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Growth and survival of silver catfish larvae, *Rhamdia quelen* (Heptapteridae), at different calcium and magnesium concentrations

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Since the relative ratios of Ca^{2+} and Mg^{2+} can vary greatly from one water body to another, and lime used for the increase of water hardness or pH can have different ratios of Ca^{2+} and Mg^{2+} in its composition, the objective of this study was to analyze the growth and survival of silver catfish, *Rhamdia quelen*, larvae at different calcium and magnesium concentrations. After fertilization, eggs were randomly divided into 4 treatments (three replicates per treatment) with different concentrations of Ca^{2+} and Mg^{2+} at hardness values of 70 mg.L^{-1} $CaCO_3$ (mg.L^{-1} : 5.2 Ca^{2+} and 14.12 Mg^{2+}; 13.11 Ca^{2+} and 7.11 Mg^{2+}; 20.26 Ca^{2+} and 2.86 Mg^{2+}; 24.95 Ca^{2+} and 0.95 Mg^{2+}) and 150 mg.L^{-1} $CaCO_3$ (mg.L^{-1} : 5.2 Ca^{2+} and 32.70 Mg^{2+}; 28.63 Ca^{2+} and 16.44 Mg^{2+}; 44.68 $Ca^{2+}$and 6.44 Mg^{2+}; 62.78 Ca^{2+} and 0.95 Mg^{2+}). There was also another group exposed to water hardness of 20 mg.L^{-1} $CaCO_3$ (Ca^{2+} 5.2 mg.L^{-1} and Mg^{2+} 0.95 mg.L^{-1}) at both experiments. The post-hatch larvae were transferred to continuously aerated 40 L polyethylene aquaria (400 larvae/tank) containing the same water as used for incubation. Samples of larvae were collected on days 0, 7, 14, and 21, and the length, weight, and specific growth rate were determined for each collection. Survival and biomass were calculated on day 21. At water hardness of 70 mg.L^{-1} $CaCO_3$, the best survival and growth of silver catfish larvae was observed at water with 20.26 mg.L^{-1} Ca^{2+} and 2.89 mg.L^{-1} Mg^{2+}, with similar results to the group exposed to water hardness of 20 mg.L^{-1} $CaCO_3$. However, compared to the group exposed to water hardness of 20 mg.L^{-1} $CaCO_3$, survival and growth were lower at 150 mg.L^{-1} $CaCO_3$. Therefore, a hardness range of 20 to 70 mg.L^{-1} $CaCO_3$ is recommended for silver catfish larviculture, but with 20.26 mg.L^{-1} Ca^{2+} and 2.89 mg.L^{-1} Mg^{2+} at 70 mg.L^{-1} $CaCO_3$. Water hardness of 150 mg.L^{-1} $CaCO_3$ is not recommended for this species.

Uma vez que as concentrações de Ca^{2+} e Mg^{2+} podem variar bastante de um corpo de água para outro, e o calcáreo utilizado para aumentar a dureza e o pH da água pode ter diferentes proporções de Ca^{2+} e Mg^{2+} em sua composição, o objetivo deste estudo foi analisar o crescimento e a sobrevivência de larvas de jundiá (*Rhamdia quelen*) em diferentes concentrações de cálcio e magnésio. Depois da fertilização, os ovos foram divididos aleatoriamente em 4 tratamentos com diferentes concentrações de Ca^{2+} e Mg^{2+} em durezas da água de 70 mg.L^{-1} $CaCO_3$ (mg.L^{-1} : 5,2 Ca^{2+} e 14,12 Mg^{2+}; 13,11 Ca^{2+} e 7,11 Mg^{2+}; 20,26 Ca^{2+} e 2,86 Mg^{2+}; 24,95 Ca^{2+} e 0,95 Mg^{2+}) e 150 mg.L^{-1} $CaCO_3$ (mg.L^{-1} : 5,2 Ca^{2+} e 32,70 Mg^{2+}; 28,63 Ca^{2+} e 16,44 Mg^{2+}; 44,68 Ca^{2+}e 6,44 Mg^{2+}; 62,78 Ca^{2+} e 0,95 Mg^{2+}). Também houve outro grupo mantido em dureza de 20 mg.L^{-1} $CaCO_3$ (5,2 mg.L^{-1} e 0,95 mg.L^{-1}) para ambos experimentos. As larvas eclodidas foram transferidas para tanques de polietileno com 40L de água e aeração constante (400 larvas/tanque) contendo a mesma água do tratamento usado para incubação. Foram coletadas amostras das larvas para avaliação de comprimento, peso e crescimento específico a 0, 7, 14 e 21 dias. A sobrevivência e biomassa foram calculadas aos 21 dias. Em dureza 70 mg.L^{-1} $CaCO_3$, a melhor sobrevivência e crescimento das larvas de jundiá foram observados na água com 20,26 mg.L^{-1} Ca^{2+} e 2,89 mg.L^{-1} Mg^{2+}, com resultados similares ao grupo exposto à dureza 20 mg.L^{-1} $CaCO_3$. Entretanto, verificou-se menor sobrevivência e crescimento em dureza 150 mg.L^{-1} $CaCO_3$ que em 20 mg.L^{-1} $CaCO_3$. Portanto, a faixa de dureza de 20 a 70 mg.L^{-1} $CaCO_3$ é recomendada para larvicultura de jundiá, mas com 20,26 mg.L^{-1} Ca^{2+} e 2,89 mg.L^{-1} Mg^{2+} com 70 mg.L^{-1} $CaCO_3$. A dureza da água de 150 mg.L^{-1} $CaCO_3$ não é recomendada para esta espécie.

