EMBRYONIC AND LARVAL DEVELOPMENT OF JUNDIÁ (*Rhamdia quelen*, QUOY & GAIMARD, 1824, PISCES, TELEOSTEI), A SOUTH AMERICAN CATFISH

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ABSTRACT

The jundiá (*Rhamdia quelen*, Quoy & Gaimard) is an endemic South American fish species. Because this species supports cold winters and grows faster during warm months, it has begun to be viewed as an ideal species for fish production in southern South America. In the present study, jundiá oocytes used were obtained by extrusion from females after hormone injection. Soon after hydration, the eggs were transferred to 50 L conic glass incubators, with constant and controlled water influx. Samples of fertilized eggs were transferred to Petri dishes and, examined under a stereoscopic microscope, were spherical, demersal, and non-adhesive with defined perivitelline space and resistant chorion. Cleavage stages occurred during the first 3.5 h. After hatching, larvae were transferred to 200 L glass fiber incubators. First signs of embryo movement were observed 21 h after fertilization; larval eclosion occurred 30.5 h after fertilization. Present findings may provide a basis for studies aimed at determining the complete ontogeny of jundiá and may be useful in eco-toxicological studies.

Keywords: embryonic development, larval development, embryology, *Rhamdia*, jundiá.

RESUMO

Estudo do desenvolvimento embrionário e larval do jundiá, *Rhamdia quelen*, (Quoy & Gaimard 1824, Pisces Teleostei)

O jundiá (*Rhamdia quelen, Quoy* & *Gaimard*) é uma espécie endêmica da América do Sul. Por ser adaptada ao frio do inverno e ter um crescimento rápido durante os meses quentes, o jundiá é uma espécie adequada para aqüicultura no sul da América do Sul. Muitos aspectos da fisiologia reprodutiva, larvicultura, hematologia, fisiologia da resposta ao estresse, têm sido recentemente estudados. Os ovócitos utilizados neste estudo foram obtidos pela extrusão de fêmeas após indução hormonal. Logo após a hidratação, foram transferidos para incubadoras cônicas de vidro com capacidade para 50 L, com fluxo de água constante e controlado. Amostras de ovos fertilizados foram colocadas em placas de Petri e examinadas através de estereomicroscópio. Os ovos eram esféricos, demersais e não-adesivos, com espaço perivitelino definido e córion resistente. Os estágios de clivagem ocorreram durante as 3,5 primeiras horas. Após a eclosão, as larvas foram transferidas para incubadoras de fibra de vidro de 200 l. Os primeiros sinais de movimento embrionário foram observados 21 h após a fertilização, e a eclosão das larvas ocorreu 30,5 h após a

fertilização. Estes resultados podem servir como base para muitos estudos, objetivando o conhecimento da ontogenia completa do jundiá, e para aplicação em estudos ecotoxicológicos.

Palavras-chave: desenvolvimento embrionário, desenvolvimento larval, embriologia, jundiá, *Rhamdia*.

INTRODUCTION

The jundiá (*Rhamdia quelen*, Quoy & Gaimard) is an endemic South American fish species that withstands cold winters and presents fast growth rate in summer. These characteristics make jundiá a suitable species for fish production in southern South America or any region with a temperate or subtropical climate. In aquaculture systems, at a density of two to four fish/m^2 jundiá reach a 600-800 g body weight in eight months (Barcellos *et al.*, 2001b). Our unpublished observations in experimental field trials and at fish farms have shown that this weight is easily reached, but high mortality rates (40-50%) might occur if small fish (1-3 g) are used to initiate the culture. However, when beginning with heavier juveniles (30-60 g), the final weight will still range from 600 to 800 g, but with mortality rates not exceeding 5-10%. Thus, the more reasonable order in jundiá culture is: hatchery, larviculture (1-6 g), nursery (from 5-6 g to 30-60 g), and termination (from 30-60 g to 600-800 g) (Barcellos *et al.*, 2001b).

