Temperature affects growth allometry and developmental patterns in the brown trout (*Salmo trutta*) fry: a multi trait approach

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Abstract

By means of a multi trait approach (survival rate, development time, morphometric characters, energetic content and ossification), we examined the development from hatching to metamorphosis of *Salmo trutta* at three temperatures (4, 6 and 12 °C). At each temperature, the analysis of allometric growth identified two different phases during the development of the fry corresponding to two groups of individuals for whom we compared the traits of development (morphology, ossification, malformation and energy allocation). The first growth phase was slower at 4 °C and the second phase of growth slower but locomotion and swimming were present. At 12 °C, fry seemed to show a disadvantage in growth (low level of ossification, and of energetic content) during the first growth phase but compensated during the second growth phase and reached a "normal" size but with low energy reserves. The different patterns of development at the different temperatures showed a developmental plasticity during the early stages of the brown trout. Moreover, this study showed that the use of morphometric characters such as L_m (total body length at metamorphosis) alone did not allow to determine precisely the stages of development and that the multi-parametric method seems the most adequated.

Key words

Life traits, morphology, osteology, energetic content, survival rate, development time, fish development.

Introduction

During early development in fish, changes in body shape lead to the formation of characteristic morphologies and allometric growth patterns. These important quantitative morphometric changes take place during early ontogeny, and are responsible for a progressive transformation of recently hatched specimens from a larval body shape to a juvenile or adult form in a relatively short time. This suggests that growth functionally optimized for survival is a common feature among fish as it was postulated by Osse & Van den Boogaart (2004). This development is regulated by gene expression and influenced by the environment (Gilbert & Bolker, 2003) resulting in different phenotypes with differential relative growth rates defined as allometry. However, under unfavorable developmental conditions, these growth patterns may also lead to structural defects affecting the development of fry and the juvenile's survival (Koumoundouros et al., 1999). In this context, the study of the allometric growth patterns of fish from hatching to the juvenile stage has proved to be a useful tool for evaluating fry development and quality under different environmental conditions (Gozlan et al., 1999; Simonović et al., 1999), because both the body and the organs respond in similar ways, either directly or indirectly, to environmental factors that regulate the rate and the duration of cell growth and division. This developmental response to the environment is a form of phenotypic plasticity, phenomenon whereby a particular genotype produces different phenotypes in different environments (Shingleton et al., 2007).

From a morphological point of view, metamorphosis is defined as a suite of abrupt or gradual changes in morphological characters, such as disappearance of fin fold, acquisition of the adult complement of fin spines and rays, adult pigmentation, squamation among others (McCormick et al., 2002; Urho, 2002; Ditty et al., 2003). From a structural and functional perspective, metamorphosis is associated with a shift in allometric growth or shape (Nikolioudakis et al., 2010). As Urho (2002) postulated, the use of a single morphological

61 trait to infer metamorphosis represents an impediment to the unambiguous definition of larval
62 life, whereas a clear definition of the transition from larval to juvenile development is very
63 important particularly when one begins to apply ontogenetic scales for inter-specific
64 comparisons of morphological, sensory and behavioral development (Nikolioudakis et al.,
65 2010). As the timing of transformation for most characters associated with metamorphosis
66 may overlap (Ditty et al., 2003), Nikolioudakis et al. (2010) suggested the use of multi-
67 character approach in addition to the classical allometric approach to precisely calculate the
68 length-at-metamorphosis (L_m, hereafter), a parameter based on scoring a suite of
69 morphological characters based on Principal Component Analysis results (Nikolioudakis et
70 al., 2010; 2014).

71 Water temperature affects ectothermic animals, through effects on the rate of
72 biochemical reactions; thus, it influences physiological characteristics such as rates of
73 development and growth, and traits associated with them (Jonsson & L'Abée-Lund, 1993).
74 Temperature can also serve as an ecological timer initiating behavioral reactions such as
75 migration from one habitat to another, as well as affecting fish feeding and spawning, and
76 finally, population recruitment. In this context, water temperature is one of the environmental
77 variables of major importance for the ecology of salmonid species (Jonsson & Jonsson, 2009).
78 Early development is a complex process that requires a large amount of energy, particularly
79 during the endogenous feeding phase when the maximum of the energy is allocated to growth
80 (Needham, 1931; Finn & Fyhn, 2010). Thus, in the present study, authors decided to evaluate
81 the effects of temperature during the early development of brown trout (*Salmo trutta*), a cold
82 stenothermal salmonid species (Réalis-Doyelle et al., 2016), on the energetic content of
83 embryos, early development (ossification of the skeleton) and allometric growth patterns of
84 different body regions associated with respiratory, feeding and swimming functions in fry.
85 This approach was conducted by the combination of bivariate and multivariate allometric

analyses on brown trout fry reared at three different water temperatures (4, 6 and 12 °C). In addition, authors decided to test the hypothesis whether different L_m could correspond to similar stages of development in this species when reared at different temperature regimes.

Material and methods

Biological material and experimental design

Brown trout specimens were obtained from the second generation of wild broodstock of trout kept in the Research Institute for Nature and Forest (INBO) in Belgium. Eggs were obtained from seven females (2.5 ± 0.4 kg in body weight and 58.0 ± 3.9 cm total length) and seven males (3.2 ± 0.8 kg, 61.0 ± 4.7 cm). Oocytes and sperm were obtained by stripping; the oocytes of each female were then fertilized with only the milt of one male. Water temperature was 8 °C and the oxygen concentration 9.0 mg $L^{-1.}$

Eggs were incubated in three recirculating water systems or hatcheries (110 × 64 × 186 cm) in the research facilities of the UR AFPA at the University of Lorraine in Nancy (France). Each hatchery unit contained 8 racks (44 × 11 × 7 cm) and had a flow rate of 4 m^3 h^{-1} and water was sterilized by means of ultraviolet lights. The experiment was performed in a controlled temperature room at 15 °C. These facilities and experimental procedures were conformed to the French legislation (agreement number C54-547-18). During the experiment, water temperature and dissolved oxygen levels were daily checked (probe ODEON, range of probe is 0.1 °C). Oxygen levels were kept above 9.0 ± 1.0 mg L^{-1}. The photoperiod was natural, increasing from December up to April. Each hatchery unit was randomly assigned to the different experimental temperatures: 4, 6, and 12 °C, that were chosen according to the global warming scenario from brown trout recently described in Réalis-Doyelle et al. (2016).

Fertilized eggs (N = 5849 ± 510, per hatchery unit) from the seven females were randomly distributed within each of the three water-temperatures. During the experiment, dead eggs and fry, uneaten food and feces were removed twice per day. Fry were fed with inert diet after their emergence (mixed feeding period). Feeding was conducted twice a day and particle size was progressively adjusted according to fish size and manufacturer's instructions [Neo supra S AL1 (pellet size: 0.3 - 0.5 mm) and AL2 (pellet size: 0.5 - 0.8 mm), Le Gouessant, France].

Fish staging

In this study, five different periods between stages were considered in order to evaluate the effect of temperature on trout development: P1, from oocyte fertilization to hatching; P2, from hatching to fry emergence; P3, from emergence to first food intake (onset of the exogenous feeding); P4: from first food intake to complete exogenous feeding (after total yolk sac completion), and P5, from exogenous feeding to metamorphosis (juvenile morphology). As embryonic and larval development is not a synchronic process, a determined stage was considered when at least 50 % of the population reached the endpoint of each period (P1-P5) (Ojanguren & Braña, 2003; Lahnsteiner, 2012). The five stages were sampling points and 150 specimens for each temperature were sampled, measured, weighed and analyzed, with the exception of the group of fish kept at 12 °C where mortality rate was higher and only 100 specimens were taken per sampling point.