Key words: hardness, water quality, larval development

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Introduction

The ionic regulation of the earlier stages (embryonic and larvae development) depends successively of the plasma membrane, blastoderm and chloride cell on the skin surface. However, subsequent ionic regulation in later stages (juveniles and adults) is done by chloride cells activated in the gills and by the intestine and kidney, whose functionality increases progressively (Alderdice, 1988). Ca^{2+} and Mg^{2+} are the principal contributors to water hardness and are important for ionic regulation of freshwater fish because both ions influence the permeability of biological membranes, and reduce diffusive flow and high ionic loss to water (Bijvelds *et al.*1998; Naddy *et al*. 2002). At elevated water hardness, fish have lower overall gill permeability and osmoregulatory costs. Hardness retards toxicant uptake and can protect against changes of several water chemistry parameters such as pH, Na^{+}, and Cl^{-} (Wood, 2001). Fish can obtain Ca^{2+} from the food by intestinal absorption (Baldisserotto & Mimura, 1995) but the main site for absorption is the gills (Hwang *et al*. 1996). However, for Mg^{2+} the intestine is the first route of absorption and the gill is the secondary one (Bijvelds *et al*. 1998). Low Ca^{2+} concentration in both food and water could limit fish growth (Rodgers, 1984). In freshwater fish, chloride cells (in the gills) appear to be the site of active Ca^{2+} (and probably other divalent ions) uptake (Perry, 1997). Chloride cells can be found in the yolk sac epithelium in the early stage of larval development (Hwang & Hirano, 1985).

Channel catfish larvae showed higher survival and initial growth with an increase of water hardness up to 100 mg l^{-1} $CaCO_3$ (Tucker & Steeby, 1993). Townsend *et al*., (2003) verified that water hardness of 30-70 mg l^{-1} $CaCO_3$ at pH 8.25 enhanced growth of silver catfish larvae compared to higher levels (150 mg l^{-1} $CaCO_3$). Molokwu & Okpokwasili (2002) recommended a water hardness range of 30-60 mg l^{-1} $CaCO_3$ for optimal normal hatching, viability and maximum larval development of *Clarias gariepinus*. However, these studies increased water hardness by adding only Ca^{2+}. Since the relative ratios of Ca^{2+} and Mg^{2+} can vary greatly from one water body to another (Naddy *et al*. 2002), and lime used for the increase of water hardness or pH can have different ratios of Ca^{2+} and Mg^{2+} in its composition, it is important to verify if the increase of water hardness by the addition of different Ca^{2+} and Mg^{2+} concentrations could change larval survival and growth. Therefore, the objective of the present study was to analyze the growth and survival of silver catfish, *Rhamdia quelen* (Heptapteridae), larvae at different calcium and magnesium concentrations.

