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Synchronizing developmental stages in Neotropical catfishes for application in germ cell transplantation

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Summary

The aim of this study was to describe the effect of temperature on the fertilization, early developmental stages, and survival rate of two Neotropical catfishes *Pimelodus maculatus* and *Pseudopimelodus mangurus*. After fertilization, the eggs were incubated at 22°C, 26°C, and 30°C, which resulted in fertilization rates of 96.95 ± 1.79%, 98.74 ± 0.76%, and 98.44 ± 0.19% for *P. maculatus* and 96.10 ± 1.58%, 98.00 ± 0.63%, and 94.60 ± 2.09% for *P. mangurus*, respectively. For *P. maculatus*, hatching occurred after 22 h 30 min post-fertilization at 22°C, 16 h 30 min at 26°C, and 11 h 20 min at 30°C, and the hatching rates were 43.87 ± 7,46%, 57.57 ± 17.49%, and 53.63 ± 16.27%, respectively. For *P. mangurus*, hatching occurred after 28 h 30 min post-fertilization at 22°C and 17 h 30 min at 26°C with respective hatching rates of 45.4 ± 21.02% and 68.1 ± 12.67%. For this species, all embryos incubated at 30°C died before hatching. Additionally, for *P. maculatus*, the larvae from the lower (22°C) and higher temperatures (30°C) presented increased abnormality rates, as observed in the head, tail and yolk regions. The lowest abnormality rate was detected at 26°C, which was considered the optimal incubation temperature for both species. The developed protocol enables the manipulation of embryonic development, which is important for the application of reproductive biotechniques, including chimerism and chromosome-set manipulation. The data obtained here are also important for the surrogate propagation of this species as *P. mangurus* was recently categorized as an endangered fish species.

Keywords: Incubation, PGC, Siluridae, Surrogate propagation, Temperature

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Introduction

The Neotropical region has one of the largest populations of ichthyofauna in the world, due to high diversity of hydrographic systems that results in a great variety of fish. Approximately 4399 freshwater species have been described and organized into two main orders. There have been 1847 Characiforms species and 2147 Siluriformes species identified with 57 Characiforms and 91 Siluriformes being considered endangered (Froese & Pauly, 2015; Brasil, 2016) only in Brazil, which has one of the biggest ichthyofauna populations in the world. In Brazil, the Paraná River basin is an important basin showing a great variety of endemism (Galves *et al*., 2009) and endangered species, as is the case with *Pseudopimelodus mangurus* (Valenciennes, 1835). This species belongs to the Siluriformes order, and was recently included in the 'red list' of endangered species in the state of São Paulo (Bressan *et al*., 2009; São Paulo, 2014). This situation demands conservation strategies for this fish species.

Some reproductive biotechniques have been established for fish conservation, including surrogate propagation (Yasui *et al*., 2011) combined with sterile fish obtained by chromosome manipulation (Piferrer *et al*., 2009; do Nascimento *et al*., 2017). For the use of these techniques, prior knowledge about the stages of embryonic development is necessary (Fujimoto *et al*., 2006). The developmental stage can be influenced by several factors, and temperature is the main limiting factor in early development (Dos Santos *et al*., 2016). Temperature manipulation during early development is also a strategy for synchronizing embryonic development of different species. In germ cell transplantation techniques, the donor and host species must be synchronized at the same developmental stage, which can be achieved with controlled temperatures in both donor and host embryos.

Embryonic development in Neotropical fishes has not been fully investigated, especially in migratory fish species (Godinho & Godinho, 2003). The aim of this study was to describe the developmental stages of two Siluriformes, to establish a protocol for germ cell transplantation. The donor species, the marbled catfish *Pseudopimelodus mangurus*, is a large bodied catfish (>8 kg) that was recently categorized as an endangered species (Bressan *et al*., 2009; São Paulo, 2014). For this species, domestication and reproduction are critical. As a host species, the spotted catfish *Pimelodus maculatus* (Lacépède, 1803) was selected as it is a small bodied catfish (~1 kg) for which domestication and breeding procedures are well established under artificial conditions. In addition, *P. maculatus* can then be used as a model host for germ cells for other endangered Siluriformes.

Materials and methods

Origin of broodstock and artificial fertilization

All procedures were conducted at the Laboratory of Fish Biotechnology (Cepta/ICMBio, Pirassununga City, São Paulo State, Brazil).

Adult males and females of *Pimelodus maculatus* were collected in the Mogi Guassu river using a cast net (5 cm mesh) and, then, transferred to 1000 m^2 earthen tanks. The fish were fed to satiation with a 6-mm commercial fish pellet (45% crude protein, 3800 kcal kg^{-1}) three times a week. These fish promptly accepted artificial feeding 5–10 days after collection. After a period of 3 to 6 months, the fish were selected for induced reproduction during the spawning season. Females are larger than males and present increased peritoneal areas, in addition to a reddish colour in the papillae area. Males were selected randomly based on the length reduced diameter of the body, in comparison with females. The females were anesthetized in clove oil 100 mg l^{-1} and induced with two doses of crude carp pituitary extract at 0.5 mg kg^{-1}, followed by a second dose of 5 mg kg^{-1} after 165 hour-degrees. Males were induced to spermiation using a single dose of crude carp pituitary at 0.5 mg kg^{-1} at the same time as the second induction of the females. Reproduction induction was performed in 500 litre tanks with constant water flow. Gamete sampling was performed by hour-degrees methodology, which is performed by summing the water temperature (°C) for each hour until spawning, resulting in 165 hour-degrees for both species. The oocytes were extruded in a glass bowl. Males were euthanized with the use of anaesthetic clove oil (100 mg l^{-1}) and sperm was collected by testicle maceration in a 15 ml tube containing 3 ml of calcium and magnesium-free Eagle's Minimum Essential Medium with the pH adjusted to 7.8 (E-MEM, Sigma, St. Louis, USA). The diluted sperm were added on the oocytes, and the gamete activation was achieved using 300 ml of hatchery water.

The marbled catfish *Pseudopimelodus mangurus* adults were collected from the Mogi Guassu river during the spawning season using hook-and-line fishing with a baitfish (hook > 5 cm). The fish were induced to spawn immediately after sampling using the same protocol described above for *P. maculatus*, but in doses of 0.6 mg kg^{-1} and 6 mg kg^{-1}, and after induction, the broodstock was maintained in 8000 litre circular tanks with constant water flow. The females were selected by the same criteria described above, including increased redness of papilla and body volume. Males were selected based on the protuberant papillae and semen release after gently abdominal stripping.

