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Phenotypic response of male and neomale of *O. mykiss* parr subjected to 8º and 16 ºC water temperature during early life stage

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ABSTRACT

Water temperature is an important modulator of variation of phenotype in poikilothermic fish and therefore of body shape. The present study explores the phenotypic response of commercial *O. mykiss parr* when they are subjected to rearing temperatures of 8º and 16ºC under controlled conditions with the aim of demonstrating, with the use of Landmark-Based Geometric Morphometrics, the influence of rearing temperature on body shape. *O. mykiss* males and neomales from eggs to parr stages, belonging to the same family and strain, were reared at different temperatures (8ºC and 16ºC) and grouped according to: Group 16ºC/neomales (n = 15), Group 16ºC/males (n = 15), Group 8ºC/neomales (n = 15) and Group 8ºC/males (n = 15). Differences in body shape among sex and rearing temperatures were examined by geometric morphometrics and traditional morphometry. At 16 weeks, geometric morphometric analysis revealed a dimorphism, which was not detected by traditional morphometry, mainly in the dorsal and caudal fin area. This dimorphism was not observed in neomales. This difference may be the product of the potential for phenotypic plasticity and adaptation to genetically controlled environmental and local fluctuations, which affects to indicators of adaptation in unfavorable environmental conditions such a biomechanical action of swimming and propulsion. In conclusion, this study demonstrates the differential effect of 8º and 16ºC water temperature on lateral body shape in male *O. mykiss* parr during the early stages of body development and in controlled conditions.

1. Introduction

The shape analysis and their relationships with the environment helps to understand the origin, nature and causes of change in the patterns of variation (Zelditch et al., 2004). The first attribute of organisms that stands out in our eyes is their morphological variability, where several biological and non-biological factors underlie such variability interacting in a complex way to model individuals, populations and species (Bosch et al., 2014). Biological factors are the product of micro- and macro-evolutionary processes interacting with the environment. Thus, the size and shape result from the interaction between the effects and the quality of life during development with the genotypic combinations inherited from the parents, in a genetic framework. Populations and species, then, acquire their own morphological traits that differentially impact their biology, behavior and body shape (Klingenberg, 2010).

Water temperature is important in the development of aquatic species. It has been defined as the *key ecological master factor* for poikilotherms, particularly due to its influence on physiology, behavior, ecology, food consumption, growth, metabolic rate, and the immune system (Bowden, 2008; Eliason et al., 2013). Its effects on ecologically important saltwater fish have been described in studies relating to the increase in water temperature mainly due to human-driven global warming. Secondly, freshwater and socio-economically important species, such as Rainbow trout (*Oncorhynchus mykiss*; Walbaum, 1792), face greater challenges, especially acute ones, due to additional stressors such as anthropogenic habitat modification, pollution of waters and particularly due to intense commercial exploitation. The variation in the thermal range of the water can result in changes in the growth curves in salmonids (Boltaña et al., 2019; Fenkes et al., 2018) in the muscular structure and by consequent on body condition (Morissette et al., 2020). Quantifying and analyzing the morphological variation that results from such interactions is the main objective of morphometry. This objective is complemented with the quantification of the co-variance between

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morphology and other variables of genetic, climatic and geographical origin. The variety of environments produces differences in form between individuals, in this context, understanding how water temperature affects early body development of *O. mykiss* is of special attention, not only in wild species, but also in intensive and commercial exploitation species.

Two approaches were proposed to measure different aspects of the body shape of *O. mykiss* from ova to parr stages to determine whether temperature induces any aspect of morphological and potentially physiological change. One approach was the analysis of the landmark-based geometric morphometrics that has been applied to several studies involving environmental, biological, physical, chemical parameters in the morphology of wild fish and even in the characterization of populations. However, to our knowledge, it has not been applied in fish populations destined for industrial commercialization, nor has it been applied to demonstrate the effects of incubation temperatures frequently used in an intensive production environment such as the aquaculture industry. Furthermore, they have been contrasted with traditional linear morphometry. Both applied on the shape of the lateral face of the body of *O. mykiss* parr. It was hypothesized that changes in body shape caused by temperature can be demonstrated by applying geometric morphometrics based on landmarks. The present study explores the phenotypic response of male and neomale of *O. mykiss* parr when they are subjected to rearing temperatures of 8° and 16°C under controlled conditions over the course of 16 weeks with the aim of demonstrating the usefulness of landmark-based geometric morphometrics to demonstrate the influence of rearing temperature on body shape.

2. Material and method

2.1. Experimental animals and rearing conditions

This study was designed, monitored and analyzed at the Institute of Biology of the Pontificia Universidad Católica de Valparaíso and carried out in a fish farm in the Region de La Araucanía, southern Chile. Animal care and handling were carried out in accordance with the Chilean Law 20.380 and the Guidelines of the Department of Fisheries and Oceans Pacific Region Animal Care Committee about protection and use of animals for scientific purposes; this research only involved the use of trouts until smolt-stage of development.

