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Impact of temperature and growth hormone on growth physiology of juvenile Atlantic wolffish (*Anarhichas lupus*)

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ABSTRACT

The effects of temperature and growth hormone (GH) implantation on growth of juvenile Atlantic wolffish (*Anarhichas lupus*) were investigated. The year-long study had three sequential experimental phases (EP) termed EP1, EP2 and EP3, lasting for 6, 9 and 37 weeks, respectively. The experimental fish were divided into four groups and reared at different target temperatures (3, 7, 11 and 15 °C) during EP1 and EP2, but at a constant temperature of 7 °C during EP3. At the beginning of EP2, half of the fish from each group was implanted with formulation of recombinant bovine GH (Posilac®), while the other half was sham-implanted with vehicle. The optimal temperature for growth ($T_{opt.G}$) of early juveniles (geometric mean weight 7.5 g) was determined as 12.1 °C during EP1, while the upper critical temperature (Tc) was concluded to be very close to 15 °C, as fish at that temperature had stunted growth, increased mortality and showed external signs of skeletal deformities. Thus, the species was found to be relatively stenothermic during the early juvenile stages and therefore vulnerable to relatively modest increases in environmental temperature above $T_{opt.G.}$ At 15 °C, GH implantation had no effects on growth rate. This indicates that the high allostatic load at this temperature leaves no scope for increased growth. In contrast, at lower rearing temperatures, the GH implantation had substantial, long-term effects on growth rate and induced remarkably similar relative growth stimulation at 3, 7 and 11 °C, suggesting a temperature-independent mechanism for the growth-promoting effects of GH.

1. Introduction

Temperature is the strongest influencing force for aquatic ectotherms, as it directly influences all biological processes in the body (Pörtner et al., 2006). Most fish species studied show a rapid increase in growth rate with increased temperature, with growth rates peaking at the optimal temperature for growth ($T_{opt.G}$) before decreasing at higher temperatures (Bett, 1979). In aquatic environments, the thermal tolerance range of ectothermic organisms is largely determined by the amplitude of thermal variation in their natural habitat. Thermal tolerance ranges are therefore generally wider in temperate species than in those inhabiting the colder and thermally stable waters at the poles (Pörtner, 2002).

The Atlantic wolffish (*Anarhichas lupus*) is a cold-water marine fish and is widely distributed in the North Atlantic Ocean. The species is demersal and sedentary, except during spawning and feeding migrations. They mainly inhabit rocky bottoms but are also frequently found in areas with soft or mixed substratum (Barsukov, 1959). In Icelandic waters, the Atlantic wolffish is most abundant at 40–180 m (Jónsson, 1982), but across their geographic range they occupy varying depth ranges from 2 to 500 m (Barsukov, 1959; Beese and Kändler, 1969). In their natural habitat in Norwegian, Icelandic and North Sea waters, they are found at temperatures as high as 11 °C (Beese and Kändler, 1969) and can withstand temperatures as low as −1.7 °C (King et al., 1989). However, prolonged exposure to 8 °C and higher during the breeding season (November – January) has been found to have negative effects on egg quality in captive Atlantic wolffish (Tveiten et al., 2001).

Atlantic wolffish are known to change their depth and geographic distribution to stay within their preferred thermal environment (Kulka et al., 2004; Dulvy et al., 2008; Nye et al., 2009). Thus, if ocean

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temperatures continue to rise as predicted by climate models, the species will in the next few decades encounter unsuitable thermal conditions, at least near the southern boundary of its geographical distribution (Brennan et al., 2016). Warming ocean temperatures are, however, not the only threat to Atlantic wolffish populations. In most fishing areas in the North Atlantic, Atlantic wolffish populations have faced declines due to overfishing, particularly in the Northwest Atlantic where the species is currently listed as a "species of special concern" by the Canadian Species at Risk Act (SARA) (McCusker et al., 2008). Moreover, in some areas, habitat destruction by bottom-trawling vessels may have caused a further decline in the species (Collie et al., 2000).

As the natural populations of Atlantic wolffish as well as its closest relative, the spotted wolffish (*Anarhichas minor*) have declined, these species have been considered as potential species for the diversification of coldwater finfish aquaculture (Le François et al., 2002; Albertsson et al., 2012). Of the two species, the spotted wolffish, grows faster and to a larger size than the Atlantic wolffish, and has therefore generally been considered a better candidate for aquaculture purposes (Moksness, 1994; Foss et al., 2004).

Growth rate is a highly heritable trait, and in coldwater fish species, breeding selection has resulted in a 10–15% increase in growth rate per generation, much higher than reported for other farm animals (Gjedrem, 2000; Gjedrem and Robertson, 2014). The phenotypic plasticity of growth traits prior to breeding selection for "new" aquaculture species, can be explored by growth hormone (GH) treatment, especially by using sustained-release GH implants which act over weeks to months (McLean et al., 1997). GH is the main endocrine regulator of growth in vertebrates, stimulating muscle and skeletal growth directly as well as indirectly though stimulation of insulin-like growth factor I (IGF−I) (Björnsson, 1997). Under laboratory conditions with ad libitum feeding regimes, GH treatment significantly increases growth rate in teleosts by stimulating appetite, foraging activity and feed intake, as well as stimulating growth of muscle and skeletal tissue (McLean et al., 1997; Leedom et al., 2002; Neregård et al., 2008; Kling et al., 2012).

Breeding selection for faster growth of Atlantic salmon is paralleled by changes in the GH–IGF-I axis of endocrine growth regulation (Fleming et al., 2002). The selection process appears to 'utilize' some or all of the GH-induced growth potential, as breeding selected Atlantic salmon (AquaGen strain) responds less to GH manipulation than wild strains (Neregård et al., 2008), a phenomenon also seen for selected fast-growing strains of rainbow trout (*Oncorhynchus mykiss*) and channel catfish (*Ictalurus punctatus*) (Silverstein et al., 2000; Devlin et al., 2001). It is therefore accepted that GH-induced growth enhancement reflects an untapped growth capacity of the organism that can be gradually recruited or exploited through long-term systematic breeding selection. GH manipulation under controlled rearing conditions can therefore be considered to be a rapid approach to explore and reveal the maximum inherent growth capacity of a given species that could potentially be recruited for aquaculture purposes, through long-term domestication and selective breeding programs.

The overall goal of the present study was to obtain data on post-hatch growth of Atlantic wolffish of wild origin from Icelandic waters and to determine growth-temperature relationships during the early juvenile phase. The specific objectives were to determine the $T_{opt.G}$ by rearing the juveniles at four different temperatures, to use slow-release GH implants to demonstrate the species full capacity for growth, e.g. after breeding selection and/or after optimization of aquaculture conditions, and to explore how stenothermic teleost growth physiology is affected at the upper thermal tolerance limits.