A study evaluating the embryonic and larval development of *Rhamdia quelen* have reported on fish captured in the wild (Ihering & Azevedo, 1936). However, paremeters related to environmental conditions that could affect embryonic and larval development were not mentioned. However, in recent years several aspects of this species have been investigated, including reproductive physiology (Barcellos *et al.*, 2001b; Barcellos *et al.*, 2002), larviculture (Piaia *et al.*, 1999; Lopes *et al.*, 2001; Townsend & Baldisserotto, 2001; Townsend *et al.*, 2003), stress response (Barcellos *et al.*, 2001a), hematology (Barcellos *et al.*, 2003, and Barcellos *et al.*, 2004b), physiology (Bello *et al.*, 2000), and transportation (Golombieski *et al.*, 2003). Experiments designed to evaluate jundiá growth, stocking density effects, and cage culture have been performed (Barcellos *et al.*, 2004a) but data on this subject are still scarce.

From the biological standpoint, uncovering each step of embryo and larval development is of great importance. In addition, in these stages most fish species may be more susceptible to changes in dissolved oxygen concentration, pH, salinity, alkalinity, turbidity, and particularly to water contaminants. A complete knowledge of embryo and larval development stages might be useful in studying the effect of even small amounts of water contaminants, mainly those of agricultural origin that eventually reach water springs or ponds used for fish culture, a quite common occurrence in agricultural areas such as those in southern Brazil. In this study, the main objective was to examine the initial stages of embryo and larval development of jundiá under controlled conditions.

MATERIAL AND METHODS

The present work was carried out at facilities of the University of Passo Fundo, Rio Grande do Sul, southern Brazil (687 m asl), using cultured females and males weighing respectively 685 ± 50 g and 500 ± 33 g. After capture, fish were quickly transferred to the laboratory and weighed. Hormonal induction of ovulation was performed using pituitary extract injection according to a routine protocol (Ittzés *et al.*, 1999). The oocytes used were extruded by females within a 9 h 30 min-10 h 55 min period after pituitary extract injection. Extracted oocyte wet weights ranged from 68 g to 118 g. Following weighing, oocytes were placed in Petry dishes. Collected by abdominal pressure, 10 mL of mixed semen from three males was added to each batch of eggs and gently mixed in; 100 mL of water was added to promote sperm activity, and egg fertilization and hydration. Soon after hydration, the eggs were transferred to 50 L conic glass incubators, with constant and controlled water influx at flow rates of 0.7 mL min^{-1} at a temperature of 24 ± 1 °C. Depending on the developmental phase, samples of fertilized eggs were taken with a plastic tube at different intervals from the incubators. During the first 2 h 43 min, egg samples were removed at approximately 15 min intervals, placed in Petri dishes, and examined under a stereoscopic

microscope (3.2 and 6.4 x magnification) equipped with a digital camera.

After hatching, the larvae of each egg batch were transferred to a 200 L glass fiber incubator filled with aerated water at an influx rate of 8-10 L min^{-1}. Larval development was analyzed from hatching until the time (92 h 45 min after hatching) that they were stocked in a previously fertilized pond (Barcellos *et al.*, 2004).

Water-quality parameters measured in incubation and larviculture were: temperature (at each sampling), dissolved oxygen, alkalinity, pH, turbidity, and total ammonia concentration (colorimetric methods and titulation, before beginning of experiment).

RESULTS

During embryonic development observation, eggs were kept in glass incubators under moderate agitation by influx at a rate of 180 L/h of water having the following characteristics: mean temperature, 24 ± 1 °C; dissolved oxygen level, 6 mg L^{-1}; alkalinity, 19 mg L^{-1}; pH, 6.8; turbidity, 3 L NTU; total ammonia concentration < 0.001 mg L^{-1} that on a pH-temperature conversion table indicated 0.0006 mg L^{-1} of un-ionized ammonia.

Initial jundiá development of was divided into two phases: embryonic and larval. A sequence of the most important events observed in each phase is shown in Figs 1-2 and Table 1.

Eggs of *R. quelen* were spherical, demersal, and non-adhesive with a clearly defined perivitelline space and resistant chorion. Cleavage stages occurred during the first 3.5 h. First signs of embryo movement were observed 21 h after fertilization; larval eclosion occurred 30.5 h after fertilization.

DISCUSSION

As previously indicated, this study showed that the eggs of *Rhamdia quelen* were spherical, demersal, and non-adhesive, similar to those described by Godinho *et al.*(1978) for another fish of the same genus, *i.e.*, *Rhamdia hilarii*, and for another Siluriform fish, the *Pseudoplatystoma coruscans* as described by Cardoso *et al.* (1995).