The water temperature of the fish farm was 10°C and was stored in a head tank. A cooling and heating circuit was used to modify it, which allowed the study of two thermal regimes. The cooling circuit consisted in the passage of water from the acclimation tank 1 (50 m^3) - by means of a water pump (40 m^3/h) - to a Water Cooler Chilled by Air (HP3, 36000 Nominal BTU; 10.5KW; 208–230 Volts; 3 Phase; Flow GPM 20 min - 40 Max), and vice versa in such a way that the water, once re-entered the tank, presented a constant temperature of 8°C and at the same time, it was directed to two growth tanks (4 m^3). The heating circuit consisted in the passage of water from the acclimation tank 2 (50 m^3) - by means of a

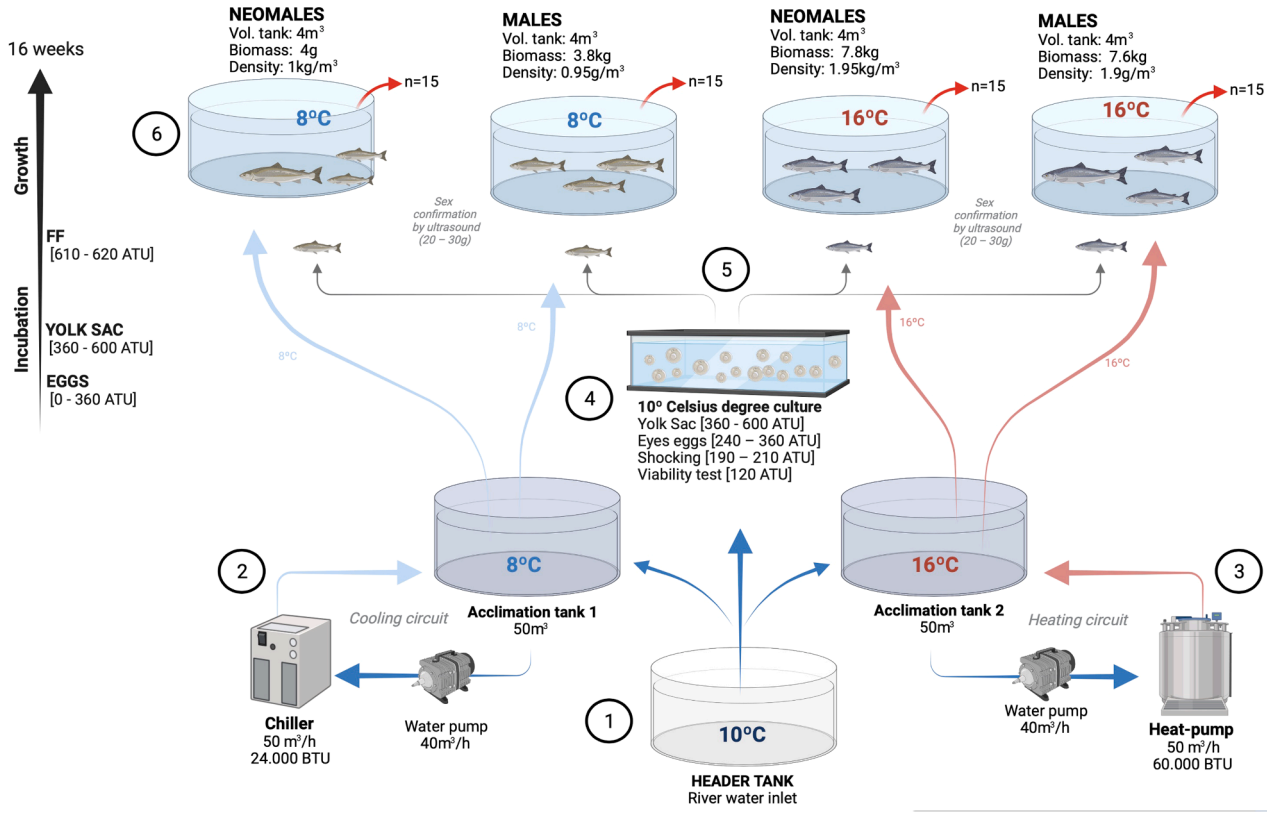

Fig. 1. Experimental design. (1) The water temperature of the fish farm was 10 °C and was stored in a head tank. (2) The cooling circuit consisted in the passage of water from the acclimation tank 1 - by means of a water pump - to a Water Cooler Chilled by Air, and vice versa in such a way that the water, once re-entered the tank, presented a constant temperature of 8°C and at the same time, it was directed to two growth tanks. (3) The heating circuit consisted in the passage of water from the acclimation tank 2 - by means of a water pump – to a Heat Pump and vice versa in such a way that the water, once re-entered the tank, presented a constant temperature of 16°C and at the same time, it was directed to two growth tanks. (4) After spawning, 2000 approx. fertilized eggs were incubated separately in both vertical flow (hatching jar) and horizontal trays incubation systems under standard industrial protocols: 10 °C water temperature, 8–12 L/min water flow through from head tank, absence of light and without mechanical actions. Eggs were given a viability tests and mechanical shock ('shocking') to separate dead from live. (5) Once the fish reached the first feeding stage (610–620 ATU) they were transferred to the growth tanks. Males and neomales were separated into two groups and incubated at 8° and 16 °C. (6) During the exposure period the fish were kept in fiberglass tanks (4 m^3) with flow through (water exchange rate of 2 times/h), natural photoperiod (16 h light) and subjected to conditions of similar feeding (type and frequency).