Materials and Methods

Silver catfish eggs were obtained from induced spawning (one spawn in December 1999- experiment 1, and in February 2000- experiment 2) at the fish culture sector at the Universidade Federal de Santa Maria, South Brazil. The brood fish received one dose of carp pituitary extract (females = 5 mg kg^{-1} and males = 3 mg kg^{-1}, according to Legendre *et al*. 1996) and the oocytes and milt were then extruded. The oocyte mass was divided into 5 equal parts and placed in 5 plastic containers. The milt was then added to each container to provide fertilization. After fertilization, eggs were hydrated and incubated in the same water to be used for treatment (to be described). Two days after hatching (end of larval yolk sac absorption) larvae were transferred and maintained in continuously aerated 40 L freshwater polyethylene aquaria for 21 days. Photoperiod was 12 h light - 12 h dark, with luminosity of 0.6 lux (measured with a LI-COR photometer model LI-185B).

Larvae were fed in excess six times a day (0800, 1000, 1200, 1400, 1600, and 1800 h) with ground dry pellets (Table 1). The granule size of the pellets was 100-200 mm (first week), 200-400 mm (second week), and 400-600 mm (third week). Stocking density was 10 larvae/L. All feces and pellet residues were removed daily by suction, and consequently approximately 10% of the water in the aquaria was replaced up to 7 days, and after that the replacement was around 50% per day to keep low ammonia levels in the water. The water for replacement was previously adjusted to the same pH and hardness of the treatments. Samples of 20 larvae were collected from each replicate on days 0, 7, 14, and 21 after transfer to the 40 L aquaria, and length and weight were measured. Specific growth rate (SGR) was calculated for each collection according to JØrgensen & Jobling (1993). On day 21 all surviving larvae were collected to determine survival and biomass (individual mean weight x number of surviving larvae).

Each experiment contained five treatments (three replicates per treatment). Larvae were exposed to different Ca^{2+} and Mg^{2+} concentrations at hardness values of 70 mg.L^{-1} $CaCO_3$ (experiment 1, concentrations mg.L^{-1}: 5.2 Ca^{2+} and 14.12 Mg^{2+}; 13.11 Ca^{2+} and 7.11 Mg^{2+}; 20.26 Ca^{2+} and 2.86 Mg^{2+}; 24.95 Ca^{2+} and 0.95 Mg^{2+}) and 150 mg.L^{-1} $CaCO_3$ (experiment 2, concentrations mg.L^{-1}: 5.2 Ca^{2+} and 32.70 Mg^{2+}; 28.63 Ca^{2+} and 16.44 Mg^{2+}; 44.68 $Ca^{2+}$and 6.44 Mg^{2+}; 62.78 Ca^{2+} and 0.95 Mg^{2+}). There was also another group exposed to water

Table 1. Composition of the ground dry pellets used to feed silver catfish larvae. 1 - Content (kg): vit. A : 75000 UI; vit. D3:15.000; vit E: 300 mg; vit K3: 25 mg; vit B1: 75 mg; vit B2: 150 mg; vit B6: 75 mg; vit B12: 250 mg; biotine: 12.5 mg; pantotenic acid: 250 mg; folic acid: 25 mg; nicotinic acid:: 875 mg; choline: 10 g; vit C: 250 mg; inositol: 4 g. 2 - Content (kg): Mg^{2+}: 50 mg.L^{-1}; S: 400 mg.L^{-1}; Mn: 40 mg.L^{-1}; Cu: 0.3 mg.L^{-1}; Fe: 7.5 mg.L^{-1}; Zn: 7 mg.L^{-1}; Co: 0.7 mg.L^{-1}; I: 20 mg.L^{-1}.

Compounds	% used
yeast	57
chicken liver	30
vitamin mixture[1]	2
mineral mixture[2]	1
rice flour	8
soybean lecithin	2
Ca^{2+}	0.3
Mg^{2+}	0.08
Crude protein (%)	41

hardness of 20 mg.L^{-1} $CaCO_3$ (laboratory water, Ca^{2+} 5.2 mg.L^{-1} and Mg^{2+} 0.95 mg.L^{-1}) at both experiments. The increase of water hardness was obtained by the addition of $CaCl_2$ and/or $MgCl_2$ to laboratory water. Waterborne Ca^{2+} and Mg^{2+} values for all the treatments were determined by flame atomic absorption spectrometry using an atomic absorption spectrometer (Perkin-Elmer Model 3030, Germany) equipped with Ca^{2+} and Mg^{2+} hollow cathode lamps (operated at 10 mA and 6 mA, respectively). The selected wavelengths were 422.7 nm and 285.2 nm (slit width of 0.7 nm) for Ca^{2+} and Mg^{2+}, respectively. A deuterium lamp was used for background correction (Eaton *et al*. 1995). Waterborne Na^+ and K^+ were measured with a Micronal B286 flame spectrophotometer, and the method of Zall et al. (1956) was used for determining Cl^- concentration.

