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Larval Development in Tropical Gar (*Atractosteus tropicus*) Is Dependent on the Embryonic Thermal Regime: Ecological Implications under a Climate Change Context

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Abstract: In ectotherm species, environmental temperature plays a key role in development, growth, and survival. Thus, determining how temperature affects fish populations is of utmost importance to accurately predict the risk of climate change over fisheries and aquaculture, critical to warrant nutrition and food security in the coming years. Here, the potential effects of abnormal thermal regimes (24, 28 and 32 °C; TR24, TR28, and TR32, respectively) exclusively applied during embryogenesis in tropical gar (*Atractosteus tropicus*) has been explored to decipher the potential consequences on hatching and growth from fertilization to 16 days post-fertilization (dpf), while effects on skeletal development and body morphology were explored at fertilization and 16 dpf. Egg incubation at higher temperatures induced an early hatching and mouth opening. A higher hatching rate was obtained in eggs incubated at 28 °C when compared to those at 24 °C. No differences were found in fish survival at 16 dpf, with values ranging from 84.89 to 88.86%, but increased wet body weight and standard length were found in larvae from TR24 and TR32 groups. Thermal regime during embryogenesis also altered the rate at which the skeletal development occurs. Larvae from the TR32 group showed an advanced skeletal development, with a higher development of cartilaginous structures at hatching but reduced at 16 dpf when compared with the TR24 and TR28 groups. Furthermore, this advanced skeletal development seemed to determine the fish body morphology. Based on biometric measures, a principal component analysis showed how along development, larvae from each thermal regime were clustered together, but with each population remaining clearly separated from each other. The current study shows how changes in temperature may induce craniofacial and morphological alterations in fish during early stages and contribute to understanding the possible effects of global warming in early development of fish and its ecological implications.

Keywords: temperature; skeletal development; ossification; morphological alterations

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1. Introduction

Climatic variations through time due to anthropogenic activities and global warming have become a significant threat to ecosystems and biodiversity (Intergovernmental Panel on Climate Change [1]). Global warming of 1.5 °C is predicted to negatively impact the natural environment, including droughts, floods, increase sea level and ocean acidification [1]. Temperature fluctuations in aquatic habitats promote changes in the development,

physiology, and behavior of fish species [2–8]. Exposure to different temperatures within and outside the optimal species-specific range during early development can specifically promote alterations in survival, growth performance, and metabolism [9,10], including changes in cortisol, sodium, potassium, glucose levels and osmolality [11] or changes in muscular development that affects swimming efficiency [12]. The potential effect on the fish skeletal development and the induced skeletal malformations are also of particular interest, as they affect the fish survival and growth [13].

The potential effect of increased global temperature and ocean acidification has been explored in different fish species, mainly in Teleost of commercial relevance. For example, changes in temperature during metamorphosis and juvenile stages of gilthead seabream (*Sparus aurata*) produce changes in gill cover, hemal lordosis, and anomalies in the caudal and dorsal fins [14]. Temperature above 18 °C during egg incubation induce the appearance of deformities in caudal vertebrae of *Solea senegalensis* when compared to those incubated at 15 °C [15]. Temperatures above 29 °C cause deformities in the mandible and vertebrae of *Trachinotus ovatus* [16]. Pimentel and co-workers [17] have shown as eggs of gilthead seabream and meagre (*Argyrosomus regius*) exposed to future ocean conditions (+4 °C in water temperature and −0.5 ΔpH of acidification) had lower hatching success and larval survival. However, while no differences in body length were observed at hatching, a significant interaction between pCO_2 and species for somatic growth length was found at a certain age (at 15 dph for *S. aurata* and at 10 dph for *A. regius*). Similar to what was reported by [14], the incidence of body malformations in *S. aurata* larvae was significantly increased under these future ocean conditions, which was suggested to affect larval performance and recruitment success, altering the abundance of fish stocks [17]. In contrast, the projected ocean acidification scenarios seemed to not affect the development of contemporary European sea bass (*Dicentrarchus labrax*) larvae when exposed to them from hatching onwards [18]. These results suggest that the effects of climate change predicted scenarios might be developmental and/or species-dependent.

As above reviewed, the effects of increased mean water temperature derived from a climate change scenario have been described for different fish species, but always considering contemporaneous specimens (i.e., not transiently exposed to temperature increase through different generations) and thus, somehow neglecting their capacity to adapt to the new environmental conditions. Climate change also predicts an increase on the frequency and intensity of extreme events such as heat waves [1]. Heat waves are defined as very high temperatures over a sustained period of days and is directly affecting the contemporary specimens. It can represent one of the most enduring effects of climate change [19]. In the last decade, heat waves have increased the mortality of aquatic and terrestrial organisms due to its correlation with physiological stress [20], limiting their ability to cope with environmental challenges [21]. However, there is scarce information available on how an altered temperature due to a heat wave might impact freshwater fish species, specially to Holostei fish species.

