Early Warming Stress on Juvenile Fish Impairs Testicular Development and Sperm Quality But Contrastingly Elicits Intergenerational Thermotolerance

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Abstract

The exposure of adult fish to warm or high temperatures is known to impair reproduction, yet the long-term reproductive impacts for treatments at early life are not well clarified. This study aimed to evaluate the effects of warm temperature (WT) during juvenile stage on gonad maturation, gamete quality, and offspring thermotolerance in rainbow trout. While the comparison of basic reproductive parameters in WT females did not reveal any kind of impairment, many WT males showed an atrophied, undeveloped gonad, or a smaller testis with lower milt volume; sperm quality parameters in WT males and deformity rates in the respective progeny were also highly affected. However, despite of such negative effects, many of the remaining progenies presented better rates of survival and growth when exposed to the same conditions as those of parental fish (WT), suggesting that thermal stress in *parr* stage males elicited intergenerational thermoresistance after a single generation . The present results support that prolonged warming stress during early life stages can adversely affect key reproductive aspects, but constrastingly increase offspring performance at upper thermal ranges. These findings have implications on the capacity of fish to adapt and to cope with global warming.

Keywords: Salmonids, germ cell, thermal adaptation, quiescent gonad, global warming

Main Text

Introduction

Water temperature comprises an important modulatory factor with critical roles on fish reproduction. During early life stages, the destiny of gonadal sex differentiation in gonochoristic species can be irreversibly driven towards either female or male by temperature, overcoming the predisposed sex determined by genotypic factors[1–4]. The appearance of sex-reversed fish and the concomitant skews in sex ratios has great implications from ecological perspectives due to their impacts on population structure[5].

Another effect of temperature on reproduction occurs through the regulation of reproductive cycle, either by promoting[6] or suppressing gametogenesis[7]. However, chronic exposure at those temperatures or acute thermal stress at even higher temperatures can cause opposite inhibitory effects on spermatogenesis[8,9]. In ovaries, although warm conditions are also able to hasten gametogenesis as in males[6], high temperatures that do show clear inhibitory effects on spermatogenesis do not necessarily induce comparable changes in oocyte development[10]. At sub-lethal, high temperature conditions, the survival of testicular somatic-supporting cells as well as the germ cells can be severely affected, whereby undifferentiated spermatogonia seems to be more tolerant to depletion by apoptosis than the differentiated ones, such as spermatocytes, spermatids, and spermatozoa[8,9]. The mechanism of heat-induced germ cell depletion is not well understood, but the Sertoli cells are likely involved, since apoptosis in these cells have been detected along with germ cells[8,9]. On the other hand, undifferentiated oogonia seems to be more susceptible than the

differentiated oocytes upon exposure to those temperatures[10], suggesting that high temperatures affect fish reproduction in a sex-specific manner.

Although the effects of thermal stress on fish reproduction have been assessed in some species, the implications on their reproductive capacity have not been well explored, especially in terms of gamete quality and offspring performance. Furthermore, the performance of offspring produced by fish exposed to warm water temperature has not been well evaluated yet. Research about how temperature acts on fish germ line, on gametes production or quality, and on progenies fitness might provide important insights for the evaluation of environmental changes (e.g., global warming) on wild populations and extensive aquaculture. On this regards, salmonids are an excellent group of fish to evaluate the effects of increasing temperature because they include several cold-water species which born in freshwater environments and then migrate downward to the river mouths until reaching the sea. Also, some species present variants that spend their entire life cycle in inland waters (landlocked) such as the rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*). But regardless of these different life cycles, salmonids have a high likelihood to experience warm temperatures and hypoxia conditions during the juvenile stage[11,12]. Some of those effects include impairment of steroidogenesis and vitellogenin synthesis[13], and advance or delay in oocyte maturation in females[14]. In case of males, impairment of spermatogenesis and reduced milt volume are reported in fish exposed to high temperature[15].

In this study, we used the rainbow trout as an experimental model to evaluate the effects of prolonged treatment at warm temperatures during juvenile stage on several reproduction parameters in female and male adults. We also compared survival and growth performances of the respective progenies under warm temperature in juveniles and the upper thermal tolerance in adults in order to investigate the intergenerational inheritance of thermotolerance.

Results

Effects of warm temperature on body growth and gonads of juveniles

At the end of the experiment (3 months) with F0 juvenile fish, warm temperature group (WT) showed lower survival rate than control group (CT) (70% and 97%, respectively), but growth parameters such as standard length (mean ± SD = 21.67 ± 9.41cm and 23.57 ± 8.76cm, respectively), and body weight (81.61 ± 28.79g and 71.73 ± 18.26g, respectively) did not differ significantly (Fig. 1A-B). The gonadosomatic index in females was lower in WT compared to CT (0.0007 ± 0.0003 vs 0.0012; $p < 0.05$) whereas no difference was found for males (Fig. 1C). Histological analyses of ovaries (Fig. 1D and 1F) and testis (Fig. 1E and 1G) did not reveal clear differences between WT and CT groups.

Reproductive parameters of adults

Females from WT group were significantly smaller than those from CT group and presented higher condition factor (K). Nevertheless, no differences were found in body weight (Suppl. Fig. 1). Fecundity rates, oocyte

mean weight, and the percentage of non-ovulated females did not differ between females of WT and CT group (Suppl. Fig. 2; Table 1).

WT males were significant smaller and had lower body weight than control males; hence, condition factor was significantly higher in WT group (Suppl. Fig. 1), as in females. Gonad dissection and histological analyses in some of those fish revealed three patterns of testis morphology. The first one consisted in a large whitish testis, similar to those of CT males (Fig. 2A). In the second pattern, a smaller whitish testis was detected and correlated with males with low relative milt volume (Fig. 2C). The third pattern was found for immature males and consisted in a thinner gonad with a reddish color (Fig. 2E). Histological analyses revealed the presence of some undifferentiated spermatogonia and a high quantity of spermatozoa in the first two patterns (Fig. 2B and 2D) whereas the third pattern was characterized by undifferentiated spermatogonia without any spermatozoa or spermatocytes (Fig. 2F and 2H), as revealed by immunohistochemistry analysis with an antibody for undifferentiated spermatogonia (Fig. 2G), which resembled the immature testis of F0 juveniles (Fig. 1E).