Gametes were collected using the same procedure described above. As the marbled catfish sperm is commonly contaminated with urine, the sperm was directly collected in a 15 ml tube containing 5 ml of calcium and magnesium-free Eagle's Minimum Essential Medium with the pH adjusted to 7.8 (E-MEM, Sigma, St. Louis, USA), used as immobilizing medium.

Collection and embryonic development

After fertilization, each spawning was divided into three batches at 22°C, 26°C, and 30°C. For each temperature, a small aliquot (30–80 embryos) was collected randomly and fixed in 2.5% glutaraldehyde in Dulbecco's phosphate-buffered saline (D-PBS) (Sigma #D5773, St. Louis, USA). The samples were collected over the following time intervals: every 2 min post-fertilization until 16 min, every 5 min until 2 h 30 min post-fertilization; every 10 min until 4 h 30 min post-fertilization; every 15 min until 7 h post-fertilization; every 20 min until 11 h post-fertilization; and every 30 min until hatching.

Embryos aliquots from each batch were observed using a stereomicroscope (Nikon SMZ 1500, Nikon, Tokyo, Japan) with a charge coupled device (CCD) camera (Nikon DS-Fi, Nikon, Japan). Digital images were captured using NIS-AR Elements software (Nikon, Tokyo, Japan). The embryonic development of *P. maculatus* and *P. mangurus* was classified into zygote, cleavage, blastula, gastrula, segmentation, and hatching stages, and each period was subdivided into phases based on previous studies (Fujimoto *et al*., 2006), (Dos Santos *et al*., 2016) and (Kimmel *et al*., 1995)

Statistics

Data are shown as the mean ± standard error. Data were obtained in triplicate for which different egg batches were considered as a replicate. Percentages of embryos in each of the developmental stages were analyzed with analysis of variance (ANOVA), followed by Tukey multiple range test. In all analyses, the software STATISTICA (7.0, StatSoft, USA) was used with the probability set at 0.05.

Results

Embryogenesis

Developmental stages of *P. maculatus* and *P. mangurus* at different incubation temperatures are presented on Tables 1 and 2. In the following topics, a detailed description of the developmental stages for both species is shown according to each temperature.

Cleavage period

After fertilization and hydration, the chorion begins to expand, inducing growth of the perivitelline space. The cytoplasm begins to migrate, forming the animal pole, initiating the formation of the blastocyst that covers the yolk in the animal pole, in which a meroblastic cleavage pattern occurs.

2-cell stage. In *P. maculatus*, embryos reached the 2-cell stage at 45 min post-fertilization when incubated at 22°C, 30 min post-fertilization when incubated at 26°C, and 25 min post-fertilization when incubated at 30°C (Fig. 1*B*). For *P. mangurus*, cleavage occurred 1 h post-fertilization when incubated at 22°C, 40 min post-fertilization when incubated at 26°C, and 25 min post-fertilization when incubated at 30°C (Fig. 2*B*).

4-cell stage. In this stage, the initial two cells (blastomeres) divide symmetrically giving rise to four cells 1 h post-fertilization when incubated at 22°C, 40 min post-fertilization at 26°C, and 35 min post-fertilization at 30°C in *P. maculatus* (Fig 1*C*). This occurred 1 h 20 min post-fertilization for *P. mangurus* when incubated at 22°C, 55 min post-fertilization at 26°C, and 35 min post-fertilization at 30°C (Fig. 2*C*).

8-cell stage. During the third stage of cleavage, the four cells of last stage divide symmetrically in a set of eight blastomeres, arranged in two 4-cell lines on the yolk. In *P. maculatus*, this stage occurs 1 h 10 min post-fertilization when incubated at 22°C, 55 min post-fertilization at 26°C, and 40 min post-fertilization at 30°C (Fig. 1*D*). For *P. mangurus*, eight cells were observed 1 h 35 min post-fertilization when incubated at 22°C, 1 h 5 min post-fertilization at 26°C, and 45 min post-fertilization at 30°C (Fig 2*D*).

16-cell stage. The fourth stage of the cleavage has 16 cells symmetrically arranged in an arrangement of four rows of four cells each. In *P. maculatus*, this stage begins 1 h 35 min post-fertilization when incubated at 22°C, 1 h 5 min post-fertilization at 26°C, and 50 min post-fertilization at 30°C (Fig. 1*E*). For *P. mangurus*, the fourth cleavage stage was observed 1 h 50 min post-fertilization when incubated at 22°C, 1 h 15 min post-fertilization at 26°C, and 1 h post-fertilization at 30° (Fig. 2*E*).

32-cell stage. The 16 cells divide again, and the blastocyst now consists of 32 blastomeres, arranged in an arrangement of four rows of eight cells each. The cluster of cells begins to overlap irregularly. For *P. maculatus*, this stage begins 1 h 55 min post-fertilization when incubated at 22°C, 1 h 15 min post-fertilization at 26°C, and 55 min post-fertilization at 30°C (Fig. 1*F*). For *P. mangurus*, this stage occurred after 2 h 15 min post-fertilization when incubated at 22°C, 1 h 25 min post-fertilization at 26°C, and 1 h 15 min post-fertilization at 30°C (Fig. 2*F*).

64-cell stage. In the last stage of cleavage, the 32 cells divide giving rise to a 64-cell blastocyst. For

Table 1 Range of embryonic development for *Pimelodus maculatus* incubated at temperatures of 22°C, 26°C and 30°C

Period	Stage	Time to stage			Fig. no.
		22	26	30	
Cleavage	2-cell	45 min	30 min	25 min	1*B*
	4-cell	1 h	40 min	35 min	1*C*
	8-cell	1 h 10 min	55 min	40 min	1*D*
	16-cell	1 h 35 min	1 h 5 min	50 min	1*E*
	32-cell	1 h 55 min	1 h 15 min	55 min	1*F*
	64-cell	2 h 15 min	1 h 25 min	1 h 5 min	1*G*
Blastula	128-cell	2 h 45 min	1 h 40 min	1 h 15 min	1*H*
	256-cell	3 h 10 min	1 h 50 min	1 h 25 min	1*I*
	512-cell	3 h 30 min	2 h	1 h 35 min	1*J*
	1000-cell	3 h 50 min	2 h 15 min	1 h 45 min	1*K*
	Elongation	4 h 5 min	2 h 30 min	1 h 55 min	1*L*
	Spherical	4 h 45 min	3 h	2 h 5 min	1*M*
	Dome	5 h 15 min	3 h 20 min	2 h 20 min	1*N*
Gastrula	25% epiboly	5 h 45 min	3 h 40 min	2 h 45 min	1*O*
	50% epiboly	6 h 20 min	4 h 5 min	3 h 5 min	1*P*
	Germ ring	6 h 20 min	4 h 10 min	3 h 15 min	1*Q*
	75% epiboly	7 h	4 h 40 min	3 h 45 min	1*R*
	90% epiboly	8 h 20 min	5 h 30 min	3 h 55 min	1*S*
	100% epiboly	10 h 30 min	6 h 30 min	5 h	2*T*
Segmentation	3 somites	12 h 15 min	8 h	6 h	3*B*
	Optic vesicle	12 h 30 min	8 h 20 min	6 h 15 min	3*D*
	Otic vesicle	14 h 30 min	9 h 20 min	7 h	3*K*
	Kupffer's vesicle	14 h	10 h 20 min	7 h 30 min	3*F*
	Kupffer's vesicle disappearance	16 h 30 min	11 h 30 min	8 h	
Hatching		22 h 30 min	16 h 30 min	11 h 20 min	5*A*