Lepisosteids are an infraclass of Actinopterygii lacking the extra whole genome duplication of Teleosts, and a key group to understand vertebrate's evolution [22]. Moreover, Lepisosteid larvae are suitable piscine models to study the early life stages of fishes due to their rapid embryonic and larval development [23,24]. In particular, the tropical gar (*Atractosteus tropicus*), one of the seven extant Lepisosteids, is found in freshwater environments such as rivers, streams, lagoons, and swamps with abundant vegetation from southeast Mexico to Costa Rica [25]. The tropical gar (known as 'pejelagarto') has an important ecological role by regulating fish and amphibian populations, but also it has a cultural and commercial significance in southeast Mexico. It is captured and cultured because of its high nutritional value, for souvenirs with handicrafts of their scales and/or whole fish, and one of the most exotic sports fishing species [26,27]. Furthermore, its environment has been under constant pressure for the past 50 years, with declining populations in Central America [28]. Indeed, habitat degradation and destruction were suggested to be responsible for the drop in the population of this species [29].

The present study aimed to evaluate the effect of a water temperature fluctuation during the embryogenesis of the tropical gar, particularly on craniofacial development and body morphology. A correct and timely precise development of the craniofacial skeleton is necessary for proper growth and survival since it is essential for efficient fish breathing and preys' capture. We hypothesize that the craniofacial development of the larvae will be influenced by the occurrence of heat waves during embryonic development. Recorded water temperature variations in the region of Tabasco (México) ranged from 25.3 ± 0.9 °C to 32.0 ± 0.8 °C [30] in the Centla wetland, and from 21 to 31 °C in the Usumacinta river (one of the main rivers in Tabasco [31]), specifically from 22.96 to 33.88 °C in the lower basin of Usumacinta [32]. Based on our results, some reflections on how heat waves might sculpt fish morphology at population level under a climate change context will be also presented at the discussion section.

2. Materials and Methods

2.1. Ethical Statement

Fish were handled in compliance with the standards for the good welfare practices of laboratory animals from the Norma Mexicana NOM-062-ZOO-1999 de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación.

2.2. Animal Acquisition and Care

Fertilized eggs of *A. tropicus* were obtained from a broodstock held at the Tropical Aquaculture Laboratory of the Universidad Juárez Autónoma de Tabasco, Mexico. One female was anesthetized with clove oil and injected with 1 mL kg^{-1} of gonadotropin releasing a hormone (GnRH, Sanfer) to artificially induce breeding. The female was deposited in a 2000 L tank with six males. Artificial substrate was introduced to mimic the natural vegetation used for egg adhesion [33]. The spawning occurred at 28 °C. Eggs were collected after the female finished laying eggs (approx. 6 h after spawning started). A total of 1800 eggs were collected and placed in 10-L tanks (60 eggs per tank) with non-chlorinated water, continuously aerated and under a natural photoperiod of 12 h light-12 h darkness. A quick adaptation to the experimental thermal regimes was performed in a frame of 2 h.

2.3. Experimental Design and Sampling

Collected eggs were submitted to three thermal regimes (TR) from egg fertilization to mouth opening: low temperature (24 °C; TR24), normal (control) temperature (28 °C; TR28) and high temperature (32 °C; TR32). For each thermal regime, 600 eggs were randomly distributed in 10 tanks of 70 L (60 fish per replicate). After mouth opening, rearing temperature was progressively (1 °C day^{-1}) increased to 28 °C in tanks from TR24 group, decreased in TR32 or remained constant in those from TR28 group and maintained at 28 °C until 16 dpf (Figure 1). At each group and experimental condition, temperature was daily monitored and maintained at the set temperature with variations of ±0.5 °C using aquarium chillers and heaters (e.g., TECO® TK-2000 and EHEIM 200).

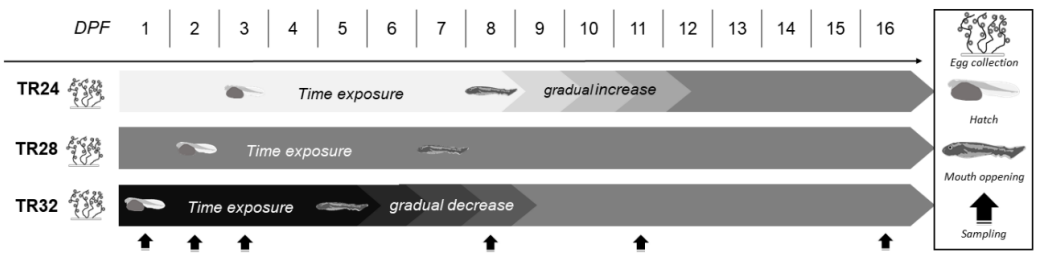

Figure 1. Experimental design to determine the effect of temperature during the early development of tropical gar (*Atractosteus tropicus*) (16 dpf). The exposure period is shown for each treatment, as well as the temperature transition and when the samplings were performed.

Larvae were fed with brine shrimp nauplii (*Artemia sp*) every 3 h from 8:00 to 17:00 h for five days. Subsequently, co-feeding of brine shrimp nauplii and trout diet (Silver Cup, 45% protein, 16% lipids) was provided to apparent satiation. One hour after every meal, dead brine shrimp and feces were removed by siphoning. Fifty percent water was renewed every 48 h. Taking into account the density of fish per tank and their airbreathing capacity, no aeration has been provided. Abiotic factors were daily monitored and recorded values were as follows: 6–8 pH and dissolved oxygen (>6 mg/L).

