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# EVALUATION OF NEOMALE *Rhamdia quelen* PROGENY GROWING AT VARIOUS STOCKING DENSITIES

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## ABSTRACT

Monosex female jundiá (*Rhamdia quelen*) generated through indirect sex reversal may have their growth influenced by the stocking density, which is an important factor for production in fish farming. Since zootechnical data from *R. quelen* offspring generated by crossbreeding between genotypic masculinized females (neomales) and normal females were never reported, the objective of this study was to compare the zootechnical indexes of neomale and normal male offspring stocked at densities of 500, 1,000 and 1,500 fish m$^{-3}$ (D1, D2, and D3) for 61 days. The initial mean weight and length of the neomale and normal male offspring were 0.90 ± 0.12 g / 4.94 ± 0.22 cm and 0.80 ± 0.12 g / 4.60 ± 0.37 cm, respectively. A completely randomized experimental design with three replicates was used, with fish stocked in 0.07 m$^3$ tanks. At the end of the experimental period, the data evaluated showed that there were no significant differences between progeny or among stocking densities in terms of survival, weight, weight gain, length, feed conversion or feed consumption. Therefore, neomale offspring presented adequate productive performance during the juvenile stage and tolerated increases in stocking density without performance loss, making the neomales suitable for *R. quelen* cultivation.

**Key words:** silver catfish; male; monosex; native species; zootechnical indices.

## DESCENDÊNCIA DE NEOMACHOS DE *Rhamdia quelen*: AVALIAÇÃO DO CRESCIMENTO EM DIFERENTES DENSIDADES

## RESUMO

Lotes monossexo feminino de *Rhamdia quelen* podem ser gerados por inversão sexual indireta e seu crescimento é influenciado pela densidade de estocagem, a qual é um fator importante no seu desenvolvimento. O estudo teve por objetivo comparar o desempenho produtivo das descendências de neomachos e de machos normais estocados nas densidades de 500, 1000 e 1500 peixes m$^{-3}$ (D1, D2 e D3), durante 61 dias, através da avaliação de índices zootécnicos. A média inicial de peso e comprimento das descendências de neomachos e machos normais, foi respectivamente, 0,90 ± 0,12 g / 4,94 ± 0,22 cm e 0,80 ± 0,12 g / 4,60 ± 0,37 cm. Utilizou-se o delineamento experimental inteiramente ao acaso com três repetições, sendo os peixes estocados em tanques de 0,07 m$^3$. Os valores finais de peso, comprimento e sobrevivência (média ± dp) foram os seguintes: Neomachos: D1 - 4,03 ± 0,50 g, 7,73 ± 0,20 cm, 98,10 ± 1,65%; D2 -: 4,08 ± 0,21 g, 7,80 ± 0,21 cm, 96,67 ± 0,82%; D3 - 4,74 ± 0,77 g, 8,18 ± 0,44 cm, 95,56 ± 3,34%; Machos: Dl - 3,67 ± 0,36 g, 7,58 ± 0,20 cm, 91,43 ± 2,86%; D2 - 3,96 ± 0,54 g, 7,86 ± 0,23 cm, 94,29 ± 3,78%; D3 - 4,23 ± 0,52 g, 8,05 ± 0,38 cm, 94,92 ± 3,85%. As diferentes descendências e densidades de estocagem testadas não apresentaram diferenças significativas para peso, ganho em peso, comprimento, sobrevivência, conversão alimentar e consumo de ração. Portanto, a descendência de neomachos apresentou desempenho produtivo adequado durante a fase de juvenis e suportou o aumento da densidade de estocagem sem prejuízos ao seu desempenho, tornando os neomachos adequados para o cultivo de jundiá.

**Palavras-chave:** espécie nativa; índices zootécnicos; jundiá; monossexo.

## INTRODUCTION

Jundiá (*Rhamdia quelen*) is an omnivorous catfish found in the Neotropical region that inhabits lakes, rivers, and reservoirs. This species is benthic and cold-tolerant, feeding also during autumn and winter (Gomes et al., 2000; Zaniboni-Filho and Schulz, 2003). The production of *R. quelen* has increased in the southern region of Brazil, since it responds well to induced reproduction, has high fertilization rates, rapid summertime growth and is adapted to the subtropical climate (Montanha et al., 2011).

Stocking density is highly relevant when considering the cultivation of a species in fish farming, since it influences the fish survival, growth, production and quality. All of these are critical factors in determining final yield (Rowland et al., 2006). The stocking density of 450 fish $m^{-3}$ is recommended for the development of *R. quelen* juveniles because they neither form territories within the tanks nor expend energy on fighting (Piaia and Baldisserotto, 2000). Therefore, stocking density is an important factor in the development of an aquaculture technology which optimizes productivity per unit area.