P. maculatus, it occurred 2 h 15 min post-fertilization when incubated at 22°C, 1 h 25 min post-fertilization at 26°C, and 1 h 5 min post-fertilization at 30°C (Fig. 1*G*). *P. mangurus* reached this stage 2 h 30 min post-fertilization when incubated at 22°C, 1 h 40 min post-fertilization at 26°C, and 1 h 30 min post-fertilization at 30°C (Fig. 2*G*).

Blastula period

The blastula period is divided into the following stages: 128 cells, 256 cells, 512 cells, over 1000 cells, elongation, spherical, and dome. The cells were now arranged irregularly and overlapping each other on the yolk.

128-cell stage. For *P. maculatus* embryos, this stage began 2 h 45 min post-fertilization, when incubated at 22°C, 1 h 40 min post-fertilization at 26°C, and 1 h 15 min post-fertilization at 30°C (Fig. 1*H*). For *P. mangurus*, the cells reached this stage 3 h 20 min post-fertilization when incubated at 22°C, 1 h 55 min post-fertilization at 26°C, and 1 h 45 min post-fertilization at 30°C (Fig. 2*H*).

256-cell stage. Pimelodus maculatus embryos achieve the second stage of the blastula after 3 h 10 min post-fertilization when incubated at 22°C, 1 h 50 min post-fertilization at 26°C, and 1 h 25 min post-fertilization at 30°C (Fig. 1*I*). For *P. mangurus*, the cells reached this stage 4 h post-fertilization when incubated at 22°C, 2 h 15 min post-fertilization at 26°C, and 2 h post-fertilization at 30°C (Fig. 2*I*).

512-cell stage. The embryos of *P. maculatus* started this stage 3 h 30 min post-fertilization at 22°C, 2 h post-fertilization at 26°C, and 1 h 35 min post-fertilization at 30°C (Fig. 1*J*). For *P. mangurus*, this stage was reached 4 h 30 min post-fertilization at 22°C, 2 h 30 min post-fertilization at 26°C, and 2 h 15 min post-fertilization at 30°C (Fig 2*J*).

1000-cell stage. Pimelodus maculatus embryos reached this stage 3 h 50 min post-fertilization when incubated at 22°C, 2 h 15 min post-fertilization at 26°C, and 1 h 45 min post-fertilization at 30°C (Fig 1*K*). *Pseudopimelodus mangurus* reached this stage 5 h post-fertilization when incubated at 22°C, 2 h 50 min post-fertilization at 26°C, and 2 h 40 min post-fertilization at 30°C (Fig 2*K*).

Elongation stage. Pimelodus maculatus embryos incubated at 22°C were observed 4 h 5 min post-fertilization, 2 h 30 min post-fertilization at 26°C, and 1 h 55 min post-fertilization at 30°C (Fig 1*L*). For *P. mangurus*, it took 5 h 45 min post-fertilization to reach this stage when incubated at 22°C, 3 h 10 min

Table 2 Range of embryonic development for *Pseudopimelodus mangurus* incubated at temperatures of 22°C, 26°C and 30°C

Period	Stage	Time to stage			Fig. no.
		22	26	30	
Cleavage	2-cell	1 h	40 min	25 min	2*B*
	4-cell	1 h 20 min	55 min	35 min	2*C*
	8-cell	1 h 35 min	1 h 5 min	45 min	2*D*
	16-cell	1 h 50 min	1 h 15 min	1 h	2*E*
	32-cell	2 h 15 min	1 h 25 min	1 h 15 min	2*F*
	64-cell	2 h 30 min	1 h 40 min	1 h 30 min	2*G*
Blastula	128-cell	3 h 20 min	1 h 55 min	1 h 45 min	2*H*
	256-cell	4 h	2 h 15 min	2 h	2*I*
	512-cell	4 h 30 min	2 h 30 min	2 h 15 min	2*J*
	1000-cell	5 h	2 h 50 min	2 h 40 min	2*K*
	Elongation	5 h 45 min	3 h 10 min	3 h	2*L*
	Spherical	6 h 30 min	3 h 50 min	3 h 20 min	2*M*
	Dome	7 h 25 min	4 h 20 min	3 h 40 min	2*N*
Gastrula	25% epiboly	7 h 40 min	4 h 30 min	4 h	2*O*
	50% epiboly	8 h 40 min	5 h 15 min	4 h 15 min	2*P*
	Germ ring	8 h 40 min	5 h 15 min	4 h 15 min	2*Q*
	75% epiboly	9 h 40 min	5 h 45 min	4 h 45 min	2*R*
	90% epiboly	10 h 40 min	6 h 30 min	5 h 30 min	2*S*
	100% epiboly	12 h 30 min	7 h 20 min	6 h	2*T*
	3 somites	14 h	9 h	–	4*B*
	Optic vesicle	15 h	9 h 20 min	–	4*G*
	Otic vesicle	16 h	11 h	–	4*L*
	Kupffer's vesicle	18 h 30 min	11 h	–	4*J*
	Kupffer's vesicle disappearance	21 h	13 h 30 min	–	
Hatching		28 h 30 min	17 h 30 min	–	5*B*

post-fertilization at 26°C, and 3 h post-fertilization at 30°C (Fig. 2*L*).

Spherical stage. The cell pellet was already organized on the yolk forming a ball shape. *P. maculatus* embryos reached this stage 4 h 45 min post-fertilization when incubated at 22°C, 3 h post-fertilization at 26°C, and 2 h 5 min post-fertilization at 30°C (Fig. 1*M*). *Pseudopimelodus mangurus* embryos took 6 h 30 min post-fertilization to reach this stage when incubated at 22°C, 3 h 50 min post-fertilization at 26°C, and 3 h 20 min post-fertilization at 30°C (Fig. 2*M*).