The perivetelline space in newly fertilized ova of *R. quelen* was relatively smaller than those of other teleosts, *e.g.*, the piabanha (*Brycon insignis* Steindachner) (Andrade-Tamelli *et al.*, 2001) and similar to that found by Godinho *et al.* (1978) for the siluriform *Rhamdia hilarii*. A larger perivetelline space in some fish species may be understood as an embryo defense against environmental adversities and contributes to higher survival rates in lotic environments (Lake, 1967; Matsuura, 1972). Thus, the characteristics of *R.quelen* ova fit this species' preferred reproduction sites, which have clear water with little flow (Gomes *et al.*, 2000).

The time required in *R. quelen* to form the first segmentation line in the ovum after fertilization (1 h 15 min), was very similar to that previously described for the same species (Ihering & Azevedo, 1936), similar to that described for *Rhamdia sapo* (Matkovic *et al.*, 1985), and also to that of *Rhamdia hilarii*, a silurid species of the genus *Rhamdia* (Godinho *et al.*, 1978). On the other hand, it was shorter than found for *Pimelodella lateristriga*, a siluriform fish of the *Rhamdia* genus (Ihering and Azevedo, 1936).

In the present work, hatching of jundiá eggs occurred 30 h 5 min after fertilization at 24 °C, which is very similar to the period found for *R. sapo* (30 h, 22-24 °C; Cussac *et al.*, 1985). In contrast, in the first documented research on embryonic and larval development of jundiá (Ihering and Azevedo, 1936), this period varied from 35 to 46 h after fertilization at 18-19 °C. In *Rhamdia hilarii*, the hatching time was 27 h at 23 °C (Godinho *et al.*, 1978). The difference in hatching time might be due to environmental conditions like water, temperature, alkalinity, pH (Cussac *et al.*, 1985), and water flow. In addition, other environmental factors not detected in the water could have affected developmental period of the embryos.

During the period in which larvae were kept in 200 L glass fiber incubators, a reduction in yolk and a marked evolution in body pigmentation was observed. The fish already had an adult appearance 123 h 15 min after from fertilization, presenting dark coloration and three well-developed pairs of barbels. A similar finding was made by Godinho *et al.* (1978), who described *Rhamdia hilarii* with an adult appearance 120 h after hatching. In *Pseudoplatystoma coruscans*, an adult appearance was detected four complete days after hatching (Santos & Godinho, 1994).

In conclusion, the observed eggs of *R. quelen* were spherical, demersal and non-adhesive with a

TABLE 1
Description of embryonic and larval development stages according to time before and after fertilization and to macroscopic characteristics found in *Rhamdia quelen*.

Stage	Phase	Time after spawning	Observed characteristics
	1	Immediately after (Fig. 1a)	Oocyte just spawned; clear yellow coloration and diameter of 1.0 mm.
		Time following oocyte fertilization	
Embryonic	2	50 min: ovum immediately after fertilization. (Fig. 1b)	Formation of blastodisc (830 μm) at the animal pole. Dark yellow coloration; formation of perivitelline space (73 μm).
	3	1 h 15 min (Fig. 1c)	Cleavage stage: first segmentation visible; 2-cell stage.
	4	1 h 30 min	Cleavage stage: 4-cell stage.
	5	1 h 40 min (Fig. 1d)	Cleavage stage: well-defined 4-cell stage.
	6	1 h 45 min (Fig. 1e)	Cleavage stage: 8-cell stage.
	7	2 h 15 min (Fig. 1f)	One pair of divisions, one on each side of 2nd cleavage line; 16-cell stage.
	8	2 h 27 min (Fig. 1g)	Cleavage stage: 32-cell stage.
	9	3 h 35 min (Fig. 1h)	Cleavage stage: 64-cell stage.
	10	4 h (Fig. 1i)	128-cell stage. Blastomere size diminished; blastoderm starts to cover yolk in the direction of vegetative pole.
	11	8 h 5 min (Fig. 1j)	Blastoderm covers 2/3 of yolk; initiates blastopore formation begins.
	12	9 h 6 min	Increasing yolk density.
	13	10 h 5 min (Fig. 1l)	Blastopore closing forming germination ring.
	14	11 h 50 min (Fig. 1m)	Elongation of yolk shape; head and tail growth.
	15	12 h 50 min	Vertebral axis development.
	16	13 h 50 min (Fig. 2a)	Cephalic organization and differentiation.
	17	15 h 22 min	Growth of caudal area.
	18	16 h 30 min (Fig. 2b)	Tail development begins.
	19	17 h 50 min	Development of the tail.
	20	19 h 20 min (Fig. 2c)	Further development of the tail.
	21	21 h (Fig. 2d)	Embryo initiates movement by repeated tail contractions; round-shaped yolk.
	22	24 h 50 min (Fig. 2e)	Spontaneous motility.
	23	25 h 10 min	Spontaneous motility.
	24	26 h 20 min	Spontaneous motility. Heart beats; visible blood circulation, although blood is colorless.
Larval	25	30 h 30 min (Fig. 2f)	Hatching. Heartbeats; visible blood circulation. Eye and yolk pigmentation begins.
	26	54 h 50 min (Fig. 2g)	Larva with opened mouth and presenting a pair of barbells; visible blood circulation; volumetric reduction of yolk presenting higher pigmentation.
	27	81 h 50 min (Fig. 2h)	Larva presenting the three pairs of barbels; pigmentation beginning in anterior region.
	28	104 h 25 min	Entire body pigmented.
	29	123 h 15 min (Fig. 2i)	Body anatomy similar to that of adult fish.