2. Materials and methods

2.1. Egg collection

On March 7th, 2016, in an annual bottom trawl survey by the Icelandic Marine and Freshwater Research Institute (MFRI), an Atlantic wolffish egg cluster was caught at a depth of 136 m off the north-west coast of Iceland (66.17°N, 25.07°W). The eggs were placed in a plastic barrel containing ~4 °C seawater which was renewed continuously with seawater pumped from 6 m depth to maintain relatively constant temperature and water quality. The eggs immediately started hatching and the cluster was completely hatched within about 24 h. Upon landing at Reykjavík harbor on March 11th, about 1800 newly hatched larvae were transported to the MFRI Aquaculture Research Station at Grindavík, southwest Iceland.

2.2. Fish, culture conditions and experimental set-up

The Aquaculture Research Station is located on a lava peninsula and pristine seawater is pumped from 50 m deep boreholes. The seawater is naturally filtered by the lava and is at 7.2 ± 0.4 °C and 31 ± 2 ppt salinity year-round. The station also has access to geothermal water (60 °C) and chilled seawater (2 °C) and thus has the capacity to perform rearing experiments over a wide range of temperatures.

The Atlantic wolffish larvae were placed in a single circular 250 l fibreglass tank with flow-through supply of oxygenated 7.2 °C seawater. They were start-fed using a mixture of 0.4 and 0.8 mm formulated dry feed pellets (56–62% protein 10–18% fat, BioMar A/S) and the first larvae started feeding about a week after hatching. Accumulated mortality during the yolk-sac and first-feeding phases was about 80% with 285 juveniles available for experimentation. The high mortality was primarily due to starvation, as most of the larvae never started eating dry feed at the end of the yolk-sac stage.

The fish were subjected to a step-wise growth experiment in three experimental phases termed EP1-EP3, which are described in detail below. During EP1 and EP2, the juveniles were reared at four different target temperatures (3, 7, 11 and 15 °C) and during EP3 they were all reared at a stable target temperature of 7 °C (daily monitoring). Mortality was monitored daily throughout all three experimental phases. Throughout the study, the fish were reared under continuous light (LD 24:0) with a light intensity of 60–80 lx at the surface. Aerated seawater was as provided in a flow-through system and adjusted so that oxygen saturation was kept close to 100%. The fish were fed commercial dry feed (Laxá Ltd. and BioMar A/S) containing 50% protein and 16–21% fat depending on fish size. Feed was provided continuously over 18–20 h per day in moderate excess by automatic feeders. All fish were routinely measured under anesthesia (MS222, tricaine methanesulphonate, 0.1 g l^{-1}).

2.3. DNA analyses

Fin clip samples were collected from 10 wolffish juveniles on April 25th, 2017 (413 dph) for species identification, i.e. if they were Atlantic or spotted wolffish. The samples were stored in EtOH and transported to the Genetic Laboratory at Matís, DNA was extracted with the" HotSHOT" method (Truett et al., 2000) and the variable cytochome oxidase subunit I (COI) gene of the mitochondria DNA (mtDNA) sequenced using universal COI primers on the ABI 3730 sequencer (Applied BioSystems). The software Sequencer v5.2.4 (Gene Codes Corporation) was used to align the forward and the reverse sequences for each sample. Since mtDNA sequencing only confirms the maternal inheritance of an individual, the samples, along with control samples of the both the Atlantic and spotted wolfish, were genotyped for a total of 16 microsatellite loci (nDNA) according to Pampoulie et al. (2012).

2.4. Pre-experimental phase

Once all initial starvation mortality had ceased, about 4 weeks after hatch, regular monitoring of growth in the hatchery tank was commenced in a pre-experimental phase, lasting from April 7th to June 22nd, 2016 (30–106 days post hatch (dph)). Temperature was kept

stable at 7 °C during this phase. Body weight (W) and length (L) were measured at 30, 34, 42, 50, 62, 70 and 86 dph on 29–84 randomly sampled fish, whereas on 106 dph, all 285 juveniles were measured. After the measurements the fish were allowed to recover from the anesthesia in aerated seawater before being returned to the tank. W was 0.32 ± 0.01 g at 30 dph and 5.26 ± 0.07 g at 106 dph.

2.5. Growth experiment

2.5.1. Experimental phase 1

Experimental phase 1 (EP1) was a six-week growth experiment, starting on June 22nd, 2016 (106 dph) and finishing on August 4th, 2016 (149 dph), aimed at determining ($T_{opt.G}$) by using four target temperatures (3, 7, 11 and 15 °C) and two replicate groups at each temperature. After the initial size measurement at 106 dph, the 285 juveniles were randomly distributed among eight rectangular 300 l grey fibreglass tanks (90 × 90 × 37 cm) with 35–36 fish per tank. While no temperature change was made for the fish on 7 °C, the rearing temperatures for the other groups were gradually adjusted over two days to 3, 11 or 15 °C in respective tanks. All fish in all tanks were measured for W and L after three weeks (127 dph) and after six weeks (149 dph). The groups are subsequently referred to as the 3C, 7C, 11C and 15C groups. During the first three weeks the mean rearing temperatures in the respective groups were 3.0 ± 0.03, 7.2 ± 0.01, 10.8 ± 0.1, and 15.1 ± 0.06 °C, and over the entire six weeks of EP1 3.0 ± 0.02, 7.4 ± 0.02, 11.0 ± 0.07 and 15.2 ± 0.06 °C.

2.5.2. Experimental phase 2

Experimental phase 2 (EP2) followed directly on to EP1, with all fish kept in their respective tanks on the four rearing temperatures established in EP1. It was a nine-week study starting on August 4th (149 dph) and finishing on October 6th, 2016 (212 dph), designed to elucidate the combined effects of temperature and GH on the growth performance. EP2 was initiated directly following the W and L measurements at 149 dph. While still under anesthesia, an approximately 5 mm long incision was made in the abdominal wall of each fish, through which a PIT-tag (Trovan passive integrated transponder tags, www.trovan.com) was manually inserted into the peritoneal cavity for future individual identification. Then, a GH- or sham-implant was delivered through the same incision. A 50 µl positive-displacement pipette (Microman; Gilson, Middleton, WI, USA) was used to implant the fish intraperitoneally with 3 µl g W^{-1} of GH or sesame seed oil vehicle. The GH implant used was a sustained-release recombinant bovine GH (rbGH) formula (Posilac®, Monsanto Co., St Louis, MO, USA), resulting in a dose of 1 mg rbGH g W^{-1} for the GH-treated fish. The sham-treated fish received a sesame-seed oil vehicle only. After the implantation, the fish were allowed to recover briefly from the anesthesia in aerated seawater after which they were returned to their respective aquaria and temperature regimes. There were no immediate mortalities associated with the implantation. Each tank contained equal number of GH- and sham-implanted fish. W and L were measured every third week as before, on August 25th, September 15th and October 6th, 2016 (at 170, 190, and 212 dph). During EP2 the mean rearing temperatures in each group were 2.8 ± 0.01, 7.4 ± 0.01, 10.9 ± 0.07, 14.8 ± 0.11 °C.