Dome stage. The group of cells began to cover the whole yolk in epiboly movement. For *P. maculatus*, this stage occurred 5 h 15 min post-fertilization when the embryos were incubated at 22°C, 3 h 20 min post-fertilization at 26°C, and 2 h 20 min post-fertilization at 30°C (Fig. 1*N*). For *P. mangurus*, embryos took 7 h 25 min post-fertilization to reach dome stage when incubated at 22°C, 4 h 20 min post-fertilization at 26°C, and 3 h 40 min post-fertilization at 30°C (Fig. 2*N*).

Gastrula period

During this period, epiboly were observed. The blastoderm converged and extended on the yolk. The stages in this period were divided according to the percentage of the yolk that was covered by the blastoderm.

25% epiboly stage. A quarter of the yolk was covered by the blastoderm. *Pimelodus maculatus* arrived at this stage 5 h 45 min post-fertilization at 22°C, 3 h 40 min post-fertilization at 26°C, and 2 h 45 min post-fertilization when incubated at 30°C (Fig. 1*O*). For *P. mangurus*, embryos were observed covering a quarter of the yolk 7 h 40 min post-fertilization when incubated at 22°C, 4 h 30 min post-fertilization at 26°C, or 4 h post-fertilization at 30°C (Fig. 2*O*).

50% epiboly stage. Half of the yolk was covered by the blastoderm in this stage. For *P. maculatus*, half of the yolk was covered 6 h 20 min post-fertilization at 22°C, 4 h 5 min post-fertilization 26°C, and 3 h 5 min post-fertilization when incubated at 30°C (Fig. 1*P*). *Pseudopimelodus mangurus* embryos were observed at this stage 8 h 40 min post-fertilization at 22°C, 5 h 15 min post-fertilization at 26°C, and 4 h 15 min post-fertilization at 30°C (Fig. 2*P*). During this stage, it was also observed that there was a germinative ring for *P. maculatus* and *P. mangurus* (Fig. 1*Q*, 2*Q*).

75% epiboly stage. Three-quarters of the yolk was covered by the blastoderm. *Pimelodus maculatus* embryos arrived at this stage at 7 h post-fertilization

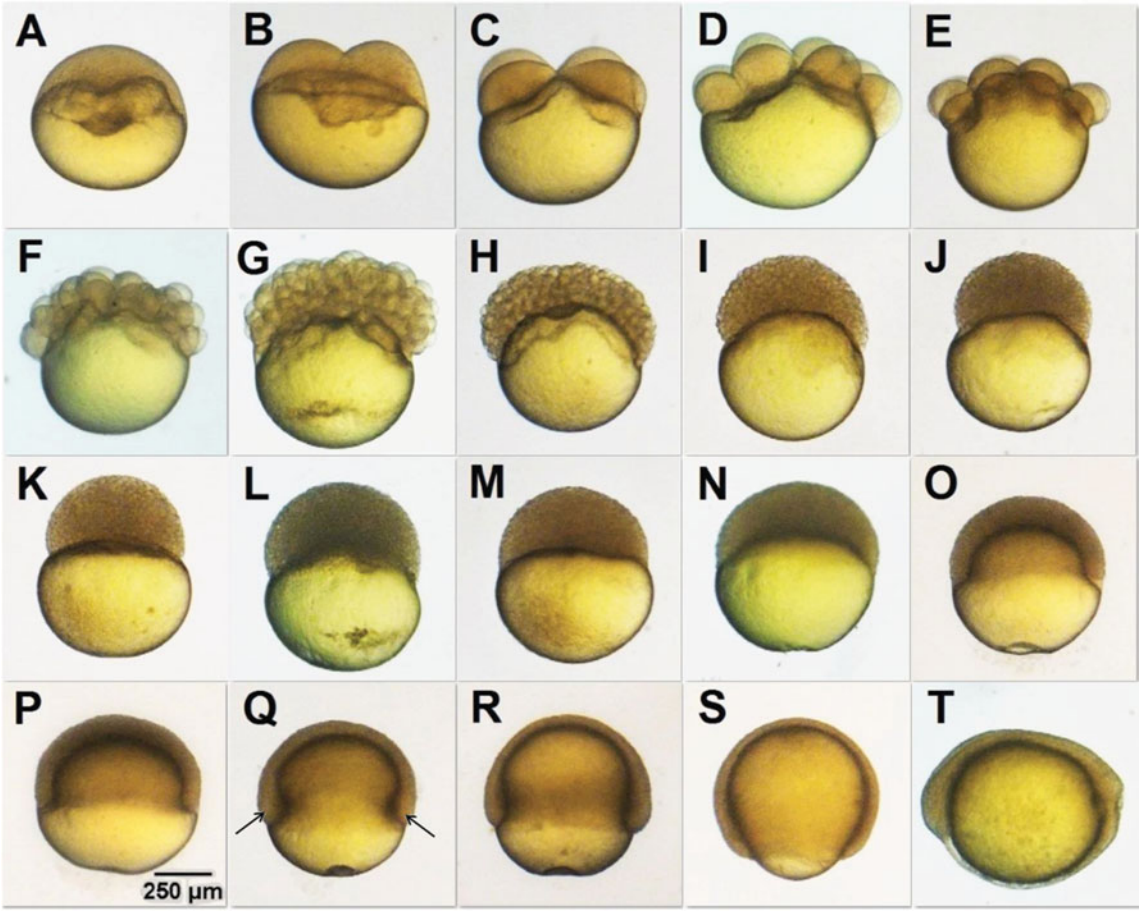

Figure 1 Embryonic development of *P. maculatus*, at period of cleavage, blastula, gastrula and initial segmentation. (*A*) Animal pole differentiation; (*B*) 2-cell stage; (*C*) 4-cell stage; (*D*) 8-cell stage; (*E*) 16-cell stage; (*F*) 32-cell stage; (*G*) 64-cell stage; (*H*) initial blastula stage with 128 blastomeres; (*I*) 256 blastomeres stage; (*J*) stage of 512 blastomeres; (*K*) stage with more than1000 blastomeres; (*L*) elongation stage; (*M*) spherical stage; (*N*) dome stage; (*O*) initial gastrula stage with 25% of epiboly; (*P*) 50% epiboly; (*Q*) germ ring stage (arrows indicate the germ ring); (*R*) 75% epiboly stage; (*S*) 90% epiboly stage; (*T*) initial segmentation stage (neurula stage). Scale bar indicates 250 µm.

when incubated at 22°C, 4 h 40 min post-fertilization at 26°C, and 3 h 45 min post-fertilization when incubated at 30°C (Fig. 1*R*). *Pseudopimelodus mangurus* embryos reached this stage 9 h 40 min post-fertilization at 22°C, 5 h 45 min post-fertilization at 26°C, and 4 h 45 min post-fertilization at 30°C (Fig. 2*R*).

