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Temperatures for fertilization and hatching and their influence on determining the sex ratio of the silver catfish *Rhamdia quelen*

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ABSTRACT. This study aimed to evaluate the effects of water temperature during fertilization and egg incubation in the determination of the sex ratio of fingerlings of silver catfish *Rhamdia quelen*. Water temperatures of 19, 25 and 30°C were used during the egg fertilizations, and the eggs were then incubated at temperatures of 19, 25 or 30°C for each fertilization temperature condition. An increase in temperature reduced the fertilization rate of *R. quelen*, while the final number of fish was reduced when a lower temperature was used during egg incubation. The temperatures of fertilization and incubation that were tested did not alter the sex ratio.

Key words: hatching time, survival.

RESUMO. Temperatura de fertilização e incubação e sua influência na determinação da proporção sexual do jundiá, *Rhamdia quelen*. O presente estudo teve como objetivo avaliar o efeito da temperatura da água de fertilização e de incubação na determinação da proporção sexual do jundiá, *Rhamdia quelen*. Para tanto, foram utilizadas três temperaturas de água de fertilização de ovos (19, 25 e 30°C), e para cada uma delas os ovos foram incubados nas temperaturas de 19, 25 e 30°C. O aumento da temperatura reduziu a taxa de fertilização de *Rhamdia quelen*, enquanto o número final de indivíduos foi reduzido quando a menor temperatura de incubação foi utilizada. As temperaturas de fertilização ou incubação testadas não alteram a proporção entre os sexos.

Palavras-chave: tempo de eclosão, sobrevivência.

Introduction

Determination and sexual differentiation have been studied for a long time in a wide variety of vertebrates, including many species of fish (NAKAMURA et al., 1998; BAROILLER et al., 1999; DEVLIN; NAGAHAMA, 2002).

The effects of environmental factors on determination and sexual differentiation have been demonstrated in several fish species; temperature is one of the important factors, as it can influence the structure and function of proteins and other macromolecules. Thus, the variation of temperatures found in different fish habitats can change the processes of development and sexual differentiation (DEVLIN; NAGAHAMA, 2002).

The effect of temperature appears to be partially mediated by the influence of the activity of aromatase, the synthesis of estradiol in females and of steroid receptors in both sexes (CREWS, BERGERON, 1994; CREWS, 1996). For *Paralichthys olivaceus*, high temperatures are associated with reduced levels of aromatase and low levels of estradiol, causing masculinization (KITANO et al., 1999). Temperature also influences the production of steroids in tilapia *Sarotherodon mossambicus* (KIME; HYDER, 1983), in trout *Salmo gairdneri* (MANNING; KIME, 1985) and in carp *Cyprinus carpio* (KIME; MANNING, 1986).

Römer and Beisenherz (1996) demonstrated that the sex ratio of 33 species of the genus *Apistogramma* is influenced by temperature and that, for most species, high temperatures increased the percentage of males. Similarly, when *Oreochromis mossambicus* is exposed to high temperatures in the early stages of development, an increase in the proportion of males is registered (WANG; TSAI, 2000). For *Oreochromis aureus*, temperature variations can also induce masculinization, but not as effectively as a constant temperature of 35°C (BARAS et al., 2000). For the channel catfish, *Ictalurus punctatus*, asymmetric sexual rates occurred, which favored the development of females when higher temperatures were applied during the critical period for sex determination (PATINO et al., 1996).

As the silver catfish males exhibit an earlier sexual maturation and a lower growth rate than females (FRACALOSSI et al., 2004), monosex female cultivation could be used for the production of larger sized fish in a lesser amount of time.

The management of the sex ratio can be extremely useful for the cultivation of some species, and hormonal treatments are in fact used to control the phenotypic sex of various fish species. As these treatments may pose risks to human health and to the environment, the identification of safe methods for sex ratio control becomes important. The present study was carried out to evaluate the effect of temperature on the determination of the sex ratio in fingerlings of the silver catfish, *Rhamdia quelen*.

Material and methods

The eggs were obtained by the induced breeding of four females and one male of wild broodstock of *Rhamdia quelen*, which were captured in the Uruguay river (Santa Catarina State, Brazil).

