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# Growth of Critically Endangered annual fish *Austrolebias wolterstorffi* (Cyprinodontiformes: Rivulidae) at different temperatures

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Given the importance of knowledge of the biology of endangered species for the planning of conservation and management efforts, the aim of this study was to investigate the influence of temperature on the growth of *Austrolebias wolterstorffi* (Ahl, 1924). To clarify the thermal influence on the growth of the species, temperatures of 16, 20, 24 , and 28°C were tested in triplicate. The present study showed that a water temperature of 28°C is detrimental to the growth of the species. Among the other tested temperatures, it was found that the optimum temperature for growth decreases as an individual ages and is slightly lower for females. It was demonstrated here that males reach a higher weight and length, and grow faster than females, reaching sexual maturity earlier. To optimize the growth of this species in captivity, the ideal temperature of the water during the initial life period is 24°C, until after puberty when the temperature should decrease to about 21°C.

Devido à importância do conhecimento da biologia das espécies ameaçadas de extinção para o planejamento de ações de conservação e manejo, o objetivo deste estudo foi investigar a influência da temperatura sobre o crescimento de *Austrolebias wolterstorffi* (Ahl, 1924). Para verificar a influência da temperatura no crescimento da espécie, as temperaturas de 16, 20, 24 e 28° C foram testadas em triplicata. O presente estudo mostrou que a temperatura da água de 28° C é prejudicial para o crescimento da espécie. Entre as outras temperaturas testadas, verificou-se que a temperatura ótima para o crescimento diminui ao longo da vida e é ligeiramente menor para as fêmeas. Foi demonstrado aqui que os machos alcançam um maior peso e comprimento e crescem mais rápido do que as fêmeas, atingindo a maturidade sexual anteriormente. Para otimizar o crescimento da espécie em cativeiro, a temperatura ideal da água durante o período inicial de vida é de 24° C, já após a puberdade, a temperatura deverá diminuir para cerca de 21°C.

**Key words:** Endangered species, Length, Maturity, Weight.

## Introduction

Annual fish are well known to exclusively inhabit shallow wetlands that become dry at certain times of year. In this type of environment, conditions are extremely variable with wide-ranging daily fluctuations in temperature and oxygen availability (Errea & Danulat, 2001; Arenzon *et al.*, 2002b; Volcan *et al.*, 2011). Annual fish live in areas of seasonal flooding and exhibit rapid growth and early sexual maturation (Arenzon *et al.*, 1999; Errea & Danulat, 2001; Volcan *et al.*, 2012). Most species bury their eggs in the substrate, where they remain until the beginning of the rains when the wetlands flood and the eggs hatch, initiating a new life cycle (Wourms, 1972; Podrabsky & Hand, 1999).

Most species of annual fish are threatened with extinction due to their restricted distributions as well as habitat loss, which has primarily occurred as a result of the conversion of habitats for human activities (Reis *et al.*, 2003; Rosa & Lima, 2008; Volcan *et al.*, 2010). Agriculture and urbanization feature prominently among these human activities (Volcan *et al.*, 2011). Faced with this situation, the "National Action Plan for the Conservation of Threatened Extinction Killifishes" was recently

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created (ICMBio, 2012). This plan stressed the importance of performing technical and scientific studies, in situ and *ex situ*, applied to killifishes and their habitats (ICMBio, 2012).

*Austrolebias wolterstorffi* (Ahl, 1924) is an annual fish of the family Rivulidae (Cyprinodontiformes) that is endemic to the Patos-Mirim lagoon system, where it occurs in isolated wetlands south of Rio Grande do Sul and east of Uruguay (Costa, 2006). Conservation concern for the species is high because it is included in the category "Critically Endangered" in Rio Grande do Sul and Brazil (Reis *et al.*, 2003; Rosa & Lima, 2008).

The study of the biology of endangered species and on techniques for culturing them is essential for the support of conservation plans, population management and restocking. In addition, these studies can offer biological and methodological information to support the maintenance of brood stocks of this important biological model for laboratory studies (Genade *et al.*, 2005; Valenzano *et al.*, 2006; Mourabit & Kudoh, 2012; Passos *et al.*, 2013).

Several studies show temperature influences in the life cycles of annual fishes in the wild or captivity (Liu & Walford, 1966; Liu *et al.*, 1975; Errea & Danulat, 2001; Arenzon *et al.*, 2002a; Vallenzano *et al.*, 2006; Volcan *et al.*, 2012, Volcan *et al.,*2013). Among the studies of *A. wolterstorffi*, most have focused on conservation, distribution and taxonomy (Costa, 2006; Porciuncula *et al.*, 2006; Quintela *et al.*, 2007; Lanés & Maltchik, 2010), but few have addressed their biology and captive maintenance (Liu & Walford, 1970; Costa, 2009).

The tolerance of *Austrolebias* species to extreme temperatures is evident based on the variation of this parameter in the fish's natural habitat (Errea & Danulat, 2001; Volcan *et al.*, 2011), but its tolerance of constant temperatures, particularly regarding the stability of its physiological functions, is not fully known.

A slow growth rate may extend the periods during which an individual is susceptible to predators and diseases and increase the risk of mortality during periods of stress (Garvey *et al.*, 2004). In captivity, the spread of diseases and parasites can be faster and exposure to stress is inevitable. Therefore, when rearing *A. wolterstorffi* in captivity, rapid growth is desired to lessen the vulnerability of the fish.