The relative milt volume was also lower in WT than CT group (Fig. 3A), with no difference in the concentration of spermatozoa (Fig. 3B). The estimated total amount of spermatozoa was reduced in about 57% in WT males (Fig. 3C). A proportion of males did not show secondary sexual characters and did not release any milt. These males were classified as immature males and they were not detected in any of CT males (Table 1). The morphological analysis of the sperm showed a 1.5-fold higher the percentage of abnormal cells in WT compared to CT group (59.99% and 39.56%, respectively) (Fig. 3D and 3E).

Computer analysis of sperm motility showed no differences for all parameters among 5 and 15 s, but at 20 s velocity was higher while wobble was lower in WT compared to CT males (Fig. 3F). At both 25 and 30 s, sperm motility was detected in 66.67% and 33.33% of CT males, respectively (Fig. 3G), whereas in WT group, no male showed sperm motility in any of these time points.

Fertilization, hatching, and abnormality rates in offspring from WT and CT broodstock

The comparative analysis of fertilization and hatching rates in crosses between CT females *vs*. CT males, CT females *vs*. WT males, and WT females *vs*. CT males showed no statistic differences for those parameters (Fig. 4A). Although the rate of abnormal fish also showed no statistic differences, the average values in the progenies derived from CT females *vs*. WT males were almost twice higher compared to other crosses (Fig. 4A).

Comparison of survival, growth, and thermal tolerance of F1 juveniles derived from WT and CT males

Survival rates of progenies from all WT groups were higher values than respective CT groups (Fig. 4B), except for WT1. Regarding growth parameters, both standard length and body weight values were significantly similar or higher in WT in relation CT groups (Fig. 4B); in the trial 3, which recorded the highest average temperature (19.2 ± 1.29 ºC), both WT5 and WT6, showed higher body weight values than respective CT groups. In the upper temperature tolerance test at 28 ºC, F1 sub-adults and adults from WT group presented a significant

superior effective time (ET), as demonstrated by the comparison between de survival curves (Fig. 4C). The LD_{50} values were 71.4% and 54.5% higher in WT group for sub-adult and adults, respectively.

Discussion

In this study, we demonstrated that exposure to warm temperature in male juveniles affect negatively not only survival, growth, and fecundity, but also the motility of spermatozoa and body formation in the respective progenies. Despite of those impacts, the progenies obtained from these males presented a remarkable high survival and growth rates when exposed to warm temperature compared to the control group, which supports an increased thermal tolerance after a single generation.

Long-term exposure to temperatures above optimal ranges causes detrimental effects on growth and survival in salmonids[14,16]. Another related impact comprises the depletion of the germ cells in juveniles and adults of many other fish species[9,10,17,18]. In our experiment, heat treatment at juvenile stage did not affect reproductive parameters in females, which was not the case of males. Those fishes showed quiescent gonads that persisted for two consecutive reproductive seasons, and reduced testes, impacts that were not observed in any fish from CT group and, to the best of our knowledge, in any teleost fish. Since the quiescent gonads possess germ cells, we can consider these fish as infertile, but not sterile. A deep analysis on the regulatory mechanisms is required to determine how this state is controlled, if these fish still possess the capacity to resume spermatogenesis, or if steroid hormones can make this fish to overcome such "quiescent" condition. The significant decrease in milt volume is likely due to spermatogonial apoptosis[9,10,18,19], because thermal treatment was performed at juvenile stage, when the immature testes are composed mainly by undifferentiated spermatogonia. Regarding the difference in heat sensitivity between females and males, similar responses have been reported in teleost and mammals[10,20,21], supporting the idea that spermatogonia exhibits higher heat-sensitivity than the oogonia.

Another intriguing effect of heat treatment was observed on motility parameters of spermatozoa, whereby WT group did not show any motility after 20 s (25 and 30 s). In addition, WT spermatozoa also had higher sperm velocity and lower wobble compared to CT group at 20 s. Such patterns could be associated with high energy consumption by WT spermatozoa at the initial 5 and 10 s, based on average motility which was about 19 to 26% higher, respectively. Thus, after 20s, WT spermatozoa would have lower available energy for motility. Reduced sperm motility after chronic heat treatment has been reported in fish and mammals[22,23]. In these cases, while thermal exposure encompassed mature adult males in which the spermatogenesis is under course, in our experiment, heat treatment was performed only during the pre-pubertal period. Therefore, the effects of temperature on spermatogonia persisted for about 18 months up to spermatozoa differentiation, affecting motility parameters, which suggest that warm temperature can compromise permanently the germ cells; in mammals, motility parameters were restored after a recovery period[22]. Those constitutive impacts observed in this study could be related to the particular thermal conditions during pre-pubertal developmental stage, which is characterized by high mitotic proliferation of spermatogonia[24].

The higher proportion of deformities in progenies from WT males could also be related to this timing, as dividing cells are more prone to errors in DNA under stress conditions[25]. Thus, spermatogonia might have acquired mutations that would be deleterious for proper development of embryos. On this regard, the analysis of sperm morphology revealed that the WT group had a higher percentage of abnormal gametes when compared to the CT group. Although we do not have evidence that deformed embryos were indeed generated by those abnormal spermatozoa, studies in mice have suggested that certain types of morphologically abnormal sperm with defects in head formation are associated with a higher frequency of chromosomal mutations[26,27]. Thus, the abnormal sperm morphology due to deleterious mutations in DNA of spermatogonia could account for higher embryo malformations in WT males-derived progenies.