The males of certain fish such as the salmonids mature before reaching commercial weight. This characteristic reduces crop uniformity, quality, and profitability (Tabata, 2000). It also interferes with *R. quelen* cultivation. The males present lower weight gain rates than the females because they prematurely divert their metabolic energy towards gamete production (Fracalossi et al., 2004).

R. quelen production can be intensified by using female monosex fish stock. According to Weiss et al. (2017), a very widespread method of monosex production in fish farming is direct or indirect sexual inversion. Various genetic techniques, temperature manipulation or hormone treatment can be used to induce sexual inversion (Almeida, 2013). Hormones can be administered by feed supplementation, injection, pellet implantation or by immersion bath (Pandian, 2013).

Female monosex batches can be produced by direct or indirect hormone administration. In the former, estrogen is supplied during the early stages of development. In the latter, genotypic females are masculinized with androgens and transformed into neomales whose semen is then used to fertilize normal oocytes (Piferrer and Donaldson, 1989).

Indirect hormone administration for sexual inversion is an environment-friendly procedure, since neomale descendants are never exposed to exogenous hormones, which reduces the use of exogenous hormones (only applied in the brood stock). In contrast, traditional tilapia cultivation involves the direct exposure of larvae to hormones during the sex reversion procedure.

According to Bila and Dezotti (2007), exogenous hormones are classified as endocrine disruptors that can interfere with the endocrine systems of humans and other animals exposed to them in the environment. Endocrine disruptors affect health, growth, and reproduction. They accumulate in adipose tissue and can reach levels there exceeding those found in the environment and the rest of the body. Some of them are degraded only very slowly and may persist in the body for several years.

Indirect sexual inversion is preferable to direct sexual inversion and hormone application. Nevertheless, to date, there is no report addressing the development of the offspring of jundiá neomales. The present study considered the importance of female monosex cultivation and the influence of stocking density on fish growth. Since zootechnical data from *R. quelen* offspring generated by crossbreeding between genotypic masculinized females (neomales) and normal females were never reported, the objectives were to compare the zootechnical indexes of *R. quelen* juveniles derived from neomales and normal females with those produced by normal males and normal females and to determine the influence of stocking density.

## MATERIAL AND METHODS

The study was carried out at the Laboratory of Biology and Cultivation of Freshwater Fish - LAPAD (27º43'45" S; 48º30'32" W), Federal University of Santa Catarina, Brazil.

### Reproduction and larviculture

Males, neomales, and females were selected from the *R. quelen* breeding facility at LAPAD. They were chosen on the basis of the gonadal maturation criteria described by Woynarovich and Horváth (1980). For induced reproduction, the fish received carp pituitary extract in single doses of 5.0 mg $kg^{-1}$ for females and 4.0 mg $kg^{-1}$ for males 12 h before manual gamete collection.

To produce and evaluate *R. quelen* offspring, aliquots (10 g) of oocytes from a single female were used in each treatment. They were each exposed either to the semen pool of three neomales (0.5 mL $neomale^{-1}$) or to that of three normal males (0.5 mL $male^{-1}$). Oocyte fertilization occurred after gamete mixing and hydration. The fertilized eggs were then placed in 10-L cylindrical-conical incubators equipped with water recirculation systems.

After hatching, *R. quelen* larvae remained in the incubators for two days to allow sufficient time for mouth opening and nearly complete yolk sac absorption. The larvae were then transferred to 100-L capacity tanks each containing 80 L of water renewed eight times per day.

For the first 15 days, the larvae were fed exclusively with enriched newly hatched *Artemia* sp. nauplii (Inve Aquaculture Inc., Salt Lake City, UT, USA). The enrichment solution consisted of 1.0 L distilled water, 12.0 g bovine liver meal (200 µm), 6.0 g casein, 2.0 g choline chloride, 10 g vitamin and mineral PREMIX, 40 mL fish oil, 60 mL soybean oil, and 8.0 g powdered milk. This enrichment protocol was used by Weiss et al. (2017). The nauplii were maintained for 30 min in an immersion bath containing 10 mL enrichment solution. In this way, the nauplii adsorbed the nutrients. The enrichment solution and the nauplii were supplied to the larvae *ad libitum* three times per day (09:00, 12:00, and 17:30).

The transition from enriched Artemia to commercial feed took place over 5 days. The Artemia was gradually reduced as the supply of microflocated feed (Alcon® Alevinos, Camboriú, Santa Catarina, Brazil) increased. For the juveniles, the mixture initially consisted of a 1:1 blend of microflocated feed with sifted bovine liver meal (250 µm). Over a 2-days period, the feed mixture was offered for one of the three daily feedings. Subsequently, the feed mixture was provided twice per day. After 30 days, the larvae received a 2.0-mm commercial feed (Guabi Pirá 40®, Guabi Nutrição e Saúde Animal SA, Campinas, São Paulo, Brazil). The larvae were maintained under these conditions for 53 days until they reached the juvenile stage. At that time, the experiment began.