There is evidence that the growth of *A. wolterstorffi* in adulthood is favoured at lower temperatures (Liu & Walford, 1970), but an increase in the growth rates of fish in higher temperature up to a heat tolerance limit, when the growth rate decreases (Imsland *et al.*, 1996) is common. It is expected that fast growth in lower thermal conditions occurs only with mature individuals of *A. wolterstorffi*, although in these cases reproductive activity and growth may overlap. In some cases, temperature can also interfere with the aging process, leading to premature aging (Liu & Walford, 1969; Utrilla & Lobon-Cervia, 1999).

Given the conservation status of this species and the paucity of studies on its biology, this study aims to investigate the influence of water temperature on the growth of *A. wolterstorffi*.

## Material and Methods

### Trials and fish precedence

The experiment was conducted at the Laboratory of Physiology Applied to Aquaculture at the University Federal de Pelotas - Brazil, from May to August 2010. The brood stock used to obtain the eggs were collected with a hand net (5mm) in temporary wetlands in Pontal da Barra (31°46'36"S 52°13'33"W), Pelotas municipality, south of Rio Grande do Sul State, Brazil and were collected under IBAMA/ICMBio license # 15108-1. Voucher specimens were deposited in the Museum of the Universidade Federal do Rio Grande (CI FURG 0051).

### Water quality

For the trials, the experimental units (EU) consisted of 30L aquariums provided with constant aeration, internal biological filters, heaters (60W) and digital thermostats (Aquaterm/FullGauge/0.1°C) for temperatures of 16, 20, 24 and 28°C. The EUs were kept at a constant room temperature (16°C) and had a photoperiod of 12 hours of light and 12 hours of darkness. The temperature was checked daily and the pH (pH meter/Lutron/0.01) and dissolved oxygen levels (oximeter/Lutron/0.1 mg/l) were observed three times per week. The total ammonia ($NH3$) concentration was measured once a week (Nutrafin ® - Hagen / 0.1 mg/l), when 50% of the water in each EU was replaced.

### Growth

The initial standard length (SL) was measured for 20 larvae selected at random soon after hatching. A total of 96 newly hatched juveniles were randomly divided into 12 EU (eight juveniles per EU), with temperature conditions of 16, 20, 24 and 28°C, in triplicates. Juveniles were fed *ad libitum* twice daily (10 and 16h). Food was offered to have enough until the next feeding. For the first 18 days after hatching (DAH), juveniles were fed first with newly hatched *Artemia* nauplii and later with native zooplankton greater than 500µm in size (composed by microcrustaceans, mainly copepods and cladocerans, with predominance of the latter).

The initial biometrics (weight 0.001g; standard length 0.1mm) were made 18 DAH and continued weekly until all specimens presented sexual dimorphism and typical mating behaviour, which defined the end of the growth experiment (Costa, 2006). To assess the length-weight relationship (LWR)

**Table 1.** Water quality parameters (mean ± standard error) throughout the growth trial of *Austrolebias wolterstorffi* reared under different temperatures. Letters denote statistical significance across parameters (ANOVA, Tukey, $p < 0.05$).

| | Temperature (°C) | pH | Dissolved $O_2$ (mg $L^{-1}$) | Total $NH_3$ (mg $L^{-1}$) |
|---|---|---|---|---|
| 16°C | 16.12 ± 0.06 a | 6.61 ± 0.06 a | 13.03 ± 0.34 a | 0.17 ± 0.05 a |
| 20°C | 19.96 ± 0.06 b | 6.62 ± 0.05 a | 11.34 ± 0.30 b | 0.03 ± 0.02 b |
| 24°C | 23.90 ± 0.08 c | 6.67 ± 0.04 a | 10.49 ± 0.35 bc | 0.08 ± 0.03 ab |
| 28°C | 27.86 ± 0.06 d | 6.66 ± 0.04 a | 9.35 ± 0.34 c | 0.03 ± 0.02 b |

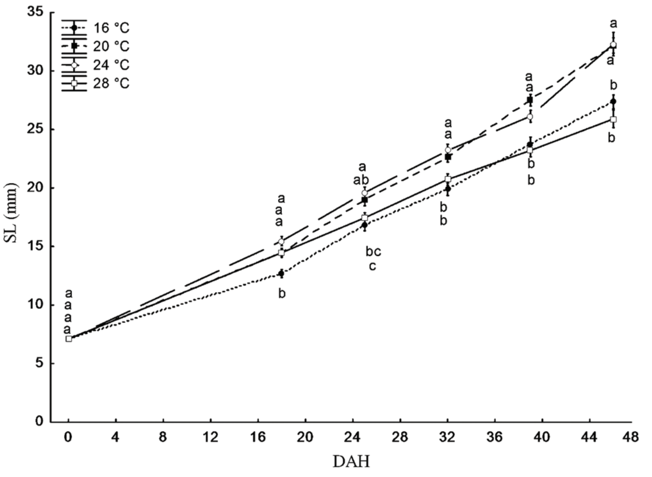


**Fig. 1.** Standard length (SL) (mean ± standard error) of *Austrolebias wolterstorffi* kept in the laboratory for 46 days after hatching (DAH) under different temperatures. Different letters represent significant mean differences between treatments (ANOVA, Tukey, $p < 0.05$)

the total weight (W) in mg and the standard length (SL) in mm were considered. W and SL were transformed by the logarithmic expression log W = log *a* + *b* log SL, where *a* represents the y-intercept and *b* is the slope. The relationship between SL and W was then determined by a linear regression. A confidence interval of 95% was calculated for *b* (95% CI) to determine whether the hypothetical value of isometry would fall within those limits (Froese, 2006).