water pump (40 m^3 / h) – to a Heat Pump (HP53, 60000 Nominal BTU; 17.5KW; 230/3 Volts *per* phase; 3 Phase; Flow GPM 30 min - 60 max) and vice versa in such a way that the water, once re-entered the tank, presented a constant temperature of 16ºC and at the same time, it was directed to two growth tanks (Fig. 1). The water temperature in the growth tanks was measured twice a day using an Oxiguard Portable System (Oxyguard Handy Polaris).

After spawning, 2000 approx. fertilized eggs were incubated separately in both vertical flow (hatching jar) and horizontal trays incubation systems under standard industrial protocols: 10 ºC water temperature, 8–12 L/min water flow through from head tank, absence of light and without mechanical actions until embryos reach eyed-stage (trout: 45 accumulated thermal units [ATU]). Then, eggs were given a viability tests and mechanical shock ('shocking') to separate dead from live. Once the fish reached the first feeding stage (610–620 ATU) they were transferred to the growth tanks. Males and Neomales were separated into two groups and incubated at 8º and 16ºC until smolt-stage of development. During the exposure period the fish were kept under standard industrial conditions, that is, in gray fiberglass tanks (4 m^3) with flow through (water exchange rate of 2 times/h), natural photoperiod (16 h light) and subjected to conditions of similar feeding (type and frequency) and water flow. The feeding rate was a range from 2.9% to 6.1% BW/day and 3.3–6.2% BW/day in fish exposed to 8 ºC and 16 ºC, respectively. Cumulative food intake at 16 weeks was 0.57 kg and 1.17 kg in fish exposed to 8 ºC and 16 ºC, respectively. The fish were weighed every 3 days. Once the parr stage had been reached and after 16 weeks of exposure, fish from each group were randomly sampled, anesthetized, weighed, measured, photographed and subjected to body shape analysis. The groups were studied according to: Group 16ºC/neomale (n = 15), Group 16ºC/male (n = 15), Group 8 ºC/neomale (n = 15) and Group 8 ºC/male (n = 15).

The masculinization procedure to induce triploidy consisted of exposing a group of fertilized eggs to a hydrostatic pressure shock (7000 psi for 3–5 min) applied at 376 ºC/m. To confirm sex in male groups and the success of masculinization in trout of the Neomales group, at 20–30 g an ultrasound evaluation of the gonads was performed, in addition, a histological confirmation of the gonadal tissue after the body shape analysis.

2.2. Image acquisition

Each *O. mykiss* parr was photographed from a side view with a 16-megapixel Canon EOS Rebel T3i. Landmark entry and measurement were performed by a single observer in order to eliminate inter-observer error. Only specimens that presented a state of conservation that allowed obtaining measurements and assigning landmarks were analyzed.

2.3. Morphological characterization and statistical analysis

Differences in body shape among sex and rearing temperatures were examined by geometric morphometrics (Zelditch et al., 2004). We digitized 19 landmarks (Fig. 2) using the software program tpsDig2 v.2.21 64 bits (Rohlf, 2015a). To determine if the variation in the shape of the data set is small enough to allow the statistical treatment of the matrix of non-linear measurements, the tpsSmall v1.33 64-bit program was used (Rohlf, 2015b). A Covariance matrix was generated for the lateral view. To eliminate the effects of translation, rotation and scale the coordinates were aligned with the Generalized Procrustes Analysis (GPA). To describe the variations of the individuals in each category a Principal Components Analysis (PCA) was performed (Fig. 3). In order to evaluate the relative amounts of variation between individuals an ANOVA of Procrustes grouped by category was applied (Tables 1 and 2). To evaluate the differences between the individuals the discriminant function analysis (DFA) was applied based on their grouping in the categories sex and temperature (Fig. 4). The canonical variation analysis

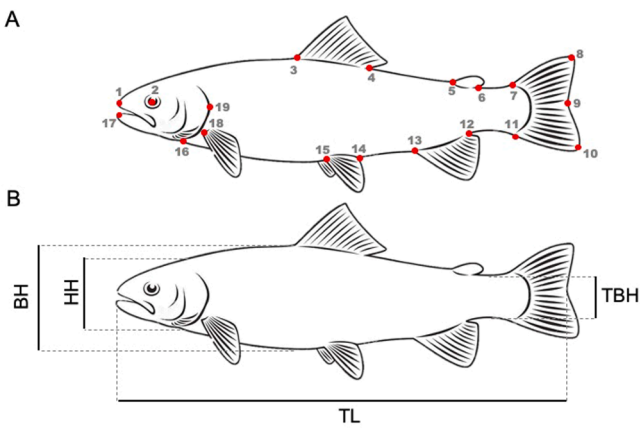

Fig. 2. *O. mykiss* illustration. A, Landmark positions (red points) on *O. mykiss* parr that were digitized twice and then averaged to minimize measurement error. B, Traditional morphometry: Head height (HH), Body height (BH), Tail-base height (TBH) and Total length (TL).