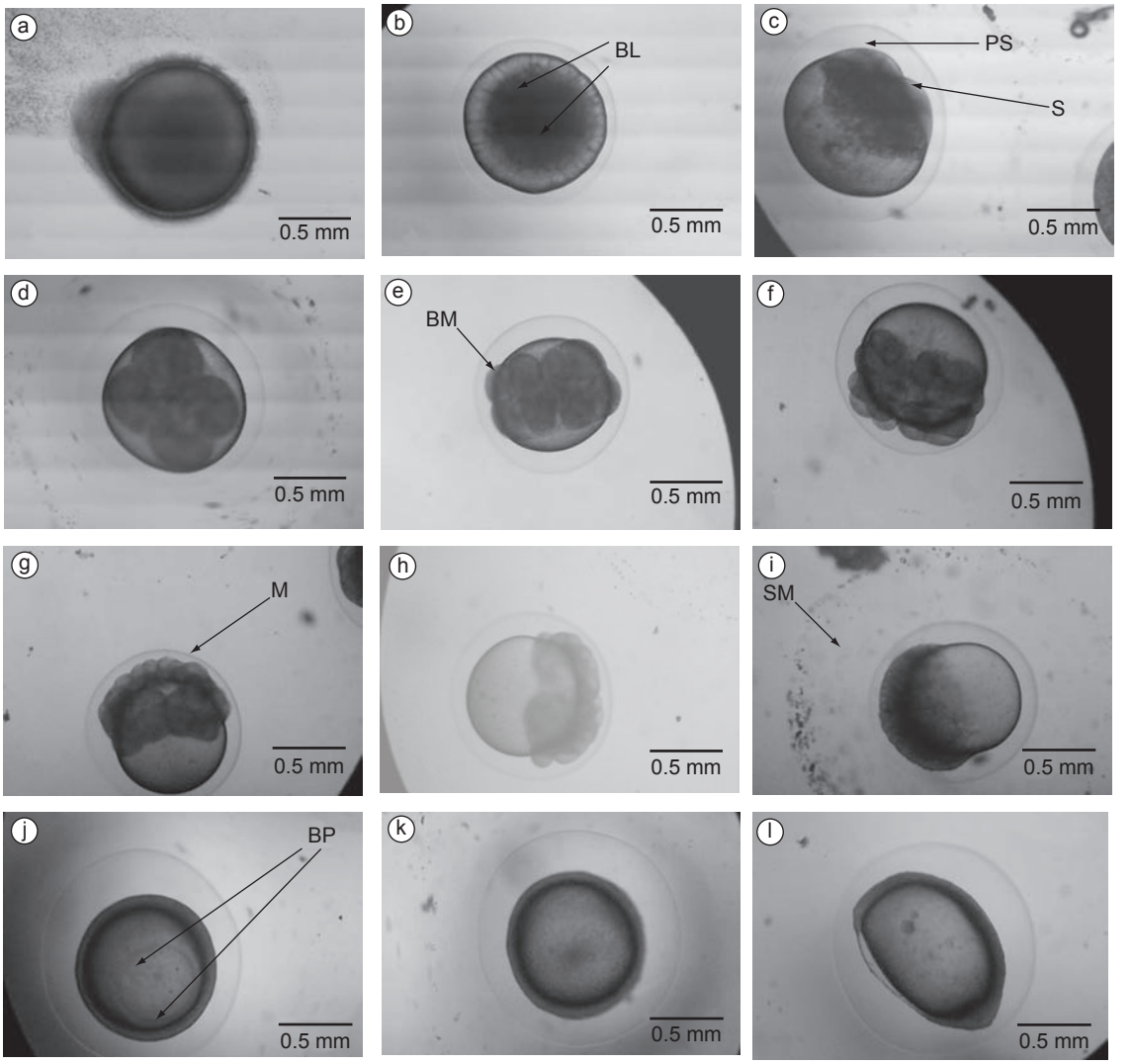

Fig. 1 — Embryonic development of jundiá (Rhamdia quelen): a) just spawned oocyte; b) blastodisc (BLl) formation; c) two-cell stage (PS – perivitelline space, S – segmentation line; d) 4-cell stage; e) 8-cell stage (BM - blastomeres); f) 16-cell stage; g) 32-cell stage (M) – ovum membrane; h) 64-cell stage; i) blastoderm covering yolk (SM – secondary membrane, 540 µm); j) blastoderm; blastopore (BP); k) yolk reduction; and l) yolk conformation change. Magnification 6.4x.