The growth performance was assessed as the specific growth rates (SGRs) for the SL and W by expressions $SGR_{SL} = (\log n\ SLf - \log n\ SLi)\ t^{-1}$ and $SGR_W = (\log n\ Wf - \log n\ Wi)\ t^{-1}$, respectively, where *Wf* is the final weight, *Wi* is the initial weight and *t* is the time interval in days.

The condition factor (*K*) was calculated as the ratio between W (g) and SL (mm) and was raised to the exponent calculated by LWR using the equation: $K = (W\ (SL^x)\ -1)*100$. For the calculation of exponent *x*, which corresponds to the slope of the linear relationship between SL and W (WLR), all measurements taken at different times and stages of development throughout the growth trial were considered.

### Statistical analysis

All results are presented as the mean ± standard error. To test for differences in the mean values of growth and K, analysis of variance (ANOVA) was carried out after the test assumptions were verified. Differences between means were evaluated by Tukey's post hoc test. The Student's t-test was used to detect differences in SL and W between males and females and a simple linear regression was used to investigate the length-weight relationship (LWR). A quadratic regression was applied to the point of inflexion of the relationship between temperature and the weights and lengths of the fish at different ages. All tests were performed considering a 95% significance level, using the program Statistica ® 7.0. Biostat 5.0 was used to calculate the point of inflexion of the regression curves.

## Results

### Water quality

The concentration of dissolved oxygen remained high for all treatments but showed an inversely proportional relationship with temperature. The mean concentration of nitrogen in the form of total ammonia was more pronounced at 16 and 24°C for part of the experiment due to defects in some of the biological filters. Despite the immediate resolution of the problem, there was a significant difference between the means for this parameter because the ammonia concentrations observed for the other treatments were low. The results for water quality are shown in Table 1.

### Growth

Immediately after hatching, the SL of the juveniles was 7.10 ± 0.40mm. The results from the 32-day experiment showed that fish growth was hampered by extreme temperatures. The average SLs of the fish maintained at 20 and 24°C remained similar and were higher than the SLs achieved at 16 and 28°C, which, in turn, were not significantly different from one another (Fig. 1).

Obvious secondary sexual characteristics began to identify the male specimens at 39 DAH, whereas females only began to develop secondary sexual characteristics at 53 DAH.

At 67 DAH, the SL of females raised at 16, 20 and 24°C were significantly higher than the females kept at 28°C (Fig. 2A). The average W of 20 and 24°C females remained higher than those of 16 and 28°C females throughout the experiment (Fig. 2B). There was an improvement for growth in SL for males throughout the study, but the extreme temperatures were unfavourable to the growth in terms of weight (Figs. 2C-D).