(CVA) was used for the simultaneous comparison of the sex and temperature categories for the total sample based on geometric morphometrics (Fig. 5). The analyzes were performed with MorphoJ software v1.05a (Klingenberg, 2011). To verify the normal distribution of the data, a Shapiro-Wilk normality test was applied, $p < 0.05$. For linear measurements, typical of traditional morphometry, the same fish were considered (without deformation) to allow an adequate comparison. Four linear measurements were analyzed (Fig. 2B): total length (TL), body height (BH), head height (HH) and tail-base height (TBH). A PCA was carried out from the data matrix of the linear variables measured. A MANOVA was performed grouping the specimens by rearing temperatures (8ºC vs. 16ºC) and sex (males vs. neomales) in order to evaluate significant differences. A non-parametric analysis of variance (two-way PERMANOVA) was performed with 9999 permutations to evaluate a probable dimorphism associated with sex (male and neomale) or temperature. Statistical analyzes and graphic representations were performed in PAST4.03 (Hammer et al., 2001) and GraphPad Prism 9.0 for Mac OS X (GraphPad Software, San Diego CA).

3. Results

3.1. Geometric morphometrics

The data presented a normal distribution. The ANOVA of Procrustes applied to *O. mykiss* showed the existence of significant differences between the specimens categorized by "temperature" (Table 1; $p < 0.0001$). The category "sex" did not present significant differences (Table 2; $p = 0.0175$). The PCA revealed that in the category "sex", the overlapping of data contained in Components 1 and 2 did not show differences with respect to the form, which can be interpreted as the absence of sexual dimorphism (Fig. 3A), while in the category "temperature" there was a slight tendency to the segregation of two sets of points with some overlap (Fig. 3B). The DFA detected differences in the category "temperature" ($p = 0.0184$). The sex category did not show differences ($p = 0.7175$) (Fig. 4). The simultaneous analysis of the category's "sex" and "temperature" by the CVA clearly separated the males exposed to 8ºC and 16ºC into two populations ($p < 0.0001$; Fig. 5; Table 3). Neomales exposed to 8 ºC and 16 ºC showed a slight overlap between specimens ($p = 0.1352$).

3.2. Traditional morphometry

The variables studied are described in Fig. 6y Fig. 7. In the linear variables studied, the univariate ANOVA detected differences between groups of the same sex exposed to different temperatures. Conversely, no

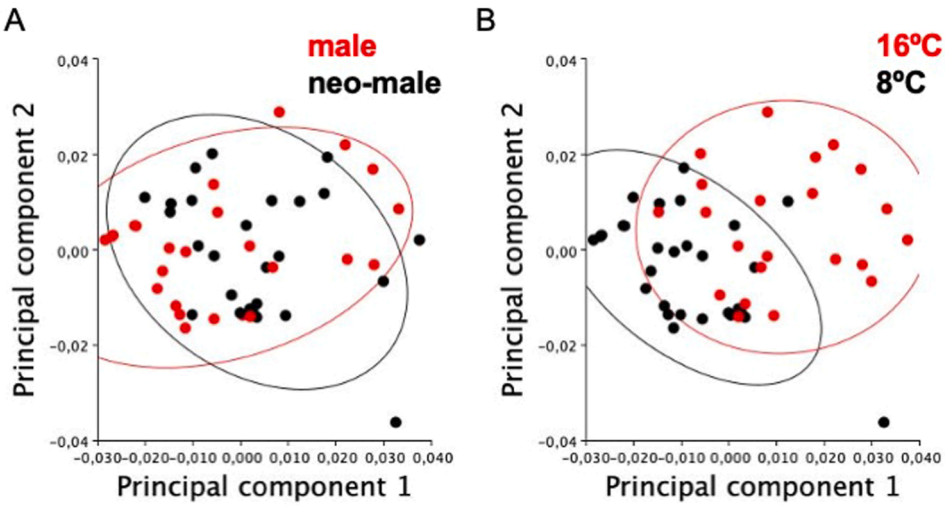

Fig. 3. Deformations of the body shape of *O. mykiss* represented in the first and second Principal Component by sex (A; n = 30) and temperature (B; n = 30).

Table 1
ANOVA of Procrustes by temperature (lateral view).

	Centroid Size				
Effect	SS	MS	df	F	*p*-values
Individual	2561447,92	2561447,92	1	23.21	< 0.0001
Residual	5407506,15	110357,26	49		
	Shape, Procrustes ANOVA				
Effect	SS	MS	df	F	*p*-values
Individual	0,00632335	0,0001756487	36	6,03	< 0.0001
Residual	0,05136419	0,0000291180	1764		

Table 2
ANOVA of Procrustes by sex (lateral view).

