O crescimento e a área e densidade das fibras musculares foram analisados quando o peixe atingiu 1, 2, 4, 6 e 12 meses. Os resultados mostram que os alevins de 1 mês sujeitos a uma temperatura mais elevada (1.5º superior) apresentaram um maior comprimento, um maior peso seco (11 mg *versus* 7 mg) e uma maior frequência de fibras com uma área compreendida entre os 100 e os 200 µm^2. Por outro lado, os alevins sujeitos a uma temperatura inferior apresentaram maior densidade de fibras (13493 mm^2 *versus* 5689 mm^2) e um maior recrutamento de novas fibras. A partir do momento em que todos os alevins passaram a estar à mesma temperatura (ao fim de 30 dias), as diferenças foram-se desvanecendo. O ponto de viragem parece ter sido aos quatro meses de idade, quando a média da área das fibras era superior no controlo (704 µm^2 *versus* 540 µm^2). De um modo geral, o recrutamento das fibras musculares diminuiu com a idade do peixe enquanto a engrossamento das fibras permaneceu constante. Este estudo mostra que o uso de diferentes temperaturas durante a ontogenia larvar pode provocar diferenças no crescimento assim como afetar a área e densidade das fibras musculares. No entanto, estas diferenças parecem ser mitigadas quando os peixes passam a estar à mesma temperatura.

Palavras-chave: corvina, crescimento, fibras musculares, hipertrofia e recrutamento de fibras, temperatura.

1 INTRODUCTION

Fish somatic growth can be affected by abiotic factors such as light regime (Krakenes *et al.*, 1991) and water temperature (López-Albors *et al.*, 2003; Lee *et al.*, 2017). Water temperature has been shown to have an important effect on muscle development on sea bass (Alami-Durante *et al.*, 2007), Atlantic salmon (MacQueen *et al.*, 2008) and Atlantic cod (Hall and Johnston, 2003; Galloway, Kjørsvik and Kryvi, 1999). Rearing temperature can affect weaning, metamorphosis, growth rates, sex, flesh quality, all of which are extremely relevant for the aquaculture industry (Abdel *et al.*, 2004; Sfakianakis *et al.*, 2013). In aquaculture, the use of higher water temperatures is commonly used to accelerate fish development. This is especially important during the larval phase where fish larvae are dependent on live feed and when mortality is high. However, the use of higher temperatures can induce changes that may only be detected in older fish (Lee et al., 2017).

The study of muscle dynamic is important because it enable us to better understand the muscle growth process and the factors that influence it (Saavedra *et al.*, 2016; Saavedra *et al.*, 2017). It is also relevant in fish farming as white muscle cellularity can affect flesh textural properties of some species (Fauconneau *et al.*, 1993; Hurling *et al.*, 1996). Muscle growth in fish

occurs through two different processes: fibre muscle hypertrophy and fibre muscle hyperplasia. The former consists of the growth of already existent fibres and only ceases when the maximum fibre area is reached (Johnston, 1999). Fibre hyperplasia occurs by recruiting new muscle fibres (Johnston *et al.*, 2003) and its more frequent during fish development and earlier life stages and less common in later stages. Fish species with large ultimate sizes tend to have higher hyperplasia rates which can be prolonged in adulthood (Weatherley *et al.*, 1988; Koumans *et al.*, 1993). On the contrary, small size species have lower percentages of hyperplasia, and, in some cases, be limited to embryo development (Alami-Durante *et al.*, 1997; Johnston, 1999). Muscle growth dynamics is the balance of muscle fibre hyperplasia and hypertrophy.

The aim of this study was to evaluate the long-term effect of using two different temperatures during larval development on meagre growth and muscle cellularity in later stages.

2 MATERIALS AND METHODS

2.1 HUSBANDRY AND EXPERIMENTAL SET-UP

This study was carried at the Aquaculture Research Station of the Portuguese Institute for the Sea and Atmosphere (IPMA) located in Olhão, in the South of Portugal.

Meagre, *Argyrosomus regius,* eggs were collected from the broodstock tanks and kept in a 200 L incubator with moderate aeration until hatching. The newly hatched larvae were distributed into six 120 L parallelepiped tanks at a density of 50 larvae/ l. The system operated in an open circuit and the water passed through a cartridge filter and UV, before entering the tanks. The tanks were randomly distributed in two different water temperature treatments: three under natural water temperature (control) and the other three under an increased water temperature (approximately 1.5º C).

Initial water flow was 0.6 L / min which was slowly increased until a maximum of 1.2 L / min. Temperature was 21.5 ± 0.9 ºC and 22.9 ± 0.6 ºC in the control and higher temperature treatment (HT), respectively. Salinity was 36 ± 1 ppt and oxygen was 5.7 ± 0.8 mg / l for both treatments. Photoperiod was 14h L / 10h D. One month after hatched (DAH), fish from both groups were transferred to separate 1500 L fibre glass tanks, all at natural water temperature (Fig. 1), until they were 12 months old. The 1500 L fibre glass tanks system operated in an open circuit and temperature and oxygen were 20.1 ± 4.1 ºC and 5.4 ± 1.4 mg/L, respectively, and salinity was 36 ± 1. The hottest month was July (average 26.5 ºC), and the coldest month was January (14.5 ºC).