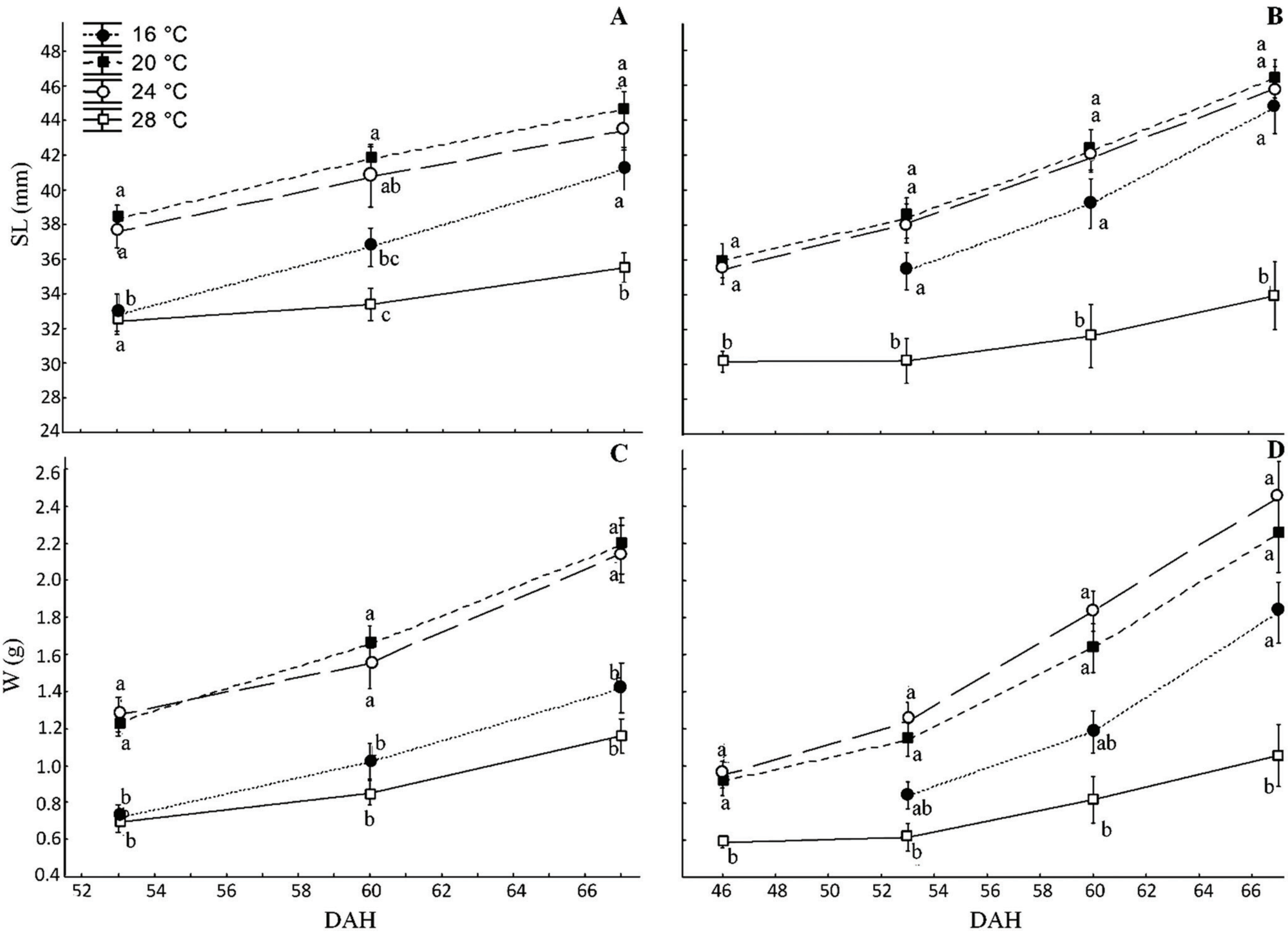


**Fig. 2.** Standard length (SL) of females (A) and males (B) and weight (W) of females (C) and males (D) (mean ± standard error) *Austrolebias wolterstorffi* kept in the laboratory for 67 days after hatching (DAH) under different temperatures. Different letters represent significant mean differences between treatments (ANOVA, Tukey, p < 0.05)

**Table 2.** Specific growth rate (mean ± standard error) from mean of the standard length ($SGR_{SL}$) and weight ($SGR_W$) of juveniles (J) (0-39 DAH), males (M) and females (F) (53-67 DAH) and final values of condition factor (*K*) (mean ± standard error) of *Austrolebias wolterstorffi* reared during 67 days under different temperatures. Letters denote statistical significance across parameters (ANOVA, Tukey, p < 0.05). DAH = days after hatching.

| | 16°C | 20°C | 24°C | 28°C |
|---|---|---|---|---|
| $SGR_{SL}$ (J) | 3.04 ± 0.04 | 3.49 ± 0.03 | 3.39 ± 0.11 | 3.05 ± 0.04 |
| $SGR_{SL}$ (F) | 1.52 ± 0.14 | 1.45 ± 0.21 | 1.42 ± 0.09 | 0.66 ± 0.38 |
| $SGR_W$ (F) | 4.94 ± 0.67 | 5.28 ± 0.72 | 4.88 ± 0.22 | 3.41 ± 0.85 |
| *K* (F) | 1.96 ± 0.06 a | 2.38 ± 0.08 b | 2.58 ± 0.07 b | 2.62 ± 0.12 b |
| $SGR_{SL}$ (M) | 1.75 ± 0.45 | 0.92 ± 0.17 | 0.71 ± 0.24 | 0.58 ± 0.11 |
| $SGR_W$ (M) | 4.75 ± 1.29 | 2.76 ± 0.25 | 2.78 ± 0.59 | 2.70 ± 0.56 |
| *K* (M) | 1.99 ± 0.11 a | 2.17 ± 0.06 ab | 2.44 ± 0.07 bc | 2.56 ± 0.11 c |

In this work it was shown that males reach a higher weight and length and grow more quickly than females, although both sexes achieve sexual differentiation at the same body size (Figs. 3A-B). The results point to a detrimental effect of the extreme temperatures on the growth of young fish, showing a tendency to reduce the optimum thermal with aging (Figs. 4-5A-B).

The weight-length relationship was highly significant (r = 0.96, p < 0.000001). Slope values slightly higher than three were observed for the regression lines for juveniles, males and females when analysed separately, determining allometric positive growth. In the present study, the estimated value of this slope was 3.04. The SGRW and $SGR_{SL}$ did not show significant differences during the study, while *K* was influenced by temperature for both sexes, demonstrating a direct relationship with the temperature in the mature fish, especially in males (Table 2).

At 53 DAH, reproductive behaviour was observed (males courting females), followed by oviposition. In the 16, 20 and 24°C treatments, eggs were deposited at the bottom of the aquarium. At the end of the experiment, only the fish in