During the breeding period, the fish were stocked in 1,000 L cages with water at a temperature of 24.7°C, with constant aeration and water exchange. For the females, hormonal inductions of two doses of carp pituitary extract were used; the first dose measured 0.25 mg kg^{-1} and the second dose 4.0 mg kg^{-1}; whereas a single dose of 5.0 mg kg^{-1} was used for the male. The females presented a mean weight of 646.3±296.0 g and the male presented a weight of 495.0 g. The time between the doses was 11h, and spawning, which was obtained by gamete extrusion, occurred 8h after the second dose of hormone. After extrusion, a pool of oocytes from the females was obtained. This pool, after being homogenized and weighed, was separated into 5.0 g portions for use in each treatment.

The eggs were mixed with 25 μL of semen, which was added to each portion of oocytes. Fertilization was done in 500 mL plastic cups, where water was added at temperatures of 19, 25 and 30°C, using three replicates for each treatment. After 3 min., the eggs were washed with water and transferred to incubators. In the incubators, in order to establish a fertilization rate, the eggs were sampled and observed every 20 min. to detect the time of blastopore closure. Observations were made on a stereomicroscope; white or malformed eggs were quantified as dead, and translucent or transparent eggs were considered viable.

The viable eggs were incubated at temperatures of 19, 25 or 30ºC, with three replicates per treatment. The incubation was performed in 16-L fiberglass, cylindrical-conical type incubators. To maintain constant temperature in the experimental units, the incubators were immersed into 1000 L cages, in which heaters were used to keep temperatures at 25 and 30°C. For the temperature of 19°C, a controlled temperature recirculation system was used. Oxygenation and egg buoyancy were maintained by artificial aeration equipment, installed at the bottom of the incubators.

Every hour, a sample of eggs was taken to identify the time of larval hatching. Upon hatching, the water volume of the incubators was reduced to 5 L, homogenized, and in order to obtain the hatching rate, three samples of 80 mL each were taken for larva counting. After this stage, the larvae were acclimated to each experimental temperature.

The larvae were held in tanks with 16 L of water in a closed recirculation system, artificially supplied with aeration and maintained at a temperature of 27.5°C. All larvae of each incubation temperature were kept separately in new experimental units. The larvae were fed four times a day during the first days after hatching with newly hatched *Artemia* sp., and after that period, with feed that contained 40% crude protein. The fish were kept in these units for two months and then transferred to larger units (50 L) until a size that was suitable for macroscopic analysis of gonads, at which time a precise identification of fish sex was established.

The temperature and dissolved oxygen concentration of water in the different treatments were measured every 8h with an oxymeter YSI-55 and a YSI-63 pH probe.

To evaluate the sex ratio of the juveniles, the gonads of all individuals within each treatment were analyzed, and sex was determined by a macroscopic exam that detected the anatomical difference between males and females. At that time, the fish presented an average weight (± standard deviation) between 2.97±1.15 and 5.07±1.53 g, and an average length between 7.55±1.00 and 8.93±1.06 cm.

To evaluate the influence of water temperature on the rates of fertilization and hatching in the different treatments, a regression analysis was applied. The final number of larvae was analyzed by ANOVA, followed by the Tukey test (ZAR, 1996) when necessary. For sex ratio analysis, the chi-square test was used (ZAR, 1996), which considered equality between the sexes (1 female: 1 male) as the null hypothesis.

Results and discussion

Temperatures were maintained within the range that was proposed for the incubation, and the concentrations of dissolved oxygen remained very

close to saturation (Table 1). Woynarovich and Horváth (1983) affirmed the oxygen consumption of eggs in the early stages is very low, but increases considerably as their development progresses. According to these authors, if the concentration of dissolved oxygen in the water after hatching reaches low levels, the larvae become weak and may not be able to recover even if the level of dissolved oxygen in the water came back to a normal condition.

The pH remained close to neutral in all treatments, although *R. quelen* is resistant to pH change and supports pH from 4.0 to 9.0 (ZAIONS; BALDISSEROTTO, 2000).

The time expended for the closure of the blastopore varied between the different incubation temperatures, with eggs that were incubated at 30°C having shown a faster embryonic development compared to the other treatments. At that temperature, the closure of the blastopore occurred 8h after fertilization; whereas at 25 and 19°C, the closure of the blastopore occurred 9 and 25h after fertilization, respectively.

Temperature showed an influence ($p < 0.05$) on fertilization rates (Figure 1), since a reduction of fertilization was recorded with an increase in incubation temperature. Eggs fertilized at 30°C showed lower rates of fertilization for the different incubation temperatures, which demonstrates the negative influence of temperature over fertilization. However, lower fertilization rates were registered with higher incubation temperatures.