Biological parameters

Developmental time and survival rate

The development time for each tested temperature (4, 6 and 12 ºC) was calculated at end of the trial by counting the time-lapse in days between fertilisation and metamorphosis, and

135 expressed in days and degree-days in order to compare data regardless of the different
136 temperatures. The relative time (RT) was calculated between two consecutive stages (in days)
137 as the percentage of time spent between them with regard to the total development time [RT =
138 number of days between two stages * 100 / global development time (days)]. Survival rate
139 (SR) was calculated for each temperature and each developmental stage as follows: SR = 100
140 - (cumulated number of death between two stages * 100) / Number of eggs at fertilisation. In
141 these formulae, unfertilised eggs were removed from the analysis. The daily survival rate
142 (DSR) was also calculated for each time period (P1-P5) by taking into account its duration:
143 DSR = 100 - (Number of death per days * 100) / Number of days of the period.
144
145 **Morphological description, growth measurements and malformation**
146 After each sampling, trout fry were anesthetized with tricaine methanesulfonate (MS-222)
147 according to Mendiola et al. (2007) and measured with a binocular magnifying glass (Optika
148 model Ni equipped with a Sony camera, CCD-Lw1235C, Sony Japan). Body morphometrics
149 were taken from digital images (1,600 dpi) using the image analysis software Archimed@
150 (Microvision Instrument, France). The morphological development of fry was described
151 according to Vernier (1969), whereas morphological data were completed with behavioral
152 observations (Supplementary data 1) in order to more precisely determine the stage of
153 development of fry. Eight different morphometric characters associated with locomotion,
154 vision and feeding were measured (Figure 1): total body length (TL), measured as the distance
155 between the snout to the tip of the tail; head length (HL), distance between the tip of the snout
156 and the opercular edge; head height (HH), the larger height of the head measured
157 perpendicularly to mid-section of the eye; eye diameter (ED), obtained as the average of the
158 maximum and minimum diameter of the eye orbit; trunk length (TrL), distance between the
159 edge of the operculum and the anal opening; myotom height (MH), the distance

perpendicularly to the body axis between the anus and the base of the dorsal fin; tail length (TaiL), the distance between the anus and the end of caudal fin; and caudal fin height (CaH), distance perpendicular to the axis of the body between the upper and lower ends of the caudal fin. All characters were measured to the nearest 0.01 cm, while deformed specimens were discarded. Three types of malformations were determined based on Holm et al. (2005) and Lahnsteiner (2012): on the vertebral column, yolk sac and others. Vertebral malformations included: scoliosis corresponding to a lateral concave curvature of the lumbar region of the skeleton, lordosis which is concave curvature of the lumbar region and kyphosis a convex curvature of the thoracic region. The yolk sac malformation is characterized by an oedema in the region separating the yolk sac from the digestive tube. Other malformations included: head malformations (jaw malformations: progmatism or retroprogmatism and no eye or more than two eyes), fin malformations (no fin or atrophied fins), shorter size, haemorrhages and twin forms (two or three fry for one yolk sac or two, three or four heads for one fry).

Energetic value

Dry weight (DW) of eggs (N = 192 per temperature) and fry (N = 24 per temperature) were measured at each of the selected five sampling points. Each sample was dried at 60 °C for 72 hours. In the case of fry, their dry weight was obtained after separating the yolk sac from the body in order to obtain two separate energetic values. Elemental analyses were performed using a Flash EA 1112 elemental analyzer (Thermo Finnigan 2003; Thermofisher Scientific, USA) at the Laboratory of Physical Measures (University of Montpellier 2, France). Each sample (25 mg) was divided in two parts: one for carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) analyses and the other to determine the total energetic content. Each sample was analyzed in triplicate (methodological replicates). The oxygen fraction (O % dry matter) was computed as follows: O = 100 - (C+H+N+S). Energetic values (J mg^{-1}dry weight) were

computed from C, H, O and S fractions and expressed as percentage of dry matter. The following formula was used to calculate the caloric value (CV): CV (J mg^{-1}) = 0.004184 * [88 * % C + 344 * (% H – 0.125 * % O) + 25 * % S] according to Kamler et al. (1998). Individual energetic value (EV ind. J^{-1}) was obtained by multiplying the calorific value (CV, J^{-1} mg) by the dry weight (DW, mg): EV ($Jind^{-1}$) = VC * DW, as described in Kamler (2002).

Skeletal ossification

In order to evaluate the effects of temperature on the skeletogenesis of trout, fry (*N* = 30 per stage) were double-stained with Alcian blue and Alizarin red according to the protocol described by Arratia & Schultze (1992) with slight modifications. In brief, fry fixed in 4 % formaldehyde were progressively dehydrated in graded series of ethanol, then samples were double stained with a buffered solution containing 0.01 % Alcian blue and Alizarin red for 24h and washed with distilled water twice. In order to remove the unspecific staining of soft tissues, fry were digested with trypsin for one to three hours, washed with distilled water three times, and bleached (50 % H_2O_2 1.5 % and 50 % KOH 0.25 %) for 5 h. Finally, fry were transferred to glycerol for their conservation in progressive graded baths of KOH and glycerol. After staining, fry were placed under a binocular equipped with a digital camera and photographed (Sony CCD-Lw1235C, Sony, Japan) for determining their degree of ossification. This parameter was calculated considering the affinity of different skeletal structures (maxillary, dentary, cleithrum and opercular from the splanchnocranium, as well as the rays of dorsal, anal, pectoral and caudal fins; Figure 1), towards the Alcian blue (cartilage) and Alizarin red (bone).

Statistical analyses

The effects of temperature and stage on morphometric measurements (TL and DW) and energetic contents (CV) were analyzed by a two-way ANOVA, followed by Tukey post hoc tests. TL, DW and CV were expressed as means ± SE. In addition, The effects of the temperature and biological stages on survival rate (SR), development time and rate of ossification were analyzed with a Chi-square test, as well as the percentage of time between two consecutive stages. At each temperature, we tested the survival curve with a Cox proportional hazards survival analysis (JMP® 10, SAS Institute Inc., US). Observed data regarding developmental time (RT) was correlated with an exponential model (Gillooly et al., 2002).

At each temperature, growth was described with regressions estimated from logarithm-transformed data for each morphometric parameter according to the allometric growth model described by Fuiman (1983): $\log(Y) = \log(a) + b \log(TL)$; where Y is the given character studied, a is the intercept, and b is the slope (allometric growth coefficient). When an isometric growth occurred, $b = 1$; allometric growth was positive when b was > 1, and negative when < 1. The inflexion points designated the value of the body character where the regression slopes changed. These inflexion points on growth curves were determined according to the method of Van Snik et al. (1997). In this method, from the log-transformed data, the (x–y) data set was sorted according to increasing TL. The inflexion point corresponded to the couple x-y that resulted in the largest t (Student t-test) value when comparing growth coefficients (b) by a Student t test.

Principal Component Analysis (PCA) was carried out using covariance matrices of the measured characters (for more details on analysis procedures see Nikolioudakis et al., 2010; Ben Khemis et al., 2013). It is generally accepted that when groups of individuals within different growth patterns are included in the PCA, PC1 summarizes the shape variation resulting from growth allometry, while PC2 summarizes the variation of divergent growth

234 trajectories. Hence, growth patterns among different stanzas are reflected as divergent PC2
235 trajectories when plotted against PC1 or TL. Piecewise linear regression, fitted with a non-
236 linear procedure, was used to estimate change in PC2 orientation: PC2 = $b_0 + b_1$ TL + b_2 (TL -
237 L_m) (TL $\geq L_m$), where b_0 is the intercept, b_1 is the slope during the 'fry' stage, b_2 is the
238 difference in slope between 'fry' and 'juvenile' appearance and L_m is the length of
239 morphometric metamorphosis, which corresponds to TL at which slope changes and growth
240 coefficients equals 1 (*i.e*. isometry) (Nikolioudakis et al., 2010; Ben Khemis et al., 2013). PC1
241 eigenvector from groups of individuals sharing common growth patterns reflects the relative
242 proportion of changes. Thus, the variable is isometric when its PC1 component score equals
243 $1/\sqrt{p}$; where p is the number of variables (LH, HH, ED, HM, TrL, TaiL, CaH) in the analysis.
244 A bootstrap method was used to estimate standard errors and confidence intervals for
245 components score comparisons with the isometric theoretical value (Nikolioudakis et al.,
246 2010). All statistical analyses were performed with R software version 3.02 (vegan 2.0–9
247 packages was used for PCA, the boot 1.3–9 for bootstrap methods, the Nortest 1.0–2 for
248 normality test analysis, and the SiZer 0.1–4 for estimating inflection points through PLR
249 analysis). Groups of fish identified were subsequently compared in relation to L_m.
250 Furthermore, the sum of character scores of each fish (index of overall change in morphology
251 associated with metamorphosis) was calculated and expressed as a percentage of maximum
252 total score. When bivariate allometric equations for individual characters were subsequently
253 examined for fish TL < L_m and TL > L_m mm separately, residuals were always randomly
254 distributed, showing no pattern or structure, which indicated that bivariate allometric
255 equations were appropriate for describing relative growth in fish with TL < L_m (Group 1 or fry
256 type) or TL > L_m (Group 2 or juvenile type). Comparisons between the two groups for
257 morphometric measurements and fry energetic contents were done with a one-way ANOVA,
258 whereas comparisons for survival rate, development time and rate of ossification, were done

with a two-way contingency table as previously. All these statistical analyses were performed with Statistica 10.