The experimental units were cleaned daily at 08:30 prior to the first feeding. Cleaning involved siphoning the bottom solids and surface residues to avoid decomposition and microbial contamination.

Experimental procedures were conducted in compliance with Protocol CEUA PP00788 and were approved by the Committee of Ethics of Animal Use of the Federal University of Santa Catarina, Brazil.

## Experimental design

The experiment lasted 61 days. It had a completely random design in a 2 x 3 factorial model with three replications. The factors were ancestry (neomale or male) and stocking density (500, 1,000 or 1,500 fish $m^{-3}$).

Juveniles derived from crosses of neomales and females (N) and from males and females (M) had mean body weight and length of 0,90 ± 0,12 g and 4,94 ± 0,22 cm, and 0,80 ± 0,12 g and 4,60 ± 0,37 cm, respectively. They were stocked at densities of 500, 1000, and 1,500 fish $m^{-3}$ (D1: 35 juveniles tank-1; D2: 70 juveniles $tank^{-1}$; D3: 105 juveniles $tank^{-1}$; equivalent to 0.29 ± 0.02; 0.59 ± 0.05 and 0.89 ± 0.07 kg $m^{-3}$, respectively). Plastic tanks of 100 L capacity were filled with 70 L salinized water (3 g $L^{-1}$) and connected to a recirculation system which renewed the water ten times daily.

All *R. quelen* juveniles were fed twice daily (09:00 and 16:00) with a balanced commercial feed (Guabi Pirá 40®, Guabi Nutrição e Saúde Animal SA, Campinas, São Paulo, Brazil). The total daily weight of the feed was 3.0% of the fish biomass. Unconsumed pellets (orts) were counted and removed from the tanks 10 min after each feeding and the daily feed intake was calculated.

Temperature, pH, electrical conductivity and dissolved oxygen (DO) were measured daily at 08:00 using a YSI Professional Plus® multiparameter probe (YSI Inc., Yellow Springs, OH, USA). The physicochemical properties of the water did not significantly differ between tanks. The temperature was 25.42 ± 0.40 °C, the pH 7.25 ± 0.16, the electrical conductivity 3,436.08 ± 742.37 µS $cm^{-1}$, and the DO 6.88 ± 0.54 mg $L^{-1}$. These parameters did not significantly vary with time and remained within the ranges conducive to fish development according to the recommendations of Gomes (2000) and Piedras et al. (2004).

## Analysis of zootechnical performance

Biometry was performed at the beginning of the experiment and every 10 days thereafter to measure the biomass stored in each experimental unit. The fish were fasted for 24 h prior to the analyses. Individual measurements were taken for 30% of the animals in each experimental unit. Survival (S), mean weight, length, weight gain (WG), apparent feed conversion (AFC), total feed consumption (TFC) and final biomass (FB), were calculated as follows:

S = (Final number of animals.Initial number of $animals^{-1}$). 100. WG = Final Mean Weight – Initial Mean Weight. AFC = Total Feed Consumption.Weight $Gain^{-1}$. TFC = Offered feed – leftover (corrected by mortality). FB = Final Number of Animal. Mean Weight.

## Statistical analyses

Data were subjected to linear regression at a significance level of 0.05. Covariance analysis was used to test the difference between the slopes of these regressions (Zar, 1999). The analyzes were performed in GraphPad Prism 4.

# RESULTS

## Survival

The stocking densities did not influence the survival rates ($P > 0.05$; Figure 1) of the different offspring (Figure 1).

## Weight

The weight and weight gain of neomale and male offspring stocked at various densities did not significantly differ ($P > 0.05$) after 61 days (Figures 2 and 3).