visually defined perivetelline space and resistant chorion. Egg cleavage, embryo movement, and eclosion occurred within the first 30 h 5 min of development. These findings may provide a basis for further studies to determine the complete ontogeny of *R. quelen* and may be useful in studies of water contaminant effects on the development and culture of jundiá, as well as research using this species as an environmental bio-indicator.

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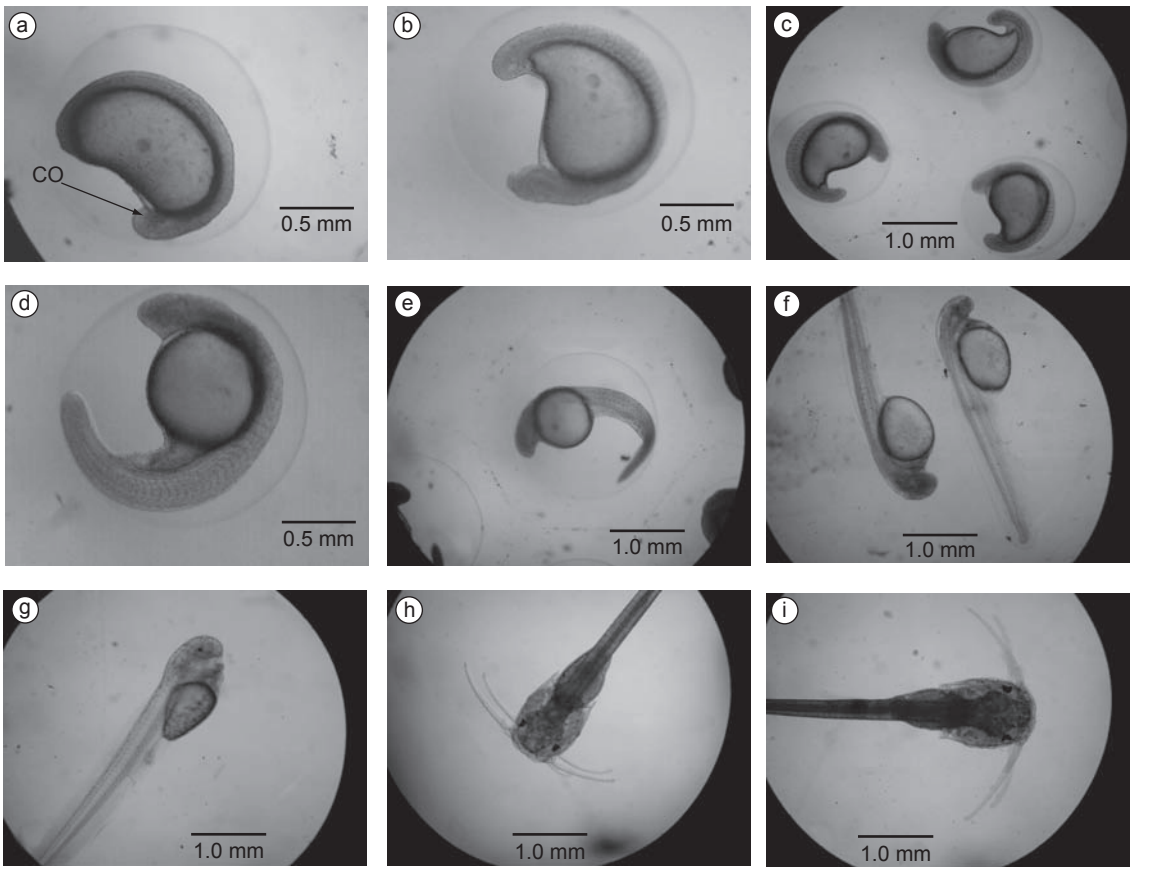