Larval feeding protocol in both groups consisted of rotifers *Brachionus sp*. at a concentration of 5 rotifers /ml enriched with Red Pepper (Bernaqua NV, Belgium) from the mouth opening to 10 days after hatched (DAH). *Artemia* metanauplii (SepArt Technology, INVE aquaculture, Belgium)

enriched with Red Pepper (Bernaqua NV, Belgium) was introduced at 8 DAH at a concentration of 0.5 *Artemia* /ml given three times a day. At 10 DAH the amount was increased to 0.6 *Artemia* /ml and, at 16 DAH, to 0.8 *Artemia* /ml per meal. Inert diet (CAVIAR, Bernaqua NV, Belgium), was given from 15 DAH *ad libitum*. The amount of inert diet fed to the larvae was quantified daily to ensure similar quantities in both treatments.

When fish were transferred to the 1500 L tanks they were already weaned and were fed an inert diet (AquaSoja, Sorgal, Portugal).

2.2 SAMPLING AND BIOCHEMICAL ANALYSIS

During the larval experimental trial, when larvae were under different temperatures, length and dry weight was quantified throughout the time at 2, 8, 15, 20 and 30 DAH (20 larvae per tank). Dry weight was determined using samples of 20 larvae stored in liquid nitrogen and later freeze-dried. Once fish was transferred to the 1500 l tanks, they were sampled at 2, 4, 6 and 12 months old, for wet weight and length determination (50 fish per tank). Five fish per tank were sacrificed at 1 (30 DAH), 2, 4, 6 and 12 months for muscle cellularity analysis.

2.3 MUSCLE CELLULARITY

Fibre area and density were determined by histology. Five fish were euthanized with phenoxyethanol, rinsed in distilled water and fixed in formalin 10% for 48 h. When using larger fish, 4, 6 and 12 months old, a cross-section of approximately 2 cm was cut in the region of the first ray of the dorsal fin and two sections were chosen. The sections or fish, depending on the size, went through a sequence of dehydrations (distilled water, alcohol 70%, 90%, and 100%) and then included in paraffin wax. From the paraffin blocks, 10 µm cuts were made which were then stained with haematoxylin and eosin. Slides were observed at a light microscope (Zeiss-Axioplan) connected to a video camera (AxioCamER5s). Photos were taken using an Image Analysis System (AxioVision Release 4.8.2. SP2). White muscle fibre area and density were determined using an image processing software (Image J). Fibre density was calculated as the number of fibres per mm^2. Four sections from each block (two in total) were analysed per fish and five individuals were used per treatment.

2.4 DATA MANAGEMENT

The relative growth rate (RGR, % DW day $^{-1}$) was calculated according to the formula: $RGR= (e^{(\ln DWt - \ln DW0) / t} -1)*100$, being DWt and DW_o the final and initial dry weights respectively and t the trial duration.

Two-way Anova were carried to identify potential differences between treatments for fish growth, weight, length and muscle cellularity. A t-test was carried to analyse the differences in fish survival between treatments.

3 RESULTS

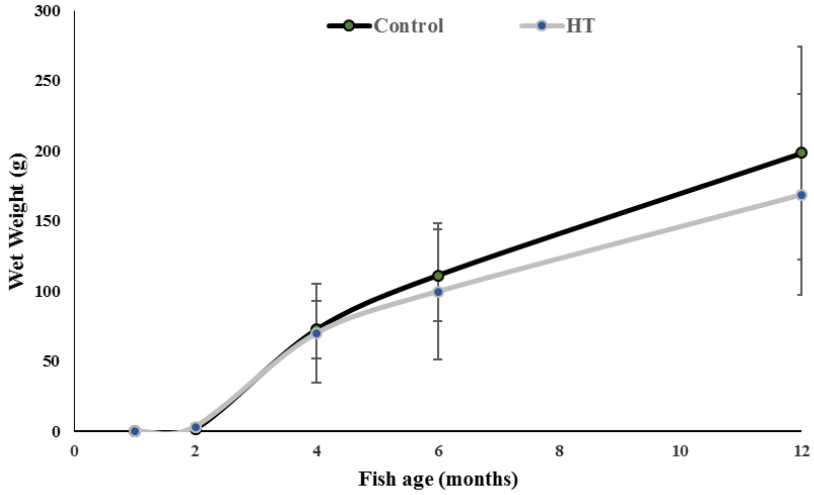
When the larval experimental trial was over, survival was 12.1 ± 1.8% and 17.2 ± 3.3%, for the HT and control treatments, respectively. Meagre larvae under a higher temperature showed higher dry weight when fish were 1 month old (11.0 mg *versus* 7.1 mg) (p=0.003) (Table 1). Significant differences were also found for larval length at 8 and 20 DAH, when larvae from the HT treatment showed higher length (4 mm *versus* 3.9 mm and 5.7 mm *versus* 7.6 mm for 8 and 20 DAH, respectively) (p=0.04 and p=0.01 for 8 and 20 DAH, respectively) (Table 1). No differences were found for the relative growth rate between treatments.