	Centroid Size				
Effect	SS	MS	df	F	*p*-values
Individual	874906,73	874906,73	1	6.04	0.0175
Residual	7094047,34	144776,47	49		
	Shape, Procrustes ANOVA				
Effect	SS	MS	df	F	*p*-values
Individual	0,00174103	0,0000483619	36	1.52	0.0243
Residual	0,05594652	0,0000317157	1764		

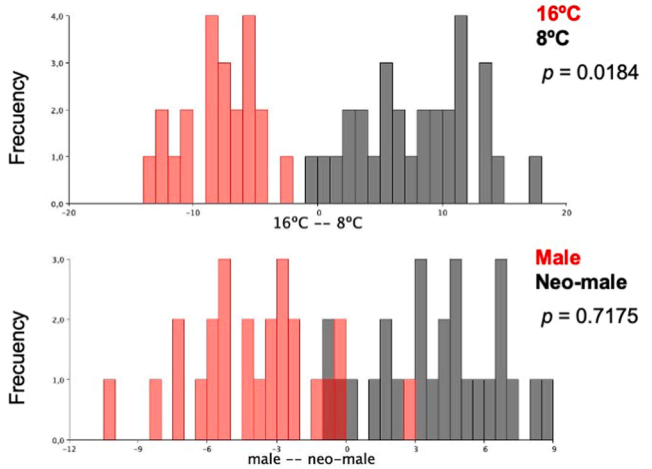

Fig. 4. Discriminant analysis function graph for the categories temperature (n = 30) and sex (n = 30) in *O. mykiss* (n = 15/group).

differences were detected between groups of different sex and exposed to the same rearing temperature (Fig. 6). Regarding the multivariate analysis, the PCA indicated that the first two components explain 93.52% of the total variance observed (Fig. 8). This indicates that *O. mykiss* grouped by sex show an overlap between specimens, while the PCA grouped by temperature distinguishes two different populations in shape, both concentrating on the axis of component 1. A MANOVA was performed by grouping the specimens according to the rearing "temperature" (8 ºC vs. 16 ºC) and sex (males and new males) categories to evaluate possible significant differences. The MANOVA did not detect significant differences between males and neomales ($p = 0.3065$), however, they did detect differences in the "temperature" category (8 ºC vs. 16 ºC; $p = 0.0000646$). Regarding dimorphism, the two-way PERMANOVA confirmed the existence of significant differences in the morphology of *O. mykiss* rearing at different temperatures ($p = 0.0001$), while it did not detect differences associated with sex ($p = 0.459$). Since there are no replicated values in each sample, it was necessary to assume that there was no interaction between variables (sex - temperature; $p = 0.1231$) i.e. the bidirectional PERMANOVA analysis assumed that temperature had the same effect, if any, in each sex.

4. Discussions

The present study demonstrated the usefulness of geometric morphometrics to describe the effect of temperature on body shape of male and neomale of *O. mykiss* parr subjected to 8º C and 16 ºC water temperature during early life stage. Males and neomales of the same progeny were exposed to 8 ºC and 16 ºC from the ova to parr stages. Subsequently, geometric morphometrics was applied and additionally traditional linear morphometry. The result of this study agrees with our hypothesis that geometric morphometrics provides substantial, robust and additional data on body shape in *O. mykiss* farmed for commercial purposes.

From the data exploration through geometric morphometrics, a significant difference between groups was determined in the category "temperature" according to the DFA and CVA. Regarding CVA, the difference was observed in males exposed to 8 ºC and 16 ºC, which was not observed in neomales. Regarding the traditional linear morphometry, the results of the MANOVA and PERMANOVA were, in general, consistent with the data obtained through geometric morphometrics, particularly in the differences in the "temperature" category, however, it was not consistent with the data obtained regarding dimorphism associated with temperature. The PCA of the linear measurements revealed that in the "sex" category, the overlapping of the data contained in