Fig. 2 — a) cephalic organization (CO); b) initiation of caudal development; c) accelerated growth of caudal region and yolk mass diminished; d) movement; e) vigorous movement of the embryo; f) hatching; g) larvae with opened mouth; h) larvae at 4.3 d (104 h); and i) larvae at 5.125 d (123 h). Magnification of 3.2 and 6.4X.

REFERENCES

ANDRADE-TALMELLI, E. F., KAVAMOTO, E. T., ROMAGOSA, E. & FENERICH-VERANI, N., 2001, Embryonic and larval development of the "piabanha", *Brycon insignis*, steindachner, 1876 (Pisces, Characidae). *Bol. Inst. Pesca, 27*(1), 21-28.

BARCELLOS, L. J. G., WOEHL, V. M., WASSERMANN, G. F., KRIEGER, M. H., QUEVEDO, R. M. & LULHIER, F., 2001a, Plasma levels of cortisol and glucose in response to capture and tank transference in *Rhamdia quelen* (Quoy & Gaimard), a South American Catfish. *Aqua. Res. 32*(3): 123-125.

BARCELLOS, L. J. G., WASSERMANN, G. F., SCOTT, A. P., WOEHL, V. M., QUEVEDO, R. M., ITTZÉS, I., KRIEGER, M. H. & LULHIER, F., 2001b, Steroid profiles in cultured female jundiá, the Siluridae *Rhamdia quelen* (Quoy and Gaimard, Pisces Telesostei), during the first reproductive cycle. *Gen.Comp. Endocrinol,, 121*: 325-332.

BARCELLOS, L. J. G., WASSERMANN, G. F., SCOTT, A. P., WOEHL, V. M., LULHIER, F., QUEVEDO, R. M., ITTZÉS, I. & KRIEGER, M. H., 2002, Plasma steroid concentrations in relation to the reproductive cycle of cultured male *Rhamdia quelen*. *J. Fish Biol., 61*(3): 751-763.

BARCELLOS, L. J. G., KREUTZ, L. C., RODRIGUES, L. B., FIOREZE, I., QUEVEDO, R. M., CERICATO, L., CONRAD, J., SOSO, A. B., FAGUNDES, M., LACERDA, L. A. & TERRA, S., 2003, Haematological and biochemical characteristics of male jundiá (*Rhamdia quelen* Quoy & Gaimard pimelodidae): changes after acute stress. *Aqua. Res., 34*: 1465-1469.

BARCELLOS, L. J. G., KREUTZ, L. C., QUEVEDO, R. M., FIOREZE, I., SOSO, A.B., CERICATO, L., FAGUNDES, M., CONRAD, J., BALDISSERA, R., BRUSCHI, A. & RITTER, F., 2004a, Nursery rearing of jundiá, *Rhamdia quelen* (Quoy & Gaimard) in cages: cage type, stocking density and stress response to confinement. *Aquaculture, 232*(1-4): 383-394.

BARCELLOS, L. J. G., KREUTZ, L. C., QUEVEDO, R. M., FIOREZE, I., RODRIGUES, L. B., SOSO, A.B., RITTER, F., CONRAD, J., CERICATO, L., FAGUNDES, M., LACERDA, L. A. & TERRA, S., 2004b, Hematological

changes in jundiá (*Rhamdia quelen* Quoy & Gaimard Pimelodidae) provoked by usual aquaculture practices, with emphasis on immunosupressive effects. *Aquaculture, 237*(1-4): 229-236.