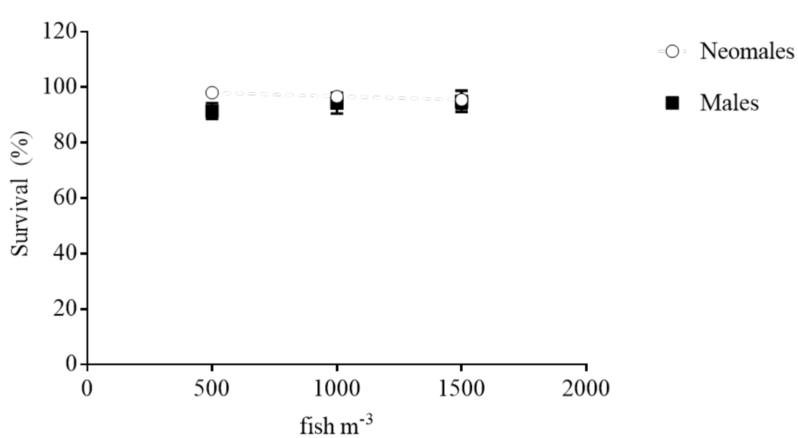
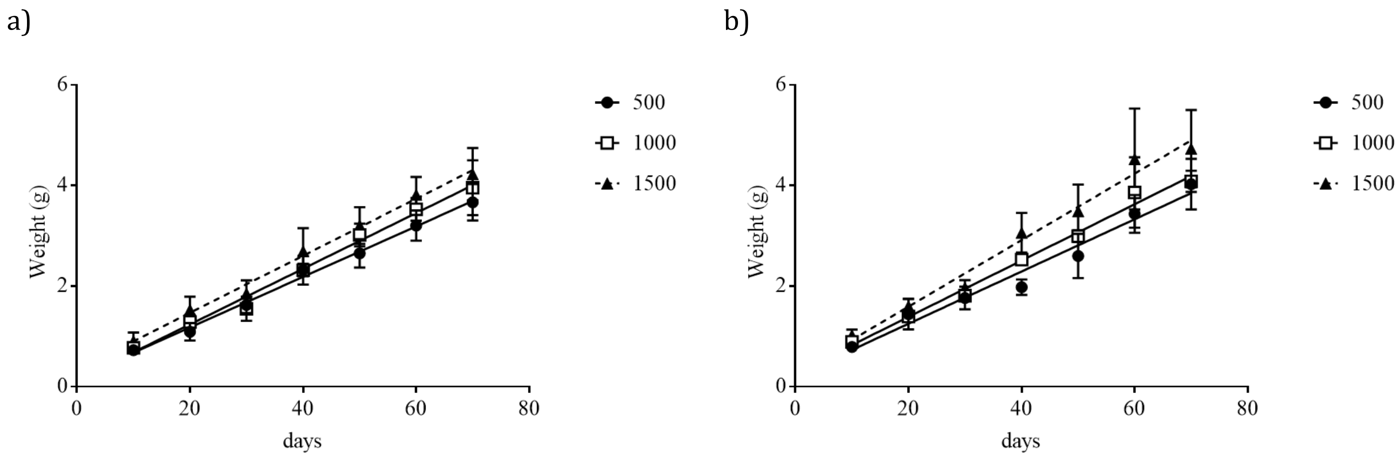



**Figure 1.** Survival of *Rhamdia quelen* juveniles descended from normal males and neomales and stocked at different densities over the course of 61 days.




● Y = 0.050X + 0.17; P<0.05; $R^2$ = 0.9953
□ Y = 0.055X + 0.13; P<0.05; $R^2$ = 0.9895
▲ Y = 0.057X + 0.34; P<0.05; $R^2$ = 0.9935

● Y = 0.052*X + 0.21; P<0.05; $R^2$ = 0.9713
□ Y = 0.056*X + 0.27; P<0.05; $R^2$ = 0.9892
▲ Y = 0.066*X + 0.27; P<0.05; $R^2$ = 0.9814

**Figure 2.** Weight of *Rhamdia quelen* juveniles descended from normal males (a) and neomales (b), stocked at different densities over the course of 61 days.

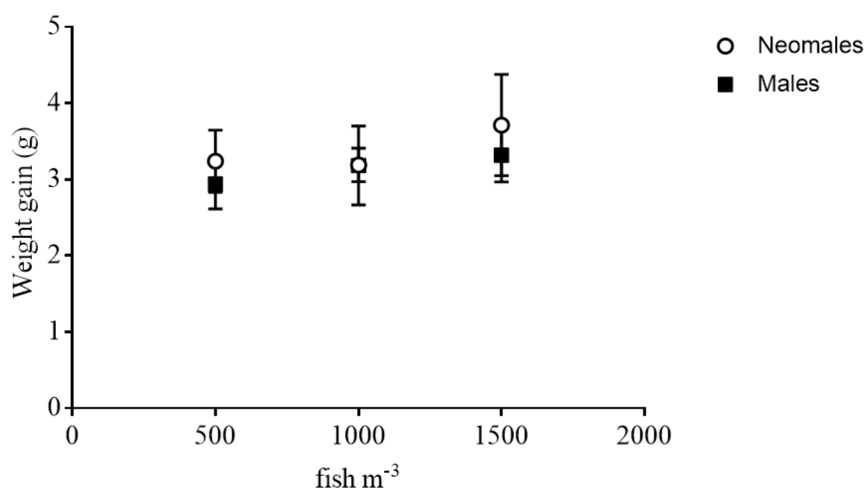



**Figure 3.** Weight gain of *Rhamdia quelen* juveniles descended from normal males and neomales, stocked at different densities over the course of 61 days.

### Length

The length of the juveniles was similar at all stocking densities and did not significantly differ (P > 0.05) after 61 days (Figure 4).