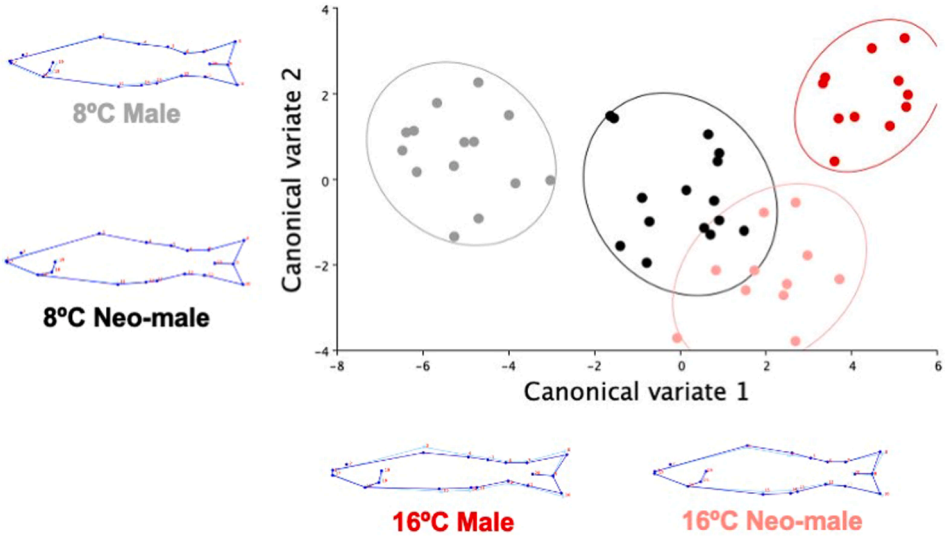

Fig. 5. Canonical variate analysis for lateral view in *O. mykiss* body shape. Explaining 93.52% of the variance of the data in its two first exes, 4 ellipses = 95%CI (n = 15/group).

Table 3
p-values from permutation tests (9999 permutation rounds) for Procrustes distances among groups: (CVA).

	16 ºC, male	16 ºC, neomale	8 ºC, male
16 ºC, neomale	0.8481		
8 ºC, male	< 0.0001	< 0.0001	
8 ºC, neomale	0.0097	0.1352	0.0006

Components 1 and 2 did not show differences between males and neomales, which suggests an absence of sexual dimorphism. On the other hand, in the category "temperature" a segregation of data was observed, forming two sets of points with no overlap, which suggests a dimorphism based on sex.

It is important to note how the morphology changes (both traditional and geometric) with the size of the fish, such relationships are different in the different treatment groups. What makes this important is that differences in temperature will lead to differences in growth rates and therefore size. *O. mykiss* exposed to 16ºC were observed robust (heavier, possibly rounder-bodied) during the course of the experiment, which may lead to better survival through an increase in energy reserve and availability and an increase in muscle mass. to improve swimming ability. The caudal fin region (relative to body length) increased as parr developed (relative to total length). Both variables showed detectable differences in the category "temperature", *id est*, an increase in the size of the area of the base of the tail fin and a change in shape induced by

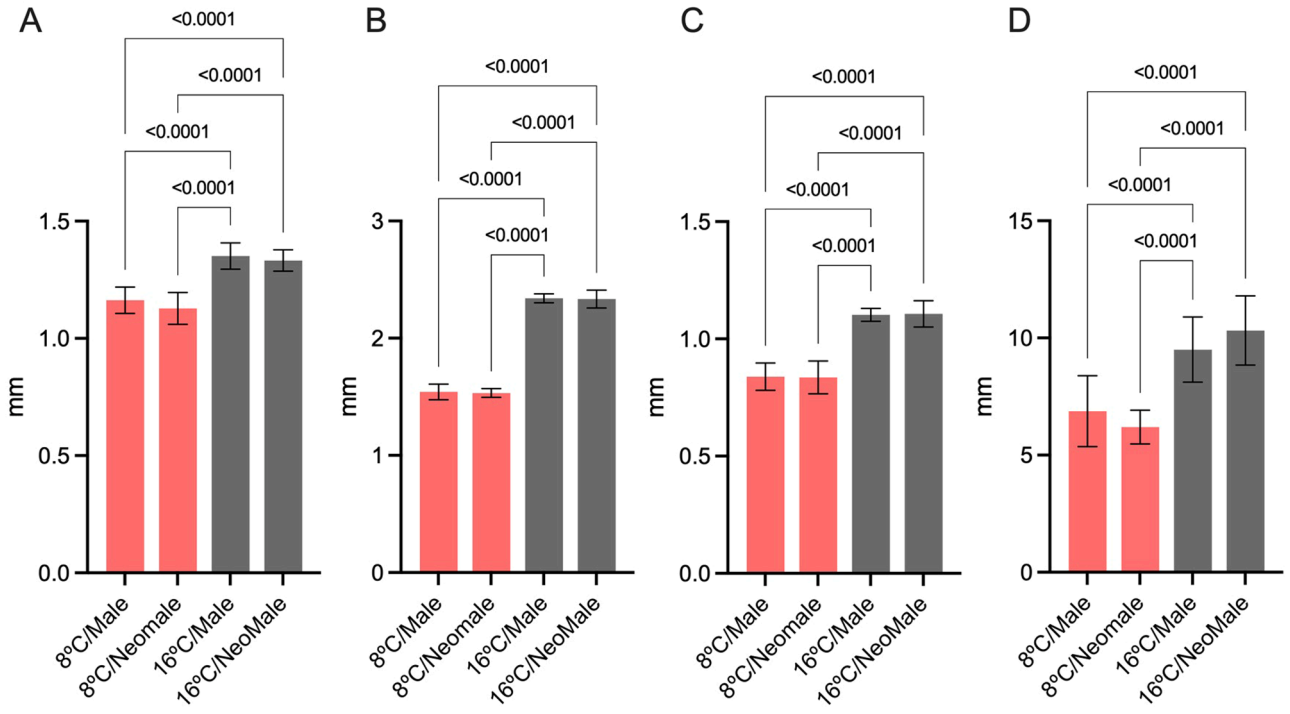

Fig. 6. Traditional morphometry in *O. mykiss*. Linear variables: A, Head height (HH). B, Body height (BH). C, Tail-base height (TBH). D, Total length (TL) (n = 15/group).