2.5.3. Experimental phase 3

Experimental phase 3 (EP3) was a direct 37-week long sequel to EP2, designed to study the long-term effects of early rearing temperature and GH manipulation. After the final EP2 size measurements at 212 dph, on October 6th, the 3C, 11C, and 15C groups were gradually acclimated to 7 °C over two days, while the 7C group remained at the same temperature. Six weeks later, on November 17th, 2016 (at 254 dph), all fish were transferred to one green rectangular fibreglass tank (2 × 2 × 0.8 m) and reared at 7 °C until June 20th, 2017 (469 dph). The individual W and L were measured eight times over the 37-week period of EP3. The interval between measurements varied between 3 and 17 weeks and increased as the fish grew larger.

2.6. Radiology

On May 11th, 2017, at 429 dph, two sham-treated fish from the 15C group, one from the 7C group and one from the 11C group were transferred live to the Innovation Centre in Reykjavík, Iceland in order to obtain X-ray images. These were taken using Phoenix Nanotom S (General Electric), with settings of 80 µA, 80 kV and 9 s exposure time, and the distance between the beam source and the X-ray film was 25 cm. No filter was placed in front of the X-ray source. The fish were anaesthetized before the images were taken and returned to seawater immediately after. As the X-ray could not capture the entire fish on a single image, two X-ray images were taken of two individuals, one from group 11C and another from group 15C. These images were combined and adjusted so that the anterior and posterior sides of the vertebral column could be included on a single image for these two individuals.

2.7. Data analysis and statistical methods

Total body length (L) was measured to the nearest 0.1 cm and body weight (W) to the nearest 0.01 g. Condition factor (CF) was calculated as $(WL^{-3})100$. The specific growth rate in body weight (SGR_W) was calculated as $G = (e^g - 1)100$, where $g = (\ln W_2 - \ln W_1)(t_2 - t_1)^{-1}$ and W_2 and W_1 are the body weights on days t_2 and t_1, respectively.

All data are presented as means ± standard error of the mean (SEM). Changes in growth rate with temperature were described with third degree polynomials.

$SGR_W = dT^3 + cT^2 + bT + a$, where SGR_W is growth rate, T is temperature, and a, b, c and d are regression constants. The polynomial regression was fitted to growth data for the first three weeks (t_1-t_2), as well as for the entire six-week period of EP1 (t_1-t_3). Geometric mean body weight for these two periods was calculated as $(W_1 \times W_2)^{1/2}$.

Normality of distribution was assessed by applying the Shapiro-Wilk test and homogeneity of variances was tested by the Levene F test. For EP1 (106 to 149 dph) data analysis, a two-way ANOVA with replicates (random) nested within temperature (fixed) was used to test for possible differences in mean W, CF and SGR_W. The same method was used to test for differences among temperature groups for the remainder of the study (EP2 and EP3), but after implantation the analyses were done separately for GH- and sham-treated sub-groups. Significant differences in the ANOVA analyses were followed by Tukey's HSD (honestly significant difference) tests.

Differences in W, CF and SGR_W between sham and GH-treated subgroups within each temperature were analyzed by Tukey's HSD tests. The replicates within each sub-group were pooled prior to analyses since there was no significant difference between them at any point in the study.

3. Results

3.1. Species identification

The cytochrome *c* oxidase subunit I mitochondrial DNA sequences from 10 randomly selected wolffish juveniles in the study were compared to registered species in the Barcode of Life Data Systems (www.barcodinglife.org) reference library (Ratnasingham and Hebert, 2007) and the spawner identified as Atlantic wolffish. The results from 16 microsatellite markers showed that species could easily be separated by their unique alleles at eight of the loci confirming that the juveniles in the study were Atlantic wolffish, not spotted wolffish nor hybrids of the Atlantic and spotted wolffish.

3.2. Mortality

The total mortality over the 484 days of the experiment, from the

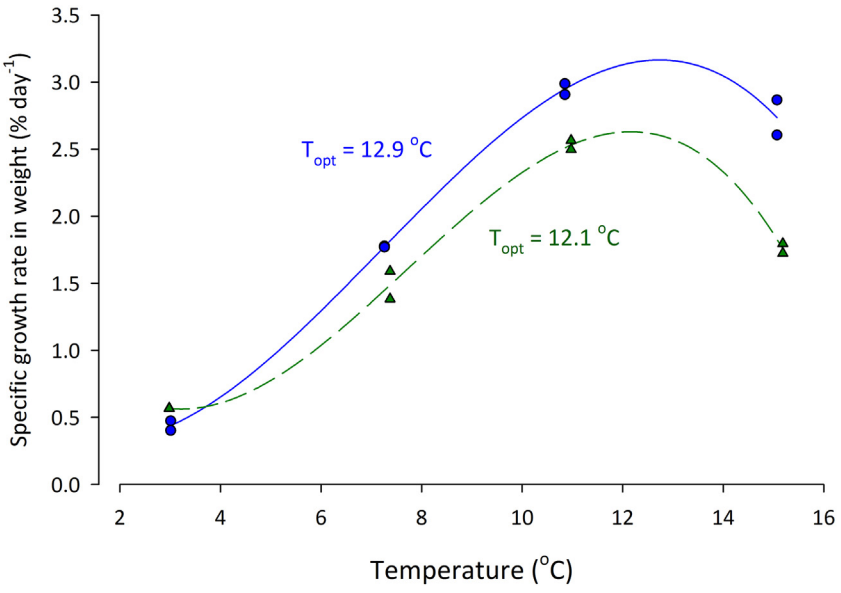

Fig. 1. Specific growth rates for weight (SGR_W) of juvenile Atlantic wolffish held in duplicate tanks at four rearing temperatures (3, 7, 11 and 15 °C). The specific growth rate is calculated over the three-week period from 106 to 127 dph (solid line and circle symbols in blue; $R^2 = 0.995$) as well as over the six-week period from 106 to 149 dph (dashed line and triangle symbols in green; $R^2 = 0.993$). The lines illustrate the relationships between temperature and specific growth rate using a third order polynomial regression line, and the symbol pairs show specific growth rates in the duplicate tanks. Optimal temperature for weight growth ($T_{opt.G}$) was calculated to be 12.9 °C for the first three weeks of exposure to different temperatures, and 12.1 °C when calculated for the longer, six-week exposure period. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

beginning of EP1 to the end of EP3, were 6, 0, 0 and 23% in the 3C, 7C, 11C and 15C groups, respectively. In the two groups where mortalities occurred, the 3C and 15C groups, about half of the mortalities occurred during EP2 and the other half during EP3. One fish of the 15C group died during EP1. Mortality rates did not differ between sham- and GH-treated subgroups during EP2 and EP3.