Water quality was analyzed daily and water pH was measured with a Hanna (HI 8424) pH meter and adjusted to pH 8.0-8.2 (with sodium hydroxide), since this is the best pH range for survival and growth of silver catfish larvae (Lopes *et al*. 2001). Total ammonia and water hardness were analyzed by the method of Greenberg *et al*. (1976), non-ionized ammonia was calculated as described by Piper *et al.* (1982), and dissolved oxygen was determined with a YSI (model Y5512) oxygen meter. Alkalinity (precision 4.0 mg.L^{-1} $CaCO_3$) and nitrite (precision 0.05 mg.L^{-1}) were estimated using a kit from Alfa Tecnoquímica (Brazil).

Survival within the treatment groups was analyzed by the chi-square test and the mean length, weight, SGR, and total biomass of treatment groups were compared by one-way ANOVA followed by the Tukey test, using the Instat Program version 2.05. Data from the treatments at 70 mg.L^{-1} $CaCO_3$ were not compared with those at 150 mg.L^{-1} $CaCO_3$ because these experiments were conducted at different times and used different fish brood. However, both experiments contained a group exposed to water hardness at 20 mg.L^{-1} $CaCO_3$ for comparison. Data were expressed as mean±SEM and the minimum significance level was set at $P<0.05$.

Results

Dissolved oxygen (7.0-7.2 mg.L^{-1}), temperature (24 °C), pH (7.9-8.2), total and non-ionized ammonia (0.2-0.6 mg.L^{-1}, 0.013-0.05 mg.L^{-1}, respectively), nitrite (maximum 0.05 mg.L^{-1}), and total alkalinity (61-75 mg.L^{-1} $CaCO_3$) did not show any significant difference among treatments or over the course of the experiments within treatment groups. The values of water hardness showed small variation (± 2 mg.L^{-1} $CaCO_3$) within the precision range of the method. Waterborne Na^+, K^+ and Cl^- levels were (mmol.L^{-1}): 0.95±0.10, 0.05±0.01, and 0.32±0.15, respectively.

Survival of larvae exposed to the treatments with 5.2 mg.L^{-1} Ca^{2+} and 14.12 mg.L^{-1} Mg^{2+} and 13.11 mg.L^{-1} Ca^{2+} and 7.11 mg.L^{-1} Mg^{2+} (hardness of 70 mg.L^{-1} $CaCO_3$) were significantly lower than those exposed to hardness of 20 mg.L^{-1} $CaCO_3$. In addition, larvae submitted to the treatment with 5.2 mg.L^{-1} Ca^{2+} and 14.12 mg.L^{-1} Mg^{2+} showed the significantly lowest survival among the treatments at hardness of 70 mg.L^{-1} $CaCO_3$. Biomass of larvae exposed to the treatments with 5.2 mg.L^{-1} Ca^{2+} and 14.12 mg.L^{-1} Mg^{2+} and 13.11 mg.L^{-1} Ca^{2+} and 7.11 mg.L^{-1} Mg^{2+} were significantly lower than those exposed to the treatment with 20.26 mg.L^{-1} Ca^{2+} and 2.89 mg.L^{-1} Mg^{2+}. Larvae submitted to the treatment with 5.2 mg.L^{-1} Ca^{2+} and 14.12 mg.L^{-1} Mg^{2+} also showed significantly lower biomass than those exposed to hardness of 20 mg.L^{-1} $CaCO_3$ Larvae exposed to hardness of 150 mg.L^{-1} $CaCO_3$ presented significantly lower survival and biomass than those maintained at hardness of 20 mg.L^{-1} $CaCO_3$ (except survival of larvae submitted to the treatment with 5.2 mg.L^{-1} Ca^{2+} and 32.71 mg.L^{-1} Mg^{2+}). Survival was significantly lower in the two treatments with the highest waterborne Ca^{2+} concentration (44.84 and 62.94 mg.L^{-1}). Biomass was also significantly lower in the treatment with 62.94 mg.L^{-1} Ca^{2+} and 0.95 mg.L^{-1} Mg^{2+} than in the treatment with 28.63 mg.L^{-1} Ca^{2+} and 16.44 mg.L^{-1} Mg^{2+} (Table 2).