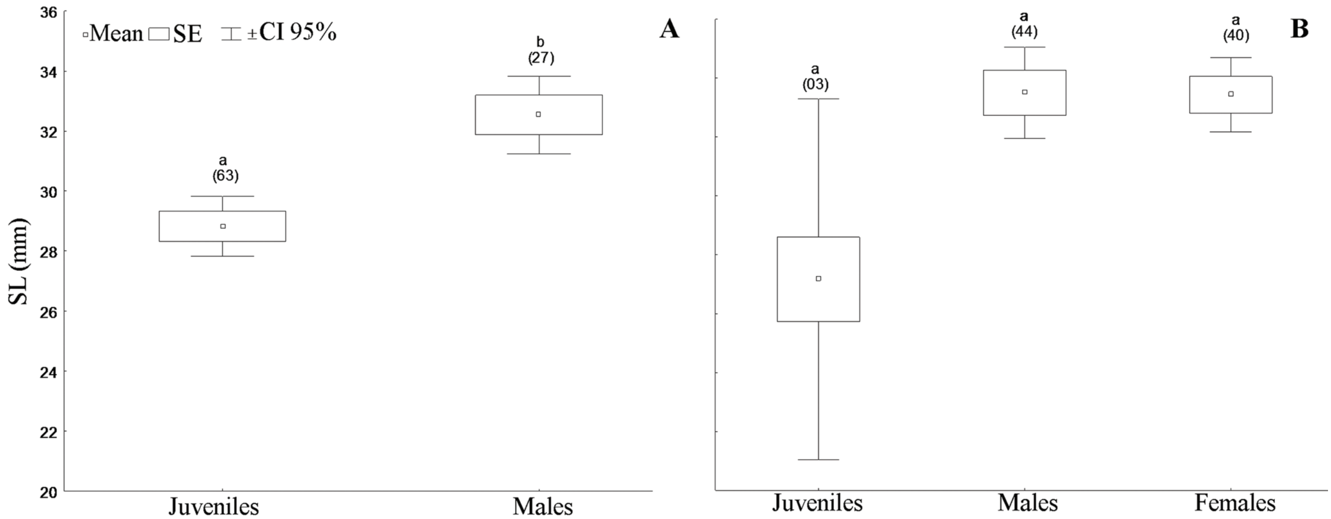


**Fig. 3.** Standard length (SL) of *Austrolebias wolterstorffi* sexually differentiated (males and females) and undifferentiated (juveniles), at 46 (A) and 53 (B) days after hatching. Different letters represent significant differences in SL means (A: t test, px < 0.05, B: ANOVA, Tukey, p > 0.05).

the 28°C treatment had not been observed ovoposition. The experiment was concluded at 67 DAH. The survival was high at approximately 90%.

## Discussion

The importance of the effects of abiotic factors on fish biology is well established. The early stages of development of annual fish are clearly adaptive in the face of environmental conditions (Haas, 1976; Errea & Danulat, 2001; Berois *et al.*, 2012). In these ephemeral environments, diverse abiotic factors face significant daily variation, temperature being the most affected parameter, which can vary by more than 20°C daily (Volcan *et al.*, 2011). In this context, it is hypothesized that annual fish have varying thermal preferences depending on their life stage (Walford & Liu, 1965). Juvenile *Austrolebias nigrofasciatus* have a higher growth rate at 22°C than at 16°C (Volcan *et al.*, 2012) and in the breeding stage of the same species, the body condition factors of individuals maintained at 25°C were impaired relative to individuals maintained at 17 or 21°C (Volcan *et al.*, 2013).

Corroborating previous research, in the present study it was evident that the optimal temperature that favours growth decreases throughout an individual's life, indicating a reversal in the negative effect that a 16°C constant temperature exhibited before puberty. However, *K* exhibited a direct relationship with temperature in the early life stages where growth is most pronounced. This relationship may reflect the higher rate of metabolic activity that occurs at higher temperatures, which, within the range of thermal tolerance of the species, can lead to increased food intake and possibly a greater accumulation of reserves (Clarke & Johnston, 1999).

In a study with *A. wolterstorffi*, the growth in length was favoured at 16°C compared to 22°C in adults. This result was due to better feed conversion at the former temperature (Liu & Walford, 1970). In contrast, is observed that for *Austrolebias viarius*, in both the natural environment and captivity, the species grows more when the temperature is high, regardless of life stage (Errea & Danulat, 2001).

In addition to the ontogenetic stages of development, there is evidence that the preferred temperature of annual fish may also vary between the sexes, with each sex selecting the thermal conditions most suitable for their survival and reproductive success. *Austrofundulus limnaeus*, when subjected to a thermal gradient from 21 to 37°C, are able to

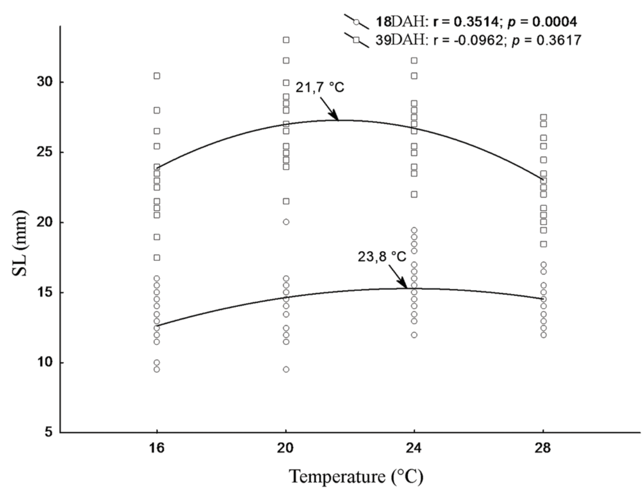


**Fig. 4.** Effect of temperature on growth in standard length (SL) of *Austrolebias wolterstorffi* under different ages (DAH = days after hatching). The arrows indicate the inflection points of curves.

accurately select different favourable temperatures (Podrabsky *et al.*, 2008). Females preferred temperatures ranging from approximately 25 to 28°C and males 23 to 26°C. The present study revealed that the optimum temperature range for the growth of males (20.1 to 20.9°C) was lower than that for females (20.7 to 21.9°C) throughout the study period.

Regardless of sex or stage of life, *A. wolterstorffi* exhibited positive allometric growth, where the changes in W were minimally higher than in SL. This is the same growth pattern observed in *A. viarius* (Errea & Danulat, 2001) and *A. nigrofasciatus* (Volcan *et al.*, 2013).