3.3. Effect of temperature growth

During EP1, the effect of temperature on SGR_W was described with a third-degree polynomial during the first three weeks (t_1-t_2), as well as for the entire six-week period of EP1 (t_1-t_3). Over the first three weeks of EP1, the 11C and 15C groups had the highest SGR_W (Fig. 1), and the mean weights of these groups were not significantly different by the end of the third week (Fig. 2A, Tukey's HSD, $p > .05$). However, during the following 3-week period the 15C group had a marked decline in SGR_W (Fig. 1) and significant differences in W were found between all groups at the end of EP1 (Tukey's HSD, $P < .05$, Fig. 2A). This delayed decline of the 15C group affects the shape of the polynomial growth curve (Fig. 1). Therefore, $T_{opt.G}$ was higher when calculated for the first three weeks (t_1-t_2 = 12.9 °C) compared with the first six weeks (t_1-t_3 = 12.1 °C).

During EP2, from tagging and implantation until the fish were returned to 7 °C, the effects of temperature on growth were similar to those found during the last three weeks of EP1. Thus, within the sham- and GH-treated subgroups, SGR_W increased with rising temperature from 3 to 11 °C but decreased rapidly at 15 °C to a level similar to that found at 3 °C (Fig. 3).

During EP3, when all fish were kept at 7 °C, the temperature induced size-differences between 3C, 7C and 11C groups were maintained. Thus, when sham- and GH-treated fish were compared separately, there were significant differences in W between the respective temperature groups on all measurement dates (Fig. 2; Tukey's HSD, $p < .05$).

Except for the initial weighing at the start of EP1, the 11C group had the highest mean W at all measurement dates throughout EP1, EP2 and EP3. The 15C group ranked a close second initially during EP1, but rapidly declining growth rates of this group resulted in the 7C group gradually becoming heavier than the 15C group during EP2. By the end of EP3, both 3C subgroups (sham- and GH-treated) had become larger than both subgroups of group 15C (Fig. 2B).

3.4. Effects of GH implantation on growth

Three weeks after the GH implant, at 170 dph, and throughout EP2 and EP3, GH-treated fish within the 7C and 11C group were significantly heavier than their sham-treated conspecifics (Fig. 2, Tukey's HSD, $p < .001$). For the 3C group, the growth-promoting effects of the GH treatment took a longer time to manifest, and a significant difference in W between sham- and GH-treated fish was first found six weeks after implant, at 190 dph (Tukey's HSD, $p < .001$). From that point onward, the GH-treated fish in the 3C group were significantly heavier than sham-treated fish (Tukey's HSD, $p < .001$). The difference in W between sham- and GH-treated fish in the 15C group was non-significant from implantation and throughout EP2 and EP3 (Tukey's HSD, $p > .05$).

Throughout most of EP2 and EP3, the relative difference in W between GH- and sham-treated subgroups (Δ_W) was roughly the same within the 3C, 7C and 11C groups (Fig. 4). For these groups, Δ_W increased rapidly during first 21–41 days post implantation (dpi). In the following weeks, the rate at which Δ_W diverged, slowed down until a maximum difference of about 30% was reached at about 105–151 dpi. From then on, the Δ_W decreased somewhat, but at the end of EP3, > 10 months after the implantation (at 320 dpi), the GH-treated fish were still about 20% heavier than their sham-treated counterparts.

For the 15C group, Δ_W peaked at 7.6%, six weeks after implanting (41 dpi) and by 151 dpi, the sham-treated fish were slightly larger than the GH-treated fish (Fig. 4).

3.5. Condition factor

CF was not significantly different among the temperature groups at the beginning of EP1 (two-way nested ANOVA, $p > .05$, Fig. 5). During EP1, there was a significant reduction of CF in the 3C and 7C groups (Tukey's HSD, $p < .05$), a slight, non-significant increase of CF in the 11C group (Tukey's HSD $p > .05$), while there was a rapid and highly significant increase of CF in the 15C group (Tukey's HSD, $p < .05$).

CF of GH-treated fish decreased significantly in all temperature groups (Tukey's HSD, $p < .05$), from the beginning of EP2 (149 dph), and until 170 dph. Thereafter, the CF of GH-treated fish began increasing, except in the 3C group which displayed a gradual decrease in CF throughout EP2.

When CF is analyzed for sham- and GH-treated fish separately, over the 15-week period of EP1 and EP2, it appears that CF increases with rising temperature from 3 to 15 °C. Further, CF was clearly influenced