Table 1. Meagre larval weight, length and relative growth rate (RGR) under natural water temperature (Control) and under a higher water temperature (HT). Values are mean and standard deviation. Different letters represent significant differences for p‹0.05.

Dry weight (mg)		
Larval age (days)	**Control**	**HT**
0	0.03 ± 0.00	0.03 ± 0.00
8	0.02 ± 0.00	0.03 ± 0.00
15	0.07 ± 0.01	0.09 ± 0.01
20	0.21 ± 0.06	0.29 ± 0.07
30	7.07 ± 1.63**a**	10.97 ± 3.77**b**
Length (mm)		
Larval age (days)	**Control**	**HT**
0	2.79 ± 0.11	2.79 ± 0.11
8	4.01 ± 0.06**a**	3.87 ± 0.05**b**
15	5.35 ± 0.10	5.32 ± 0.25
20	5.74 ± 0.40**a**	7.55 ± 0.57**b**
30	16.52 ± 1.05	18.70 ± 2.09
RGR		
Larval age (days)	**Control**	**HT**
0-8	-2.67 ± 1.39	-1.22 ± 1.52
8-15	16.63 ± 2.91	13.51 ± 0.18
15-20	23.26 ± 6.95	25.45 ± 6.04
20-30	42.01 ± 3.28	43.61 ± 4.65

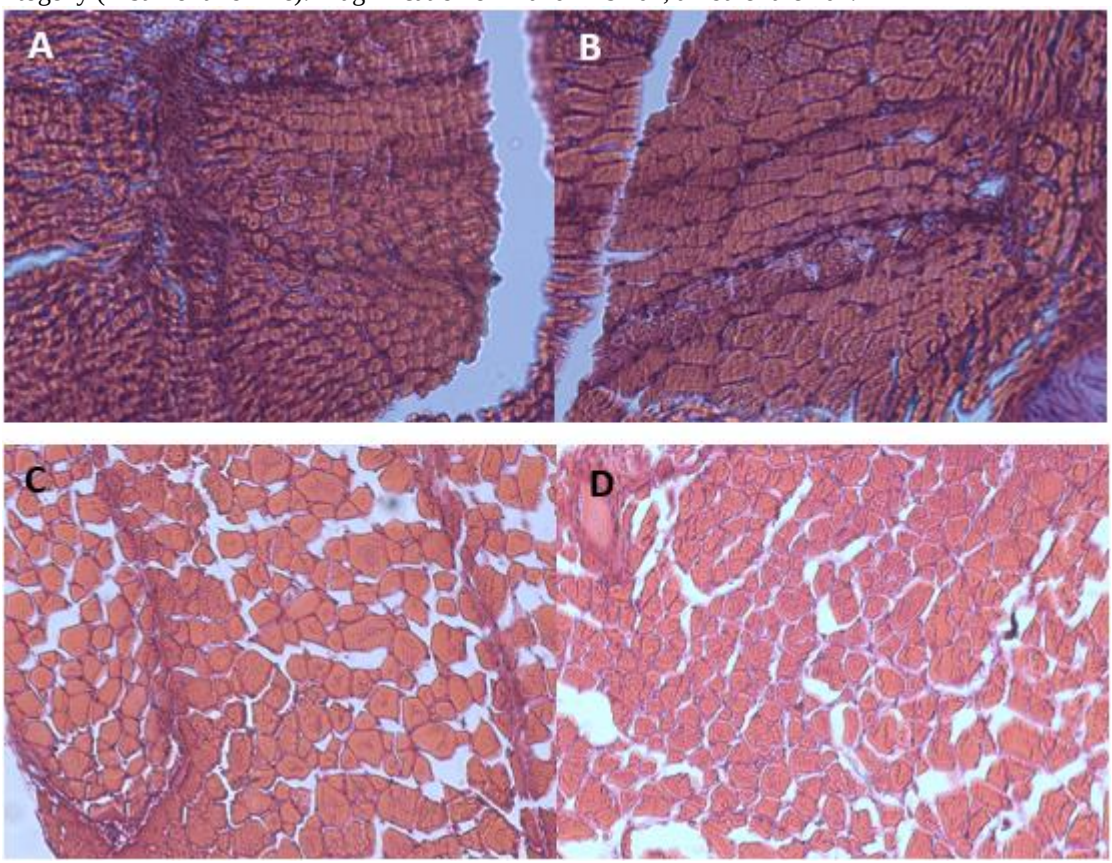
When fish from both treatments were under the same water temperature, no significant differences were found in fish weight between treatments (Fig. 1).