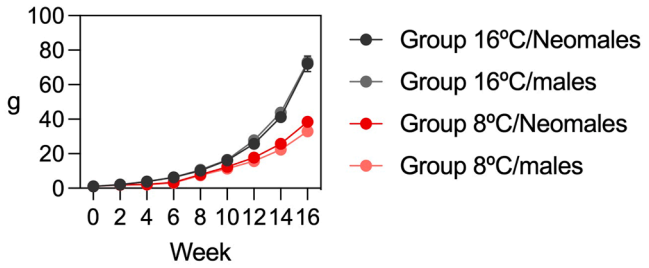

Fig. 7. Average weight of *O. mykiss* reared at two temperatures. Error bars represent plus and minus one standard deviation from the mean weight (n = 15/group).

temperature in the lateral face of *O. mykiss*. Pakkasmaa and Piironen (2000) attribute these phenotypic changes to the specific reaction rules of each species. Although this is probably the case, and given the effect of temperature on the physicochemical characteristics of water, it is also possible that its effect on the availability of dissolved oxygen in the water confounds our results. Therefore, there may be fundamental differences in the norms of reaction of salmonids to environmental variation (e.g. water velocity and oxygen availability) depending on the age and stage of development of the subjects (Grünbaum et al., 2008). It is possible that the sample size of n = 15 individuals in each study group was too small to detect the real effect of temperature on body shape using geometric morphometrics methods and this possible limitation should be considered when interpreting the results.

The difference between groups of *O. mykiss* reared at 8ºC and 16ºC in size and body shape was also observed in the linear metric variables and in the contributions of Components 1 and 2 of the PCA. The variable TL is the most important and it was the one that presented the highest data variability followed by TBH. These results can be evidenced in the canonical variables 1 and 2 of the AVC mainly by the morphological variation of the region of the base of the dorsal and caudal fins. The shape of the fins is related to the biomechanics of swimming and the force exerted at the base of the tail during propulsion (Anttila et al., 2014), which could be explained by the differences in body weight. It should be noted that in swimming fish, such as *O. mykiss*, the lateral shape of the body represents the size of the main propelling surface and is therefore considered an indicator of the efficiency and maneuverability of the propulsion (Webb, 1984).

At higher water temperature, the metabolic rate of fish increases concomitantly with growth (Leonard et al., 2000; Lee et al., 2003; Steinhausen et al., 2008). These effects are closely related to muscle development, so it is not surprising that morphometric differences are observed in the O. mykiss studied. The growth rate of fish depends on the accumulation of muscle mass, which constitutes more than half of the total body weight and contains mainly protein (Weatherly and Gill, 1987; Mommsen, 2001; Bureau et al., 2006; Johnston et al., 2011). Studies indicate that high temperatures produce an increase in the rate of muscle development without affecting the relative time of formation of anatomical structures (Elliott and Elliott, 2010; Réalis-Doyelle et al., 2016). Even the thermal impression during the early stages of development affects somatic growth and the distribution of the number and size of muscle fibers in juvenile fish, however, the underlying mechanisms are unknown (Macqueen et al., 2008; Johnston et al., 2009). In this regard, it is important to highlight the role of temperature on the endocrine system and growth, where the exact mechanism in which temperature affects hormone synthesis is unknown, it is estimated that environmental temperature promotes growth through an effect direct on GH secretion leading to an elevation of plasma IGF1, the effect of which occurs only when fish are in optimal nutritional conditions (Gabillard, 2005; Kawauchi and Sower, 2006). Recent studies indicate that high water temperatures (16–18 °C approx.) accelerate linear growth and therefore body weight in *O. mykiss* promoting the turnover of muscle proteins, both by synthesis and by protein degradation, particularly by the calpain-dependent pathway (Lysenko et al., 2020). All these effects of temperature, together with other abiotic and biotic environmental factors, have a considerable effect on skeletal muscle, which can be expressed in microscopic morphological differences, either in density or size of the muscle fiber, and therefore have a macroscopic impact.