BELLO, A. R., FORTES, E., BELLO-KLEIN, A., BELLO, A. A., LLESUY, S. F., ROBALDO, R. B. & BIANCHINI, A., 2000, Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen. Dis. Aquat.Organisms, 42*(3): 233-236.

CARDOSO, E. L., ALVES, M. S. D., FERREIRA, R. M. A. & GODINHO, H. P., 1995, Embryogenesis of the neotropical freshwater Siluriforme *Pseudoplatystoma coruscans*. *Aquat. Living Resour., 8*: 343-346.

CUSSAC, V. E., MATKOVIC, M. & MAGGESE, M. C., 1985, Desarrollo embrionario de Rhamdia sapo (Valenciennes, 1840) Eigenmann y Eigenmann, 1888 (Pisces, *Pimelodidae*) II. Organogénesis media, organogénesis tardia y eclosión. *Rev. Bras. Biol., 45*(1-2): 149-160.

GODINHO, H. M., FENERICH, N. A. & NARAHARA, M. Y., 1978, Desenvolvimento embrionário e larval de Rhamdia hilarii (Valenciennes, 1840) (Siluriformes, Pimelodidae). *Rev. Bras. Biol., 38*(1): 151-156.

GOLOMBIESKI, J. I., SILVA, L. V. F., BALDISSEROTTO, B. & DA SILVA, J. H. S., 2003, Transport of silver catfish (*Rhamdia quelen*) fingerlings at different times, load densities, and temperatures. *Aquaculture, 216*(1-4): 95-102.

GOMES, L. C., GOLOMBIESKI, J. I., REGINA, A., GOMES, C. & BALDISSEROTO, B., 2000, Biology of *Rhamdia quelen* (Telesostei, Pimelodidae). *Ciência Rural, 30*: 179-185.

IHERING, R. VON & AZEVEDO, P., 1936, A desova e a hypophysação dos peixes. Evolução de dois Nematognathas. *Arch. do Inst. Biológico, 7*: 107-120.

ITTZÉS, I., QUEVEDO, R. M., BARCELLOS, L. J. G. & WOEHL, V., 1999, Uso de hipófise de carpa húngara (*Cyprinus carpio*) na indução à reprodução de fêmeas e machos de jundiá (*Rhamdia quelen*). Efeito de diferentes faixas de peso. *In*: Anais da 51ª. Reunião Anual da SBPC, 1999.

LAKE, J. S., 1967, Rearing experiments with species of Australian freshwater fishes. II. Morphogenesis and ontogeny. *Aust. J. Mar. Freshwater Res., 18*: 155-173.

LOPES, J. M., SILVA, L. V. F. & BALDISSEROTTO, B., 2001, Survival and growth of silver catfish larvae exposed to different water pH. *Aqu. Int., 9*(1): 73-80.

MATKOVIC, M., CUSSAC, V. E., CUKIER, M., GUERRERO, G. A. & MAGGESE, M. C., 1985, Desarrollo embrionario de *Rhamdia sapo* (Valencieness, 1840) Eigenmann y Eigenmann, 1888 (Pisces, Pimelodidae). I. Segmentación, morfogénesis y organogénesis temprana. *Rev. Bras. Biol.*, *45*(1-2): 149-160.

MATSUURA, Y., 1972, Egg development of scaled sardine Harengula pensacolae Goode & Bean (Pisces Clupeidae). *Bol. Ints. Oceanogr., 21*: 129-135.

PIAIA, R., TOWNSEND, C. R. & BALDISSEROTTO, B., 1999, Growth and survival of fingerlings of Silver catfish exposed to different photoperiods. *Aqua. Int., 7*(3): 201-205.

SANTOS, J. E. & GODINHO, H. P., 1994, Morfogênese e comportamento larvais do surubim (Pseudoplatystoma coruscans Agassiz, 1829) sob condições experimentais. *Arq. Bras. Med. Vet. Zootec., 46*(2): 139-147.

TOWNSEND, C. R., SILVA, L. V. F., & BALDISSEROTTO, B., 2003, Growth and survival of *Rhamdia quelen* (Siluriformes, *Pimelodidae*) larvae exposed to different levels of water hardness. *Aquaculture, 215*: 103-108.

TOWNSEND, C. R. & BALDISSEROTTO, B., 2001, Survival of silver catfish fingerlings exposed to acute changes of water pH and hardness. *Aqua. Int., 9*(5), 413-419.