Results

Mean values of TL and DW in brown trout fry differed significantly among water rearing temperatures, stages, and their interaction ($F_{1, 8}$ = 14.65, P < 0.0001, $F_{1, 8}$ = 107.86, P < 0.0001, for TL and DW, respectively).

Developmental time, survival rate, skeletal ossification and energetic content

The total developmental time from fertilization to metamorphosis decreased with increasing water temperature (Figure 2). The developmental time that fry needed to reach each stage was significantly different between stages (χ^2 = 544.79, df = 4, P < 0.05), rearing temperatures (χ^2 = 1154.18, df = 2, P < 0.05), as well as the interaction between these two parameters (χ^2 = 1141.45, df = 8, P < 0.05) (Figure 2). The relative time (RT) between each stage was significantly different among environmental temperatures (χ^2 = 11.91, df = 4, P < 0.005; Fig. 2). Survival rates at metamorphosis were significantly affected by water temperature (χ^2 = 34.65, df = 2, P < 0.001; Figure 3). The survival curves did not differed from the Cox proportional analysis model for all temperatures; in particular, mortality significantly increased after hatching in all cases (Fisher test: P < 0.05 for each temperature), although its magnitude varied depending on the temperature considered.

The degree of ossification of selected skeletal structures of the splanchnocranium and fins was significantly affected by the environmental temperature (χ^2 = 98.10, df = 4, P < 0.005) and developmental stages (χ^2 = 142.19, df = 4, P < 0.001). Both temperature and developmental stage significantly affected the energetic content of fry (ANOVA, $F_{1, 2}$ = 4.78,

$P < 0.0001$, $F_{1, 4} = 8.86$, $P < 0.0001$), as well as the stage × temperature interaction (ANOVA, $F_{1, 8} = 19.25$, $P < 0.0001$). At hatching, fry emergence, onset of exogenous feeding and metamorphosis stages, the energetic content was lower in fry reared at 4 and 12 °C compared to those kept at 6 ºC. In contrast, at the end of the mixed nutritional period, energetic content values were lower in fry reared at 4 and 6 ºC with regard to those kept at 12 ºC.

Multivariate and bivariate allometric analyses of morphometric parameters

Results from the PCA on log-transformed morphometric characters measured from hatching to exogenous feeding at 4, 6 and 12 ºC, showed that all characters were interdependent and significantly correlated among themselves (Pearson's coefficient: $r = 0.92$; $P < 0.001$, for all paired comparisons). In addition, PCA results also revealed significant changes in the oblique orientation of PC2 scores when plotted against PC1 scores or TL (Supplementary data 2); thus, indicating shifts in the allometric growth of the organism, the largest being observed at 4 and 6 °C. The fit of the piecewise linear regression model between PC2 scores and TL allowed to estimate two different growth stanzas for the three water rearing temperatures, but the estimated L_m of fry differed depending on the water temperature considered, from a maximum observed in cold temperature (4 ºC), namely 2.72 ± 0.85 cm in TL (mean ± SE) at 4 ºC, to 2.50 ± 0.23 cm in TL and 2.22 ± 0.56 cm in TL at 6 and 12 ºC, respectively (Figure 4). Consequently, the development of brown trout could be divided in two distinct phases separated by the above-mentioned L_m; these two phases corresponded to two groups of fish (Group 1 and Group 2, hereafter).

Estimates of the PC1 component scores were quite stable within both Groups (1 and 2), as indicated from their small standard errors (Figure 5); thus, showing the robustness of the groups generated by L_m values provided by the PCA. At 4 ºC, the PC1 component scores for all morphometric characters related to the horizontal axis of the body (ED, HH , HL, TrL,

MH) in Group 1 were close to the theoretical value of multivariate isometry, namely 0.378, and hence, exhibiting isometric growth (Figure 5). In contrast, HL, HH and TrL in Group 2 and TaiL and CaH in both fish stanzas exhibited negative allometry, whereas only MH showed positive allometry. A similar pattern was observed in fry kept at 6 ºC, where all measured characters for Group 1 exhibited an allometric growth value close to isometry, whereas in Group 2, HL, ED and TaiL showed the highest PC1 component scores (*i.e*. positive allometry), and HH, MH and CaH showed the lowest PC1 components scores, indicating a negative allometric growth pattern of the above-mentioned parameters. At 12 °C, all measured characters exhibited larger PC1 components scores and closer to isometry in Group 1 than in Group 2, except for TaiL, which exhibited lower PC1 components scores in Group 1.

The examination of the residuals from the fitted bivariate allometric equations revealed a structured (*i.e*. non-random) distribution for all the morphometric characters examined (ED, HH, HL, TaiL, TrL, CaL, CaH) in relation to TL, indicating a shift in their relative growth (Figure 6). Results from the bivariate allometric analysis of different body regions are shown in the supplementary data 3, whereas the allometric coefficients and their respective inflection points are shown in Figure 5.

Development time, survival rate, ossification and energetic content considering the groups generated by the PCA (L_m)

The distribution of the relative time between different stages of Groups 1 and 2 was similar (Chi-square test; $\chi^2 = 0.56$, $df = 1$, $P = 0.58$) at 6 ºC (69 and 31 % for Group 1 and 2, fry and juveniles respectively) and 12 °C (61 and 39 %, respectively), but differed significantly at 4 °C (89 and 11 % for Group 1 and 2; $P < 0.001$).

Nevertheless, the survival rate of the Group 1 differed among water rearing temperatures (38 % at 12 °C, 76% at 6 °C and 75 % at 4 °C). For this group, the survival analysis showed that there was an impact of temperatures (Cox proportional analysis: at 4 °C $\chi^2 = 67.64$; $df = 2$, $P < 0.001$; at 6 °C $\chi^2 = 40.08$; $df = 2$, $P < 0.001$; at 12 °C $\chi^2 = 167.09$; $df = 2$, $P < 0.001$). In contrast, the temperature did not affect the survival rate of the Group 2, regardless of the temperature considered (Cox proportional analysis; Figure 3).

At 4 and 6 °C, the level of ossification of selected skeletal structures did not differ among Groups ($\chi^2 = 0.55$, $df = 1$, $P = 0.59$), since the ossification of cranial structures (cleithrum, dental, opercula and maxillaries) took place during the first growth phase (in Group 1 when TL < 2.22 ± 0.56 cm). In contrast, the ossification of fin elements and vertebral column occurred at LT values comprised between Groups 1 and 2. At 12 °C, the ossification of all skeletal structures occurred later than in the other experimental groups, at TL > 2.22 ± 0.56 cm; Group 2) (4 - 12 °C $\chi^2 = 4.61$, $df = 1$, $P < 0.05$; 6 - 12°C $\chi^2 = 4.76$, $df = 1$, $P < 0.05$; Table 1).

The energetic content of brown trout fry significantly differed between Group 1 and 2 (ANOVA, $F_{1, 1} = 5.59$, $P < 0.05$) and among temperatures ($F_{1, 2} = 7.19$, $P < 0.05$), whereas there was also a slightly marginal effect of their interaction ($F_{1, 2} = 5.33$, $P = 0.06$). At the end of the first growth phase (Group 1), the energetic value was similar at 4 and 12 °C, and it was up to 1.6 times higher in fry reared at 6 °C (Table 2). At the end of second growth phase (Group 2), the energetic value was also larger at 6 °C and lowest at 12 °C (Table 2).