In spite of many negative effects on reproductive parameters of F0 males, the juveniles of the respective progenies (F1) presented remarkable superior survival and growth performances when exposed to the same conditions as those of parental fish. Moreover, sub-adults and adults progenies showed longer effective time under sublethal temperature, supporting improved thermotolerance of F1 generation. These data may imply that warm temperature may have selected the F0 fish with genotypes that confer better thermal tolerance (WT group showed higher mortality), similarly to the reports of thermo-tolerant rainbow trout generated after several generations, as described both in the wild[28] and captivity[29]. Alternatively, beneficial mutations may have been inserted into spermatogonia's genome or, considering the fast acquisition of thermal tolerance, epigenetic modifications (methylation or non-coding RNAs) may have been "imprinted" onto spermatogonia in response to thermal stress and those "signatures" would be carried by spermatozoa DNA to F1 progeny. Moreover, the survival rates in the first trial did not differ between WT and CT males, which was caused by the appearance of white spot disease. However, in the second and third trials, WT males presented survival rates above 87% and superior growth rates compared to CT group, suggesting that chronic thermal exposure during juvenile stage may cause long-term effects on spermatozoa and on respective progenies. Whether such tolerance is brought about by simple selection of genotypes with high tolerance or as mentioned previously, by epigenetic modifications in the germ cell genome or transcriptome has to be further explored, but regardless of the mechanism, our results open a new perspective on the capacity of organisms to overcome long-term stressful conditions within a short-term scale. Although the final quantity of gametes and viable progenies can be severely reduced upon thermal stress, the remaining ones are able to acquire genotypes or epigenotypes that confer higher thermal tolerance into existing populations or even to replace completely these populations, thus establishing new populations.

In conclusion, this study showed that warm temperature exposure in juveniles causes deleterious effects on germ cells that persist even in adults by affecting gamete production in males. Apart from germ cell degeneration, germ cell quiescence or functional sterility has to be considered as another new impact of thermal stress in fish with impairment on reproductive capacity. Nevertheless, these negative effects may be counteracted by improved survival and growth performances of progenies at warm temperature, suggesting a clear tradeoff between parental fecundity and offspring thermal resistance. These results have implications on the adaptive capacity of wild populations to cope with chronic thermal stress associated to global climate change[30,31].

198
199 **Materials and Methods**
200 All experiments were conducted following the protocols approved by the Sao Paulo Fisheries Institute under
201 the CEEAIP 07/2018. The procedures for the care and use of experimental fish were also approved by the
202 institution's committee under the same CEEAIP 07/2018. This study was carried out in compliance with the
203 ARRIVE guidelines.
204
205 **Fish rearing conditions.**
206 This study was conducted at the governmental research hatchery Salmonid Experimental Station (SES) and
207 in a commercial trout farm, respectively located at neighboring Campos do Jordão and Pindamonhangaba
208 municipalities, Sao Paulo State, Brazil with a distance of 10.7 km between sites. These two sites were chosen with
209 the aim to provide a similar water source and at conditions that approximate to those of natural environments. The
210 hatchery (control water) is supplied by a stream running at the top of the Mantiqueira mountain at 1520 m above
211 mean sea level (AMSL), while the farm (warm water) is supplied by a stream descending abruptly to the base of that
212 mountain at 715 m (AMSL); both streams run through rainforest on governmental protected areas.
213 F0 fish used in this experiment were produced and maintained at SES until the beginning of experiment. 6-
214 months-old *parr* stage juveniles (body weight 22.62 ± 8.94 g; standard length 10.08 ± 1.38 cm; mean ± SD) were
215 divided in two groups (100 per group), whereby the group transferred to the trout farm corresponded to warm
216 temperature (WT) group (19.22 ± 1.65 ºC; mean±SD) and the one maintained at SES was the control temperature
217 (CT) group (14.74 ± 1.63 ºC; mean ± SD). The decision on applying thermal manipulation in juveniles instead of
218 larvae or adults was based on the higher likelihood of warm or high temperatures exposure at this stage, which
219 coincides with summer, as wild salmonids born in headwaters and generally migrate downward to the sea during this
220 season. Juveniles from both experimental groups were reared in 0.25 m^3 round tanks during 90 days with constant
221 water flow under natural photoperiod conditions. After this period, all animals were individually tagged (30 in WT
222 and 36 in CT group) and transferred to a single 2m^3 round tank at SES for an additional period of 15 months, when
223 most of fish reached sexual maturity. Gonad samples were collected during juvenile and adult stages for histological
224 analyses (see scheme in Fig. 5). Animals were fed commercial diet (45% protein) twice a day *ad libitum* during the
225 entire experiment.
226
227 **Histological analysis of juvenile and adult gonads.**
228 Both juvenile and adult fish were sacrificed with an overdose of benzocaine (0.2 g/L). Gonad samples were
229 dissected, fixed in Bouin's solution overnight at room temperature, and stored in ethanol 70% until further
230 processing. Samples were dehydrated in ascending ethanol series, embedded in paraffin, sectioned transversally at 5
231 µm thickness, and stained with hematoxylin-eosin (HE). For immunohistochemistry analysis sections were
232 deparaffined, re-hydrated, and submitted to antigen retrieval with citrate buffer (pH 6.0) in microwave for 10 min.
233 Endogenous peroxidase activity was blocked using 3% H_2O_2 solution in 0.1M PBS at room temperature during 30

min. Sections were rinsed in 0.1M PBS and blocked with horse serum from ImmPRESS Universal reagent (Vector Laboratories). Sections were incubated with the primary antibody #189, specific for undifferentiated spermatogonia (1:500 dilution)[32], during 16 h at 4 °C, rinsed in 0.1M PBS, and incubated with ImmPRESS Universal secondary antibody (Vector Laboratories) during 30 min at RT. Then, sections were rinsed in 0.1M PBS, incubated with ImmPACT DAB HRP substrate (Vector Laboratories), counter-stained with hematoxylin and mounted.

Analysis of growth and reproductive parameters in two-years-old fish.

Total body weight (BW; g) and standard length (SL; cm) were measured for each fish. The condition factor K was calculated as $K=100xBW/SL^3$ and the gonad weight (GW; g) was also measured for some fish to calculate the gonad-somatic index (GSI=GW/BWx100). Reproductive parameters were weekly screening by gentle abdominal pressure for ovulated females and spermiating males. Fish were anesthetized in benzocaine solution and the maximum quantity/volume of gametes were collected. This process was repeated every week only for males and for this reason the milt volume measurement was performed more than once in some fish, with the highest values considered as the milt volume for each male. Spermatozoa concentration was measured in a Neubauer chamber. For females, the total weight of ovulated oocytes and mean oocyte weight were counted. Those parameters were used to calculate the relative fecundity, which in females was presented as the oocyte number per gram of fish whereas in males it was presented as the milt volume in mL per g of fish.