90% epiboly stage. At this stage, 90% of the yolk was covered by the blastoderm. The embryos of *P. maculatus* arrive at this stage at 8 h 20 min post-fertilization when incubated at 22°C, 5 h 30 min post-fertilization at 26°C, and 3 h 55 min post-fertilization at 30°C (Fig. 1*S*). *P. mangurus* embryos arrived at this stage 10 h 40 min post-fertilization at 22°C, 6 h 30 min post-fertilization at 26°C, and 5 h 30 min post-fertilization at 30°C (Fig. 2*S*).

100% epiboly stage. At this stage, 100% of the yolk was already covered by the blastoderm. *P. maculatus* embryos reached this stage 10 h 30 min post-fertilization when incubated at 22°C, 6 h 30 min post-fertilization at 26°C, and 5 h post-fertilization when incubated at 30°C (Fig. 1*T*). For *P. mangurus*, the embryos took 12 h 30 min post-fertilization to arrive at this stage at 22°C, 7 h 20 min post-fertilization at 26°C, and 6 h post-fertilization at 30°C (Fig. 2*T*).

Segmentation period

The segmentation period began in the neurula stage with the appearance of somites (Fig. 3*A*, 4*A*) and differentiation of head and tail (Fig. 3*B*, 4*B*), and it ended with hatching (Fig. 5*A*, 5*B*). During this stage, the embryo began to develop its morphological structures, such as the optic vesicle, otic vesicle, Kupffer vesicle, and the onset of somatogenesis. Somites development occurs from the trunk to the tail of the embryo. This period is defined by the structures observed and by the number of somites.

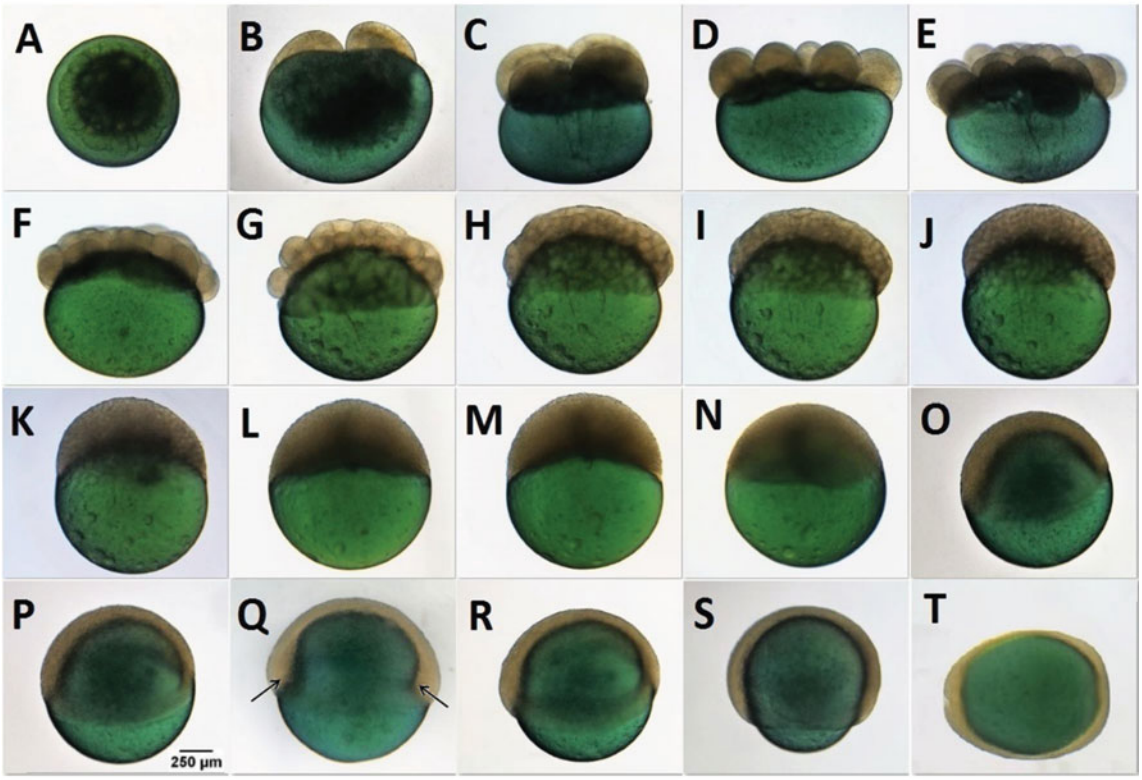

Figure 2 Embryonic development of *P. mangurus*, at period of cleavage, blastula, gastrula and initial segmentation. (*A*) Animal pole differentiation; (*B*) 2-cell stage; (*C*) 4-cell stage; (*D*) 8-cell stage; (*E*) 16-cell stage; (*F*) 32-cell stage; (*G*) 64-cell stage; (*H*) initial blastula stage with 128 blastomeres; (*I*) 256 blastomeres stage; (*J*) stage of 512 blastomeres; (*K*) stage with more than1000 blastomeres; (*L*) elongation stage; (*M*) spherical stage; (*N*) dome stage; (*O*) initial gastrula stage with 25% of epiboly; (*P*) stage with 50% epiboly; (*Q*) germ ring stage (arrows indicate the germ ring); (*R*) 75% epiboly stage; (*S*) 90% epiboly stage; (*T*) initial segmentation stage (neurula stage). Scale bar indicates 250 µm.

During this period, a high mortality rate occurs during *P. mangurus* embryo incubation, resulting in 100% mortality when embryos are incubated at 30°C.

In the *P. maculatus* embryos, the first somites appeared 12 h 15 min post-fertilization when incubated at 22°C, 8 h post-fertilization at 26°C, and 6 h post-fertilization when incubated at 30°C. For the *P. mangurus*, the first somites appeared 14 h post-fertilization when incubated at 22°C and 9 h post-fertilization at 26°C.

The segmentation period ended at hatching. At this moment, the larvae broke out of the chorion and started free swimming

Hatching period

The embryonic development ended when the larvae broke out of the chorion. Larvae of *P. mangurus* incubated at 22°C hatched 28 h 30 min post-fertilization, presenting 37 somites; 17 h 30 min post-fertilization when incubated at 26°C with 35 somites; and no larvae hatched at 30°C (Fig. 5*B*). *Pimelodus maculatus* larvae hatched 22 h 30 min post-fertilization when incubated at 22°C, presenting 32 somites, 16 h 30 min post-fertilization when incubated at 26°C with 29 somites; and 11 h 20 min post-fertilization at 30°C, having 27 somites (Fig. 5*A*).