Table 2. Survival and biomass of silver catfish larvae after 21 days as a function of Ca^{2+} and Mg^{2+} concentrations in the water. Means (±SEM) with different letters in the same column and experiment are significantly different ($P < 0.05$) as determined by ANOVA and by the Tukey test. First line of each experiment is the treatment with water hardness of 20 mg.L^{-1} $CaCO_3$.

Ca^{2+} (mg.L^{-1})	Mg^{2+} (mg.L^{-1})	Survival (%)	Biomass (g)
Experiment 1			
5.2	0.95	94.13 ± 1.65^{a}	40.11 ± 0.82^{ab}
5.2	14.12	85.85 ± 3.43^{b}	25.39 ± 0.71^{c}
13.11	7.11	90.91 ± 6.55^{c}	33.13 ± 7.80^{bc}
20.26	2.89	92.45 ± 0.87^{ac}	46.37 ± 0.82^{a}
24.95	0.95	92.58 ± 1.59^{ac}	28.16 ± 1.12^{bc}
Experiment 2			
5.2	0.95	40.25 ± 2.08^{a}	8.23 ± 0.27^{a}
5.2	32.71	36.79 ± 3.55^{ab}	4.46 ± 0.41^{bc}
28.63	16.44	32.10 ± 1.20^{b}	5.52 ± 0.84^{b}
44.84	6.43	23.81 ± 3.10^{c}	4.27 ± 0.86^{bc}
62.94	0.95	22.38 ± 1.36^{c}	2.26 ± 0.16^{c}

Larval length was significantly lower on the 7th day at hardness of 70 mg.L^{-1} $CaCO_3$ (except the treatment with 20.26 mg.L^{-1} Ca^{2+} and 2.89 mg.L^{-1} Mg^{2+}) compared to control. At the end of the experiment larval length at this water hardness was lower at the two highest Mg^{2+} concentrations (7.11 and 14.12 mg.L^{-1} Mg^{2+}) and at the highest Ca^{2+} concentration (24.95 mg.L^{-1} Ca^{2+}). At water hardness of 150 mg.L^{-1} $CaCO_3$, larval length was significantly reduced after 7 days at the the two highest Ca^{2+} concentrations compared to those exposed to hardness of 20 mg.L^{-1} $CaCO_3$, but at days 14 and 21 there was no significant difference among treatments (Table 3).

Larvae exposed to water with the highest Mg^{2+} concentration at 70 mg.L^{-1} $CaCO_3$ showed a significantly lower weight than those exposed to hardness of 20 mg.L^{-1} $CaCO_3$ after 14 and 21 days (Table 4). At water hardness of 150 mg.L^{-1} $CaCO_3$, larvae exposed to the treatment with 62.94 mg.L^{-1} Ca^{2+} and 0.95 mg.L^{-1} Mg^{2+} and the treatment with 5.2 mg.L^{-1} Ca^{2+} and 32.71 mg.L^{-1} Mg^{2+} showed significantly lower weight after 21 days compared to those exposed to hardness of 20 mg.L^{-1} $CaCO_3$

Table 3. Length (mm) of silver catfish larvae as a function of Ca^{2+} and Mg^{2+} concentrations in the water. Means (±SEM) with different letters in the same column and experiment are significantly different ($P < 0.05$) as determined by ANOVA and by the Tukey test. First line of each experiment is the treatment with water hardness of 20 mg.L^{-1} $CaCO_3$.