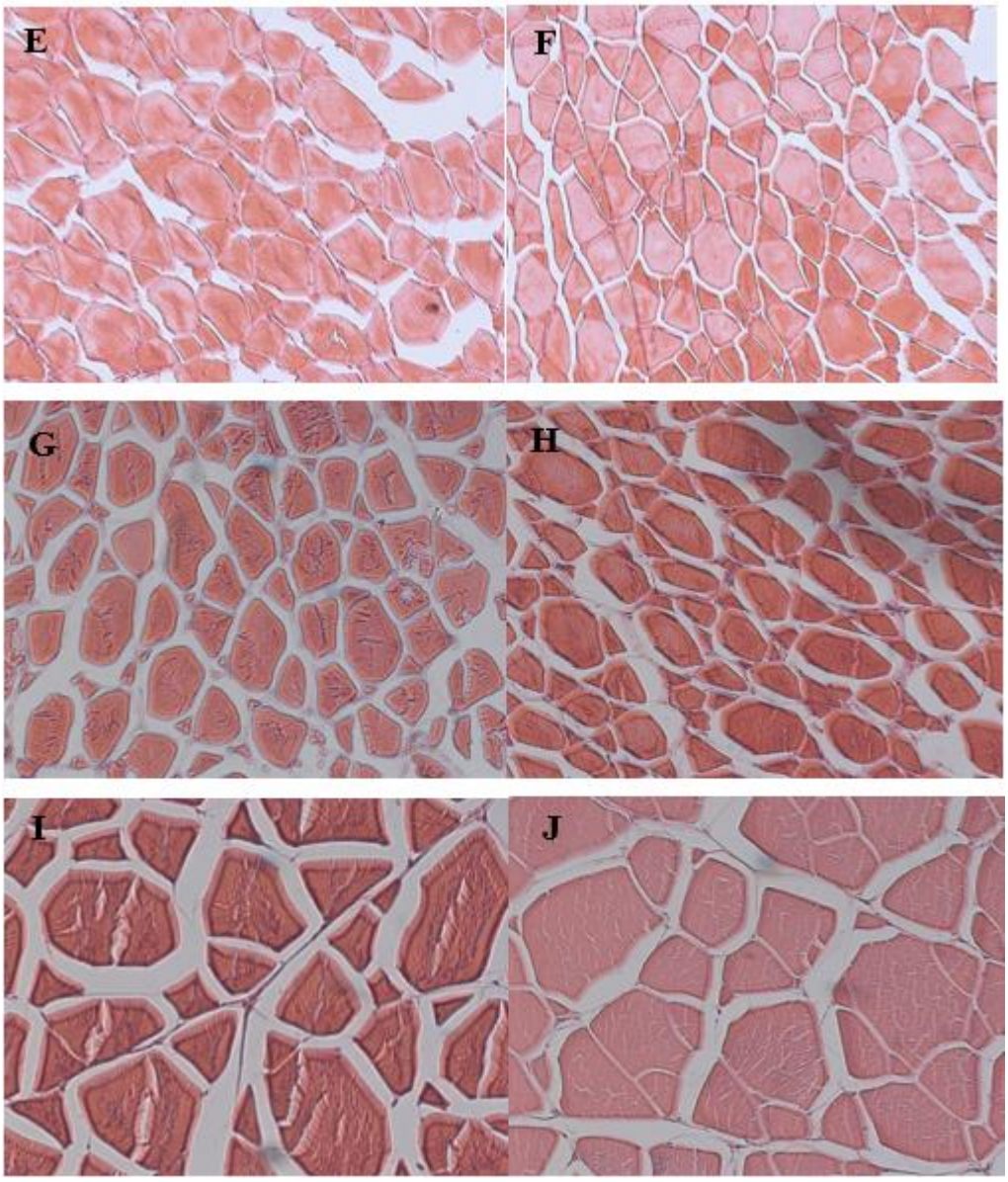
Fig. 1. Meagre wet weight from 1 to 12 months, after being subjected to natural water temperature (Control) and a higher temperature (HT) during larval ontogeny (first 30 days). Values are mean and standard deviation. Different letters represent significant differences for $p<0.05$.



Regarding muscle fibre area, significant differences between fish age were found, in both treatments (Fig. 3).

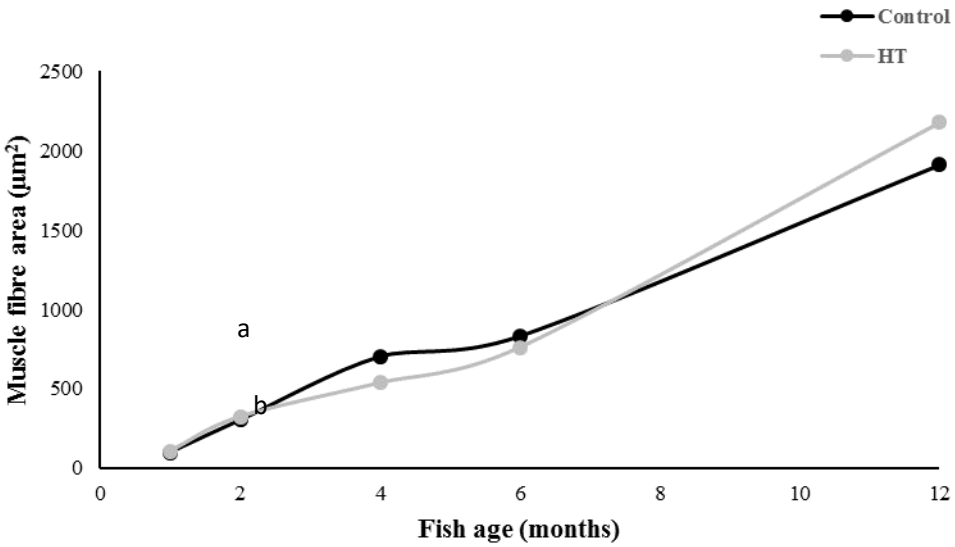
Fig. 2. Images of white muscle sections, showing the muscle fibres, when meagre was 30 DAH (A- control, B- HT), 2 months (C- control, D-HT), 4 months (E- control, F- HT), 6 months old (G- control, H- HT) and 12 months old (I- control, J- HT) after being under a natural water temperature (Control) and a higher water temperature (HT) during larval ontogeny (first month of life). Magnification of A and B is 40x, all other are 20x.