Fifteen larvae were randomly collected at hatching, 8, 11 and 16 dpf from each treatment for standard length (SL) and wet body weight (WBW) individual assessment. For skeletal development and body morphology analysis, 10 larvae were collected from each sampling time, 40 per thermal group. For both analytical purposes, fish were first euthanized with an overdose of MS-222. After, larvae for skeletal development were fixed in 4% paraformaldehyde (PFA) with 1× phosphate-buffered saline (PBS) at pH 7.4 for 24 h. Then, larvae were rinsed in 1× PBS for 15 min and progressively dehydrated in absolute ethanol-PBS solutions (25:75, 50:50 and 75:25 *v*/*v*) and finally preserved in 100% absolute ethanol until processing.

2.4. Survival and Growth

Survival was assessed by counting the number of dead larvae every day from every TR. Growth performance was evaluated as changes in WBW, SL and Fulton's condition factor (K). WBW (in milligrams) of the larvae was accounted using an analytical balance; SL was measured using a digital caliper to the closest millimeter. Condition factor was calculated as $K = (WBW \div SL^3) \times 100$ [34].

2.5. Skeletal Development Assessment and Body Morphology Biometrics

To analyze the degree of development of skeletal structures in the tropical gar, the acid-free double stain protocol described by [35] was previously adapted and conducted.

Processed larvae were observed under a dissecting microscope SMZ 25 to analyze osteological development and high-resolution photographs were taken. The degree of skeletal development was evaluated as the proportions of 'red pixels' (for bone) or 'blue pixels' (for cartilage) of the total surface of the fish (in pixels). Photographs and measurements were analyzed in the software Image J (Version 1.50i, https://imagej.nih.gov/ij/download.html (accessed on 1 December 2021)).

To explore how the exposure to different TRs determined the body morphology, different biometric measures (including pre-orbital length, body depth at cleithrum, prepectoral length, pre-pelvic length, pre-anal length, head length and width, jaw length and width, distance between ceratohyals at ossification front and length of ossified ceratohyal) were assessed.

Since fish metabolism is temperature dependent, as well as skeletal development is growth dependent, biometric data was normalized with SL and °C day^{-1}. Data normalization procedure consisted in dividing the corresponding values by the respective SL of the specimen and the °C day^{-1} at each sampling point. Data on bone skeletal development degree (ratios) was normalized by the °C day^{-1} at each sampling point. This procedure allowed us to reduce and/or avoid the potential effect of sampling individuals at different stages (temperature dependent) and effect of interindividual variability within each experimental group [36].

2.6. Statistical Analysis

Otherwise indicated, results are given as mean values ± standard deviations. All data were previously checked for normality (Kolmogorov–Smirnov test) and homoscedasticity of variance (Bartlett's test). Results were compared by means of one-way ANOVA to detect differences among experimental groups at each sampling time, and when detected, the post-hoc Tukey's multiple comparisons test was performed using GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA). A Principal Component Analysis using

SPSS was performed with the biometric data. Since data followed normal distribution and equal variance, data was not log-transformed. The matrix of covariance was analyzed. In all analysis, statistical significance was set at $p < 0.05$.

3. Results

3.1. Hatching Rate, Survival and Growth Performance

Hatching occurred at 1-, 2- or 3-days post-fertilization (dpf) in eggs under TR32, TR28 and TR24, respectively. Hatching rate has been significantly affected by the thermal regime applied (Figure 2). Hatching rate was significantly higher when *Atractosteus tropicus* eggs were incubated at 28 °C than at 24 °C (90.17 ± 4.67% vs. 85.17 ± 5.12%; ANOVA, $p < 0.05$). Hatching rate at 32 °C (89.83 ± 2.88%) was not significantly different from both TR24 (85.16 ± 5.11%) and TR28 (90.16 ± 4.67%) groups. Increased temperature also advanced fish development, particularly regarding the timing of mouth opening, taking place at 8, 6 and 5 dpf in TR24, TR28 and TR32 larvae (results not shown), respectively. In contrast, no significant differences were observed in the survival rate of *A. tropicus* larvae at the end of the trial (16 dpf; Figure 3; ANOVA, $p > 0.05$), with values ranging from 84.89 to 88.86%.

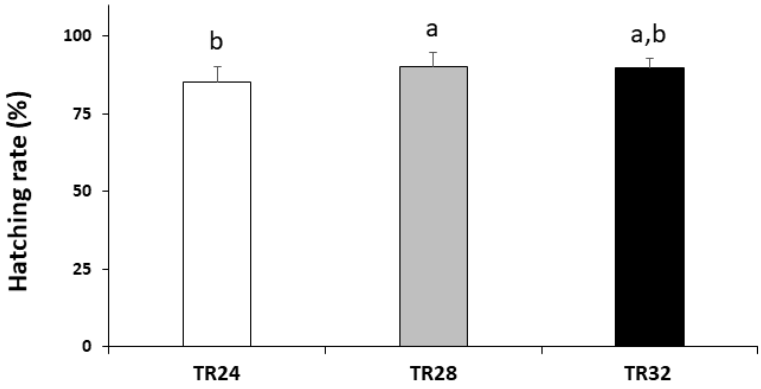

Figure 2. Hatching rate (mean ± standard deviation) of *Atractosteus tropicus* larvae when embryos were incubated at different thermal regimens. Lowercase letters at the top of each bar indicate statistical differences among experimental groups (ANOVA, $p < 0.05$; $N = 10$).