The milt was diluted in buffered formaldehyde in the proportion of 1:1000 (milt: diluent solution) for spermatozoa morphological analysis. Samples were stained with Rose Bengal dye (3%) and analyzed under the microscope [33]. A total of 200 spermatozoa per male (n = 6 for each group in duplicate) were randomly selected and scored as "normal" or "abnormal", based on ovoid or triangular head shape, respectively[34].

Computer analysis of sperm motility.

The activation process for fresh milt was performed using 1 µL of milt and 400 µL of 0.01% $NaHCO_3$ (25 °C, 0.0 mOsm.kg-1), as activator solution. Six spermiating-males for each of WT and CT groups were randomly selected. Images and videos were captured as described in previous studies[35–37] and processed following the description of the components required for CASA application through a free software[35], with settings adapted from Lahnsteiner, F., Mansour, N. & Plaetzer, K, 2010[38]. Spermatozoa that presented curvilinear velocity (VCL), average path velocity (VAP), velocity in straight line (VSL), above 20, 10, and 3 µm s-1, respectively, were considered as motile. Besides those parameters the motility rate (MOT), straightness (STR), wobble (WOB), and progression (PROG) were also measured. Those analyses were performed for 1 s (100 images) at different post-activation times (5, 10, 15, 20, 25, and 30 s), with three videos for each male.

Evaluation of fertilization, hatching, and abnormality rates in progenies.

In the analysis using 2 years-old-fish, a total of 12 WT males were crossed with 13 CT females and conversely 6 WT females were crossed with 11 CT males. For those crosses, 27 g of oocytes were inseminated with

2 mL of milt. In the following year, three-years-old males from WT (n = 8) and CT (n = 12) were crossed with eggs from a single CT female, in order to evaluate whether the effects observed in progenies from two-years-old-fish were restricted to fish from that age. Spermatozoa were activated using a sodium bicarbonate solution (0.01%). Eggs were then hydrated with rearing water during 20 min. and incubated at 11 °C in UV-treated water under constant flow, following conditions described in a previous study[39]. The percentage of fertilization at eyed-egg stage, hatching rate, and abnormality in eleuteroembryos were quantified for each cross. Embryos with body deformations such as twisted body or with circular movements were considered as abnormal.

Survival and growth performance of progenies derived from WT males.

The survival and growth performances of F1 progenies derived from WT males were compared with those from CT males. Milt from 2 WT males and 2 CT males was used to artificially inseminate the same pool of oocytes for each trial. Egg incubation and larvae rearing followed the same procedure described in the previous section. For challenge experiments under warm temperature, 300 fingerlings were randomly selected per cross. This procedure was repeated in the second trial and third trial, with 2 WT and 2 CT males each. Fish were transferred to the same commercial trout farm at Pindamonhangaba and maintained for 3 months at the same rearing conditions used for thermal treatment in broodstock fish. The temperatures in the first, second, and third trials were 17.92 ± 1.57 °C, 18.74 ± 1.59 °C, and 19.20 ± 1.29 °C (mean ± SD) at the same rearing conditions as those of WT F0 males.

Analysis of upper temperature tolerance

Upper temperature tolerance was compared between CT and WT groups using 1-year-old sexually immature fish (n= 32 per group; BW ± SD = 305.8 ± 48.9 g and 295.5 ± 39.3 g, respectively) and 2 years-old maturing females (n = 15 per group; BW ± SD = 952 ± 184.8 g and 801.3 ± 295.1 g, respectively) from F1 generation. Fish were transferred into the temperature trial tank 24 hours prior to the beginning of the experiment for acclimation. Temperature was raised from ambient (16-17 °C) to upper lethal temperature of 28 °C, following the methodology described by Jackson *et al.* 1998[40]. Water flow was held constant and dissolved oxygen levels were maintained at a minimum of 8.0 mg/L. The time when fish started to lose the capacity to maintain the equilibrium was recorded for all individuals and considered as the 'effective time' (ET) in the zone of thermal resistance. LT_{50} (median lethal temperature) was calculated for each curve.

Sex genotyping by *sdY* amplification

Total genomic DNA was extracted from caudal fin using saline buffer method[41] and used for PCR amplification of *sdY* gene (sexually dimorphic on the Y chromosome). Primers and amplification conditions followed[42] and PCR products were electrophoresed in 1% agarose gel stained with Ethidium bromide.

Statistical analysis

Data were analyzed in GraphPad Prism (v. 5.02, GraphPad Software, USA) using the analysis of variance (One-way ANOVA), followed by Tukey's multiple comparisons test. All data were presented as mean ± S.E.M. The GSI in juveniles and spermatozoa parameters were compared by the t-student test. The comparison of effective time was performed by a survival test, with the curves compared based on Gehan-Breslow-Wilcoxon test. Differences were considered as significant for $p \leq 0.05$.

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Author Contributions

Conceived and designed the experiments: RSH, YAT, CO, AJB. Performed the experiments: RSH, YAT, AJB, TTY, ODMA, EAS. Analyzed the data: RSH, AJB, TTY, JIF, EAS. Wrote the paper: RSH, AJB, TTY. Reviewed the manuscript for content: RSH, JIF, CO.

Competing interests.