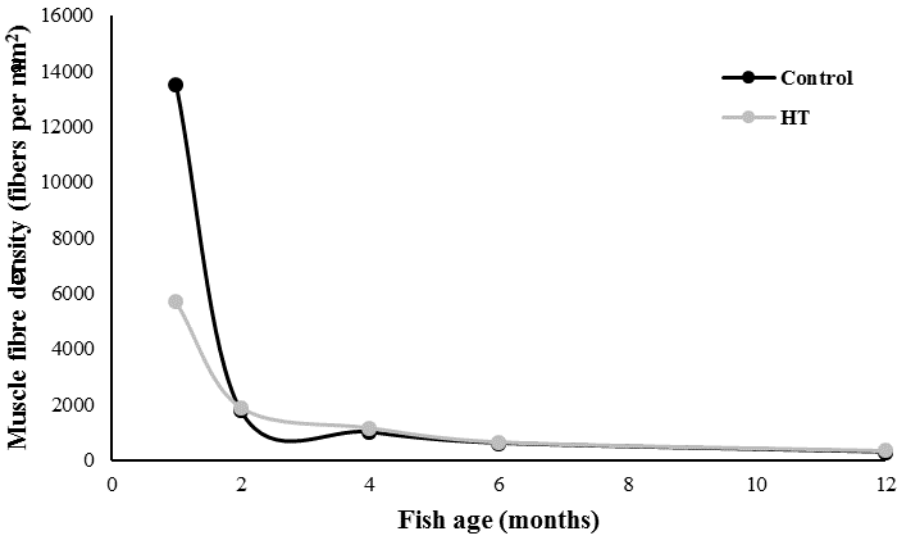
Muscle fibre area increased from approximately 100 µm^2, when fish were 1 month old, to aproximately 2000 µm^2 when fish were 12 months. The only significant difference between treatments was found when fish was 4 months old. At this age, meagre from the control treatment showed a higher fibre area (704 µm^2 compared to 540 µm^2) (p=0.006) (Fig. 3).

Fig. 3. Meagre muscle fibre area from 1 month to 12 month after being under a natural water temperature (Control) and a high water temperature (HT) during larval ontogeny. Values are mean. Different letters represent significant differences for p p‹0.05.



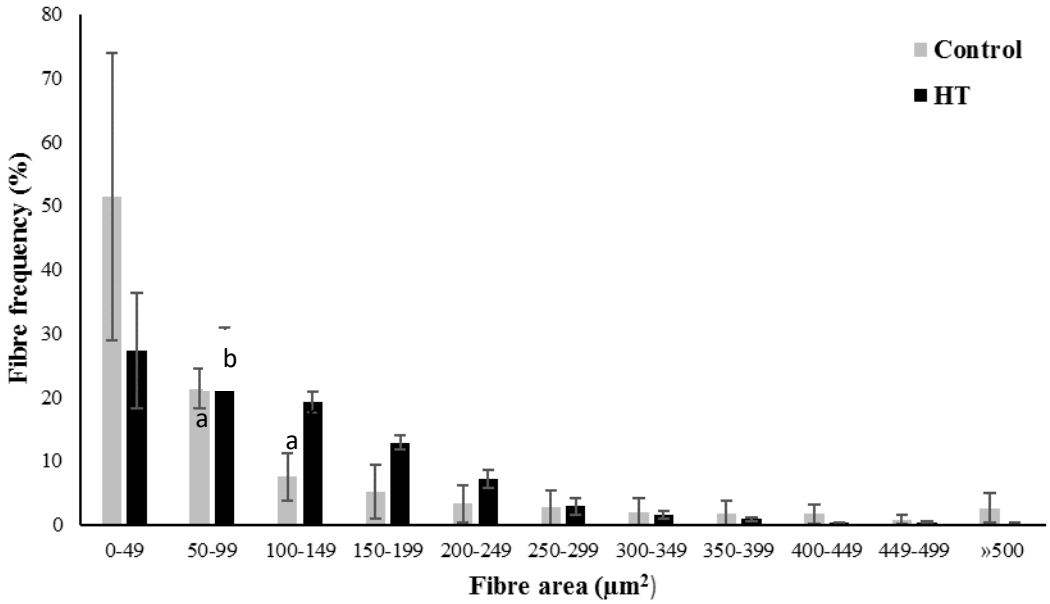
Significant differences were found at the end of larval ontogeny, when fish was 1 month old. Larvae from the control group showed higher fibre density compared to larvae from the HT group (13493 fibres per mm^2 compared to 5689 fibers per mm^2) ($p < 0.001$). No further differences were found for fibre density in later stages (Fig. 4). Muscle fibre density significantly decreased ($p<0.001$) from aproximately 13500 fibers per mm^2 to approximately 300 fibers per mm^2 in the control treatment and from approximately 6000 fibers per mm^2 to 300 fibers per mm^2 in the HT treatment.