Oocyte size

For *P. mangurus*, the size of oocytes non-hydrated, hydrated and perivitelline space was 1217.16 ± 13.35, 1790.95 ± 18.38 and 356.87 ± 19.21 µm, respectively. While for *P. maculatus* it was 792.26 ± 18.69, 1134.76 ± 19.46, and 259.67 ± 12.89 µm, respectively.

Effect of temperature on embryonic development

There was a large difference in embryo development time between the two species when incubated at 22°C, 26°C, or 30°C. During *P. maculatus* incubation, the embryos incubated at 30°C hatched 11 h 10 min faster compared with the embryos incubated at 22°C and 5 h 10 min when compared with embryos incubated at 26°C (Table 1). In *P. mangurus*, the difference was 11 h between the temperatures of 22°C and 26°C

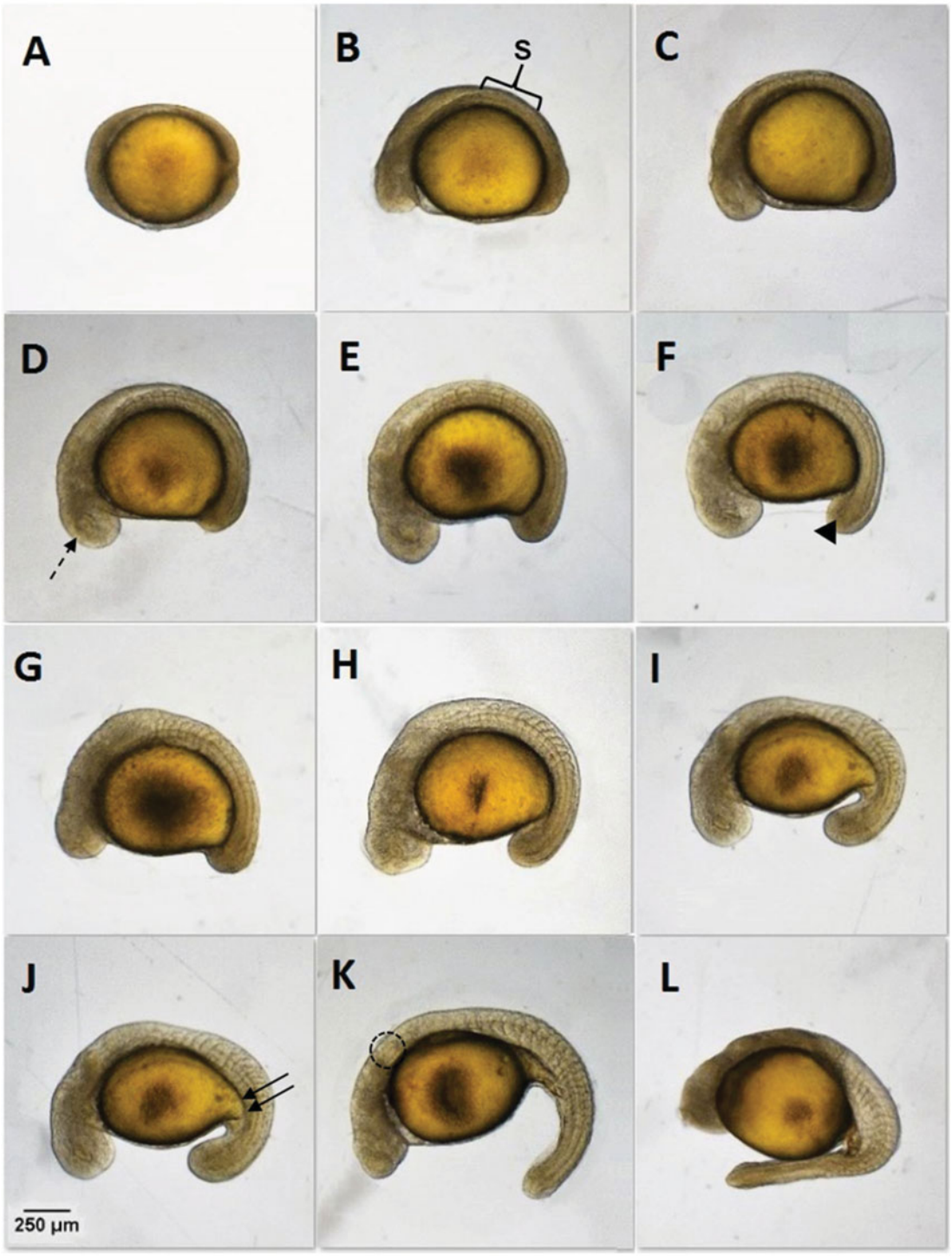

Figure 3 Embryos of *P. maculatus* during segmentation period, when incubated at 26°C. (*A*) Neurula stage; (*B*) 3-somite stage, brackets indicates the first somites (S); (*C*) 5-somite stage; (*D*) 8-somite stage, dashed arrow points the optic vesicle; (*E*) 10-somite stage; (*F*) 12-somite stage (arrowhead indicates the Kupffer's vesicle); (*G*) 14-somite stage; (*H*) 15-somite stage; (*I*) 20-somite stage; (*J*) 21-somite stage, double arrows indicate the elongation of the yolk; (*K*) 24-somite stage, dashed circle indicates otic vesicle; (*L*) 28-somite stage. Scale bar indicates 250 µm.