Discussion

Our results confirmed that the temperature significantly affects the overall development of fry during the early stages of life. As Osse & van den Boogaart (2004) postulated, during this period, important morphological, osteological and energetic changes

359 linked to the transformation of fry into juveniles were observed, and confirmed the
360 stenothermy of brown trout during its early life stages of development. In this context, a
361 collapse of the population of brown trout fry reared at 12 °C was observed, with a dramatic
362 drop in survival (> 50 %) occurring during the first 25 days, which was in accordance to
363 previous studies in this salmonid species (Réalis-Doyelle et al., 2016). In addition, the
364 development time was strongly affected by water temperature, which is well known in
365 poikilothermic organisms (Ojanguren & Braña, 2003; Lahnsteiner, 2012; Réalis-Doyelle et
366 al., 2016). The effect of temperature on the incubation time was relatively similar if we
367 compared it with other studies performed on brown trout embryos, especially at 6 °C,
368 regardless of the fact that strains had different geographical origins (Ojanguren & Braña,
369 2003). Thus, present results showed that the proportion of time between different stages of
370 development varied with temperature, confirming that development time was not linear, as it
371 was also observed by different authors (Cossins & Bowler, 1987; Blaxter, 1992; Kamler,
372 2002; Ojanguren & Braña, 2003). Contrary to what it was expected, this study also
373 highlighted the shortest time in DD that brown trout fry needed to the reach emergence stage
374 when kept at 4 ºC in comparison to the other tested temperatures (6 and 12 ºC), which might
375 be indicative of the stenothermic condition of this species at early life stages of development,
376 as well as an optimal early rearing temperature of 4 ºC. Thus, fry kept at 4 ºC would be more
377 developed than at other temperatures, because they spent longer time during the incubation
378 period (embryogenesis) and had the largest size in TL at hatching, as the energy contained in
379 the yolk sac was used more efficiently (Kamler, 2002). During the endogenous period, the so-
380 called lecitotrophic stage, fry spend a lot of energy contained in yolk-sac reserves to ensure its
381 organogenesis and mainly allocate energy to the formation of new tissues and organs (Elliott,
382 1981; Cossins & Bowler, 1987; Kamler, 1992; Ojanguren & Braña, 2003). The energetic
383 content at the end of this first phase of growth was significantly different between 4 and 6 °C

(32 and 54 J ind^{-1}, respectively). The lower energetic content of fry at 4 °C at the onset of exogenous feeding could be explained by their longer development time, and consequently, a minor content in yolk-sac reserves in these specimens. In natural environments at 4 and 6 °C, fry leave the gravel in the river, when their yolk reserves were consumed and they began to swim in the water column (emergence phase) (Klemetsen et al., 2003). This behavior is energy consumer and fry must make a trade-off between swimming for finding food and for allocating energy for growth and tissue formation affecting some vital biological functions (e.g., feeding and locomotion) that must be functional (Osse & Van den Boogaart, 1995; Simonović et al., 1999; Russo et al., 2007; Ben Khemis et al., 2013, Gisbert et al., 2014).

In our study, with the multivariate approach we were able to highlight changes in allometric growth patterns in post-hatched brown trout fry reared at different. This analysis showed that they existed two phases of development after hatching, results that were similar to most of the studies using this statistical approach (Nikolioudakis et al., 2010, 2014; Ben Khemis et al., 2013; Gisbert et al., 2014). However, the shift between the two phases occurred at different fry sizes, which decreased with water temperature (L_m = 2.72 ± 0.85 cm at 4 °C, 2.50 ± 0.23 cm at 6 °C and 2.22 ± 0.56 cm at 12 °C). These sizes corresponded to different developmental stages, for instance, in fry kept at 4 ºC the L_m values corresponded to the onset of exogenous feeding, whereas in fry reared at 6 ºC the L_m values corresponded to the mixed nutrition period, and in fish kept at 12 ºC with fry emergence from the gravel. Similar results regarding different values for L_m were found with three sparid species of the genus *Diplodus*, which developed under different temperature regimes. In particular, *D. sargus and D. puntazzo*, which developed in summer or spring, respectively, had similar L_m values, but different to *D. vulgaris* that developed in winter (Nikolioudakis et al., 2014). As in fish, the influence of temperature on growth is well known (Koumoundouros et al., 2001; Sfakianakis et al., 2004; Johnston & Temple, 2002; Jordaan et al., 2005), and in this regard, Nikolioudakis

et al. (2014) concluded that synchronization of allometric changes might be higher at low temperatures than at high temperatures in altricial marine fish species, which seemed to be confirmed by the results from the present study in a precocial freshwater fish species, suggesting that the above-mentioned developmental patterns were not only exclusive of fish species with altricial larvae, as it was originally postulated (Osse & Van den Boogaart, 2004; Nikolioudakis et al., 2014).

During the first phase of growth in brown trout, the developmental time was 4-times higher in fry reared at 4 °C than in those kept 6 and 12 °C, whereas the survival rate during this first growth phase mainly impact the specimens kept at 4 °C, but not those at 6 and 12 °C: it could be due to the fact that this first phase took relatively longer time at 4 °C than at the other temperatures. Moreover, during the first phase of growth at 4 ºC ($1.96 < L_m < 2.72$ cm in TL), at 6 ºC ($1.83 < L_m < 2.50$ cm in TL), and at 12 °C ($1.54 < L_m < 2.22$ cm in TL), the head variables (HL, HH, ED), the myotom height, and the length of the trunk grew in a positive allometric relationship with regard to fry TL. In particular, the positive growth of the eye may be associated with the maturation of the visual organ during this period, since vision is one of the most important sensory systems in fry at early stages of development (Packard & Wainwright, 1974). In addition, the positive allometric growth of the head might also allow fry to predate in larger prey items, since larger prey are energetically more favorable than smaller ones (Mathias & Li, 1982), as well as improving their respiratory capacity by enlarging the branchial cavity and gill apparatus (Higgs & Fuiman, 1998; Osse & Van den Boogaart, 2004). Considering that starvation and predation are the major mortality factors at early life stages of development (Chambers & Trippel, 1997), a rapid development of the locomotor, respiratory and sensory organs could improve both prey detection and avoidance from predators, enhancing fry survival and population's recruitment (Osse & Van den Boogaart, 2004; Gisbert et al., 2014). However, fry energetic values at the end of this first

phase were significantly higher in fish kept at 6 °C (53.91 ± 0.05 J.ind^{-1}) than those at 4 °C (32.23 ± 0.07 J.ind^{-1}) and 12 °C (35.37 ± 0.07 J.ind^{-1}), such differences in their energetic content could be explained by a different consumption of the yolk-sac reserves (Pepin et al., 1997; Johnston & McLay, 1997; Galloway et al., 1998; Ojanguren & Braña, 2003; Jaroszewska & Dabrowski, 2010). If it is evident that energetic consumption was higher at high temperature, but for the embryos of cold stenothermal species as trout or arctic char (*Salvelinus alpinus*), the high length of development during incubation could lead to a higher energy consumption and the late late-emerging larvae will also have consumed more yolk reserves, as it has been reported by Régnier et al. (2012). At 12 °C, high temperature disrupted the process of ossification of intramembranous bones in brown trout fry, and they showed a lesser degree of ossification of selected skeletal structures than fry reared at 4 and 6 °C. In addition to a delay in ossification, we have seen a large increase in the incidence of skeletal deformities at 12 °C (82% ± 5.68 %), principally scoliosis. At these last temperatures, fry showed a positive allometric growth of the anterior part of the body that was also correlated with the ossification of several cranial structures (*i.e.*, cleithrum, maxillary, premaxillary and dentary), and skeletal elements of the pectoral and caudal fins. In this sense, among the main risk factors invoked for the onset of skeletal anomalies in salmonids species, temperature seems to be one of the most potent (Boglione et al., 2013). In particular, high temperatures in Atlantic salmon (*Salmo salar*) have been correlated with the appearance of skeletal deformities when a temperature shock (from 6 to 12 °C in 24 h) was induced in embryos, whereas defective skeletogenesis in the splanchnocranium and fin elements were observed in rainbow trout (*Onchorhynchus mykiss*) reared at high temperatures (Baeverfjord et al., 2009). Thus, under present experimental conditions, the delay in ossification of several bones from the cranium, pectoral girdle and caudal fin in brown trout fry reared at 12 ºC could make them more prone to develop abnormally and result in skeletal disorders. As the bone

459 tissue does not develop as fast as other "soft" tissues in the organism (Schoenau, 2005), it
460 could potentially result in skeletal deformities in those structures (Boglione et al., 2013),
461 which are key elements involved in foraging (Osse & Van den Boogaart, 2004) and
462 swimming behaviour (Arendt & Wilson, 2000; Mabee et al., 2002; Ott at al., 2012), and
463 ultimately, affecting fry recruitment.