Fig. 4. Meagre muscle fibre density from 1 month to 12 month after being subjected to a natural water temperature (Control) and a high water temperature (HT) during larval ontogeny. Values are mean. Different letters represent significant differences for p‹0.05.



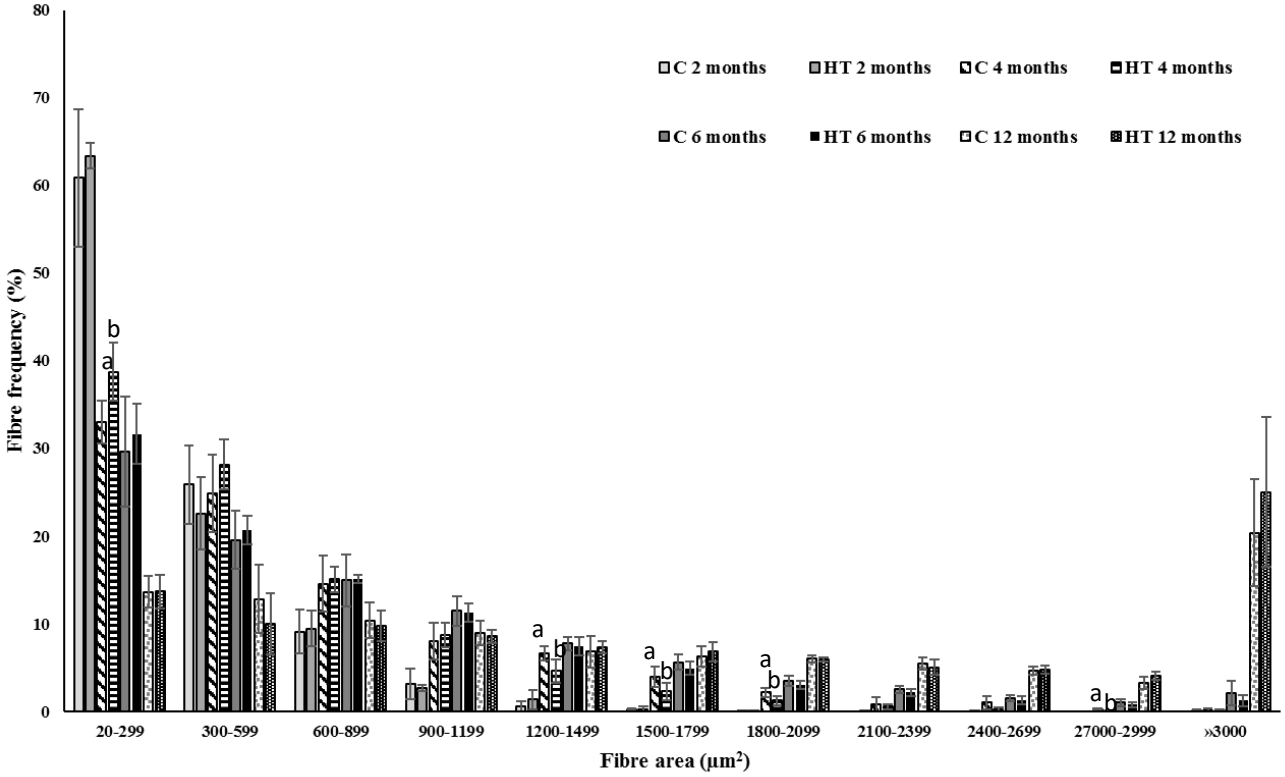
At the end of the larval experimental trial, when larvae were 1 month old, significant differences were found for muscle fibre areas between the two treatments. Larvae under a higher temperature during larval ontogeny showed higher frequency of fibres with an area between 100 and 199 μm^2 (Fig. 5).

Fig. 5. Frequency of muscle fibre areas of 30 DAH meagre larvae subjected to a control (Control) and a high temperature (HT). Values are mean and standard deviation. Different letters represent significant differences for $p<0.05$.