For fish, it is common for sexual maturity to be influenced by size (length or body weight) and age (Osmundson, 2006). The results suggest that to *A. wolterstorffi* body size is more influential than age in the puberty. In this study, it was observed that the males showed more pronounced initial growth than females, as the sexes of this species attain a similar SL and W, but the females grow more slowly and attain puberty two weeks later them males, but with similar length and weight. Males also grow faster than the females in other species of the *Austrolebias* genus (Calviño, 2005; Volcan *et al.*, 2012). Considering the trade off pattern of puberty time in fish, some studies indicated that a size or energy storage threshold must be surpassed for maturation to occur and that faster growing individuals matured at an earlier age (Silverstein *et al.*, 1997).

In teleosts, sex can be determined by ambient temperature (Strüssmann & Ito, 2005). Rearing *A. nigrofasciatus* at 16°C can result in a higher proportion of males (Volcan *et al.*, 2012b). In the present study, temperature had no observable effect on the sex ratio.

Although 28°C was not favourable for growth after the first two weeks of life, the first specimens to sexually differentiate were those maintained at 24 and 28°C. At 39 DAH, only one specimen reared at 16°C was sexually differentiated, indicating that higher temperatures accelerate the maturation process. *Austrolebias nigrofasciatus* specimens kept at 22°C attain sexual differentiation earlier than those reared at 16°C (Volcan *et al.*, 2012). Under laboratory conditions, *Austrolebias viarius* shows sexual differentiation at 75 DAH when kept at 25°C, but at 15°C this phenomenon occurs only at 119 DAH (Errea & Danulat, 2001).

Usually, the onset of puberty is marked by a process of morphological sexual differentiation in species that exhibit dimorphism; however, the first sign of maturity may not be morphological (Okusawa, 2002). Thus, reproductive behaviour can indicate responses that are directed by the sex steroids produced in maturing or mature gonads, which also mark puberty (Hermelink *et al.*, 2011). In this study, the first maturation behaviour sign was competition between males and courting females, behaviours that occur only in sexually differentiated specimens.

Early maturation is common in annual fish (Liu & Walford, 1970). In *Nothobranchius guentheri* sexual maturity is reached in less than four weeks (Haas, 1976). *Simpsonichthys boitonei* takes approximately two months to reach adulthood (Shibatta, 2005). In its natural environment, *A. viarius* requires between eight and 18 weeks to mature (Errea & Danulat, 2001). In captivity, *Cynopoecilus melanotaenia* can take three months to reach sexual maturity (Arenzon *et al.*, 1999). In the laboratory, sexual maturation of *A. nigrofasciatus* occurred in the fourth week at 22°C, or the fifth to sixth week at 16°C (Volcan *et al.*, 2012). In the present study, the first signs of sexual maturity were observed in the fourth week, but ovoposition was only just observed in the ninth week, excluding at 28°C. At higher temperatures, ovoposition was not observed until the end of the study period.

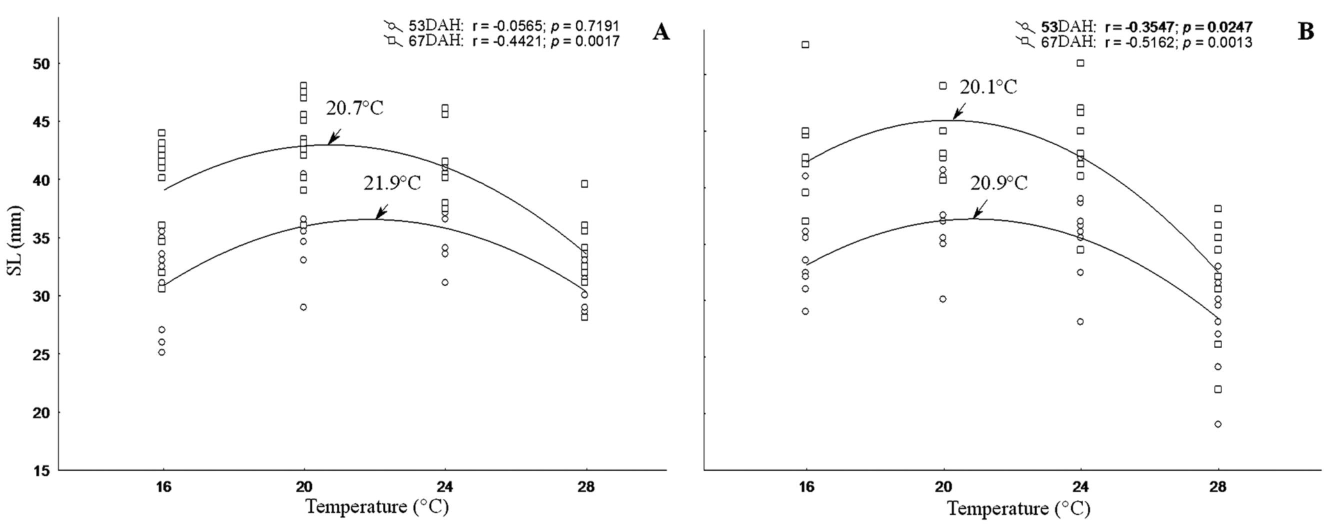


**Fig. 5.** Effect of temperature on growth in standard length (SL) of female (A) and male (B) of *Austrolebias wolterstorffi* under different ages (DAH = days after hatching). The arrows indicate the inflection points of curves.