### Apparent feed consumption and conversion

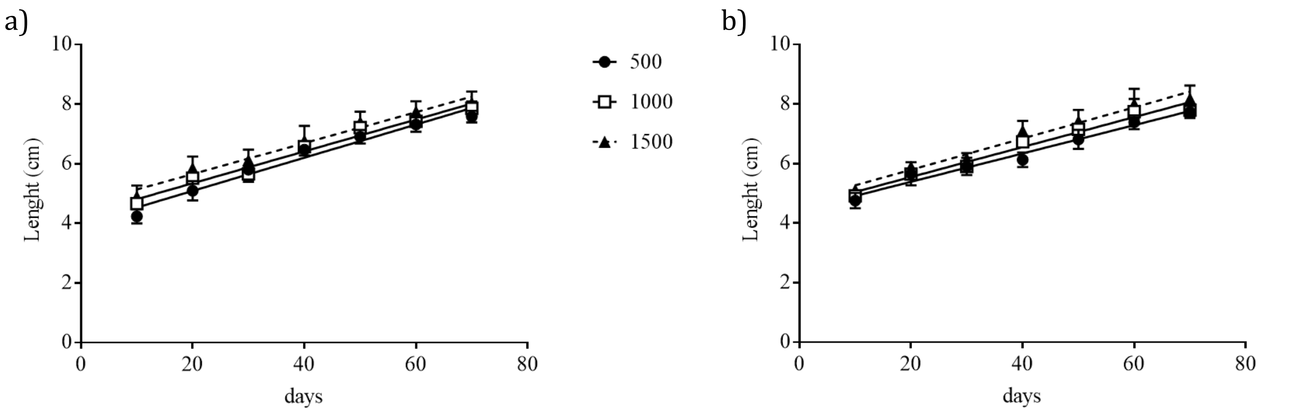
Apparent feed intake and conversion of jundiá juveniles did not significantly differ among the various offspring and stocking densities after 61 days (Figure 5).

### Final biomass

Final biomass of the male and neomale offspring significantly varied (P < 0.05) with stocking density (Figure 6).

## DISCUSSION

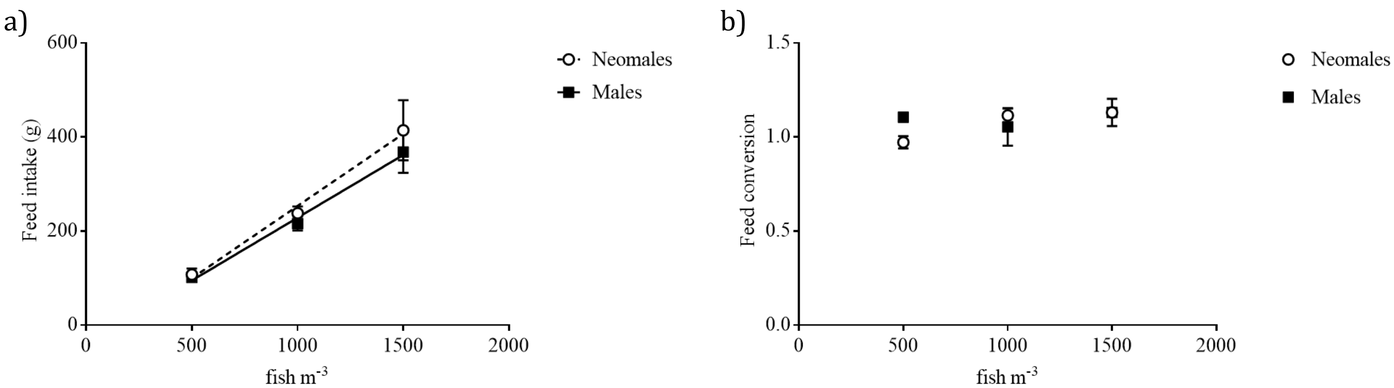
Jundiá tolerates environmental variation since it is an indigenous rustic species that tolerates the following physicochemical ranges: temperature, 17-27 °C; DO, 5.0-8.0 mg $L^{-1}$; pH, 4.0-9.0




● Y = 0.056X + 3.98; P<0.05; $R^2$ = 0.9673
□ Y = 0.053X + 4.28; P<0.05; $R^2$ = 0.9729
▲ Y = 0.051X + 4.62; P<0.05; $R^2$ = 0.9766