Hatching time varied among the different temperatures, with larvae that were incubated at 30°C hatching faster (26h after fertilization) than those incubated at the temperatures of 25°C or 19°C, for which the hatchings occurred 32 and 77h after fertilization, respectively.

Similar results were reported by Baldisseroto and Radünz Neto (2004), who found that eggs of *R. quelen* that were incubated at 24°C can extend their hatching time from 27 to 36h after fertilization. For the same species, Mardini et al. (1981) showed that eggs incubated at 16°C hatched around 72h after fertilization and took about 24h when incubated at 24°C. A similar pattern was reported by Luz et al. (2001) for eggs of the *Pimelodus maculatus* that showed blastopore closure after 5h 5 min. and hatched 21h 20 min. after fertilization when an average temperature of 23.1°C was used.

As cooler temperatures prolong the duration of the embryonic and larval stages of *R. quelen*, the stocking time for the production of larvae is higher, which makes the time for using the production facilities longer. Hatching rates were not influenced by incubation temperature ($p > 0.05$), although the hatching time in the treatment with the coldest temperature was higher.

Pavlidis et al. (2000) studying Dicentrarchus labrax, tested the hypothesis that sex determination could be affected by the incubation temperature during the early stages of embryonic development. They concluded that, for larvae, the incubation temperature is a crucial factor in the process of sexual differentiation, and that low temperatures (13 or 15°C) favor the development of female fish. Regarding Paralichthys olivaceus, however, higher and lower temperatures induced male single sex populations, while intermediate temperatures produced a sexual ratio of 1:1 (YAMAMOTO, 1999).

The combination of genetic and environmental mechanisms in sex determination has been examined for the Atlantic silverside, *Odontesthes bonariensis*. For this species, Strüssmann et al. (1996) showed that the critical period for sex determination occurs between the 28th and 49th day after hatching, and that the proportion of females and males ranged from 100% between 17-19°C to 0% at 29°C. However, the prolonged exposure of the juveniles to 29°C produced completely sterile individuals (STRÜSSMANN et al., 1997). For the Atlantic silverside larvae incubated at higher temperatures, there was an increase in the proportion of males compared to females (CONOVER, KYNARD, 1981). In this species, sex determination occurred in the first half of the larval period, and temperature variation in subsequent periods had no effect on the sex ratio (CONOVER, FLEISHER, 1986).

Table 1. Water temperature (T,°C), pH and dissolved oxygen (DO) concentration (mg L^{-1}) (mean ± standard deviation) during silver catfish *Rhamdia quelen* egg incubation period.

		Fertilization temperature (°C)								
		19			25			30		
		T	pH	DO	T	pH	DO	T	pH	DO
Incubation temperature (°C)	19	19.0±0.2	7.2±0.1	9.3±0.2	19.0±0.1	7.2±0.1	9.3±0.1	19.1±0.7	7.3±0.2	9.3±0.1
	25	25.2±0.6	6.8±0.1	8.2±0.1	25.2±0.6	6.9±0.1	8.1±0.9	25.2±0.9	6.8±0.4	8.2±0.1
	30	30.3±0.3	6.8±0.2	7.2±0.2	30.4±0.3	6.8±0.2	7.2±0.3	30.2±0.1	6.8±0.3	7.2±0.1

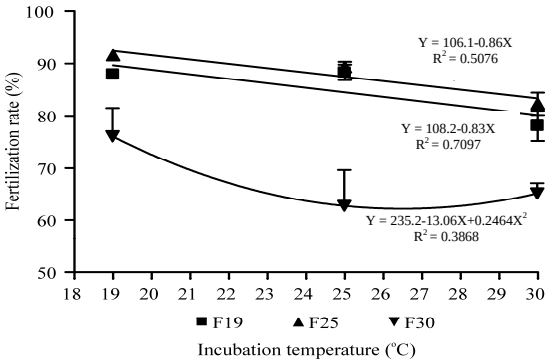

Figure 1. Fertilization rate (mean ± standard deviation) of silver catfish *Rhamdia quelen* eggs at different temperatures of fertilization (F19 = 19ºC; F25 = 25ºC and F30 = 30ºC) and incubation.