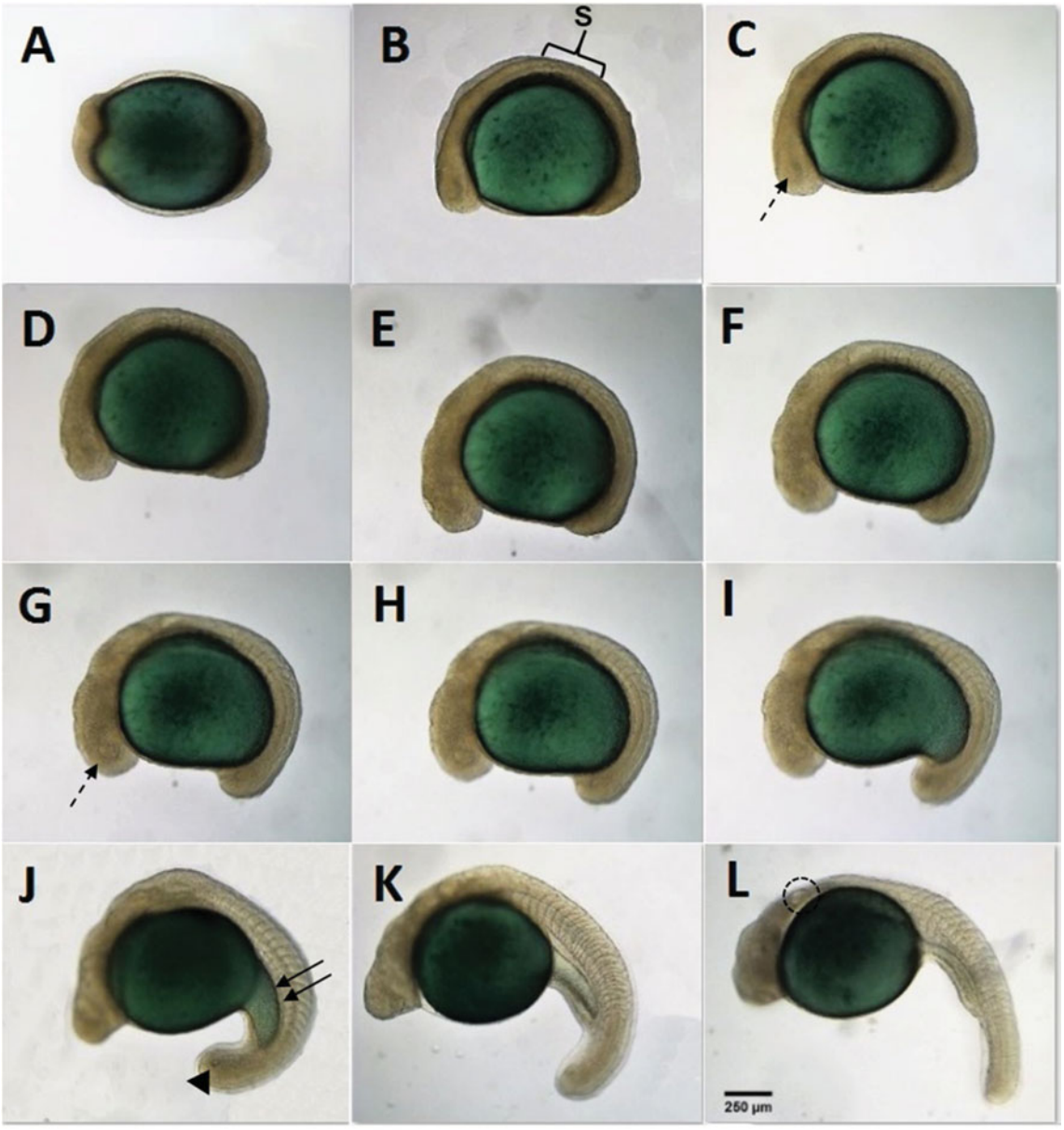

Figure 4 Embryos of *P. mangurus* during segmentation period when incubated at 26°C. (*A*) Neurula stage; (*B*) 3-somite stage, brackets indicates the first somites (S); (*C*) 5-somite stage; (*D*) 8-somite stage; (*E*) 10-somite stage; (*F*) 12-somite stage; (*G*) 14-somite stage, dashed arrow points the optic vesicle; (*H*) 15-somite stage; (*I*) 20-somite stage; (*J*) 21-somite stage, arrowhead indicates Kupffer's vesicle and double arrows indicate the elongation of the yolk; (*K*) 24-somite stage; (*L*) 28-somite stage, dashed circle indicates otic vesicle. Scale bar indicates 250 µm.

(Table 2). Warmer temperatures (30°C) accelerated embryo development time in incubation, however, this led to a higher rate of abnormality among the *P. maculatus* larvae and caused the death of all *P. mangurus* embryos up to the segmentation period.

Temperature influenced embryonic development in *P. maculatus* and *P. mangurus* that were submitted to treatment, and higher and lower temperatures accelerate and decreased, respectively, embryonic development. Additionally, for both species, the larvae from the lower (22°C) and higher temperatures (30°C) also presented increased abnormality rates, as observed in the head (Fig. 6*E*, 6*F*), tail (Fig. 6C, 6*D*) and yolk regions (Fig. 6*E*, 6*F*). The lowest abnormality rate was observed at 26°C, which was considered the optimal incubation temperature for both species. *P. mangurus* embryos showed tolerance to temperatures with a 45.4 ± 21.02% survival and 2.3 ± 1.73% larvae abnormality when incubated at 22°C, and presented 68.08 ± 12.67% survival and 3.3 ± 1.86% larvae abnormality

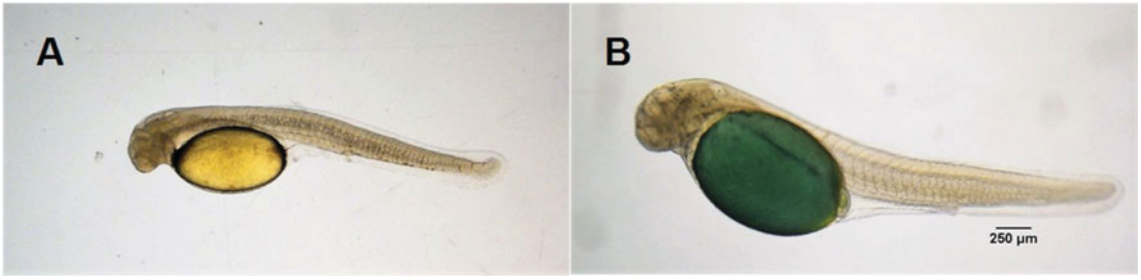

Figure 5 (*A*) Larvae of *Pimelodus maculatus*; (*B*) *Pseudopimelodus mangurus* after hatching. Scale bar indicates 250 µm.