Days after yolk sac absorption					
Ca^{2+} (mg.L^{-1})	Mg^{2+} (mg.L^{-1})	0	7	14	21
Experiment 1					
5.2	0.95	5.05 ± 0.05	8.90 ± 0.01^{a}	12.30 ± 0.25	19.60 ± 0.25^{a}
5.2	14.12	4.97 ± 0.03	8.20 ± 0.11^{b}	11.25 ± 0.16	15.75 ± 0.35^{b}
13.11	7.11	4.97 ± 0.03	8.30 ± 0.03^{b}	11.78 ± 0.18	17.10 ± 0.55^{bc}
20.26	2.89	4.99 ± 0.01	8.52 ± 0.02^{ab}	11.82 ± 0.27	18.70 ± 0.22^{ac}
24.95	0.95	4.93 ± 0.04	8.35 ± 0.18^{b}	11.50 ± 0.13	16.69 ± 0.46^{b}
Experiment 2					
5.2	0.95	5.03±0.04^{ab}	8.30 ± 0.09^{a}	10.03 ± 0.18	15.18 ± 0.26
5.2	32.71	4.97±0.02^{ab}	8.13 ± 0.01^{ab}	9.91 ± 0.12	13.02 ± 0.41
28.63	16.44	5.07±0.07^{a}	8.08 ± 0.11^{ab}	10.68 ± 0.12	14.80 ± 0.78
44.84	6.43	5.05±0.05^{ab}	7.70 ± 0.25^{bc}	10.31 ± 0.77	14.48 ± 0.93
62.94	0.95	4.90±0.02^{b}	7.27 ± 0.08^{c}	9.31 ± 0.02	13.15 ± 0.38

Table 4. Weight (mg) of silver catfish larvae as a function of Ca^{2+} and Mg^{2+} concentrations in the water. Means (±SEM) with different letters in the same column and experiment are significantly different ($P < 0.05$) as determined by ANOVA and by the Tukey test. First line of each experiment is the treatment with water hardness of 20 mg.L^{-1} $CaCO_3$.

Days after yolk sac absorption					
Ca^{2+} (mg.L^{-1})	Mg^{2+} (mg.L^{-1})	0	7	14	21
Experiment 1					
5.2	0.95	1.59 ± 0.09	8.65 ± 0.35	29.50 ± 0.50^{a}	118.55 ± 0.95^{ab}
5.2	14.12	1.97 ± 0.01	6.79 ± 1.08	21.57 ± 1.50^{b}	82.50 ± 5.47^{c}
13.11	7.11	1.53 ± 0.03	6.90 ± 0.56	26.97 ± 1.56^{ab}	97.50 ± 12.50^{abc}
20.26	2.89	1.67 ± 0.12	8.47 ± 0.44	25.83 ± 2.20^{ab}	128.50 ± 6.40^{a}
24.95	0.95	1.63 ± 0.29	7.26 ± 0.26	23.30 ± 0.26^{ab}	92.93 ± 4.42^{bc}
Experiment 2					
5.2	0.95	2.23±0.23	7.47 ± 0.37	14.50 ± 0.90	76.50 ± 1.80^{a}
5.2	32.71	2.30±0.35	6.53 ± 0.67	16.13 ± 0.45	49.27 ± 5.23^{b}
28.63	16.44	1.80±0.10	7.07 ± 1.03	17.33 ± 1.48	69.93 ± 7.14^{ab}
44.84	6.43	2.50±0.50	5.45 ± 0.75	12.15 ± 2.45	81.30 ± 0.20^{a}
62.94	0.95	2.20±0.31	6.37 ± 1.01	12.73 ± 0.67	47.70 ± 4.18^{b}

(Table 4). The SGR decreased over time, but there was no significant difference among treatments at 70 mg.L^{-1} $CaCO_3$ either at 20 mg.L^{-1} $CaCO_3$. However, at 150 mg l^{-1} $CaCO_3$ the SGR was significantly lower at day 21 in the larvae exposed to treatment with 5.2 mg.L^{-1} Ca^{2+} and 32.71 mg.L^{-1} Mg^{2+} than those exposed to hardness of 20 mg.L^{-1} $CaCO_3$. In addition, larvae exposed to treatment with 44.84 mg.L^{-1} Ca^{2+} and 6.43 mg.L^{-1} Mg^{2+} showed significantly higher SGR than those exposed to treatment with 5.2 mg.L^{-1} Ca^{2+} and 32.71 mg.L^{-1} Mg^{2+} and to treatment with 62.94 mg.L^{-1} Ca^{2+} and 0.95 mg.L^{-1} Mg^{2+} (Table 5).