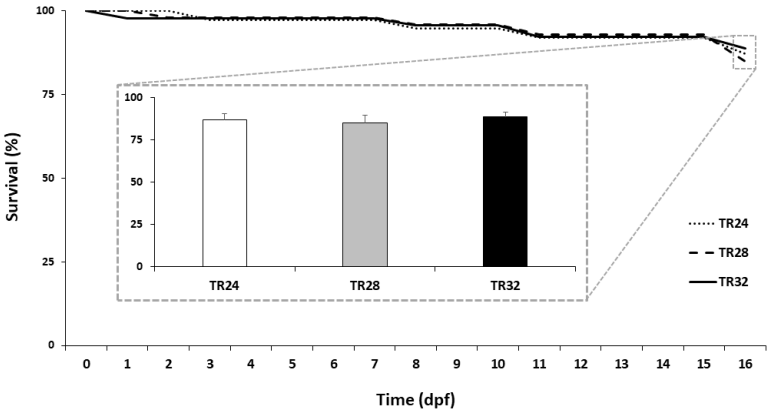

Figure 3. Mean survival of *Atractosteus tropicus* larvae along and at the end of the experimental trial (16 dpf) when embryos were incubated at different thermal regimens. The values of survival represented in the histogram bars are the mean ± standard deviation at 16 dpf.

Fish growth in terms of wet body weight (WBW; Figure 4a) and standard length (SL; Figure 4b) increased progressively during larval development, while the Fulton's condition factor (K) sharply decreased after hatching (Figure 4c). No differences in WBW were found at hatching. At 16 dpf, larvae from TR24 and TR32 reached similar WBW (108.20 ± 20.58 mg and 107.18 ± 15.85 mg, respectively) and significantly higher than that

of larvae from TR28 (82.20 ± 8.91; ANOVA, $p < 0.05$). Although larvae from TR32 showed higher WBW than those from TR24 and TR28 (ANOVA, $p < 0.05$) at 8 and 11 dpf, such differences might be related to the differential yolk-sac resorption status which the larvae from these two last experimental groups might be at. At hatching, SL were significantly higher in *A. tropicus* from TR24 (9.37 ± 0.49 mm) than that of larvae from TR28 and TR32 (ranging from 8.96 ± 0.14 to 9.00 ± 0.01 mm; Figure 4b; ANOVA, $p < 0.05$). However, these differences were not maintained along larval development. Indeed, larvae from TR32 start to show greater length than the other thermal regimes, and maintained such differences with larvae from TR28 until the end of the trial (ANOVA, $p < 0.05$), reaching 31.00 ± 2.16 mm. In line with WBW results, larvae from TR24 achieved similar SL to that of larvae from TR32 (29.79 ± 1.41 mm; ANOVA, $p > 0.05$), while SL and condition factor at 8 and 11 dpf might be also influenced by different rates on yolk-sac resorption in TR24 and TR28 larvae. At 16 dpf, Fulton's condition factor in TR24 (0.41 ± 0.02) was significantly higher than the one of larvae from TR32 (0.36 ± 0.03; ANOVA, $p < 0.05$), with larvae from TR28 showing intermediate values (0.37 ± 0.03; Figure 4c).

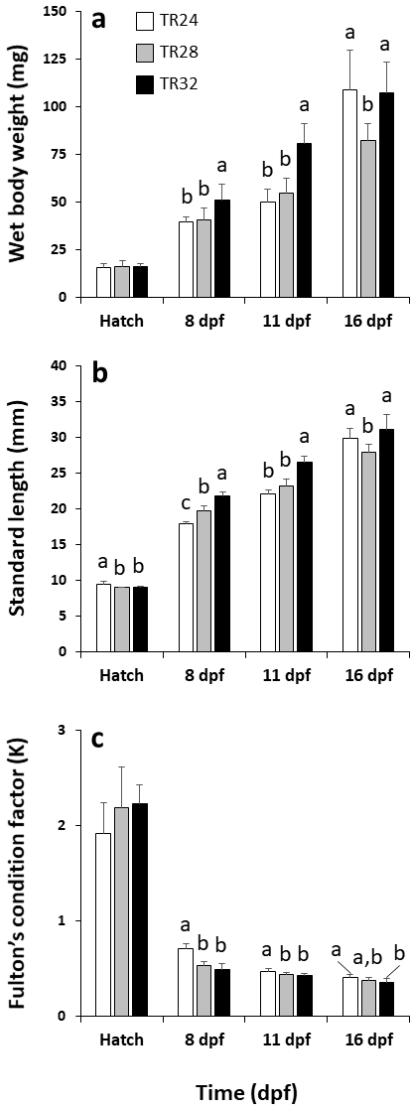

Figure 4. Growth performance (mean ± standard deviation) of *Atractosteus tropicus* larvae when embryos were incubated at different thermal regimens. Wet body weight (**a**), Standard length (**b**), and Fulton's condition factor (**c**). Lowercase letters at the top of each bar indicate statistical differences among experimental groups at the specific developmental sampling time (ANOVA, $p < 0.05$; $N = 10$). White bars, TR24; grey bars, TR28; and black bars, TR32.