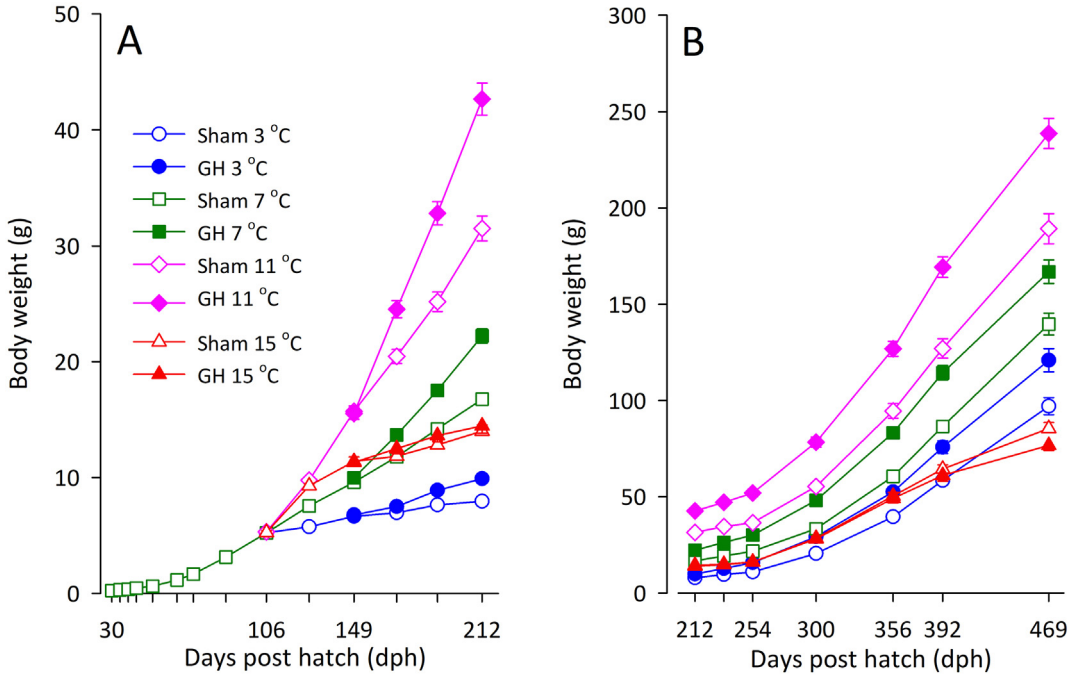

Fig. 2. The effects of rearing temperatures and growth hormone (GH) implants on body weight (W) of Atlantic wolffish from 30 to 469 dph (April 7th, 2016 to June 20th, 2017). Panels A and B illustrate W during the early (A) and late (B) part of the study. (A) From 30 to 106 dph, all the post-hatched larvae were reared together at 7 °C. At 106 dph, the fish were randomly divided among four different temperature regimes, 3 °C (●), 7 °C (■), 11 °C (◆) and 15 °C (▲) and reared at these temperatures until 212 dph. During this period, at 149 dph, each temperature group was subdivided into two groups, one implanted with GH (GH-group; filled symbols) and the other with vehicle (sham-group; open symbols). (B) At 212 dph, all groups were placed back on a 7 °C temperature regime. Statistical effects of GH on W (Tukey's HSD, $p < .001$) for the different temperature regimes are: At 3 °C, GH increased W from 190 dph onwards. At 7 °C and 11 °C, GH increased W from 170 dph onwards. At 15 °C, GH did not affect W (Tukey's HSD, $p > .05$).

by the direction and rate of temperature change during the first six weeks after the groups were returned to 7 °C (EP3). During this period, fish subjected to reduction in temperature from 11 and 15 °C to 7 °C exhibited a notable decline in CF, while the opposite effect was found for fish moved from 3 to 7 °C, and CF remained stable for fish kept at 7 °C throughout (Fig. 5). Thus, after being returned to 7 °C, the CF of sham- and GH-treated fish within all temperature groups began converging, and after six weeks at 7 °C (254 dph), there was no significant difference in CF between the 3C, 7C and 11C groups (Tukey's HSD, $p > .05$).

After the initial decline in CF following transfer to 7 °C, the sham- and GH-treated 15C groups entered a second growth stanza characterized by a rapid increase in CF. Thus, the CF of the 15C group increased from 0.99 ± 0.02 to a plateau at 1.30 ± 0.04 during a period of 20 weeks. After six weeks at 7 °C, there was also a notable increase in CF among GH-treated fish in the 3C, 7C and 11C groups, and throughout EP3, the GH-treated fish within the respective temperature groups had significantly higher CF than their sham-treated conspecifics (Tukey's HSD, $p < .05$). CF was not significantly different (Tukey's HSD, $p > .05$) between sham and GH-treated fish within group 15C at

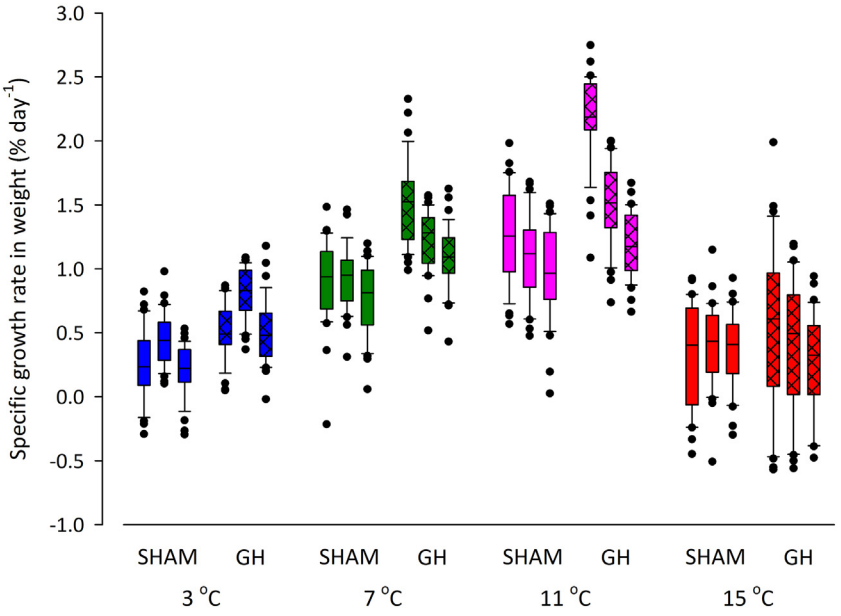

Fig. 3. The distribution of specific growth rates in weight (SGR_W) of sham-implanted (clear boxes) and growth hormone (GH)-implanted (cross-hatched boxes) Atlantic wolffish reared at four different temperature regimes over three successive 3-week periods; from the time of GH/sham-implantation at 149 to 170 dph, from 170 to 190 dph, and from 190 to 212 dph. The temperature regimes and number of fish at each regime were 3 °C (■; $n = 35$), 7 °C (■; n = 35–36), 11 °C (■; 35–36), and 15 °C (■; $n = 30–32$). Box (interquartile range, IQR), whiskers (1.5 × IQR), black line (median), black dots (outliers). Comparison between corresponding sham- and GH-treated groups shows that GH increased SGR_W significantly ($p < .001$, Tukey's HSD) at all three periods for the 3C, 7C and 11C fish, but did not affect SGR_W for the 15C fish.

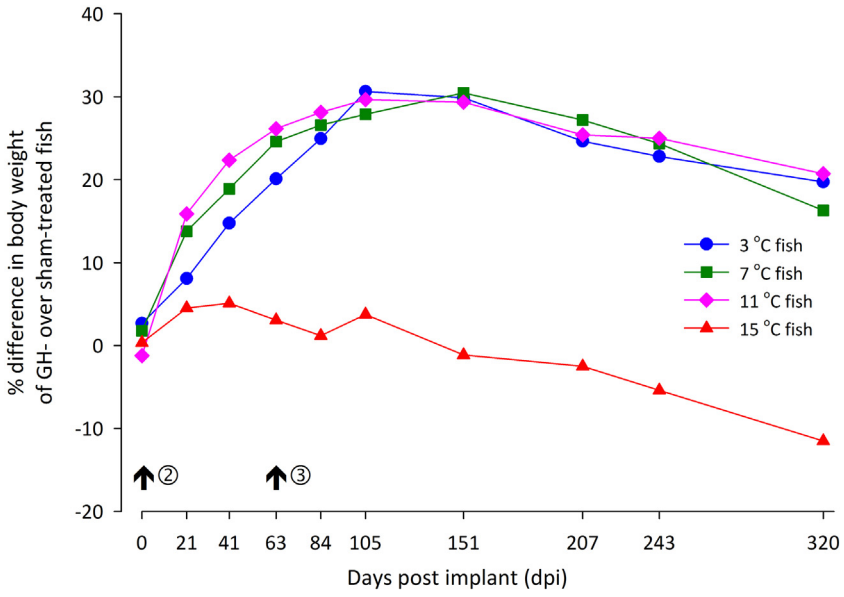

Fig. 4. Body weight (W) of growth hormone-implanted Atlantic wolffish relative to sham-implanted conspecifics. The fish were implanted on 149 dph (②), when the fish were at four different rearing temperatures; 3 °C (●), 7 °C (■), 11 °C (◆) and 15 °C (▲). Nine weeks later (63 dpi, ③), all fish were returned to their initial rearing temperature of 7 °C.

any point during the study.