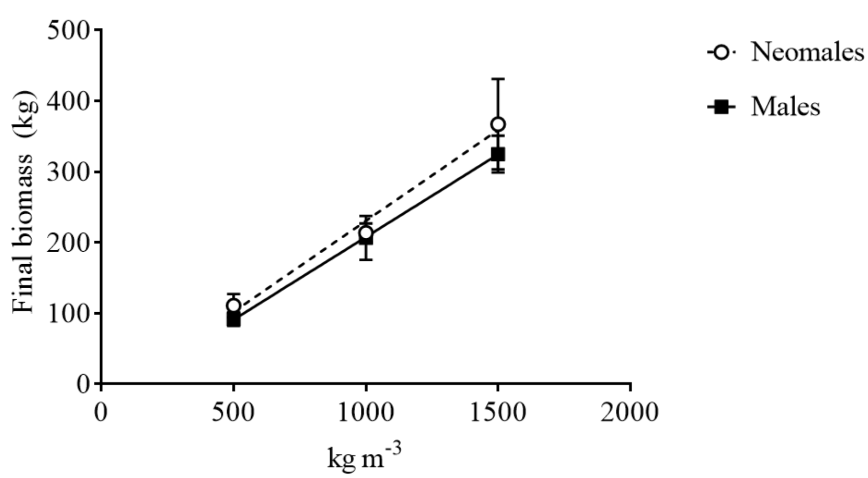
● Y = Y = 0.047X + 4.44; P<0.05; $R^2$ = 0.9760
□ Y = 0.050X + 4.55; P<0.05; $R^2$ = 0.9734
▲ Y = 0.052X + 4.75; P<0.05; $R^2$ = 0.9753

**Figure 4.** Length of *Rhamdia quelen* juveniles descended from normal males (a) and neomales (b), stocked at different stocking densities over the course of 61 days.




○ Y = 0.307X - 53.58; P<0.05; $R^2$ = 0.9927
■ Y = 0.267X - 38.42; P<0.05; $R^2$ = 0.9933

**Figure 5.** Feed intake (a) and apparent feed conversion (b) of *Rhamdia quelen* juveniles descended from normal males and neomales, stocked at different densities over the course of 61 d.




○ Y = 0.256X - 25.86; P<0.05; $R^2$ = 0.9870
■ Y = 0.233X - 25.44; P<0.05; $R^2$ = 0.9999

**Figure 6.** Final biomass of *Rhamdia quelen* juveniles descended from normal males and neomales, stocked at different densities over the course of 61 days.

(Baldisserotto and Radünz Neto, 2004). In the present study, the physicochemical properties of the water did not significantly vary and stay within the indicated ranges for the species.

Survival rates were not influenced by stocking densities for males or neomales descendants. The increase in stocking density did not adversely affect the survival of some catfish species. This result was similar to that recorded in different stocking densities for *R. quelen* (8 - 32 fish $m^{-3}$, Menezes et al., 2015) and *Salminus brasiliensis* (30 – 300 fish $m^{-3}$, Braun et al., 2010). However, Piaia and Baldisserotto (2000) showed a direct correlation between survival rate and stocking density for *R. quelen*. They reported that at higher stocking densities, *R. quelen* rapidly ingested all the feed offered, whereas at lower stocking densities ingestion was interrupted by periods of inactivity or aggression.

Neither the weight nor the length of the jundiá juveniles significantly differed ($P > 0.05$) among the various offspring tested at different densities over 61 days. Similar results were found for tambaqui (*Colossoma macropomum*). Their weight and length did not significantly differ over 30 days at stocking densities of 200, 300, 400 and 500 fish $m^{-3}$. Nevertheless, by day 60, both weight and length had significantly increased with decreasing stocking density. Fish production per unit area was significantly higher at 400 and 500 $m^{-3}$ (0.56 ± 0.20 g, Brandão et al., 2004).

A polyculture with jundiá and Hungarian carp (24.39 ± 0.54 g and 27.73 ± 0.22 g, respectively) was conducted over a 4-week period by Corrêia et al. (2010). Early-stage fish raised at 57 $m^{-3}$ and 143 $m^{-3}$ did not significantly differ in terms of final weight. Martinelli et al. (2013) studied jundiá (57.48 ± 3.34 g) for 60 days and observed that there were no significant differences between the 50 $m^{-3}$ and 150 $m^{-3}$ stocking densities in terms of final biomass, average daily gain, mass gain, total length, survival, or specific growth rate. These results were attributed to the low densities tested and the short experimental period. Piaia and Baldisserotto (2000) reported that a stocking density of 454 $m^{-3}$ is preferable for jundiá juveniles. In the present study, no significant differences in weight, length, weight gain, feed conversion, or feed intake were observed between treatments at stocking densities higher than those recommended for jundiá juveniles.

Brandão et al. (2005) evaluated the effects of increasing stocking density on matrinxã (*Brycon amazonicus*) grown in cages (200 – 500 fish $m^{-3}$; 0.24 ± 0.01g). The authors found no significant differences in the growth variables among the various stocking densities after 60 days. At this stage of development, the relative availability of space did not influence growth. Jundiá cultivated at various stocking densities for 61 days did not significantly differ in terms of most of the growth variables tested. Therefore, the availability of space had no adverse effect on their growth at that stage.