Despite the results observed in males, this study does not provide evidence of a differential effect of rearing temperature on morphological characteristics in neomales. Studying why the new males present homogeneous populations, in terms of body size and shape, when exposed to different rearing temperatures (8ºC and 16ºC) is relevant. The absence of a morphological effect on the lateral form of the new male suggests that the phenotypic adaptability induced by water temperature was limited. The neomales are used in intensive production in order to homogenize growth until reaching a desirable productive size, since rapid growth is associated with an increase in the early maturation rate, particularly in males (Berejikian et al., 2010; Guiry et al., 2010). The evidence obtained in the present study shows homogeneity in the body shape of the newborn, which is maintained even in the presence of variable environmental factors, such as the differentiating effect of temperature. Keeley et al. (2007) concluded that phenotypic variability is mainly caused by genetics, while environmental heterogeneity plays a secondary role in the presentation of morphological differences between populations. In non-wild life fish, such as those used in the present study, this phenotypic variability is probably diminished. This would explain the high degree of adaptability observed in wild fish under local environmental conditions, but ultimately detrimental under commercial or intensive production conditions. This raises the question whether captive-bred or domesticated *O. mykiss*, such as those used in the present study, retain the same potential for phenotypic plasticity and adaptability to recently induced environmental fluctuations (e.g., conditions of temperature variability).

The biochemical, physiological and growth profiles in males and neomales demonstrated dissimilar results and could explain the difference in body shape observed in *O. mykiss* reared at 8ºC and 16ºC. Studies report that males and neomales have similar sex hormone profiles, fertilization rates, and weights (Hopkins and Todd, 1991; Nynca et al.,

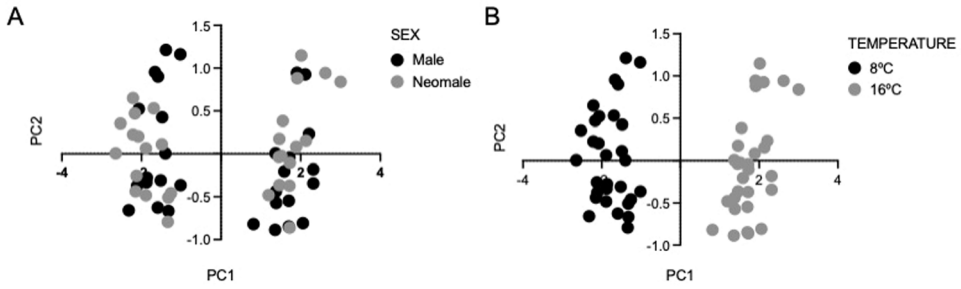

Fig. 8. Analysis of Principal Components Analysis of linear variables. Component 1 and Component 2 for the lateral view in *O. mykiss* in categories sex (A) and temperature (B).

2012b). Males present lower concentrations of 20β-dihydroxy-4-pregnen-3-one (Espinosa et al., 2011) and higher antitrypsin, lactate dehydrogenase, protein and osmolality activity than those described in neomales (Nynca et al., 2012a, 2012b). The main growth regulators, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-I), are related to water temperature (Gabillard et al., 2003, 2005). Additionally, metabolic activity and therefore oxygen demand increase positively with temperature and negatively with size and age in fish (Burton et al., 2011).

Variations in water temperature affect the concentration of dissolved oxygen in the water, with a lower concentration being observed in warm waters, resulting in low gill uptake and periods of hypoxia, influencing productive performance and consequently body growth (Burton et al., 2011; Sambraus et al., 2018), generating periods of stress that trigger inflammatory cascades in the intestine, responsible for failures in the absorption of nutrients and probably the appearance of secondary infections, which will induce an unfavorable conversion food, and modifying the metabolism and therefore, altering the energy cost, influencing the food consumption and therefore the growth rate. Finally, it should be considered that the increase or decrease in temperature will influence according to the age and reproductive status of each species, and the productive performance will vary, according to the evolutionary and adaptive process (Myrick and Cech, 2005).

This study is, to our knowledge, the first to demonstrate by landmark-based geometric morphometry the phenotypic response of males and neomales of *O. mykiss* parr subjected to 8º and 16 ºC water temperature during early life stage, under controlled and productive conditions. Our results provide clear evidence that *O. mykiss* grown at different temperatures exhibit body dimorphism. The temperature affected the growth rate and morphology irrespective of the sex. This dimorphism was observed only in males grown at different temperatures and was not detected in neomales. Evidence of the influence of water temperature on body shape with a robust morphometric tool is relevant to validate the technique for diagnostic, research, nutritional or pharmacological purposes. In conclusion, this study demonstrates the differential effect of 8º and 16ºC water temperature on lateral body shape in males *O. mykiss* parrs during the early stages of body development and in controlled conditions. According to the evidence, these temperatures would not have a differential effect on the shape of masculinized *O. mykiss*.

Declarations

Ethics approval

The approval of the research ethics committees was not required to achieve the objectives of this study since the fish used were euthanized for causes unrelated to this research.

Authors' contribution

Study conception, design, data collection and morphometrics analysis were performed by Paulo Salinas. The first draft of the manuscript was written by Fernanda Molina, Carlos Sandoval and Nicolás Hernández and all authors commented and contributed on final versions of the manuscript. All authors read and approved the final manuscript. PS was responsible for supervision of study.

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Code availability

Not applicable.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Field collection of fish was conducted under according to guidelines of the Department of Fisheries and Oceans Pacific Region Animal Care Committee and Chilean Law nº20.380 about protection and use of animals for scientific purposes. This article does not contain any studies with human participants.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data Availability

Not applicable.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2021.100996.

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