3.2. Skeletal Development

Development of the skeletal structures in *A. tropicus* larvae progressed quite rapidly, particularly those composing the cranial region. Skeletal structures development (expressed as median values of the ratio of cartilage and bone surfaces over total larval surface per °C day^{-1}; Supplementary Data S1) was determined by the thermal regime during egg incubation and is presented in Figure 5. At hatching, higher quantity of cartilage was observed in larvae from TR32 group (0.51) than in larvae from TR24 (0.23) and TR28 (0.33; Figure 5a; ANOVA, $p < 0.05$). In contrast, at 16 dpf, the quantity of cartilage decreased in *A. tropicus* larvae, and statistical differences were again observed among the experimental groups. Larvae from TR32 groups exhibited the lowest cartilage quantity (median surface ratio per °C day^{-1} of 0.06), larvae from TR28 showed an intermediate value (0.07) and larvae from TR24 revealed the lowest value (0.09; Figure 5b; ANOVA, $p < 0.05$).

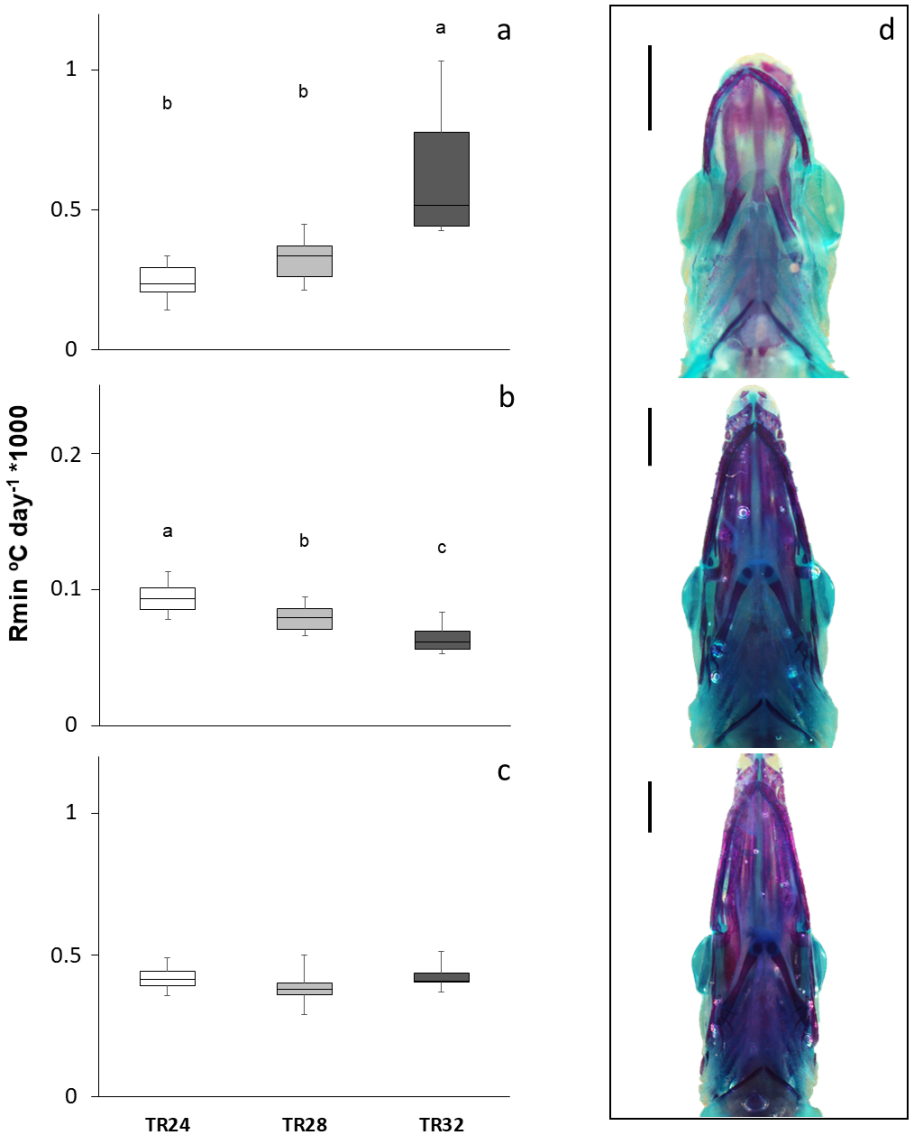

Figure 5. Skeletal development degree rates (expressed as median ± max/min values) per °C day (Rmin °C day^{-1} × 1000) in *Atractosteus tropicus* larvae when embryos were incubated at different thermal regimens. Cartilage quantity (ratio of blue stained surface over total larval surface) at hatching (**a**) and 16 (**b**) days post-fertilization (dpf); and bone structures (ratio of red stained surface over total larval surface) at 16 (**c**) dpf. Examples of tropical gar juveniles stained with alcian blue and alizarin red (ventral view) showing progressive ossification of cranial bones (**d**). Different letters at the top of the boxes denote significant differences ($p < 0.05$). Scale bar = 1 mm.

Regarding the bone ossification of the skeletal structures, it reflected the normal progression of increased ossification along larval development. At hatching, none of the skeletal elements showed bone ossification regardless the experimental group considered. First bone structures to start to be ossified were those involved in respiration and live prey capture (e.g., cleithrum and jaws; results not shown). Higher bone ossification was observed in *A. tropicus* larvae along larval development, and no differences were observed among the experimental groups at 16 dpf (median values ranging from 0.37 to 0.41, respectively; Figure 5c).