Discussion

In the present study the best survival of silver catfish larvae was observed at a hardness of 20 mg.L^{-1} $CaCO_3$ (control) in both experiments and also at water hardness of 70 mg.L^{-1} $CaCO_3$ with the two highest Ca^{2+} concentrations. However, larvae exposed to the highest Ca^{2+} concentration at water hardness of 70 mg.L^{-1} $CaCO_3$ also presented lower length and weight than those maintained at 20 mg.L^{-1} $CaCO_3$. These results are in agreement with Silva *et al*. (2003), who demonstrated that the hatching rate of silver catfish eggs were higher at water hardness of 70 mg.L^{-1} $CaCO_3$ independently of the Ca^{2+} and Mg^{2+} concentrations used. However, the same authors showed that increase of waterborne Ca^{+2} above 20 mg.L^{-1}, irrespective of water hardness, was not recommend for incubation of silver catfish eggs because it reduced post-hatch (2 days after hatching) survival.

Townsend *et al*. (2003) also obtained a higher survival with larvae of this species exposed to water hardness of 30 mg.L^{-1} $CaCO_3$, followed by 70 mg.L^{-1} $CaCO_3$ (increased with Ca^{2+}). The same authors verified that survival at higher water hardness was very low: 8.7%, 1%, and 0% for 150, 300, and 600 mg l^{-1} $CaCO_3$, respectively. Probably the higher larval survival obtained in our experiments at water hardness of 70 and 150 mg.L^{-1} $CaCO_3$ than that reported by Townsend *et al*. (2003) at the same water hardness could be due to the fact that in our study egg hydration and incubation were performed in the same water as used for the treatments. The better survival and growth of larvae hatched at higher water hardness may reflect a better adaptation to the medium conferred by transfer prior to hatching, but this hypothesis still needs to be proved experimentally.

The highest larval survival (71%) for *Clarias gariepinus* was observed at 60 mg.L^{-1} $CaCO_3$, and a similar water hardness range (30-60 mg.L^{-1} $CaCO_3$) is recommended for optimal normal hatching, viability and maximum larval development of this species (Molokwu & Okpokwasili, 2002). Channel catfish swim-up fry exposed to 0, 0.4, 2, 4, or 40 mg.L^{-1} Ca^{2+} (0, 1, 5, 10, and 100 mg.L^{-1} $CaCO_3$) showed best growth at 4 and 40 mg.L^{-1} Ca^{2+} (Tucker & Steeby, 1993). The same authors observed an abnormal behavior (fry appeared lethargic and were spread out over the bottom) in water with low Ca^{2+} concentration (below 2 mg.L^{-1} Ca^{2+}). There are no experiments with silver catfish larvae exposed to such low water hardness, but interestingly, this kind of abnormal behavior was observed in our experiments in some larvae only at high water hardness (150 mg.L^{-1} $CaCO_3$).

It is well known that SGR decreases with fish size (Jobling, 1994). This pattern was observed in the present experiment and was not influenced by Ca^{2+} and Mg^{2} concentrations at water hardness of 70 mg.L^{-1} $CaCO_3$, but at water hardness of 150 mg.L^{-1} $CaCO_3$, the larvae exposed to the treatment with the highest Mg^{2+} concentration (5.2 mg.L^{-1} Ca^{2+} and 32.71 mg.L^{-1} Mg^{2+}) showed lower SGR than those exposed to the water hardness of 20 mg.L^{-1} $CaCO_3$. The SGR obtained were higher than those verified by Townsend *et al*. (2003) with larvae of the same species, may be because the larvae of the present experiment were born in water of the same hardness as that used for the experiment. However, this hypothesis needs additional experiments to be proved, as explained for survival.

Table 5. Specific growth rate (SGR, %.day^{-1}) of silver catfish larvae as a function of Ca^{2+} and Mg^{2+} concentrations in the water. Means (±SEM) with different letters in the same column and experiment are significantly different ($P < 0.05$) as determined by ANOVA and by the Tukey test. First line of each experiment is the treatment with water hardness of 20 mg.L^{-1} $CaCO_3$.