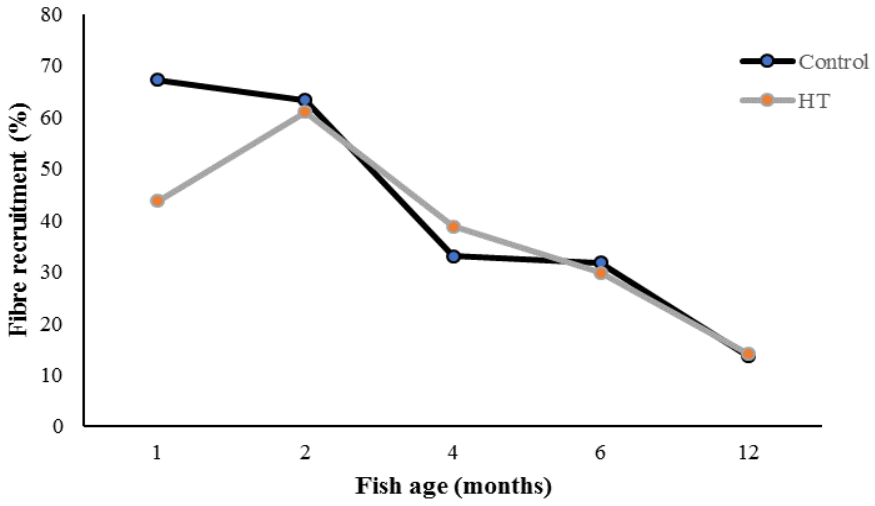
Once meagre from the control and HT treatment were reared at the same temperature, significant differences were observed when fish were 4 months old for fibre areas between 20-299 μm^2 , 1200-2099 μm^2 and 2700 to 2999 μm^2 (Fig. 6).

Fig. 6. Frequency of muscle fibre areas of meagre of 2, 4, 6 and 12 month old meagre after being subjected to a control (C) and a high temperature (HT) during larval ontogeny. Values are mean and standard deviation. Different letters represent significant differences for $p<0.05$.



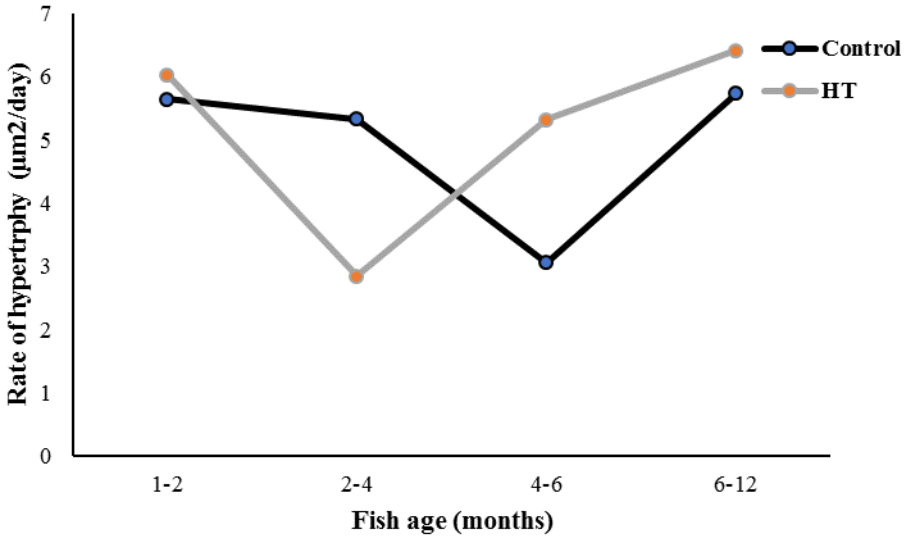
Fibre recruitment decreased throughout meagre life cycle (Fig. 7). At the end of the larval stage, fibre recruitment was around 70 % and 40 % for control and HT treatment. This percentage was decreased to 10 % when fish was 12 months old.

Fig. 7. Fibre recruitment in 1, 2, 4, 6 and 12 months old meagre after being subjected to a control (C) and a high temperature (HT) during larval ontogeny. Values are mean.



Muscle growth in terms of hypertrophy varied between 3 and 7 μm^2 /day (Fig. 8). Between 2 and 4 months, hypertrophy rate decreased in the HT treatment. In the control treatment, the decreased in the hypertrophy rate occurred later, between 4 and 6 months.

Fig. 8. Variation of the rate of muscle fibre hypertrophy from 1 to 12 months old meagre after being subjected to a control (C) and a high temperature (HT) during larval ontogeny. Values are mean.



4 DISCUSSION

Temperature can have an important impact and a permanent effect on fish growth pattern if exposure occurs in early stages of development because of high growth rate, often exceeding 50 % DW/day (Conceição *et al.*, 1997). One of these effects may be observed on muscle development (Johnston, 1999; Macqueen et al., 2008; Valente *et al.*, 2013).