3.3. Body Morphology and Biometric Lengths

In order to decipher how advanced skeletogenesis under different thermal regimes during egg incubation might affect body growth and development, we further evaluated different body (including SL, body depth at cleithrum, and pre-orbital, pre-pectoral, pre-pelvic and pre-ana lengths) and cranial (including head length, head width, jaw length, jaw width, distance between ceratohyals at ossification front and length of ossified ceratohyal) biometric measures in *A. tropicus* (Figure 6; Supplementary Data S2).

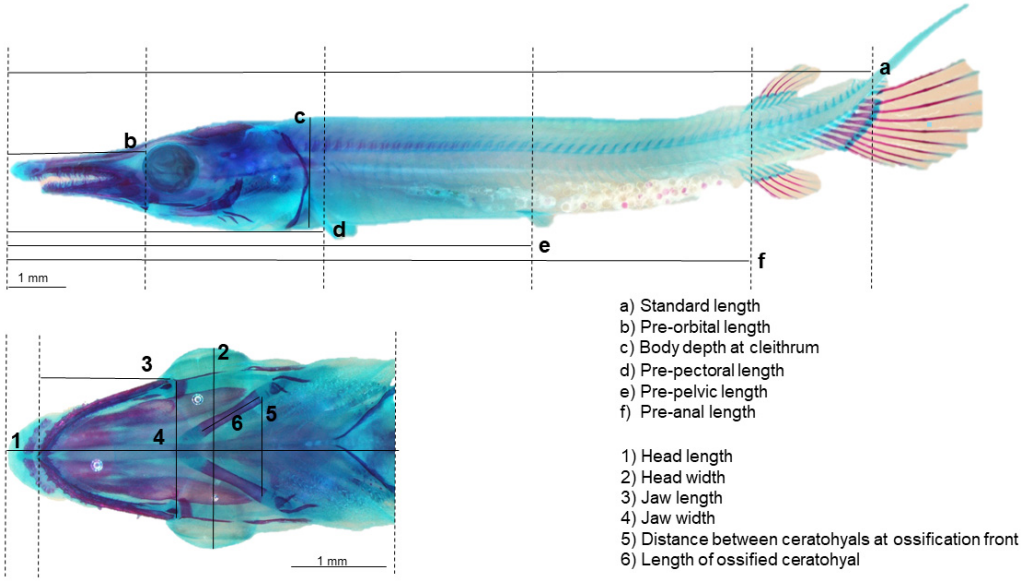

Figure 6. Different biometric measurements performed in the body and head of tropical gar (*Atractosteus tropicus*) larvae when embryos were incubated at different thermal regimens.

A principal component analysis (PCA) was conducted to identify the variables that explain the differences between larvae from the thermal groups at hatching and 16 dpf (Figure 7; Supplementary Data S3). Results showed how along the larval development, biometrics are altered depending on the TR to which *A. tropicus* embryos were exposed to. At hatching, Component 1 explained the 95.35% of the variability observed between TRs, and larvae from TR32 were clearly clustered separately from TR24 and TR28 larvae (Figure 7a). At the end of the trial (16 dpf), variance was mostly explained by Component 1 (74.37%), and includes pre-anal, pre-pectoral and head lengths as well as head width as the main contributing variables (Figure 7b).