The attainment of puberty by fish often promotes a decline in growth due to the allocation of energy to reproduction, as established to annual killifish *Austrofundulus limnaeus* (Podrabsky *et al.*, 2008). Annual fishes feed continuously even when they are reproducing because after puberty, they spawn daily until aging or death (Wourms, 1972; Haas, 1976).

The present study has shown that maintaining *A. wolterstorffi* at 28°C is detrimental to its growth. Among the tested temperatures, it was found that the optimum temperature for growth decreases throughout life. It is concluded that to optimize growth in this annual fish in captivity, the optimal water temperature is 24°C for the initial growth period, reaching temperatures around 21°C after puberty.

It is hoped that knowledge about the best conditions for the maintenance of *A. wolterstorffi* in captivity will facilitate subsequent studies with this species and contribute to understanding the biology of this endangered group of fishes.

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## Literature Cited

Arenzon, A., A. C. Peret & M. B. C. Bohrer. 1999. Reproduction of the annual fish *Cynopoecilus maelanotaenia* (Regan, 1912) based on a temporary water body population in Rio Grande do Sul State, Brazil. Hydrobiologia, 411: 65-70.

Arenzon, A., C. A. Lemos & M. B. C. Bohrer. 2002a. The influence of temperature on the embryonic development of the annual fish *Cynopoecilus melanotaenia* (Cyprinodontiformes: Rivulidae). Brazilian Journal Biology, 62: 743-747.

Arenzon, A., C. A. Lemos & M. B. C. Bohrer. 2002b. Culture parameters of the annual fish, *Cynopoecilus melanotaenia* (Regan, 1912) based on a temporary water body characteristics (Cyprinodontiformes, Rivulidae). Journal American Killifish Association, 35: 133-136.

Berois, N., M. J. Arezo, N. G. Papa & G. A. Clivio. 2012. Annual fish: developmental adaptations for an extreme environment. WIREs Developmental Biology, 1: 595-602.

Calviño, P. A. 2005. *Austrolebias toba* (Cyprinodontiformes: Rivulidae), una especie nueva de pez anual de la Argentina. Revista do Museu Argentino de Ciencias Naturais, 7: 183-190.

Clarke, A. & N. M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology, 68: 893-905.

Costa, W. J. E. M. 2006. The South American annual killifish genus *Austrolebias* (Teleostei: Cyprinodontiformes: Rivulidae): phylogenetic relationships, descriptive morphology and taxonomic revision. Zootaxa, 1213:1-162.

Costa, W. J. E. M. 2009. Trophic radiation in the South American annual killifish genus *Austrolebias* (Cyprinodontiformes: Rivulidae). Ichthyological Exploration Freshwaters, 20: 179-191.

Errea, A. & E. Danulat. 2001. Growth of the annual fish, *Cynolebias viarius* (Cyprinodontiformes), in the natural habitat compared to laboratory conditions. Environmental Biology of Fishes, 61: 261-268.

Froese, R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. Journal Applied Ichthyology, 22: 241-253.

Garvey, J. E., K. G. Ostrand & D. H. Wahl. 2004. Energetics, predation, and ration affectsize-dependent growth and mortality of fish during winter. Ecology, 85: 2860-2871.

Genade, T., M. Benedetti , E. Terzibasi, P. Roncaglia, D. R. Valenzano, A. Cattaneo & A. Cellerino. 2005. Annual fishes of the genus *Nothobranchius* as a model system for aging research. Aging Cell, 4: 223-233.

Haas, R. 1976. Sexual selection in *Nothobranchius guentheri* (Pisces-Cyprinodontidae). Evolution, 30: 614-622.

Hermelink, B., S. Wuertz, A. Trubiroha, B. Rennert, W. Kloas & C. Schulz. 2011. Influence of temperature on puberty and maturation of pikeperch, *Sander lucioperca*. General and Comparative Endocrinology, 172: 282-292.

ICMBio, Instituto Chico Mendes de Conservação da Biodiversidade. 2012. Sumário executivo do plano de ação nacional para a conservação dos peixes Rivulídeos ameaçados de extinção. Brasília. Available: http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/plano-de- acao/2833-plano-de-acao-nacional-para-a-conservacao-dos-rivulideos.html. (May 2013).

Imsland, A. K., L. M. Sunde, A. Folkvord & S. O. Stefansson. 1996. The interaction between temperature and size on growth of juvenile turbot. Journal of Fish Biology, 49: 926-940.

Lanés, L. E. K. & L. Maltchik. 2010. Discovery of the critically endangered annual killifish, *Austrolebias wolterstorffi* (Ahl, 1924) (Rivulidae: Cyprinodontiformes) in Lagoa do Peixe National Park, Rio Grande do Sul, southern Brazil. Journal Threatened Taxa, 2: 1282-1285.

Liu, R. K. & R. L. Walford. 1966. Increased growth and life-span with lowered ambient temperature in the annual fish *Cynolebias adloffi*. Nature, 212: 1277-1278.

Liu, R. K. & R. L. Walford. 1969. Laboratory studies on life-span, growth, aging, and pathology of the annual fish *Cynolebias bellottii* Steindachner. Zoologica, 54: 1-16.

Liu, R. K. & R. L. Walford. 1970. Observations on the lifespans several species of annual fishes and of the world's smallest fishes. Journal Experimental Gerontology, 5: 241-246.