464 During the second phase of growth (L_m > 2.72 cm in TL at 4 ºC; L_m > 2.50 cm in TL at
465 6 ºC and L_m > 2.22 cm in TL at 12 ºC), the development time in DD was four times higher at
466 4 °C, whereas it was similar between 6 and 12 °C. During this second growth phase, at 4 ºC
467 (L_m > 2.72 cm in TL), at 6 ºC (L_m > 2.50 cm in TL), and at 12 °C (Lm > 2.22 cm in TL), the
468 eye diameter, the trunk and tail showed a positive allometric growth, that could be associated
469 with an increase of swimming abilities. At 4°C, the positive growth of trunk myotom could be
470 also correlated to an enhanced swimming performance and locomotion, whereas the growth
471 rates of the head length, trunk and parameters of fins (caudal fin height and tail length),
472 showed a negative growth. The positive allometric growth of the tail and the complete
473 ossification of the caudal fin rays may improve the tail's propulsive power and its swimming
474 efficiency by reducing dragging energetic costs (Osse & Van den Boogaart, 1995, 2004). The
475 development of propulsion is crucial both for catching prey items and avoiding predators,
476 whereas early swimming induces an earlier onset of ossification in fish (Fiaz et al., 2012),
477 which may affect, in addition to water temperature, the developmental plasticity of the
478 skeleton, as it has been reported by Grünbaum et al. (2012) in newly-hatched Arctic charr
479 (*Salvelinus alpinus*). Moreover, the ossification of rays of all fins, in particular the tail fin, led
480 to a change in the swimming mode of brown trout fry based on the oscillation of the caudal
481 region (Osse & Van den Boogaart, 1995). In fact, after the emergence the trout fry swim
482 freely in the water column and are confronted with changes of environmental conditions (i.e.,
483 flow, turbulence, velocity gradients, rocks) that may affect their swimming performances

(Liao, 2007). Complementary, as a positive allometric growth of the head could influence the respiratory system (Ben Khemis et al., 2013; Gisbert et al., 2014), the growth of the head during the second phase might have favored the respiratory function at this developing temperature. During the second growth phase, the head of fry reared at 12 ºC had a positive allometric growth, whereas the tail length and eye diameter had an isometric one. These growth characteristics reflected the acquisition of a juvenile-like aspect. Indeed, this isometric growth has been already described for other species. For instance, in beluga (*Huso huso*) the isometric growth of the trunk coincided with the completion of the digestive system (Asgari et al., 2014) and for three sturgeon's species by the differentiation of the paired and unpaired fins (Gisbert 1999). The isometric growth of tail length could be corresponding for three sturgeon's species at the differentiation of their paired and unpaired fins that improve their swimming capacities (Gisbert, 1999). In larvae at 12 °C, ossification did not follow the classical order in contrast to other temperatures: this delay of ossification could be due at a higher swimming activity than fry at 4 and 6 °C (Colchen et al. (2016). Indeed, Fiaz et al. (2012) showed on zebrafish (*Danio rerio*) that swim training induced an earlier onset of both chondrogenesis and osteogenesis, as well as an impact on the formation of skeletal elements in the median fins. In summary for fry at 12°C, the second growth phase was fast and when the structures involved in feeding, respiration and swimming developed, which could increases the chance of survival and to escape predation (Fiaz et al., 2012).

Conclusions

A first conclusion was that we observed different patterns of fry development between our three temperatures in terms of allometric, osteological and energetic growth, while the morphological parameters (Vernier table, 1969) appeared similar. Thus we can speculate that

508 the morphological parameters alone are not sufficient to determine the stage of development
509 and a multivariate approach seems more appropriate.

510 Secondly, our study highlights the use of a multi-trait approach for evaluating
511 the impact of rearing temperature on the development of early life stages of brown trout. The
512 allometric growth of different morphological variables, the degree of ossification of different
513 osteological parameters, the energetic content of fry, and their relative development time and
514 survival rates were significantly impacted by different rearing temperatures. Our study
515 showed a developmental plasticity in response to variation of temperature during early stage;
516 indeed, during their first growth phase fry at 12 °C had a very slow growth characterized by a
517 delay of ossification and a small size at hatching; they had to manage additional energy
518 expenditure due to the high rearing temperature (i.e., impact on breathing and maintenance
519 processes). While during the second growth phase, the fry showed positive growth for the
520 morphological characteristic involved in swimming and isometric for the others (eye diameter
521 and tail length). This isometric growth appeared to indicate a rapid growth, which culminated
522 rapidly in a juvenile facies at 12 °C. Unlike, fry at 4 °C showed a longer phase of growth
523 influencing some morphological structures involved in swimming. The development
524 characteristics between these extremes temperatures lead to different behavior and could have
525 ecological consequences, affecting their performance and recruitment.

526

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689

690 **Table 1**: Evolution of ossification development for each structure on Group 1 and 2. We took
691 into account the smallest and the largest size (TL, mm) fry for each skeletal structure.
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	4 °C			6 °C			12 °C		
	SL Start	SL End	Group	SL Start	SL End	Group	SL Start	SL End	Group
Cleithrum	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Opercular	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Frontal	23.33	25.89	1	23.17	25.94	1 < > 2	24.05	30.05	2
Dentary	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Maxillary	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Dorsal fin (rays)	23.33	27.80	1 < > 2	26.31	27.76	2	27.51	34.78	2
Anal fin (rays)	27.72	30.05	2	26.31	27.76	2	32.76	34.79	2
Caudal fin (lepidotrichia and epurals of the caudal fin)	20.60	24.85	<TL>	22.35	25.94	1 < > 2	24.05	27.71	2
Pectoral fin (rays)	23.33	26.35	1	22.35	27.76	1 < > 2	23.38	30.05	2
Vertebral centra	27.72	30.05	2	26.31	27.76	2	32.76	34.79	2

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698 **Table 2**: Means (± SD) of the Energetic values (J.ind $^{-1}$) for each group (1 and 2, see Material
699 and Methods for definition) at each stage of development (H: hatching; EM: emergence, MF:
700 mixed feeding; M: metamorphosis).
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702

	4°C		6°C		12°C	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Stages	H-EF	M	H-MF	EF-M	H-EM	MF-M
TL (mm)	TL < 2.72 ± 0.85	2.72 ± 0.85 < TL < 2.81 ± 0.73	TL < 2.50 ± 0.23	22.50 ± 0.23 < TL < 2.77 ± 0.56	TL < 2.22 ± 0.56	2.22 ± 0.56 < TL < 4.78 ± 0.56
Energetic content (J.ind^{-1})	32.23 ± 0.07	72.38 ± 1.93	53.91 ± 0.05	98.91 ± 3.34	35.57 ± 0.04	55.95 ± 2.55

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Legends of the figures

Fig.1. Morphometric characters measured on *Salmo trutta* larvae from hatching to metamorphosis. ED, eye diameter; HH head height; HL, head length; TL total length; TaiL, tail length; TrL, trunk length; CaH caudal fin height ; MH myotome height.
Orange spot indicated the maxillary, blue spot the dentary, pink spot the cleithrum, green spot the opercular from the splanchnocranium, brown spot the frontal. As well as the light blue spot indicated the rays of dorsal fins, the black spot indicated the rays of anal fins, the yellow spot indicated the rays of pectoral fins, the red spot indicated the rays of the caudal fins, and the light orange spot indicated the vertebral column.

Fig.2. Effect of temperature during development time:
a) Total number of days from fertilization to metamorphosis
b) Percentage of relative development time between each biological stage.

Fig.3. Effect of temperature on survival rate calculated for each day. The blue line was for 4 °C, the light blue line for 6 °C and the red line for 12 °C (Error bars = SD).

Fig.4. Piecewise regression of PC2 scores-on-standard length (TL mm) for estimating mean length-at-metamorphosis in *Salmo trutta* larvae (size-at-change in oblique orientation of scores a) at 4° C , b) at 6° C, c) at 12° C.

Fig.5. Multivariate allometry on morphometric characters of *Salmo trutta;*

a) at 4 °C (Dashed line indicates multivariate isometry (0.34). Diamond represented fish <2.73 mm, and square fish >2.73 mm.
b) at 6 °C (Dashed line indicates multivariate isometry (0.35). Diamond represented fish <2.50 mm, and square fish >2.50 mm diamond represented fish < 2.50 mm.
c) at 12 °C Dashed line indicates multivariate isometry (0.35). Diamond represented fish <2.22 mm, and square fish >2.22 mm.
d) Error bars = 95% bootstrapped confidence intervals.

Fig.6. Residuals plots revealed a non-random (structured) distribution of residuals for all characters examined (ED, HH , HL, TaiL, TrL, MH, CaH) in relation to TL by fitting them in the allometric equation (a) At 4 °C; b) at 6 °C and c) at 12 °C).