3.6. Effect of temperature on skeletal deformity

Radiological analyses carried out during EP3, 31 weeks after the fish were returned to 7 °C (at 429 dph, 217 dpi), showed no skeletal deformity in the fish from the 7C or 11C groups (fish from group 3C were not X-rayed). However, the two randomly sampled individuals from the 15C group had severe skeletal malformations, revealing a direct temperature effect on skeletal structure. Fig. 6 shows a photograph and a corresponding radiograph of two representative juveniles from the 11C and 15C groups. The 11C fish has a normal body shape (Fig. 6A) and skeletal structure (Fig. 6B), while the 15C fish has a axially compacted body shape (Fig. 6C) and a severely deformed skeletal structure (Fig. 6D). Only a few vertebrae on the posterior end of the vertebral column of the deformed fish seem to have symmetrical structure (Fig. 6C), while all other vertebrae are asymmetric, compressed and/or ankylosed. The neural and haemal spines in the compacted fish are also severely deformed, some having an s-shaped appearance. In contrast, the spines in the normally growing juvenile are regularly shaped (Fig. 6A and B).

Concerning skin coloration, both fish on Fig. 6 are representative of the fish in the experiment. They have pale brown color with dark sports on the head, as well as on the dorsal- and caudal fins. From the head to the caudal fin, the spots form dotted lines. These lines are particularly distinctive on the normal fish from the 11C group, but on the fish from the 15C group, the spaces between the stripes are vaguer and the stripes more densely packed together. With increasing size and age, the skin color of the fish gradually changed from pale brown to grey during EP3. Further, the spotted pattern of the stripes became less prominent and they turned darker and more solid.

4. Discussion

The current study has monitored the long-term development and growth of Atlantic wolffish juveniles over 469 days from hatching.

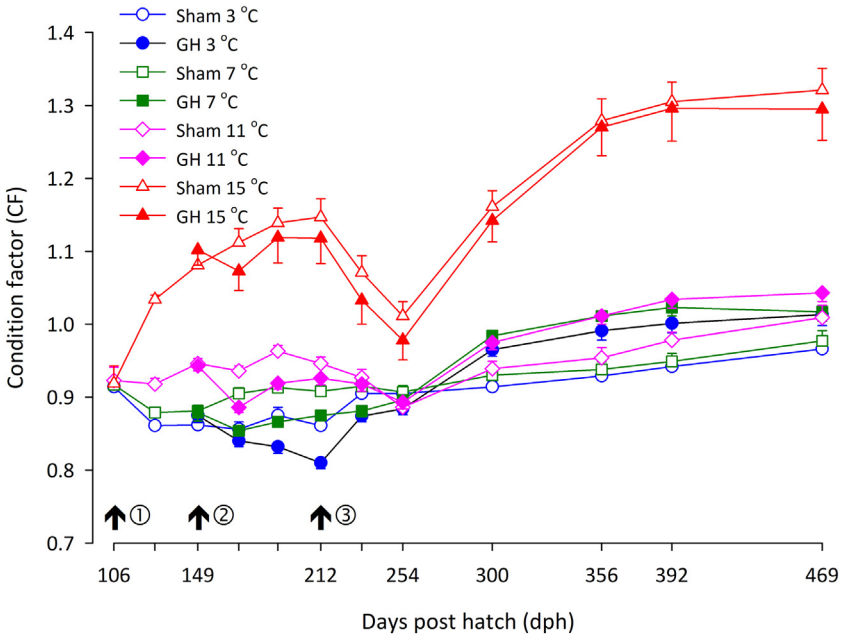

Fig. 5. Condition factor (CF) of Atlantic wolffish reared at four different temperatures, 3 °C (●), 7 °C (■), 11 °C (◆) and 15 °C (▲) from 106 dph (①). At 149 dph (●), the fish were sham-implanted (open symbols) or growth hormone-implanted (closed symbols). At 212 dph (③), all groups were placed back on a 7 °C temperature regime.

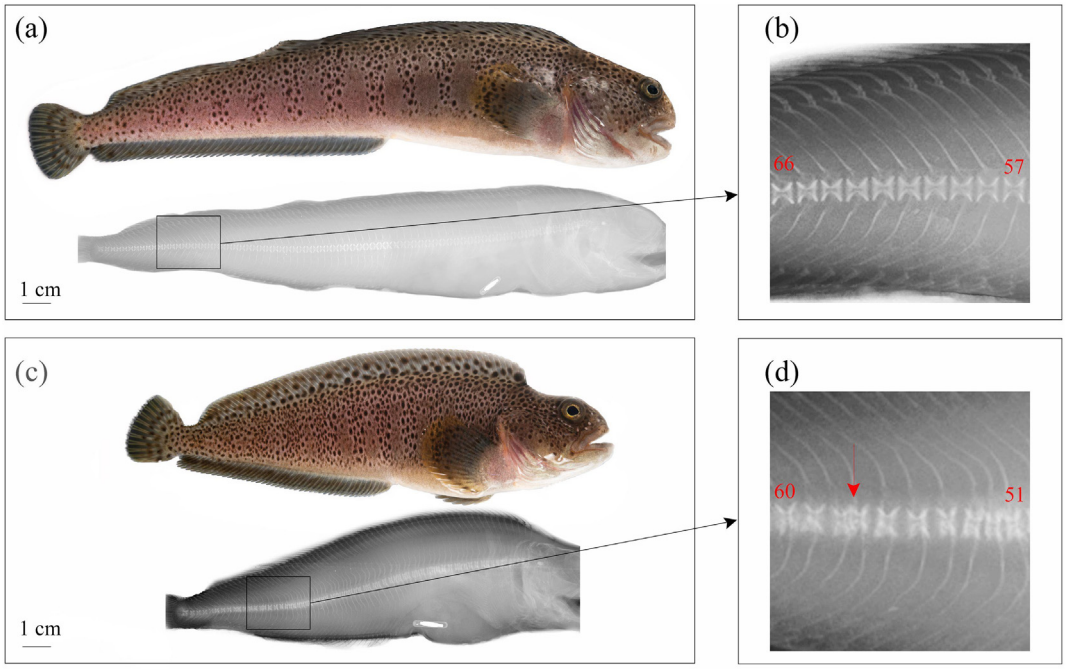

Fig. 6. Photograph and corresponding radiograph of a representative (a) normally growing Atlantic wolffish exposed to 11 °C and a representative (c) deformed Atlantic wolffish exposed to 15 °C. The insert (b) shows normal spinal patterning between vertebrae 57 and 66, whereas insert (d) shows non-symmetric and compressed structure of vertebrae 51–60, with ankylosis between vertebrae 57 and 58 (arrow). Photo: S. Egilsdóttir, MFRI.