The authors declare no competing interests**.**

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Figures and Tables

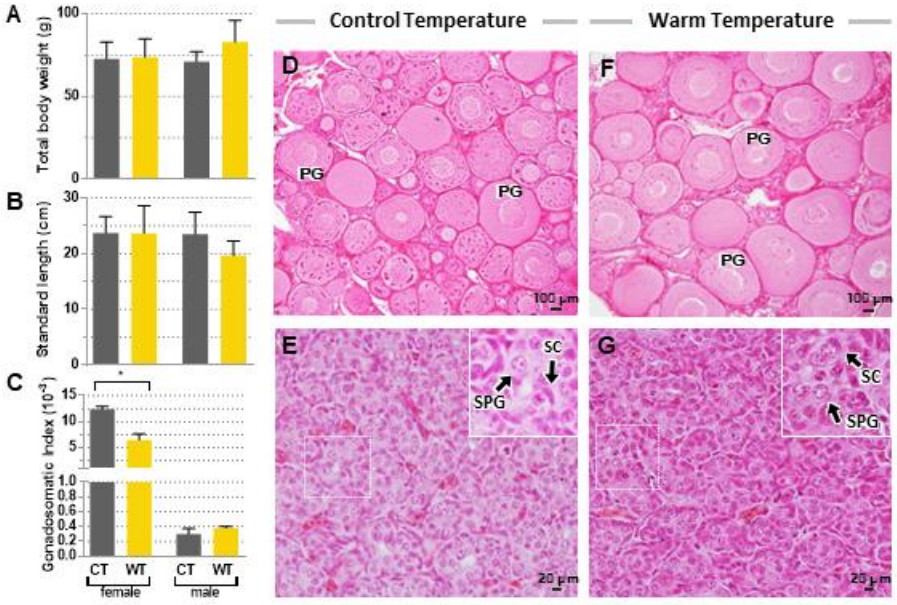


Figure 1. Growth and reproductive parameters of F0 juveniles from control and warm temperature groups. (**A**) Total body weight, (**B**) standard length, and (**C**) gonadosomatic index in females and males after thermal treatment. (**D** and **F**) Ovary and (**E** and **G**) testis histology from F0 juvenile fish three months after thermal treatment. The enlarged images in E and G refer to the respective dotted boxes. **PG**: primary growth oocytes; **SPG**: spermatogonia; **CS**: Sertoli cells

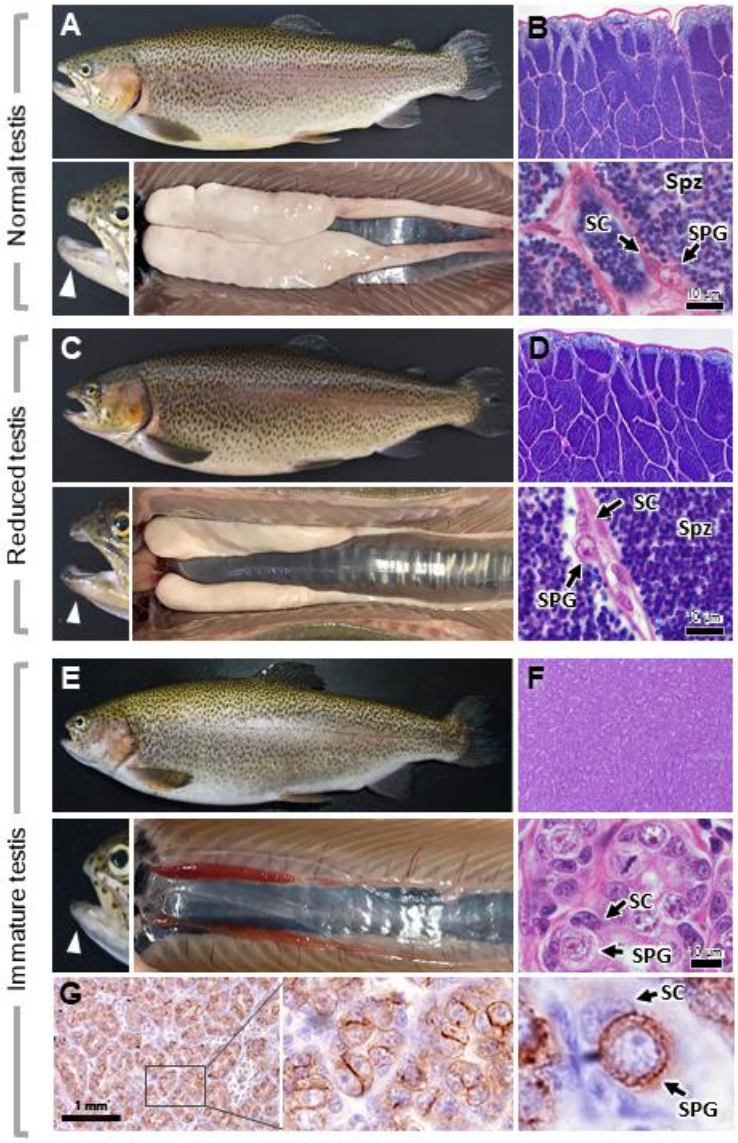
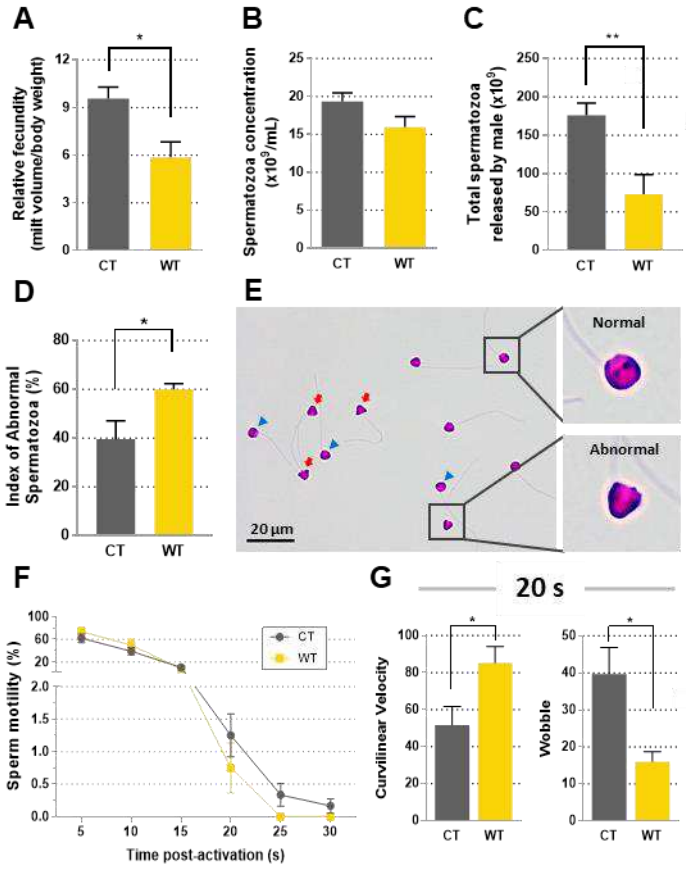