Kocmarek et al. (2015) compared growth rates and gene expression profiles among the descendants of neomale and normal male rainbow trout. The authors did not detect any significant differences in length or weight among offspring groups. However, the amplitudes of these variables were lower for the neomale offspring than the male offspring.

For *Cynoglossus semilaevis*, the weight and length of the offspring of the males at days 300, 600, and 720 were significantly higher than those for the offspring of the neomales (Hu et al., 2014). In the present study, however, the offspring did not significantly differ in terms of weight or length by the end of the experiment. This discrepancy may be explained by the relative difference in cultivation time between the two trials.

*R. quelen* feed intake increased with stocking density because of the relative increase in the number of juveniles. However, no significant differences were found among the various offspring at the same stocking density. Therefore, it is preferable to use a higher stocking density because it improves spatial utilization, production, and cultivation efficiency.

The duration of the experiment may have been too short to demonstrate the effects of offspring or stocking density on zootechnical performance. This limitation could also explain the lack of significant difference among treatments in terms of these zootechnical parameters.

## CONCLUSION

The survival rates, weight, length, feed consumption and feed conversion did not significantly differ between the offspring of *R. quelen*. Therefore, neomale offspring tolerated increases in stocking density without performance loss. Although neomales are modified females, it was verified in the present study that their offspring grow normally and could, therefore, potentially be used in large-scale cultivation.

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## REFERENCES

Almeida, F.L. 2013. Endocrinologia Aplicada na reprodução de peixes. Revista Brasileira de Reprodução Animal, Belo Horizonte, 37(2): 174-180. <**http://ainfo.cnptia.embrapa.br/digital/bitstream/item/89485/1/pag174-180-RB455.pdf**> Acesso em: 15 jun. 2019.

Bila, D.M.; Dezotti, M. 2007. Desreguladores endócrinos no meio ambiente: efeitos e consequências. Química Nova, 30(3): 651.

Baldisserotto, B.; Radünz Neto, J. 2004. Criação de Jundiá. Santa Maria, SC, Brasil: Editora UFSM: p. 73-92.

Brandão, F.R.; Gomes, L.C.; Chagas, E.C.; Araújo, L.D. 2004. Densidade de estocagem de juvenis de tambaqui durante a recria em tanques-rede. Pesquisa Agropecuária Brasileira, 39(4): 357-362. **http://dx.doi.org/10.1590/S0100-204X2004000400009**.

Brandão, F.R.; Gomes, L.C.; Chagas, E.C.; Araújo, L.D.; Silva, A.L.F. 2005. Densidade de estocagem de matrinxã (*Brycon amazonicus*) na recria em tanques-rede. Pesquisa Agropecuária Brasileira, 40(3): 299-303. **http://dx.doi.org/10.1590/S0100-204X2005000300014**.

Braun, N.; de Lima, R.L.; Baldisserotto, B.; Dafre, A.L.; Nuñer, A.P.O. 2010. Growth, biochemical and physiological responses of *Salminus brasiliensis* with different stocking densities and handling. Aquaculture, 301(1-4), 22-30. **https://doi.org/10.1016/j.aquaculture.2010.01.022**.

Corrêia, V.; Neto, J.R.; Rossato, S.; Maschio, D.; Martinelli, S.G. 2010. Efeito da densidade de estocagem e a resposta de estresse no policultivo de jundiá (*Rhamdia quelen*) e carpa húngara (*Cyprinus carpio*). Revista da FZVA, Uruguaiana, 17(2): 170-185. <**http://revistaseletronicas.pucrs.br/ojs/index.php/fzva/article/viewFile/7575/6012**> Acesso em: 15 jun. 2019.

Fracalossi, D.M.; Meyer, G.; Santamaria, F.M.; Weingartner, M.; Zaniboni-Filho, E. 2004. Desempenho do jundiá, *Rhamdia quelen*, e do dourado, *Salminus brasiliensis*, em viveiros de terra na região sul do Brasil. Acta Scientiarum. Animal Sciences, 26(3): 345-352. **http://dx.doi.org/10.4025/actascianimsci.v26i3.1806**.

Gomes, L.C.; Golombieski, J.I.; Gomes, A.R.C.; Baldisserotto, B. 2000. Biology of *Rhamdia quelen* (Teleostei, Pimelodidae). Ciência Rural, 30(1): 179-185. **http://dx.doi.org/10.1590/S0103-84782000000100029**.

Hu, Q.; Chen, S. L.; Gao, F.; Li, Y.; Liu, S.; Liu, F.; Yang, J.; Du, M. 2014. Differences in sex reversion and growth between normal and neomale stock in half-smooth tongue sole, *Cynoglossus semilaevis*. Aquaculture International 22(4): 1437-1449. **https://doi-org.ez49.periodicos.capes.gov.br/10.1007/s10499-014-9757-7**.