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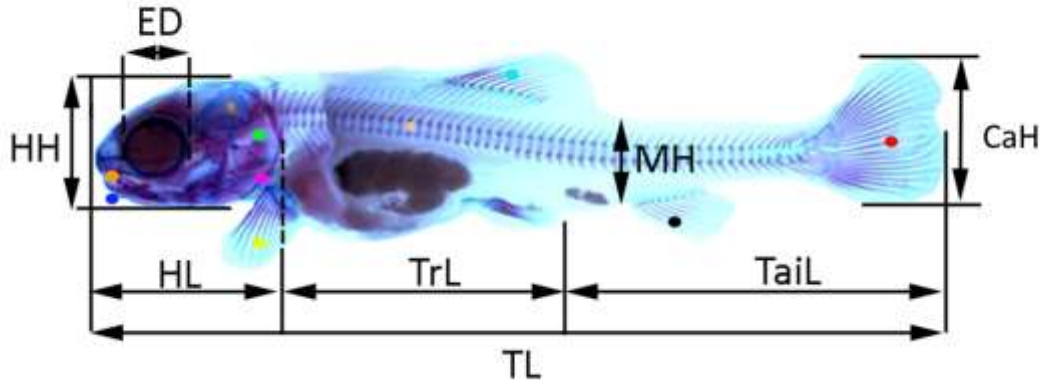

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747 **Figure 1**

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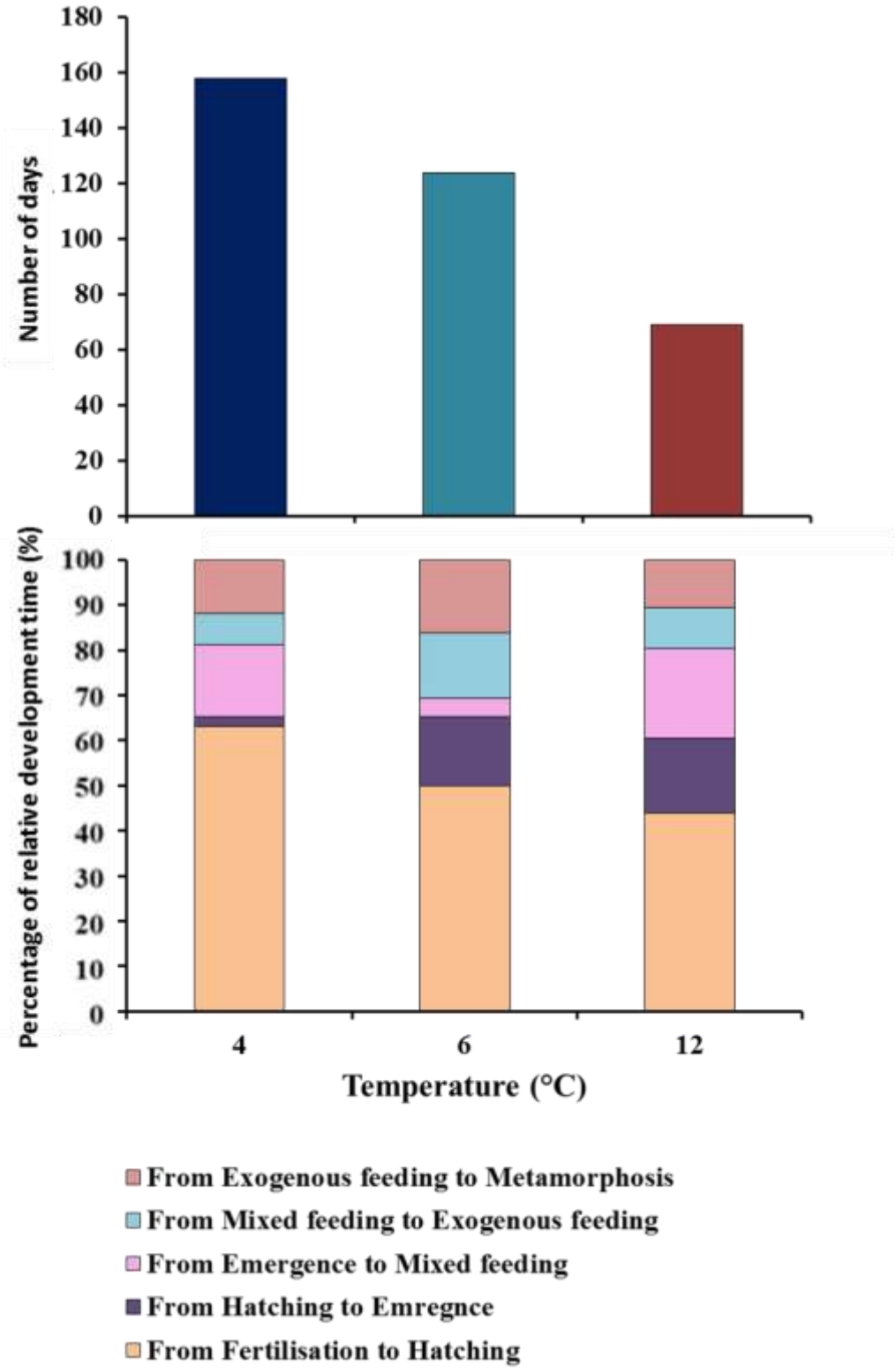

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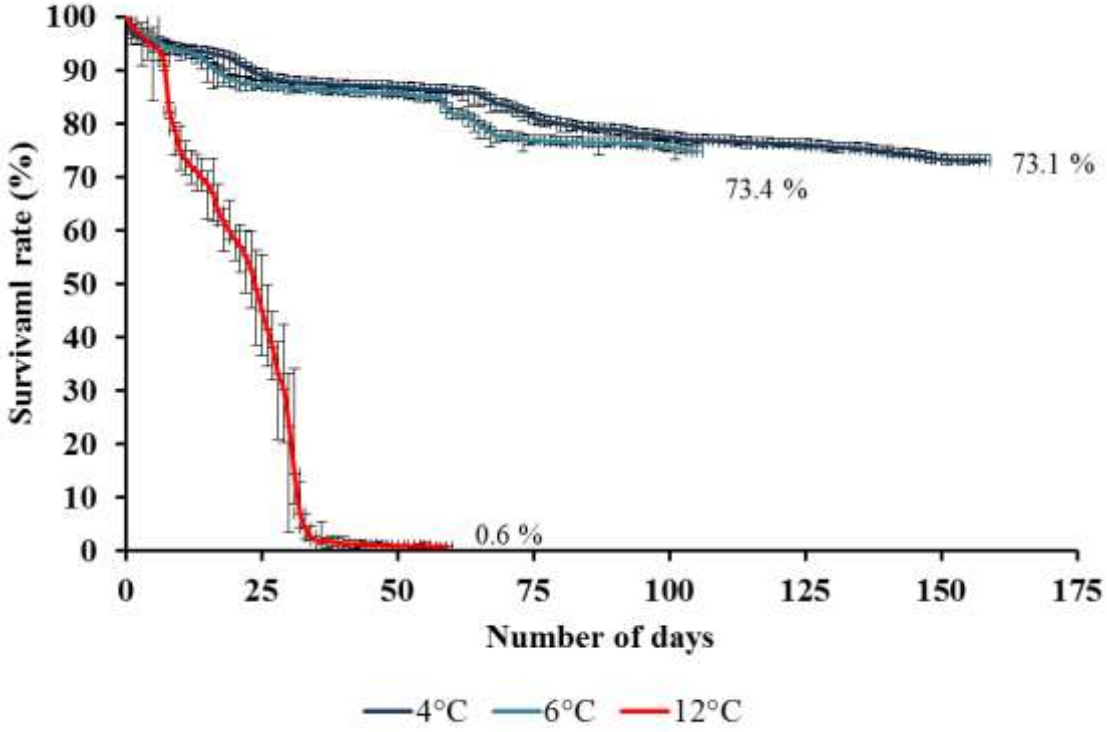
751 **Figure 2**