Figure 2. External body appearance and morpho-histological analysis of gonads in warm and control temperature males. (**A**-**B**) Adult trout with typical male phenotype presenting a hook-like jaw, a large whitish testis with abundant spermatozoa; spermatogonia and Sertoli cells are also present. (**C**-**D**) Adult trout with typical male phenotype as in (**A**), but with a smaller testis; testis histology presents similar aspect to (**B**). (**E**, **F**) Adult trout without male-specific secondary sexual characteristics, presenting a thin, reddish gonad, without spermatozoa; most germ cells are spermatogonia. **SPG**: spermatogonia; **CS**: Sertoli cells; **Spz**: spermatozoa. Arrowheads indicate the jaw, which has a hook-like morphology in **A** and **C** (mature males), but not in **E** (immature male). (**G**) Immunohistochemical detection of undifferentiated spermatogonia in immature testis with a cell surface marker.



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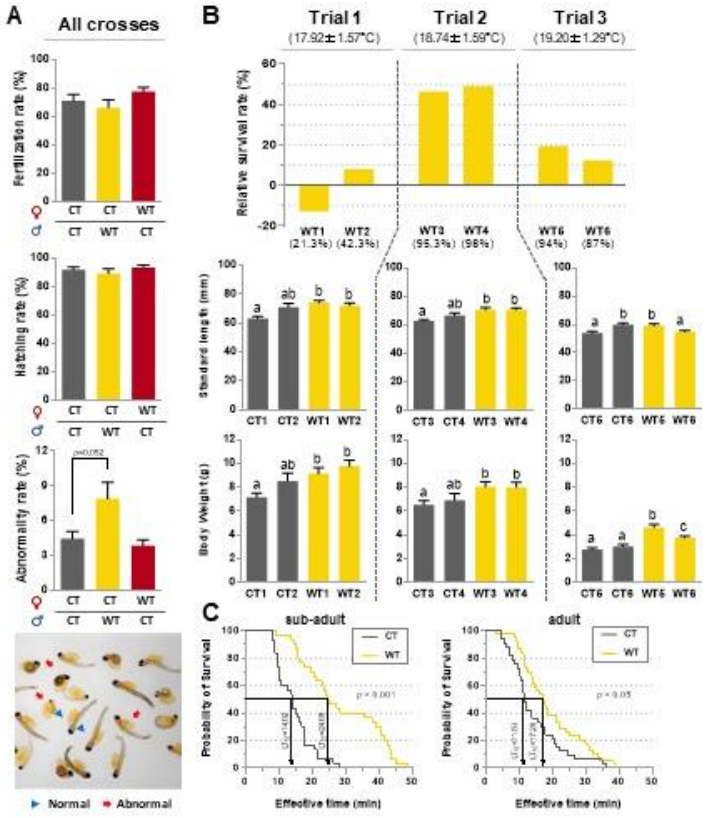

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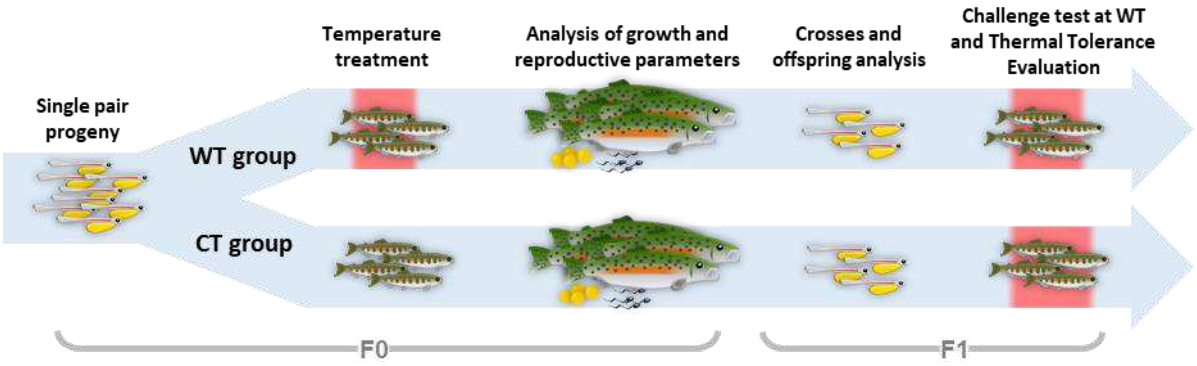