Kocmarek, A.L.; Ferguson, M.M.; Danzmann, R.G. 2015. Comparison of growth-related traits and gene expression profiles between the offspring of neomale (XX) and normal male (XY) rainbow trout. Marine Biotechnology, 17(2): 229-243. **https://doi-org.ez49.periodicos.capes.gov.br/10.1007/s10126-015-9612-5**.

Martinelli, S.G.; Neto, J.R.; Silva, L.P.; Bergamin, G.T.; Maschio, D.; Flora, M.A.L.D.; Nunes, L.M.C.; Possani, G. 2013. Densidade de estocagem e frequência alimentar no cultivo de jundiá em tanques-rede. Pesquisa Agropecuária Brasileira, 48(8): 871-877. **http://dx.doi.org/10.1590/S0100-204X2013000800009**.

Menezes, C.; Ruiz-Jarabo, I.; Martos-Sitcha, J.A.; Toni, C.; Salbego, J.; Becker, A.; Loro, V.L.; Martínez-Rodríguez, G.; Mancera, J.M.; Baldisserotto, B. 2015. The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): a metabolic and endocrine approach. Aquaculture, 435, 257-264. **https://doi.org/10.1016/j.aquaculture.2014.09.044**.

Montanha, F.P.; Nagashima, J.C.; Kirnew, M.D.; Astrauskas, J.P.; Pimpão, C.T. 2011. Características fisiológicas e reprodutivas do *Rhamdia quelen*. Revista Científica Eletrônica de Medicina Veterinária, 17(1): 1-8. <**http://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/fK7r1IMtpK7LUbr_2013-6-27-15-24-53.pdf**> Acesso em: 15 jun. 2019.

Pandian, T.J. 2013. Endocrine sex differentiation in fish. Boca Raton, FL: CRC Press. p. 75-212.

Piaia, R.; Baldisserotto, B. 2000. Densidade de estocagem e crescimento de alevinos de jundiá *Rhamdia quelen*. Ciência Rural, 30(3): 509-513. **http://dx.doi.org/10.1590/S0103-84782000000300024**.

Piedras, S.R.N.; Moraes, P.R.R.; Pouey, J.L.O.F. 2004. Crescimento de juvenis de jundiá (*Rhamdia quelen*), de acordo com a temperatura da água. Boletim do Instituto de Pesca, 30(2): 177-182. <**https://www.pesca.sp.gov.br/boletim/index.php/bip/article/view/piedras30_2**> Acesso em: 15 jun. 2019.

Piferrer, F.; Donaldson, E.M. 1989. Gonadal differentiation in coho salmon, *Oncorhynchus kisutch*, after a single treatment with androgen or estrogen at different stages during ontogenesis. Aquaculture 77(2-3): 63-251. **https://doi-org.ez49.periodicos.capes.gov.br/10.1016/0044-8486(89)90207-X**.

Rowland, S.J.; Mifsud, C.; Nixon, M.; Boyd, P. 2006. Effects of stocking density on the performance of the Australian freshwater silver perch (*Bidyanus bidyanus*) in cages. Aquaculture 253(1-4): 301-308. **https://doi.org/10.1016/j.aquaculture.2005.04.049**.

Tabata, Y.A. 2000. Atualização sobre métodos e procedimentos para obtenção de populações monossexo de peixes: Produção de Fêmeas. Agência Paulista de Tecnologia dos Agronegócios, São Paulo. <**http://www.aquicultura.br/trutas/info/wd/producaodefemeas.doc**> Acesso em: 15 jun. 2019.

Weiss, L.A.; Bernardes, J.J.; Nuñer, A.P.O. 2017. Identification of neomales in South American catfish *Rhamdia quelen* on the basis of the sex ratio in the progeny. Animal Reproduction 14: 1285-1291. **https://doi.org/10.21451/1984-3143-AR0031**.

Woynarovich, E.; Horvath, L. 1980. The artificial propagation of warm-water finfishes — a manual for extension. FAO Fisheries Technical Paper, No. 201. <**http://www.fao.org/3/AC742E/AC742E00.htm#TOC**> Acesso em: 15 jun. 2019.

Zaniboni-Filho, E.; Schulz, U.H. Migratory fishes of the Uruguay River 2003. In: Carolsfeld, J.; Harvey, B.; Baer, A.; Ross, C. Migratory fishes of South America: Biology, Fisheries and Conservation Status. IDRC / World Bank / World Fisheries Trust, Ottawa. p. 157-194. <**http://documents.worldbank.org/curated/pt/607691468753260624/pdf/29828011552510114100Migratory0fish.pdf**> Acesso em: 15 jun. 2019.

Zar, J.H. 1999. Biostatistical analysis. 4th ed. Upper Saddle River, NJ: Prentice-Hall. 929 p.