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754 **Figure 3**

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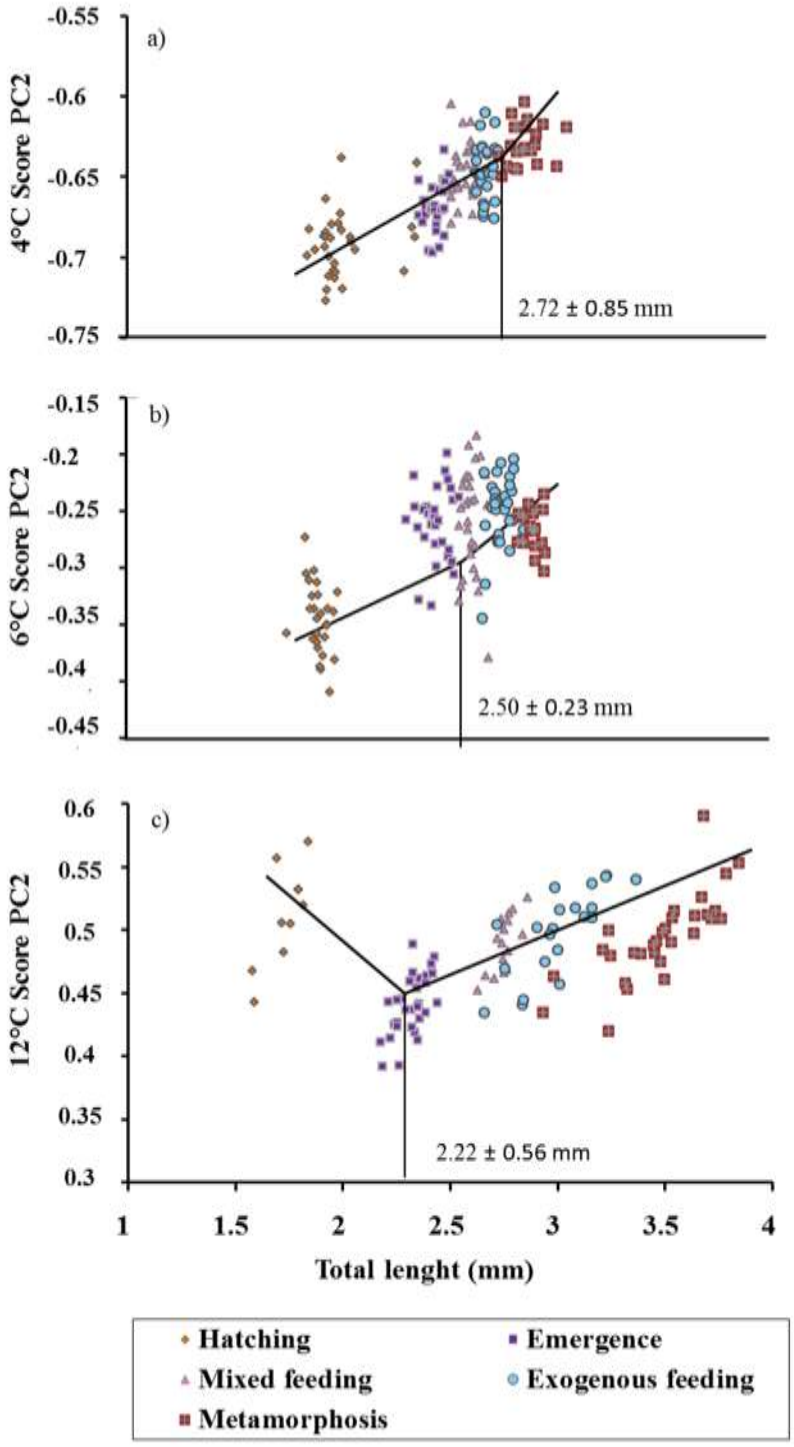
a)
4°C Score PC2
-0.55
-0.6
-0.65
-0.7
-0.75
2.72 ± 0.85 mm
b)
6°C Score PC2
-0.15
-0.2
-0.25
-0.3
-0.35
-0.4
-0.45
2.50 ± 0.23 mm
c)
12°C Score PC2
0.6
0.55
0.5
0.45
0.4
0.35
0.3
2.22 ± 0.56 mm
1
1.5
2
2.5
3
3.5
4
Total lenght (mm)
Hatching
Emergence
Mixed feeding
Exogenous feeding
Metamorphosis

Figure 4

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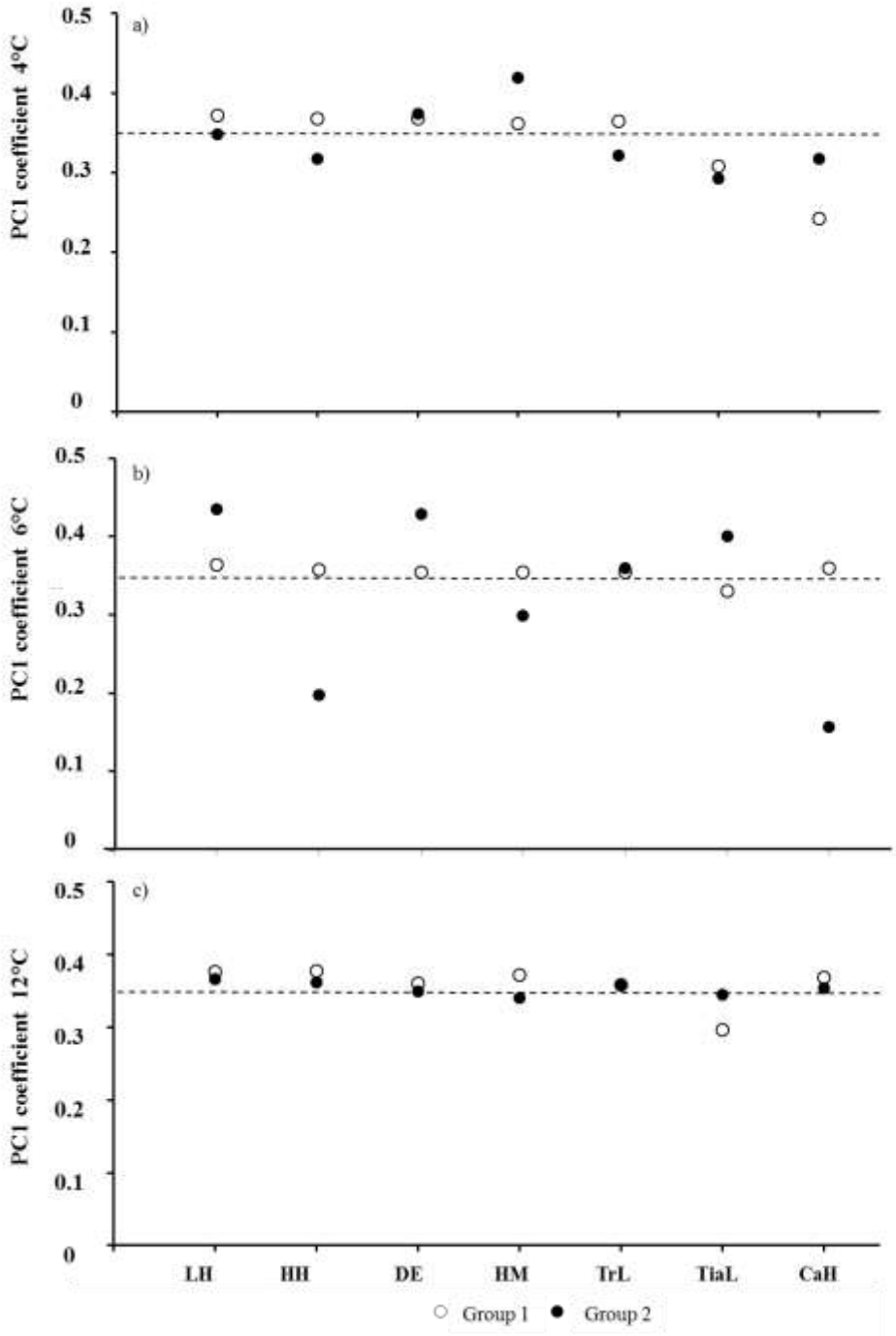

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761 **Figure 5**

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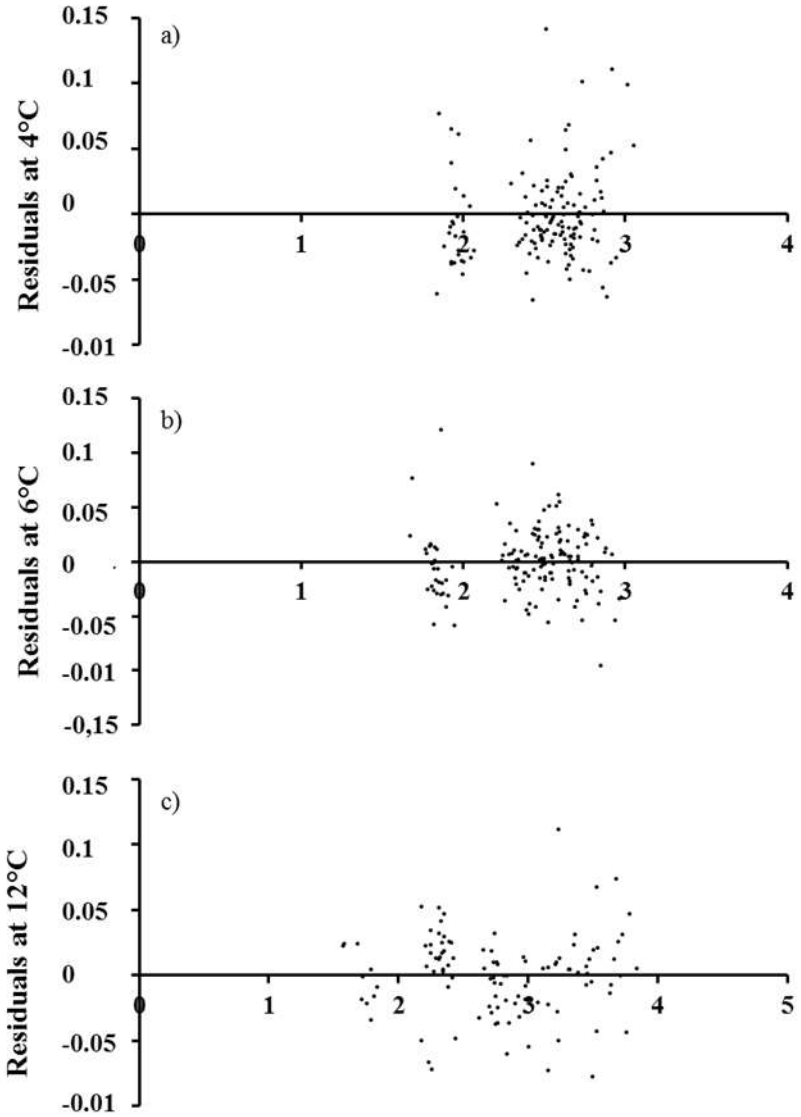