In the present study, determination of the sex ratio was not influenced by the temperatures for fertilization or hatching (Table 2), since the male:female ratio was not different from 1:1 ($p > 0.05$). In addition to the absence of a direct influence of temperature on sex ratio determination, other conditions, like the use of temperature outside the period of gonadal differentiation and/or the application of inappropriate temperatures can also be important factors. At the end of the experiment, the number of fish was correlated with temperature (Figure 2), since the lowest number of individuals, among the incubation temperatures tested ($p < 0.05$), was found at the lowest incubation temperature (19ºC).

Table 2. Final number of silver catfish *Rhamdia quelen* males and females at day 90 after being fertilized and incubated at different temperatures.

	Fertilization temperature (ºC)						
	19		25		30		
	males	females	males	females	males	females	
Incubation	19	7	8	5	6	7	6
temperature	25	34	34	28	18	29	25
(ºC)	30	25	40	23	15	23	27

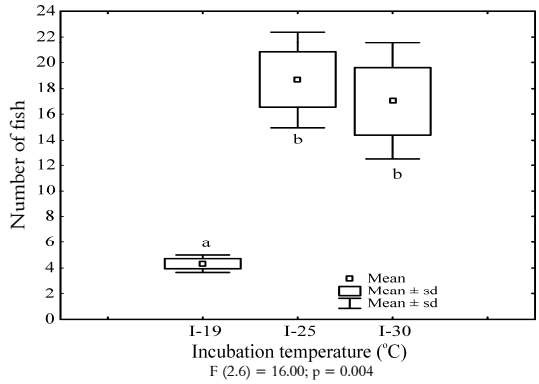

Figure 2. Number (mean ± standard deviation) of silver catfish *Rhamdia quelen* after fertilization and incubation at temperatures 19 (I-19), 25 (I-25) and 30ºC (I-30). Means followed by different letters indicate statistical difference (Tukey test; $p < 0.05$).

Conclusion

The increase in temperature reduced the rate of fertilization of *R. quelen* eggs. While the final number of larvae was reduced when the lower temperature for incubation was used, temperatures for fertilization and incubation, however, did not alter the sex ratio.

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References

BALDISSEROTO, B.; RADÜNZ NETO, J. **Criação de Jundiá**. Santa Maria: UFSM, 2004.

BARAS, E. E. BARAS, E.; PRIGNON, C.; GOHOUNGO, G.; MÉALARD, C. Phenotypic sex differentiation of blue tilapia under constant and fluctuating thermal regimes and its adaptive and evolutionary implications. **Journal of Fish Biology**, v. 57, n. 1, p. 210-223, 2000.

BAROILLER, J. F.; GUIGEN, Y.; FOSTIER, A. Endocrine and environmental aspects of sex diferentiation in fish. **Cellular and Molecular Life Sciences**, v. 55, n. 6-7, p. 910-931, 1999.

CONOVER, D. O.; FLEISHER, M. H. Temperature-sensitive period of sex determination in the Atlantic silverside, *Menidia menidia*. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 43, n. 3, p. 514-520, 1986.

CONOVER, D. O.; KYNARD, B. E. Environmental sex determination: interaction of temperature and genotype in a fish. **Science**, v. 213, n. 4507, p. 577-579, 1981.

CREWS, D. Temperature-dependent sex determination: the interplay of steroid hormones and temperature. **Zoological Science**, v. 13, n. 1, p. 1-13, 1996.

CREWS, D.; BERGERON, J. M. Role of reductase and aromatase in sex determination in the red-eared slider (*Trachemys scripta*), a turtle with temperature-dependent sex determination. **Journal of Endocrinolology**, v. 143, n. 2, p. 279-289, 1994.

DEVLIN, R. H.; NAGAHAMA, Y. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. **Aquaculture**, v. 208, n. 3-4, p. 191-364, 2002.

FRACALOSSI, D. M.; MEYER, G.; SANTAMARIA, F. M.; WEINGARTNER, M.; ZANIBONI FILHO, E. Desempenho do jundiá, *Rhamdia quelen*, e do dourado, *Salminus brasiliensis*, em viveiros de terra na região sul do Brasil. **Acta Scientarium. Animal Sciences**, v. 26, n. 3, p. 345-352, 2004.

KIME, D. E.; HYDER, M. The effect of temperature and gonadotropin on testicular steroidogenesis in *Sarotherodon mossambicus* (tilapia) in vitro. **General and Comparative Endocrinology**, v. 50, n. 1, p. 105-115, 1983.