Liu, R. K., E. Leung & R. L. Walford. 1975. Effect of temperature transfer on growth of laboratory populations of a south American annual fish *Cynolebias bellotti*. Growth, 39: 337-343.

Mourabit, S. & T. Kudoh. 2012. Manipulation and imaging of *Kryptolebias marmoratus* embryos. Integrative and Comparative Biology, 52: 761-768.

Okuzawa, K. 2002. Puberty in teleosts. Fish Physiology and Biochemistry, 26: 31-41.

Osmundson, D. B. 2006. Proximate causes of sexual size dimorphism in Colorado pikeminnow, a long-lived cyprinid. Journal of Fish Biology, 68: 1563-1588.

Passos, C., B. Tassino, M. Loureiro & G. G. Rosenthal. 2013. Intra- and intersexual selection on male body size in the annual killifish *Austrolebias charrua*. Behavioural Processes, 96: 20-26.

Podrabsky, J. E., D. Clelen & L. I. Crawshaw. 2008. Temperature preference and reproductive fitness of the annual killifish

*Austrofundulus limnaeus* exposed to constant and fluctuating temperatures. Journal of Comparative Physiology, 194: 385-393.

Podrabsky, J. E. & S. Hand. 1999. The bioenergetics of embryonic diapause in annual killifish, *Austrofundulus limnaeus*. The Journal of Experimental Biology. 202: 2567-2580.

Porciuncula, R. A., F. M. Quintela & D. Loebmann. 2006. Pisces, Cyprinodontiformes, Rivulidae, *Austrolebias minuano* Costa and Cheffe, 2001 and *Austrolebias wolterstorffi* (Ahl, 1924): new species records at Rio Grande city, Rio Grande do Sul state, Brazil. Check List, 2: 44-46.

Quintela, F. M., R. A. Porciuncula, M. V. L. Condini, J. P. Vieira & D. Loebmann. 2007. Composição da ictiofauna durante o período de alagamento em uma mata paludosa da planície costeira do Rio Grande do sul, Brasil. Panamjas, 2: 191-198.

Reis, R. E., Z. M. S. Lucena, C. A. S. Lucena & L. R. Malabarba. 2003. Peixes. In: Fontana, C. S., G. A. Bencke & R. E. Reis (Eds.). Livro vermelho da fauna ameaçada de extinção no Rio Grande do Sul. Porto Alegre, Edipucrs.

Rosa, R. S. & F. C. T. Lima. 2008. Peixes. In: Machado, A. B. M., G. M. Drummond & A. P. Paglia (Eds.). Livro vermelho da fauna brasileira ameaçada de extinção. Brasília, Ministério do Meio Ambiente.

Shibatta, O. 2005. Reprodução do pirá-brasília, *Simpsonichthys boitonei* Carvalho (Cyprinodontiformes, Rivulidae), e caracterização de seu habitat na Reserva Ecológica do Instituto Brasileiro de Geografia e Estatística, Brasília, Distrito Federal, Brasil. Revista Brasileira Zoolologia, 22: 1146-1151.

Silverstein, J. T., H. Shimma & H. Ogata. 1997. Early maturity in amago salmon (*Oncorhynchus masu ishikawai*): an association with energy storage. Canadian Journal of Fisheries and Aquatic Sciences, 54: 444-451.

Strüssmann, C. A. & L. S. Ito. 2005. Where does gonadal sex differentiation begin? Gradient of histological sex differentiation in the gonads of pejerrey, *Odontesthes bonariensis* (Pisces, Atherinidae). Journal of Morfology, 265: 190-196.

Utrilla, C. G. & J. Lobón-Cerviá. 1999. Life-history patterns in a southern population of Atlantic salmon. Journal of Fish Biology, 55: 68-83.

Walford, R. L. & R. K. Liu. 1965. Husbandry, life-span and growth rate of the annual fish, *Cynolebias adloffi*. Experimental Gerontology, 1: 161-171.

Wourms, J. P. 1972. The developmental biology os annual fishes. I. Stages in the normal development of *Astrofundulus myersi* Dahl. Journal of Zoology, 182: 143-168.

Valenzano, D. R., E. Terzibasi, A. Cattaneo, L. Domenici & A. Cellerino. 2006. Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish *Nothobranchius furzeri*. Aging Cell, 5: 275-278.

Volcan, M. V., L. E. K. Lanés & A. C. Gonçalves. 2010. Threatened fishes of the world: *Austrolebias univentripinnis* Costa and Cheffe 2005 (Cyprinodontiformes, Rivulidae). Environmental Biology of Fishes, 87: 319-320.

Volcan, M. V., A. P. Fonseca, M. R. C. Figueiredo, L. A. Sampaio & R. B. Robaldo. 2012. Effect of temperature on growth of the threatened annual fish *Austrolebias nigrofasciatus* Costa & Cheffe 2001. Biota Neotropica, 12: 68-73.

Volcan, M. V., A. P. Fonseca & R. B. Robaldo. 2011. Reproduction of the threatened Annual Killifish *Austrolebias nigrofasciatus* (Cyprinodontiformes: Rivulidae), confined in a natural environment. Journal Threatened Taxa, 3: 1864-1867.

Volcan, M. V., L. A. Sampaio, D. C. Bongalhardo & R. B. Robaldo. 2013. Reproduction of the annual fish *Austrolebias nigrofasciatus* (Rivulidae) maintained at different temperatures. Journal of Applied Ichthyology, 29: 648-652.

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