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766 **Figure 6**

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		Rearing temperature		
Stage of development		**4 °C**	**6 °C**	**12 °C**
Hatching	TL (mm)	19.58 ± 0.20 a	18.30 ± 0.16 a	15.41 ± 0.18 b
	DW (mg)	0.30 ± 0.01 a	0.31 ± 0.02 a	0.28 ± 0.02 b
	DD	364	372	360
	Vernier's stage	31. Melanophores cover the entire body. Pectoral fin rays are present. The curving radiala of the anal and dorsal fins are distal to the basalia. The primordial fin musculature is no longer visible. The terminal end of the caudal vein disappears, and all the blood in the tail passes through the caudal fin. The slit in the choroid is no longer visible.		30. Melanophores orienting along the posterior border of the somites and the onset of their invasion of the yolk sac and the dorsal and anal fins. A capillary network is and anal fins artery and caudal vein established at the extremity of the caudal
	Behavioral trait	Fry on the bottom	Fry on the bottom	Fry on the bottom
	Survival (%)	81.14 a	83.00 a	31.51 b
	Energetic content (J ind^{-1})	54.76 ± 0.06 a	56.42 ± 0.05 b	51.51 ± 0.04 c
Emergence	TL (mm)	24.36 ± 0.22 a	23.52 ± 0.12 a	23.18 ± 0.32 a
	DW (mg)	0.32 ± 0.02 a	0.35 ± 0.02 b	0.26 ± 0.01 c
	DD	376	486	492
	Vernier's stage	32. The melanophores extend half way down the yolk sac. The terminal end of the caudal artery disappears, and the pinnal artery exists alone.		
	Behavioral trait	Fry start swiming		
	Survival (%)	80.50 a	77.91 a	2.62 b
	Energetic (J ind^{-1})	39.74 ± 2.70 a	52.81 ± 1.98 b	35.57 ± 2.13 c
Mixed feeding	TL (mm)	25.62 ± 0.32 a	23.62 ± 0.25 b	27.93 ± 0.20 c
	DW (mg)	0.32 ± 0.02 a	0.35 ± 0.02 a	0.25 ± 0.01b
	DD	516	516	648
	Vernier's stage	33. A few melanophores on the anal fin. Caudal fin well delineated.		

	Behavioral trait	Larva starting exogenous nutrition activity, predation activity		
	Survival (%)	78.71 a	77.84 a	2.18 b
	Energetic content (J ind^{-1})	34.312 ± 1.93 a	53.92 ± 2.06 b	33.61 ± 1.96 a
Exogenous feeding	TL (mm)	26.37 ± 0.38 a	25.87 ± 0.23 a	30.05 ± 0.22 b
	DW (mg)	0.40 ± 0.01 a	0.36 ± 0.02 b	0.34 ± 0.01 b
	DD	564	624	732
	Vernier's stage	36. Yolk sac absorbed. Adipose fin in its final form.		
	Behavioral trait	Larva feeding exclusively exogenous way and swimming subcarangiform		
	Survival (%)	74.93 a	74.82 a	0.83 b
	Energetic content (J ind^{-1})	32.23 ± 1.99 a	31.15 ± 1.96 a	35.23 ± 1.85 b
Metamorphosis	TL (mm)	28.17 ± 0.24 a	22.77 ± 0.16 b	34.78 ± 0.10 c
	DW (mg)	0.51 ± 0.02 a	0.71 ± 0.01 b	0.61 ± 0.02 ac
	DD	632	744	828
	Vernier's stage	37. Yolk sac almost entirely covered with chromatophores. Edges of the dorsal and anal fins are serrated. Height of the pelvic fin is twice that of the remaining embryonic fin fold		
	Behavioral trait	Larva having an and swimming rapidly and hide		
	Survival (%)	73.12 a	73.42 a	0.66 b
	Energetic content (J ind^{-1})	72.38 ± 3.21 a	98.91 ± 2.50 b	55.95 ± 3.68 c

Supplementary data 1: Morphometric measurements (total length (TL) and weight), time of development (degree days (DD) before fertilization), behavior, survival rate and energetic content for each stage of development (letter indicated significant different by Tukey test) (We added the information from Vernier 1969).

	4°C		6°C		12°C	
All fish	PC1	PC2	PC1	PC2	PC1	PC2
LH	0.36	0.04	0.36	0.19	0.36	-0.24
HH	0.36	-0.09	0.35	-0.14	0.353	0.17
DE	0.36	-0.10	0.34	-0.56	0.35	0.38
MH	0.35	0.10	0.35	0.14	0.34	0.61
TrL	0.36	-0.05	0.35	-0.39	0.35	$0.1.10^{-2}$
TiaL	0.31	0.85	0.34	0.66	0.34	-0.56
CaH	0.34	-0.25	0.35	0.04	0.35	-0.23
Group 1						
LH	0.37	0.07	0.36	0.11	0.37	0.03
HH	0.36	-0.07	0.35	-0.22	0.37	-0.04
DE	0.36	-0.06	0.35	-0.14	0.36	-0.14
MH	0.36	0.06	0.35	-0.11	0.37	0.12
TrL	0.36	-0.04	0.35	-0.23	0.35	-0.18
TiaL	0.30	0.85	0.33	0.90	0.29	0.79
CaH	0.24	-0.38	0.35	-0.18	0.31	-0.54
Group 2						
LH	0.34	-0.08	0.43	-0.17	0.36	-0.23
HH	0.31	-0.20	0.19	0.15	0.36	0.02
DE	0.37	-0.28	0.42	0.72	0.34	0.28
MH	0.41	0.52	0.29	-0.08	0.34	0.79
TrL	0.32	0.18	0.35	0.53	0.35	-0.02
TiaL	0.29	0.53	0.40	-0.28	0.34	-0.36
CaH	0.31	-0.53	0.15	-0.21	0.35	-0.19

Supplementary data 2: First (PC1) and second (PC2) principal factor loadings for the morphometric parameters studied in trout *(Salmo trutta*) larvae. Rescaled components scores can be obtained by dividing raw scores with the character standard deviation (for the signification of the morphometric parameters see Figure 1 legend).

	4°C			6°C			12°C		
	Start	End	Group	Start	End	Group	Start	End	Group
Cleithrum	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Opercular	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Frontal	23.33	25.89	1	23.17	25.94	1 <TL< 2	24.05	30.05	2
Dentary	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Maxillary	21.34	24.22	1	20.20	23.72	1	23.15	23.48	2
Dorsal fins (Rd)	23.33	27.80	1 <TL< 2	26.31	27.76	2	27.51	34.78	2
Anal fin (Ra)	27.72	30.05	2	26.31	27.76	2	32.76	34.79	2
Caudale fin (Ec)	20.60	24.85	1<TL<2	22.35	25.94	1 <TL< 2	24.05	27.71	2
Pectoral fin (Rp)	23.33	26.35	1	22.35	27.76	1 <TL< 2	23.38	30.05	2

Supplementary data 3: Bivariate measurement for allometric growth for seven morphological parameters. *b* is the slope measured as the allometric growth coefficient: b_1 is a slope for first phase of growth and b_2 is a slope for second phase. When an isometric growth occurred $b = 1$; allometric growth was positive when *b* was superior to 1, and negative when *b* was less 1. The inflexion points (TL) designated the value of the total body length, where the regression slopes changed significantly.