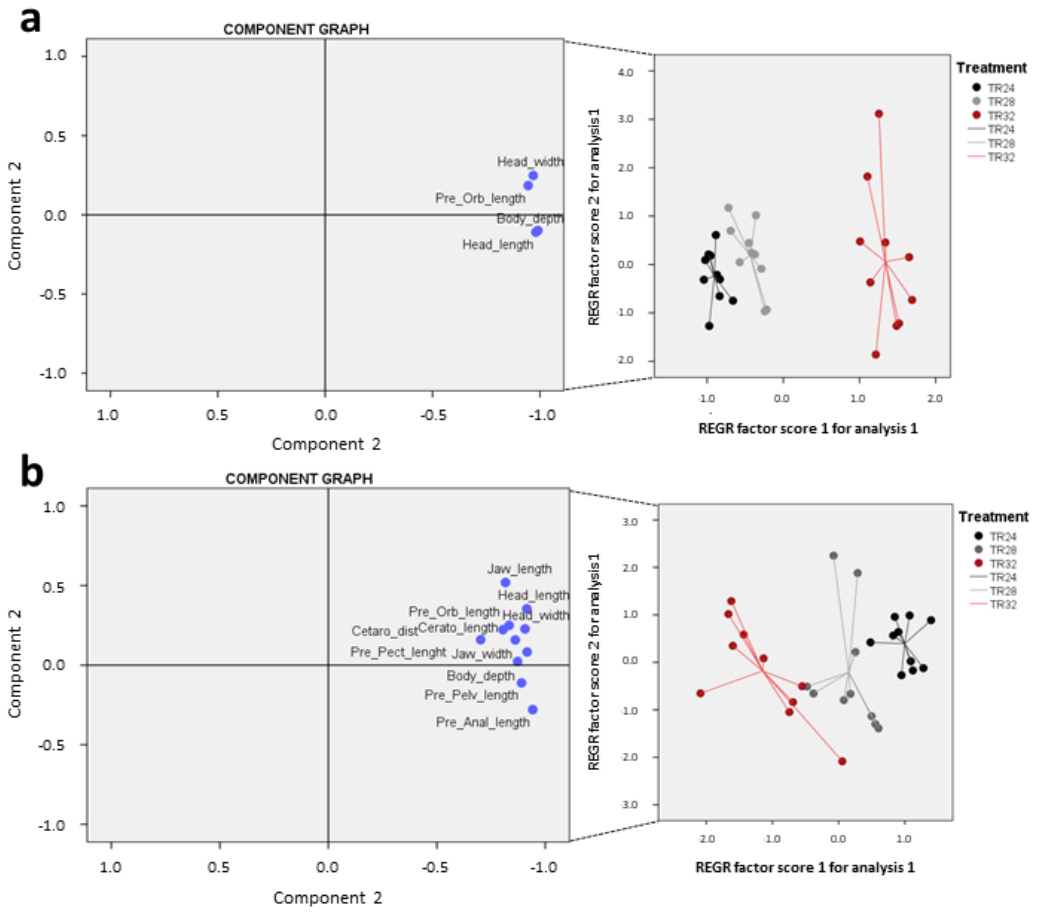

Figure 7. Scatter plot of experimental groups (with centroid distribution of the replicates) separated by the two principal components obtained from a principal component analysis (PCA), to reduce the dimensionality of the dataset, when embryos of *A. tropicus* were incubated at different thermal regimens. Representation is according to normalized biometric data at hatching (**a**) and 16 days post-fertilization (dpf) (**b**).

4. Discussion

Experimental approaches where the natural occurring alterations of the environmental conditions are mimicked more precisely are essential to unveil their potential consequences and for an accurate risk assessment. This is the case of studies focused on establishing the potential effects of the climate change over fish species. Most studies on this issue exposed contemporary populations of fishes to global warming (higher mean water temperature and ocean acidification) that progressively will take place in the coming years [14–18,37], neglecting the capacity of fish species to adapt to this environmental condition through the different generations. Here, we explored the effect of a more extreme event (although shorter in time, only lasting some days) related to climate change, the heat waves, a contemporary event which frequency and intensity are predicted to be increased [1]. This extreme climatic event has been previously suggested to represent one of the most enduring effects of climate change [19], increasing the mortality of aquatic and terrestrial organisms [20]. In this sense, the potential effects of a short alteration of the water temperature during the embryogenesis of tropical gar, an emblematic fish species in Central America, was explored to envisage their potential consequences in the short-term (16 dpf).

In the last decade, greater interest and efforts to increase aquaculture production of tropical gar have been placed due to the reduced capture of this species in the natural environments [28,29]. These efforts have been translated in a broader knowledge on the optimal rearing conditions and husbandry practices [23,24,38–42]. The optimal rearing temperature is 26–28 °C (Álvarez-González et al., unpublished results); thus, an alteration of just 4 °C

seemed to be a quite realistic approach to explore the effects of a heat wave, considering that broader thermal alterations were registered in freshwater bodies [43]. Good hatching rate and survival at 16 dpf (both > 80%) from the Control group (TR28) suggests that present results are reliable and not driven by low egg quality and/or suboptimal rearing conditions. Moreover, although slight differences were found in hatching rate when different thermal regimes were applied during embryogenesis, all experimental groups exhibited good hatching rates and survival, evidencing a great thermal tolerance (Δ8 °C) of *A. tropicus* embryos. Other fish species were shown to be more sensitive to thermal alterations. For example, in European eel (*Anguilla anguilla*) larvae, increasing temperature from 18 °C (suggested to be the optimal temperature) to 22 °C (Δ4 °C) affected hatching success, survival and growth, and accelerated larval development [44]. Additionally, increased temperature, from 15 to 21 °C (Δ6 °C), during embryonic development until hatching in Senegalese sole (*Solea senegalensis*) leads to an increased incidence of skeletal deformities [15]. Therefore, these results are in line with the reported high resistance of *A. tropicus* larvae to suboptimal rearing conditions with low requirements for water quality (regarding pH, dissolved oxygen, and pollutants, and with high ammonia tolerance) [24,45–47]. Nevertheless, thermal variation during embryonic development was shown to induce sublethal effects in fish growth, skeletal development, and body morphology.

Alteration of rearing temperature has been shown to dramatically alter fish metabolism, growth potential, muscle development, immune response, and even sex differentiation, among other processes [48–54]. Here, a different thermal regime during *A. tropicus* embryogenesis induced an altered growth in terms of WBW and SL that varied along with the larval development. Although encountered differences among experimental groups at 8 and 11 dpf might be due to differences in developmental stages (e.g., differences in the rate of yolk-sac resorption) and/or differences in the current rearing temperature; this might not be the case at hatching (where all the specimens from the different experimental groups are at the same developmental stage) or at 16 dpf, where the thermal regime in all groups has been restored to a common situation (water temperature at 28 °C). In general, the higher the temperature of egg incubation, the higher growth in all developmental stages analyzed unless at 16 dpf. The general trend of higher growth in larvae from TR32 is in line with previous studies showing higher growth with increased rearing temperatures [44]. The rationale behind the equal growth reached in larvae from TR24 and TR32 at the end of the trial (16 dpf) remains unknown; although the lower hatching rate, leading to higher availability of food and less competence within congeners in the tanks and/or a kind of compensatory growth, might partially explain how these two extreme groups reached similar growth (in terms of WBW and SL).