During the three sequential experimental phases of the study, manipulation through use of temperature and GH treatment has helped elucidate novel aspects of growth physiology of a marine coldwater teleost species, especially in relation to its upper thermal tolerance limits.

As the experimental fish were hatched from a single wild-caught egg cluster, it was initially unclear if they were Atlantic or spotted wolffish. Phenotypic examination was not conclusive, and the genotyping was therefore carried out to ascertain their species identity as Atlantic wolffish.

The fish were twice subjected to changes in rearing temperature; at the beginning of EP1, fish were transferred from 7 °C to both warmer (11 and 15 °C) and colder (3 °C) water, and at the beginning of EP3, the reverse transfer was carried out. The initial changes in SGR_W following changes in rearing temperature show clearly the thermal-dependence of growth, with all transfers to colder or warmer water resulting in decreased or increased SGR_W, respectively. However, while most of these changes in SGR_W were sustained, transfer from 7 to 15 °C revealed a dual thermal response, with a dramatic decrease in SGR_W following the initial increase in SGR_W. The dual growth response of the 15C group affects the calculation of $T_{opt.G}$ during EP1, being 12.9 °C when calculated for the first three weeks (t_1-t_2) whereas being 12.1 °C when calculated for the full six-week duration of EP1 (t_1-t_3). Thus, although a downshift in optimal temperatures for growth is frequently observed with increasing body sizes in fish (e.g. McCarthy et al., 1998; Árnason et al., 2009a), the small difference in geometric mean weight between periods $t_1 - t_2$ and $t_1 - t_3$ (6.49 and 7.46 g, respectively) suggests the change in $T_{opt.G}$ between the respective periods may almost entirely be attributed to the delayed growth inhibition of the 15C group.

Therefore, the $T_{opt.G}$ over the shorter growing period is likely overestimated, and $T_{opt.G}$ over the six-week growing period gives a more reliable estimate. In a previous study, McCarthy et al. (1998) estimated $T_{opt.G}$ for two size-classes of Atlantic wolffish juveniles. According to their study, the $T_{opt\cdot G}$ for early juveniles (~0.2 g) was 14 °C, whereas 50–60 g juveniles grew maximally at 11 °C. Assuming, the same rate of reduction with log-increased body size, the $T_{opt.G}$ for 7 g juveniles would be about 12 °C, which is similar to the $T_{opt.G}$ for same-sized fish in the current study.

The present study shows that the intraperitoneal implant of the rbGH formulation Posilac® has a strong, long-term growth-promoting effect on the Atlantic wolffish at rearing temperatures from 3 to 11 °C. A similar effect as has been demonstrated earlier for several teleost species, including salmonids (McLean et al., 1997; Biga et al., 2005; Neregård et al., 2008; Kling et al., 2012), tilapia (Leedom et al., 2002) and channel catfish (Peterson et al., 2004, 2005) at standard hatchery rearing temperatures. The strong growth responses to GH implantation across many different species illustrate that, even under optimal rearing conditions, most farmed fish do not grow to their full capacity. The GH treatment is generally believed to reveal a large part of the inherent, untapped growth potential of that species. For the Atlantic wolffish of the present study, there is thus clearly a potential for faster growth, which could likely be realized through breeding selection and domestication programs.

The growth-promoting effects of GH are the combined result of multiple physiological and behavioral mechanisms. Thus, GH treatment encourages caloric intake by stimulating appetite and foraging behavior. It also enhances lean weight gain through promoting muscle protein accretion and lipid mobilization, a mechanism which improves feed conversion efficiency. GH treatment also stimulates hepatic secretion of insulin-like growth factor I (IGF−I) (Duan and Plisetskaya, 1993; Moriyama et al., 1994), a hormone recognized to be of specific importance for the endocrine control of skeletal growth (Björnsson, 1997). The GH-treatment protocol employed in the current study is known to elevate plasma IGF-I levels in rainbow trout (Kling et al., 2012) and channel catfish (Peterson et al., 2005). This is a likely endocrine mechanism for the proportionally greater stimulation of skeletal (length) growth than muscle (weight) growth, resulting in a decreased CF for the Atlantic wolffish in this study, as for other fish species studied (Leedom et al., 2002; Biga et al., 2005; Peterson et al., 2005; Neregård et al., 2008; Kling et al., 2012).

The current study is the first to demonstrate the qualitative and quantitative effects of GH-stimulation at different rearing temperatures. At temperatures (3, 7 and 11 °C) lower than the upper thermal tolerance limit, the GH implant resulted in a remarkably similar 30% growth enhancement, when expressed as a maximum weight difference over

the sham-treated fish reared at the same temperature. This suggests a temperature-independent mechanism for the growth-promoting effects of GH.

While most studies using similar GH implants as used in the current study, report growth stimulation over experimental periods limited to 4–8 weeks, McLean et al. (1997) reported a sustained increase in SGR of Posilac®-implanted coho salmon juveniles over 20 weeks. These were about 8 g in initial mean weight, implanted with three doses (ca. 0.5, 1.5 and 5 mg rbGH g^{-1}) and kept at 10.3 °C throughout the study. Plasma analysis carried out on the high-dose implanted fish revealed significant plasma rbGH levels over the entire 20-week study. As that study is comparable with the current study in terms of fish size, water temperature and dose of rbGH implant, it is plausible that the GH stimulation of the Atlantic wolffish was sustained for as long as four months. However, the GH-treated Atlantic wolffish were still significantly larger than the sham-treated conspecifics (about 20%) at the end of the study, or 320 days after the fish were GH-implanted. This shows that growth advantage obtained during a limited period at an early stage can remain for a relatively long time.