Understanding how muscle growth occurs and how it can be affected is of extremely importance for farmed fish not only to optimize fish growth but, as well, to improve flesh quality, mainly textural properties (Weatherley, 1990; Johnston *et al.*, 1998). In this study, meagre larvae were subjected to two different water temperatures for 1 month: natural water temperature (control) and a higher water temperature (1.5 ºC higher). Later, fish continued to be reared until they were 12 months old, all under the same regime, natural water temperature. Larvae under a higher temperature showed higher final dry weight and higher length at 20 DAH. A high heterogeneity in fish size, commonly observed in this species (Saavedra *et al.*, 2015a; Saavedra et al. 2016, 2017), was possibly responsible for the lack of further differences. This size heterogeneity often leads to cannibalism (Saavedra *et al.*, 2016). Lee *et al.* (2017) obtained similar results when exposing sablefish to different water temperatures. Lee *et al.* (2017) observed that, although the higher temperature treatment led to a higher fish weight, it also led to a lower survival rate. In the present

study no differences were found for survival rate, but the standard deviation was relatively high. Survival rate was similar to the one obtained by Saavedra *et al.* (2015a).

Muscle cellularity, which is defined by fibre area distribution and fibre density in the myotomes, may significantly vary during the life cycle of fish (López-Albors *et al*., 2003). Meagre mean muscle fibre area when fish were 1 month old was lower compared to other published studies using meagre of approximately same age (100 µm^2 *versus* 150 and 200 µm^2 in Saavedra *et al.*, 2016 and 2017, respectively). This must have influenced the fibre density which was, in the current study, much higher compared to the values obtained by Saavedra *et al.* (2016, 2017) (approximately 6000 fibres per mm^2 compared to 3000 and 1500 fibres per mm^2). In fact, more than 50% of the fibres, in the control treatment, and 30 % of the fibre, in the HT treatment, had an area lower than 80 µm^2. The 80 µm^2 is commonly set as the maximum area of the recruited fibres (Alami-Durante *et al.*, 1997; Galloway *et al.,* 1999; López-Albors *et al.,* 2003). This high percentage of newly recruited fibres suggests that meagre, as other species such as carp (Alami-Durante *et al.,* 1997) and cod (Galloway *et al*., 1999), have their initial muscle growth mainly by hyperplasia. This is consistent with Saavedra *et al.* (2016, 2017), although, in the current study, this is more evident. Other studies have shown that higher rearing temperatures during larval ontogeny led to an increase of muscle fibers (Johnston & Andersen, 2008). The opposite was observed in the current study, where the control treatment showed a higher percentage of smaller fibres when fish were 1 month old. Similar results were found by Macqueen *et al.* (2008) who obtained a reduction on muscle fibre density when fish were exposed to high temperatures.

Once larval metamorphosis ended, at 30 DAH, fish were transferred to larger tanks and all kept under a natural water temperature. The effects of early temperature exposure faded one month later and no more significant differences were found in fish weight or length. During early life is common the existence of a compensatory growth after thermal treatment and has already been described for several fish species such as sea bass (López-Albors *et al.*, 2003), sablefish (Lee *et al.*, 2017) and other teleosts (Johnston et al., 2003). For muscle cellularity, the turnover period seemed to be at 4 months old, when mean fibre area became higher in the control treatment, and where several differences were found in the frequency of fibres with areas between 1200 and 3000 µm^2. These differences in the fibre area frequencies can be the result of a previous fibre boom, which was observed, for example, at 30 DAH, when fibre density was significantly higher in the control treatment. Those fibres, in two months (from 1 to 4 month old), must have grown through hypertrophy and moved to larger fibre classe.

In terms of muscle growth dynamics, it was possible to observe that, as expected, the hyperplasia decreased throughout the fish life cycle. During the first two months, meagre growth was due mainly to fibre hyperplasia as the newly recruited fibres accounted for more than 50 % of all muscle fibres. From 2 to 4 months an accentuated decrease was observed in both treatments, and the percentage of newly recruited fibres was reduced to approximately 30 %. There are several possible explanations for this. The first is that, in some fish species, has been reported that fibre recruitment is affected by seasons and that, for example in halibut, fibre recruitment occurs mainly in the winter (Haugen *et al.*, 2006). In the current study, the 2 and 4 months old period corresponds to June and August, which agrees with the results found by Haugen *et al*. (2006) for halibut. Another possible explanation is that from this phase onwards, muscle fibre hyperplasia becomes naturally less important. Fibre hypertrophy seems to be more constant throughout meagre life cycle, apart from a decrease observed between 2 and 4 months for the HT treatment, and between 4 and 6 months for the control treatment. These results suggest that the importance of this process is kept until meagre reaches adulthood. In the wild, meagre can reach a weigh of 50 kg (Hernandéz *et al*, 2009) and, therefore, hypertrophy is expected to have a relevant role in adult fish growth. Nevertheless, hyperplasia will not cease in adulthood as Saavedra *et al.*, (2015b), obtained recruitment percentages of approximately 18 % in 2500 g meagre.

In conclusion, this study shows that, in the case of meagre, the use of different water temperatures during larval ontogeny induced differences in fish weight, length as well as muscle fibre density and fibre area frequency. These changes may be an advantage when farming this species as the larval phase can be reduced. However, once the water temperature became similar in both treatments, the differences observed faded in older meagre and they did not seem to have repercussions in fish muscle dynamic.

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