How the embryo and its skeleton are formed in gars have been previously described, particularly the head region, and mainly in the spotted gar (*Lepisosteus oculatus*) [55–62]. Some descriptions of the early development of *A. tropicus* have been published in the last decade regarding the buccal cavity [23]. In general, the skeletal development of *A. tropicus* was similar to its closest sister species, the Cuban gar (*Atractosteus tristoechus*), which has been particularly described in [62]. In contrast to other studies, no specific skeletal deformities were found in *A. tropicus* regardless of the thermal regime applied. This might be related to the higher thermal tolerance of the species or to the early developmental stage here evaluated (larvae of only 16 dpf). Unless for severe alterations of the skeletal development, in order for any disequilibrium to be translated in such a process in specific skeletal anomalies, a longer rearing time or a longer thermal alteration exposure (not only during embryogenesis) might be needed. In fact, skeletal deformities are regularly detected at larval or juvenile stages [63,64]. The axial skeleton of our larvae was not fully ossified at 16 dpf, this occurring at later stages (e.g., at 118 days post-hatching in *A. tristoechus*; [62]). Previous studies found that abnormal thermal regimes during embryogenesis induced skeletal deformities in *S. senegalensis*, mainly in the axial and caudal complex skeletons [15]. However, this species is already known to be prone to show a high incidence of skeletal deformities [65]. Indeed, skeletal deformities were only induced

with extreme temperatures during embryogenesis [66,67], or with moderated changes of thermal regimes applied during both embryogenesis and larval development [44] or larval development [14,16,17,68,69]. These effects on the skeleton have been suggested to be induced through a disruption in the armonic development of bone structures and muscle growth, with higher temperatures, faster muscle growth, and the subsequent increased mechanical load over the skeleton [70,71]. Another plausible hypothesis of increased temperature inducing skeletal deformities is that it also advanced the development of the skeletal structures, as shown in European eel [44]. Although skeletal development is a process with some plasticity [72], advanced or delayed ossification has been suggested to induce the appearance of skeletal deformities [73]. In the present study, an advanced skeletal development was observed in larvae from TR32, with increased cartilage quantity at hatching, and lowest at 16 dpf. These events might be related to an advanced endochondral ossification, the process by which several skeletal structures develop through a cartilaginous anlagen that will be finally replaced by bone tissue [74]. Other abiotic and biotic factors have been shown to advance skeletal development [13,75] that finally will induce abnormal skeletogenesis. Nevertheless, if the thermal alteration here performed during embryogenesis induced any skeletal deformity afterward remains to be deciphered.

Higher temperatures accelerate the rate of development, resulting in the appearance of some structures at smaller larval sizes [76], as it has been observed for fin formation and metamorphosis [77–79]. Higher temperature during embryogenesis induced an early hatching and mouth opening (1 or 2 days earlier than eggs incubated at 24 °C; please see Figure 1), as well as an advanced skeletal ossification at 16 dpf in *A. tropicus*. A similar effect has been recorded in the sister species *A. tristoechus* when eggs were incubated at 26–30 °C [80]. Further altered development related to the thermal regime applied in the *A. tropicus* embryos were recorded here. Biometric data showed how tropical gars from each thermal regime have a common body morphology, but distinct from each other. At 16 dpf, pre-anal, pre-pectoral, jaw, head and length of ossified ceratohyal as well as body depth at cleithrum and head width were the biometric variables that mainly explained the variability observed between larvae from different thermal regimes. Similarly, Cuban gar larvae reared at increasing temperatures (from 26 to 30 °C) also showed an accelerated inflexion points of different morphometric characters [81]. Since the survival of gars has been intrinsically related with their ability to catch prey, with a proper head elongation (longer heads with reduced heights and widths) during early stages warranting an efficient food capture [80]; present results suggest that heat wave occurrence during *A. tropicus* embryogenesis might be a risk factor for natural populations. In the long-term, alteration of the characteristic allometric growth along development of the gars due to increased temperature (specifically during embryogenesis) might alter the larval survival capacity of the wild populations.

Recently, long lasting effects of early temperature exposure have been identified in metamorphosing gilthead seabream, including decreased critical swimming speed and the incidence of caudal-fin abnormalities [82]. Although further research is needed to decipher the specific implications of the long-term heat waves during tropical gar embryogenesis, present results showed how a thermal regime alteration during embryogenesis induced lower hatching rate, increased growth, advanced skeletal development, and modified body morphology of tropical gar. These results suggest that heat waves related to climate change might be a source of biodiversity loss. Furthermore, due to the relevance of this species in Central America, the currently predicted climate change scenarios might have a detrimental economic and social impact on their communities.

5. Conclusions

In the present study, the effects of an altered thermal regime (mimicking an extreme event related to climate change, the heat waves) during *A. tropicus* embryogenesis were described. Through the comparison of larval performance, skeletal development and body morphometrics of larvae at the same developmental stages (hatching) and after the time

exposure to thermal alteration (16 dpf), alterations on hatching rate, skeletal development and body morphometry have been evidenced. Fish incubated during embryogenesis at higher temperatures exhibited an advanced skeletal development that was translated in a distinct body morphometry. Although to decipher the long-term implications of these alterations over fish survival and the population dynamics further research efforts are needed, present results anticipate climate change as an additional risk for wild fisheries conservation of *A. tropicus*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fishes7010016/s1, Supplementary Data S1: normalized mineralization degree. Supplementary Data S2: normalized biometrics for PCA. Supplementary Data S3: Data from larvae at hatching and at 16 dpf.

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Data Availability Statement: The data that support the findings of this study are available upon request from the authors.

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