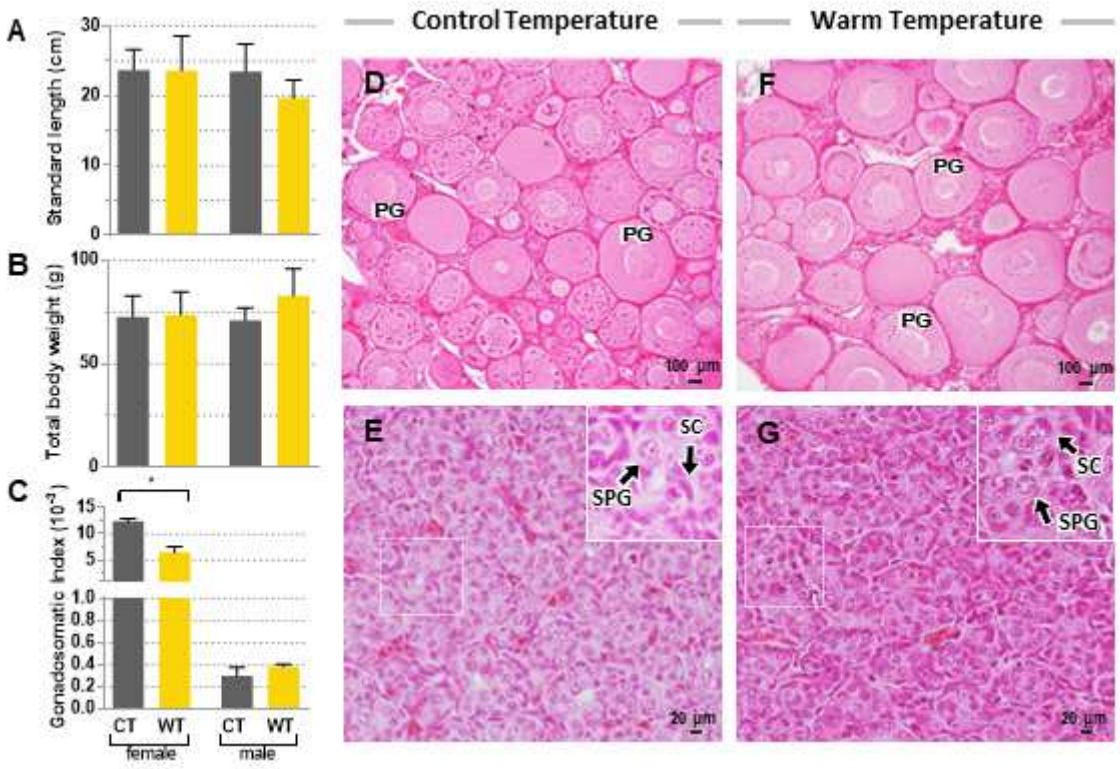
Figure 5. Overview representation of the experimental design. Rainbow trout juveniles obtained from a single pair cross were divided in two groups. At six-months, one group was exposed to warm temperature (WT) for three months in a local fish farm (Pindamonhangaba) while control group was maintained at Salmonid Experimental Station (Campos do Jordao). After this period, the treated group returned to control temperature until reaching sexual maturity for the analysis of growth and reproductive parameters. Finally, survival and growth performance of respective progenies at WT and upper thermal tolerance were evaluated.

Table 1. Number of mature and immature adult fish at both control (CT) and warm temperature groups 16 mo after treatment. Number in parentheses represent the frequency of animals in each group.

Maturity Stage	**Females**		**Males**	
	WT	CT	WT*	CT
Matures	6 (75%)	13 (81.25%)	12 (60%)	17 (100%)
Immatures	2 (25%)	3 (18.75%)	8 (40%)	0 (0%)

* Represent significant difference for Fisher's test considering $p < 0.05$.

Figures



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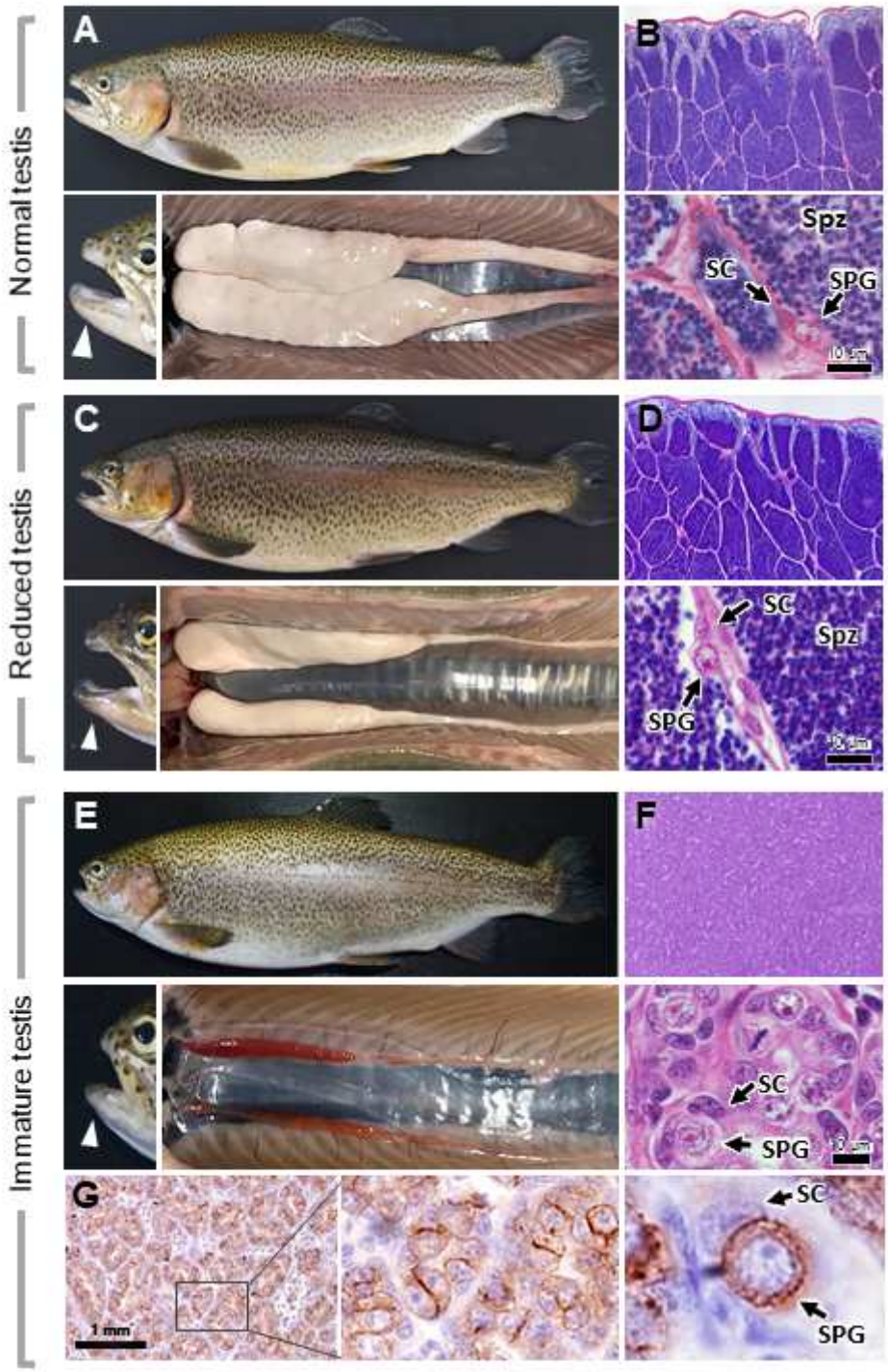