Although most of the 15C fish survived the exposure to 15 °C, this can be assumed to be their upper critical temperature (T_C) as there was about 12% mortality during EP2. Mortalities continued in the 15C group even after it was returned to 7 °C (EP3), resulting in a total mortality of 23% in the 15C group, compared with 0% mortality in the 7C and 11C groups. The welfare of the surviving fish was also seriously compromised during the exposure to 15 °C, as they had suppressed growth rate and developed severe skeletal deformities, as discussed below. As most individuals within the 15C group did not lose weight during EP2, it can be assumed that they were feeding, even if this was not measured and quantified. However, as standard metabolic rate increases with temperature, while the temperature-dependent capacity of ventilation and circulation limits oxygen supply and aerobic scope, their energy intake must have been reallocated from growth processes to vital organismic and cellular maintenance (Pörtner, 2001, 2002; Pörtner et al., 2006). In terms of the concept of oxygen- and capacity-limited thermal tolerance, the current study provides strong support for the assumed shift in energy use from growth processes to allostatic maintenance (Ramsay and Woods, 2014). The otherwise potent growth stimulation of the rbGH implant, which increased the body weight of the 3C, 7C and 11C fish by 30%, completely failed to elicit increased growth of the 15C fish.

Although both muscle- and skeletal growth of vertebrates is under stimulatory endocrine control by the GH-IGF-I system, the detailed control mechanisms may vary, e.g. with local IGF-I production being of particular importance for skeletal growth. Soon after being exposed to 15 °C, most fish started to show external indications of skeletal deformity. Spinal kyphosis and lordosis were observed in many fish, and almost all 15C individuals developed a compressed body shape, reflected in rapidly increasing CF (see Fig. 5). This prompted an X-ray assessment of two representative fish at 429 dph which revealed that these 15C fish had developed severe skeletal deformities, affecting most of the vertebral column as well as neural and haemal spines. The current results on the 15C fish directly support the conclusion by Moberg (2000) that the biological cost of coping with stress may shift energy away from normal biological functions such as growth. When this happens, the animal experiences distress, and enters a pre-pathological state during which it is susceptible to a number of pathologies.

Although the skeletal deformities in the current study were likely the result of prolonged process that developed over the 109 days at 15 °C, the rapid increase in CF during the first three weeks at 15 °C, indicates that transient exposure to temperatures close to the upper thermal limits of a species can have rapid, severe and permanently detrimental effects on developmental processes such as juvenile skeletal growth. Similar effects of temperature on CF have been observed in 8–16 g Atlantic cod juveniles, with a rapid increase in CF after transfer from 10 to 20 °C (Árnason et al., 2009b).

Although the current study suggests that 15 °C is close to the upper thermal tolerance limit for Atlantic wolffish juveniles (> 5 g), it is important to note that fish can shift their lower and upper thermal tolerance limits during acclimation (Pörtner, 2002). The time needed for fish to change their lethal thermal limits has been found to range from 1 to 20 days depending on the species and the direction and magnitude of the temperature change (Beitinger and Bennett, 2000). In the present study, Atlantic wolffish juveniles were kept at 7 °C prior to the experiment, and were adapted over two days to 3, 11 and 15 °C. It is therefore likely the fish would have performed better if they had been given a longer acclimation period, especially at the lower and higher ends of the temperature spectrum (3 °C and 15 °C).

However, it is also important to consider that thermal tolerance limits among ectothermic species are determined by the amplitude of temperature fluctuation in their natural habitat (Pörtner, 2002). Although limited information exists on the distribution and habitat use of Atlantic wolffish juveniles in their natural environment, it is unlikely that juveniles encounter temperatures as high as 15 °C for any length of time. In Newfoundland waters, spawning of Atlantic wolffish is known to occur in shallow (5–15 m) water as well as in deep offshore areas (Kulka et al., 2004). However, Keats et al. (1986) concluded that Atlantic wolffish juveniles do not inhabit shallow spawning areas (< 30 m) off the coast of Newfoundland, and the most likely habitat is offshore in deeper water, which in turn suggests colder and more thermally stable habitats. Furthermore, based on data from autumn bottom trawl surveys in Icelandic waters (October 1995–2017), 6–12 cm juveniles inhabit a similar thermal range (0.7–10.2 °C) as the fishing biomass (> 60 cm), although the juveniles are on average found in somewhat lower temperatures (5.1 °C) than the fishing biomass (7.1 °C) (MFRI, unpublished data). In captivity, Atlantic wolffish are mostly pelagic during the larval (1.9–2.7 cm) and early juvenile stage (2.7–5.0 cm), but juveniles over 6 cm prefer to spend time in shelters and become almost exclusively bottom dwelling at around 10 cm or 10 g (Moksness and Pavlov, 1996). Therefore, it seems likely the demersal and sedentary lifestyle in deeper and colder waters has allowed Atlantic wolffish juveniles to become relatively stenothermic, with upper thermal tolerance limits only 3–4 degrees °C above their $T_{opt.G}$. In this context, the benthopelagic Atlantic cod, a fish with a similar geographic distribution as the Atlantic wolffish, has a considerably wider temperature tolerance range than the Atlantic wolffish (see Björnsson et al., 2007).

From an aquaculture perspective, the growth performance of the Atlantic wolffish is relatively poor and even the fastest growing groups in the present study are predicted to require about three years from hatch to grow to the mean weight of about 1.5 kg. Such a modest growth rate may demand relatively high investment costs from a farming venture but, on the other hand, the wolffish has several important advantages for farming such as low optimal temperatures, high viability, high fillet yield and valuable byproducts (Moksness and Pavlov, 1996; Le François et al., 2002). Further, the product price is the single most important feasibility factor and a high price can easily outweigh the negative effects of slow growth performance. It is beyond the scope of this study to evaluate the feasibility of farming the Atlantic wolffish, but the species clearly demonstrates potential for growth promotion through domestication and selective breeding.

5. Conclusions

The relatively narrow thermal span from the $T_{opt.G}$ to T_C indicates that this species may be particularly vulnerable in the perspective of current and predicted future rise in ocean temperatures. We hypothesize that the high allostatic load at the T_C demands all the energetic resources of the fish, leaving no scope for increased growth following the GH implant. Further, the temporary exposure to T_C, led to severe skeletal abnormalities and permanent growth disturbances. In contrast, at temperatures at or below $T_{opt.G}$, the GH implant had strong and long-

lasting growth promoting effects, demonstrating a potential for establishing populations of faster growing Atlantic wolffish in aquaculture through breeding selection.

6. Post-study note

Soon after the study was ended on June 20th, 2017 (at 469 dph), the 15C fish were euthanized as they were permanently damaged by the exposure to 15 °C. The three other groups have been kept and raised together in a single tank as described for EP3. On November 7th, 2018 (at 974 dph) the body weight of the sub-groups was: 3C-sham 544 ± 37 g, 3C-GH 635 ± 37 g, 7C-sham 766 ± 43 g, 7C-GH 849 ± 41 g, 11C-sham 992 ± 41 g and 11C-GH 1132 ± 40 g. Thus, two years after the implants, the GH-treated fish were still significantly heavier (10–15%) than corresponding sham-treated fish.

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