Ca^{2+} (mg.L^{-1})	Mg^{2+} (mg.L^{-1})	Days after yolk sac absorption 7	14	21
Experiment 1				
5.2	0.95	24.20 ± 0.23	8.75 ± 0.39	6.62 ± 0.10
5.2	14.12	17.33 ± 2.82	8.41 ± 1.05	6.41 ± 0.46
13.11	7.11	21.33 ± 1.26	9.79 ± 0.99	6.29 ± 0.29
20.26	2.89	23.30 ± 1.15	7.93 ± 0.91	7.65 ± 0.34
24.95	0.95	21.84 ± 3.17	8.36 ± 0.19	6.59 ± 0.18
Experiment 2				
5.2	0.95	17.38 ± 1.91	4.74 ± 0.10	7.92 ± 0.23^{ab}
5.2	32.71	15.05 ± 3.31	6.55 ± 0.60	5.25 ± 0.42^{c}
28.63	16.44	19.28 ± 2.14	6.51 ± 1.31	6.62 ± 0.67^{abc}
44.84	6.43	11.29 ± 1.00	5.66 ± 2.45	9.17 ± 0.97^{a}
62.94	0.95	15.10 ± 3.70	5.11 ± 0.87	6.21 ± 0.44^{bc}

Juveniles of *Sciaenops ocellatus* (euryhaline fish) showed higher survival at 25 mg.L^{-1} Ca^{2+} in the water when compared with 50 or 100 mg l^{-1} Ca^{2+} (obtained with $CaCl_2$), but the 1.5 to 268 mg.L^{-1} waterborne Mg^{2+} concentration range (obtained with $MgCl_2$) did not alter survival of this species (Wurts & Stickney, 1989). There were conflicting results in a growth experiment of callichthyid catfish (*Megalechis personata*) larvae in low mineral freshwater (2.92 mg.L^{-1} Ca^{2+} and 0.36 mg.L^{-1} Mg^{2+}) and high mineral freshwater (2.36 mg.L^{-1} Ca^{2+} and 47.14 mg.L^{-1} Mg^{2+}), with the best growth being obtained in low mineral freshwater in one replicate, while in the other replicate the opposite result was obtained (Mol *et al*. 1999). The same authors also observed a higher rate of Ca^{2+}uptake in low mineral water than in high mineral water, while the rate of magnesium accumulation did not differ.

The increase of water hardness with $MgSO_4$ up to 400 mg.L^{-1} $CaCO_3$ (with $MgSO_4$) reduced survival of *Ictalurus punctatus* juveniles to 0%, but when $CaCO_3$ was used to increase water hardness survival was higher (95%) (Perschbacher & Wurts, 1999). Silver catfish juveniles are also able to survive abrupt transfer to high water hardness (from 30 to up 600 mg.L^{-1} $CaCO_3$, increased by adding only $CaCl_2$) without problems. In addition, an increase of water hardness to 70 mg.L^{-1} $CaCO_3$ is enough to improve survival even at pH 3.75, and an additional increase is ineffective. On the other hand, at extremely alkaline pH's such as 10.0 and 10.5 it is necessary to increase water hardness up to 300 mg.L^{-1} $CaCO_3$ to improve survival significantly (Townsend & Baldisserotto, 2001). According to Grizzle & Mauldin (1994), the ability to regulate the internal medium improves with development, a fact that could explain the different effect of water hardness on the survival of *R. quelen* larvae and juveniles.

According to our results, the best water hardness for hatching and larviculture of *Rhamdia quelen* is in the 20 - 70 mg.L^{-1} $CaCO_3$ range, but with a waterborne Ca^{2+} concentration of 20.26 mg.L^{-1} and a Mg^{2+} concentration of 2.89 mg.L^{-1} at 70 mg.L^{-1} $CaCO_3$. The different ratio of these ions at this water hardness would result in a higher waterborne Ca^{2+} or Mg^{2+} concentration, both situations impairing hatching rate and/or larviculture of this species. Similar water hardness levels are also recommended for *C. gariepinus* larvae (Molokwu & Okpokwasili, 2002) and channel catfish swim-up fry (Tucker & Steeby, 1993), but experiments with different waterborne Ca^{2+} and Mg^{2+} proportions are still missing for these species.

In conclusion, the best survival and growth of *Rhamdia quelen* larvae was observed at water hardness of 20 mg.L^{-1} $CaCO_3$ and water hardness of 70 mg.L^{-1} $CaCO_3$ with 20.26 mg.L^{-1} Ca^{2+} and 2.89 mg.L^{-1} Mg^{2+}. Magnesium concentrations over 7.11 mg.L^{-1} at water hardness of 70 mg/L $CaCO_3$ are not recommended for *Rhamdia quelen* larviculture. Water hardness of 150 mg.L^{-1} $CaCO_3$ is not recommended for *Rhamdia quelen* larviculture, regardless of Ca^{2+} and Mg^{2+} concentrations.

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