KIME, D. E.; MANNING, N. J. Maturational and temperature effects on steroid hormone production by testes of the carp, *Cyprinus carpio*. **Aquaculture**, v. 54, n. 1-2, p. 44-55, 1986.

KITANO, T.; TAKAMUNE, K.; KOBAYASHI, T.; NAGAHAMA, Y.; ABE, S. I. Suppression of P450 aromatase gene expression in sex-reversed males produced by rearing genetically female larvae at a high water temperature during a period of sex differentiation in the Japanese flounder (*Paralichthys olivaceus*). **Journal of Molecular Endocrinology**, v. 23, n. 2, p. 167-176, 1999.

LUZ, R. K.; REYNALTE-TATAJE, D. A.; FERREIRA, A. A.; ZANIBONI FILHO, E. Desenvolvimento embrionário e estágios larvais do mandi-amarelo *Pimelodus maculatus*. **Boletim do Instituto de Pesca**, v. 27, n. 1, p. 49-55, 2001.

MANNING, N. J.; KIME, D. E. The effect of temperature on testicular steroid production in the rainbow trout, *Salmo gairdneri*, in vitro and in vivo. **General and Comparative Endocrinology**, v. 57, n. 3, p. 377-382, 1985.

MARDINI, C. V.; SILVEIRA, M. A.; BARENHO, D. H. L. **Técnica de indução da desova em jundiá (*Rhamdia quelen)* empregada na estação experimental de piscicultura da Lagoa dos Quadros**. Porto Alegre: Secretaria da Agricultura, 1981.

NAKAMURA, M.; KOBAYASHI, T.; CHANG, X. T.; NAGAHAMA, Y. Gonadal sex differentiation in teleost fish. **Journal of Experimental of Zoology**, v. 281, n. 5, p. 362-372, 1998.

PATINO, R.; DAVIS, K. B.; SCHOORE, J. E.; UGUZ, C.; STRUESSMANN, C. A.; PARKER, N. C.; SIMCO, B. A.; GOUDIE, C. A. Sex differentiation of channel catfish gonads: normal development and effects of temperature. **Journal Experimental of Zoology**, v. 276, n. 3, p. 209-218, 1996.

PAVLIDIS, M.; KOUMOUNDOUROS, G.; STERIOTI, A.; SOMARAKIS, S.; DIVANACH, P.; KENTOURI, M. Evidence of temperature-dependent sex determination in the European sea bass (*Dicentrarchus labrax L.*). **Journal of Experimental of Zoology**, v. 287, n. 3, p. 225-232. 2000.

RÖMER, U.; BEISENHERZ, W. Environmental determination of sex in *Apistogramma* (Ciclidae) and two other freshwater fishes (Teleostei). **Journal of Fish Biology**, v. 48, n. 4, p. 714-725, 1996.

STRÜSSMANN, C. A.; TAKASHIMA, F.; TODA, K. Sex differentiation and hormonal feminization in pejerrey *Odontesthes bonariensis*. **Aquaculture**, v. 139, n. 1, p. 31-45, 1996.

STRÜSSMANN, C. A.; SAITO, T.; USUI, M.; YAMADA, H.; TAKASHIMA, F. Thermal thresholds and critical period of thermolabile sex determination in two atherinid fishes, *Odontesthes bonariensis* and *Patagonina hatcheri*. **Journal of Experimental Zoology**, v. 278, n. 3, p. 167-177, 1997.

WANG, L. H.; TSAI, C. L. Effects of temperature on the deformity and sex differentiation of tilapia, *Oreochromis mossambicus*. **Journal of Experimental Zoology**, v. 286, n. 5, p. 534-537, 2000.

WOYNAROVICH, E.; HORVÁTH, L. A. **A propagação artificial de peixes tropicais**. Brasília: FAO/Codevasf/CNPq, 1983.

YAMAMOTO, E. Studies on sex-manipulation and production of cloned populations in hirame, *Paralichthys olivaceus* (Temmink et Shlegel). **Aquaculture**, v. 173, n. 1-4, p. 235-246, 1999.

ZAIONS, M. I.; BALDISSEROTTO, B. Na^+ and K^+ body levels and survival of fingerlings of *Rhamdia quelen* (Siluriformes, Pimelodidae) exposed to acute changes of water pH. **Ciência Rural**, v. 30, N. 6, p. 1041–1045, 2000.

ZAR, J. H. **Biostatistical analysis**. New Jersey: Prentice Hall, 1996.

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