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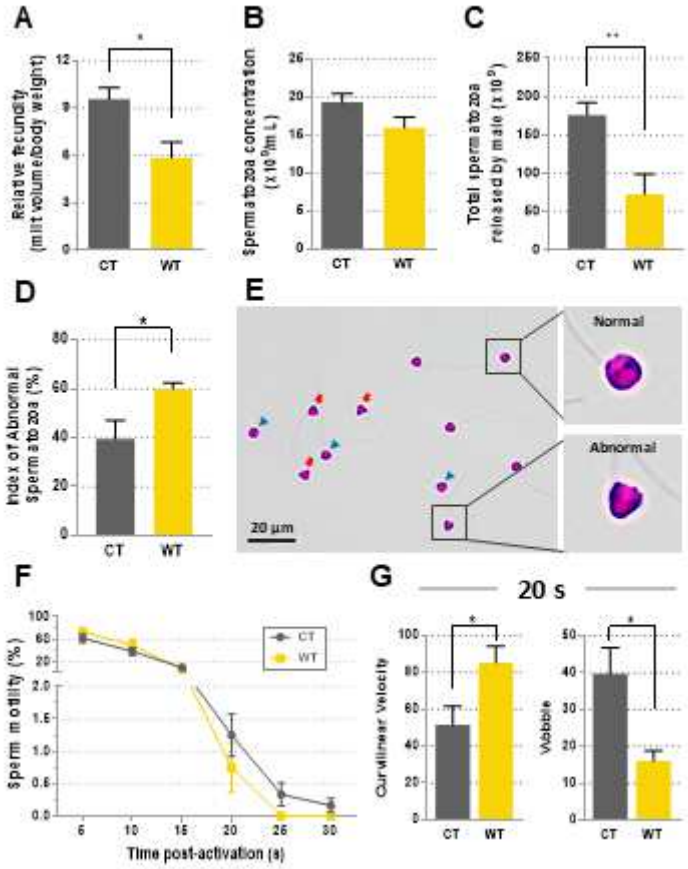

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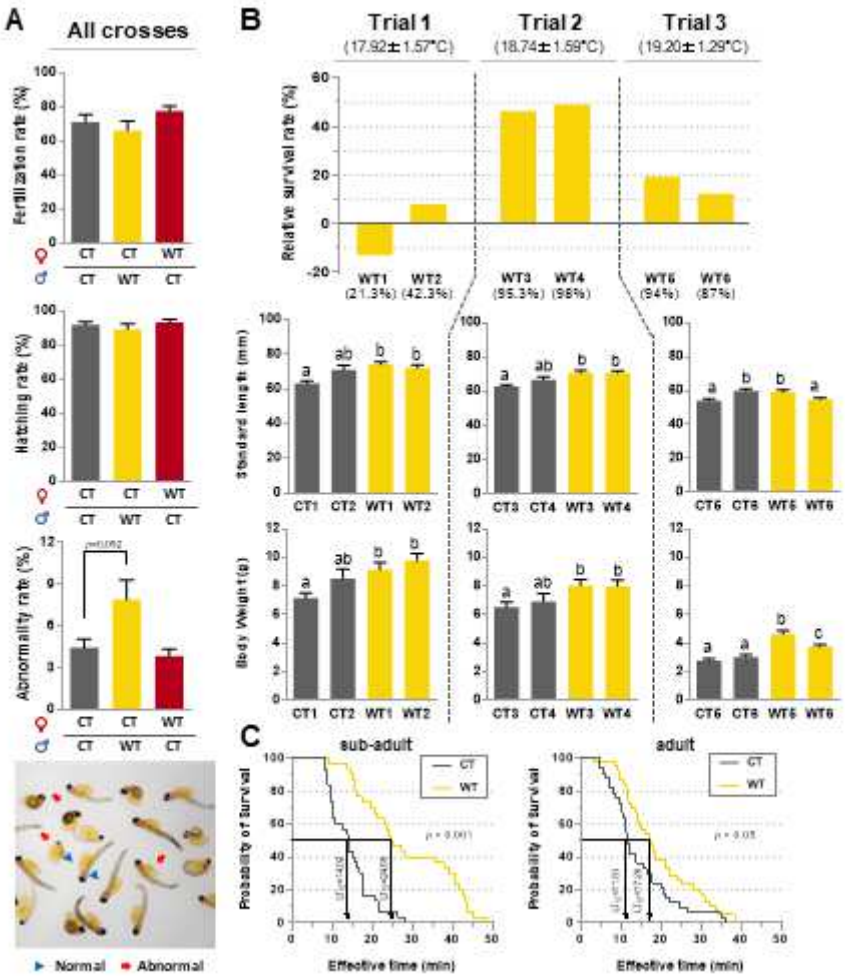

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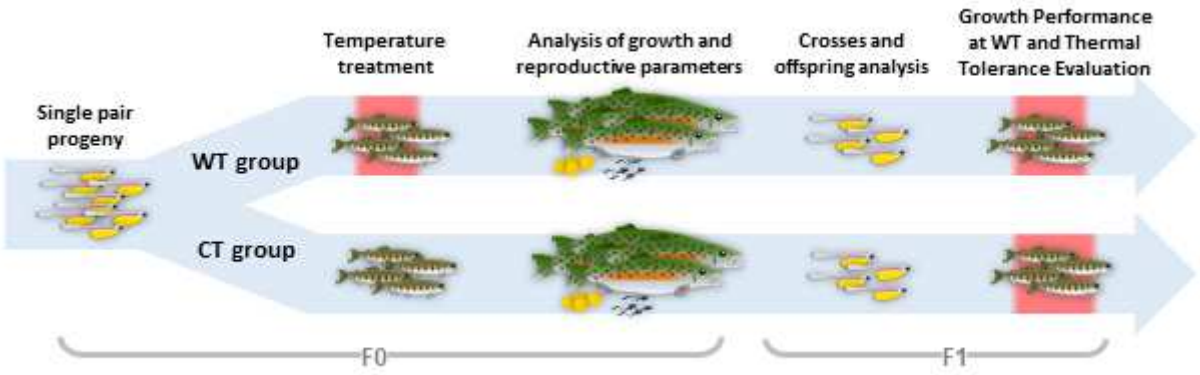

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