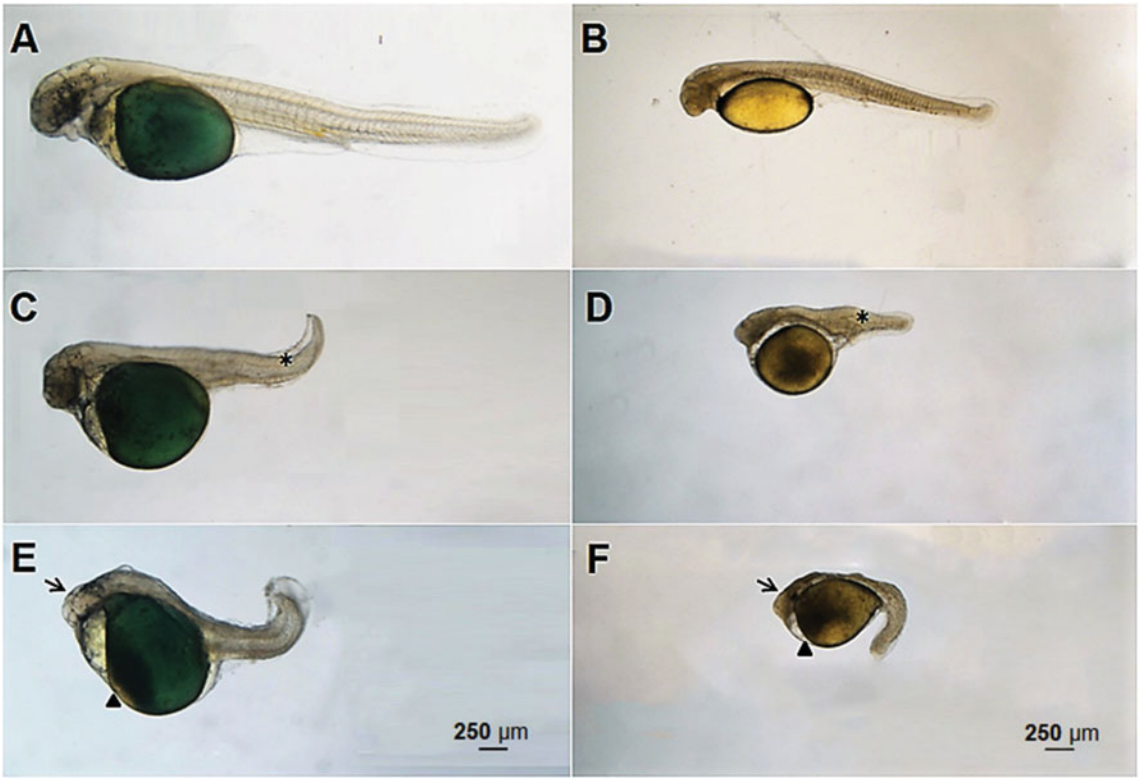

Figure 6 (*A*) Normal larvae of *Pseudopimelodus mangurus*. (*B*) Normal larvae of *Pimelodus maculatus*. (*C*) Larvae of *Pseudopimelodus mangurus* containing abnormality in caudal region (asterisk indicates abnormal tail). (*D*) Larvae of *Pimelodus maculatus* containing abnormality in caudal region (asterisk indicates abnormal tail). (*E*) Larvae of *Pseudopimelodus mangurus* containing abnormality in yolk and head region (arrow indicates the abnormal head and arrowhead indicates abnormal yolk region). (*F*) Larvae of *Pimelodus maculatus* containing abnormality in yolk and head region (arrow indicates the abnormal head and arrowhead indicates abnormal yolk region). Scale bar indicates 250 µm.

when incubated at 26°C. When incubated at 30°C, all embryos died before hatching (Table 3). Conversely, *P. maculatus* embryos hatched at all temperatures with a 20.13 ± 19.8% survival rate and 21.84 ± 2.37% larvae abnormality when incubated at 22°C. When incubated at 26°C, they presented a 57.57 ± 17.49% survival rate and 10.79 ± 4.17% larvae abnormality. At 30°C, they presented a 53.63 ± 16.27% survival rate and 20.76 ± 7.45% larvae abnormality (Table 4).

Discussion

Defining the optimal temperature range for embryo incubation is a preliminary step for artificial propagation in fish species. Improvement in egg hatchability with less malformations is among the main parameters for evaluation of incubation temperatures (Pepin, 1991; Jordaan, 2002). As ectotherms, fish embryos may develop over a wide range of temperatures, in which

Table 3 Percentage of main qualitative stages of embryonic development of *Pseudopimelodus mangurus* incubated at 22°C, 26°C or 30°C

	Development stage (%)							
Temperature	Unfertilized	2-cell	Blastula	Gastrula	Segmentation	Hatching	Normal	Abnormal
22°C	3.91 ± 1.58	96.09 ± 1.58	95.19 ± 1.74	88.69 ± 5.18	50.24 ± 22.4[a]	45.36 ± 21.02[a]	43.02 ± 20.02	2.34 ± 1.73[a]
26°C	2 ± 0.63	98 ± 0.63	95.85 ± 1.57	90.09 ± 5.01	72.66 ± 12.7[a]	68.08 ± 12.67[a]	64.76 ± 11.95	3.30 ± 1.86[a]
30°C	5.37 ± 2.09	94.63 ± 2.09	94.06 ± 2.54	84.42 ± 7.22	2.98 ± 1.62[b]	0 ± 0[b]	0 ± 0	0 ± 0[b]
P-value	0.464	0.661	0.936	0.804	0.004	0.0003	0.246	0.0002

[a,b]Values with different superscripts differ significantly.

Table 4 Percentage of main qualitative stages of embryonic development of *Pimelodus maculatus* incubated at 22°C, 26°C or 30°C

	Development stage (%)							
Temperature	Unfertilized	2-cell	Blastula	Gastrula	Segmentation	Hatching	Normal	Abnormal
22°C	3.05 ± 1.79	96.95 ± 1.79	88.45 ± 9.43	82.51 ± 8.74	66.67 ± 7.11	43.87 ± 7.46	20.13 ± 19.8	21.84 ± 2.37
26°C	1.26 ± 0.76	98.74 ± 0.76	96.42 ± 0.59	92.88 ± 1.17	72.42 ± 13.04	57.57 ± 17.49	46.78 ± 21.47	10.79 ± 4.17
30°C	1.55 ± 0.19	98.45 ± 0.19	90.35 ± 8.04	88.80 ± 7.98	64.35 ± 17.89	53.63 ± 16.27	32.87 ± 21.9	20.76 ± 7.45
P-value	0.786	0.786	0.899	0.612	0.929	0.777	0.257	0.649

warmer temperatures increase the velocity of development and embryo formation (Dos Santos *et al*., 2016). However, such a range of incubation temperature is well known to be species specific, as observed in *Atractosteus tristoechus* (26°C) (Comabella *et al*., 2014), *Lota lota* (2°C) (Lahnsteiner *et al*., 2012), *Rhamdia quelen* (21–30°C) (Galdino, 2013; Rodrigues-Galdino *et al*., 2010), *Oncorhynchus nerka* (10°C) (Velsen *et al*., 1980), *Brevoortia tyrannus* (15–25°C) (Ferraro, 1980), *Limanda ferruginea* (8–14°C) (Laurence & Howell, 1981), *Anguilla anguilla* (20°C) (Davidsen, 2012), *Cynopoecilus melanotaenia* (20°C) (*Arenzon et al*., 2002), *Coregonus clupeaformis* (0.5–6°C) (Price, 1940), *Hexagrammos otakii* (12–16°C) (Hu *et al*., 2015) and *Ctenopharyngodon idella* (26–28°C) (Korwin-Kossakowski, 2008).

The emerald green oocytes of the *P. mangurus* were larger than the yellowish oocytes of the *P. maculatus* and other Neotropical Characiformes species such as *Prochilodus scroffa* (1111 µm non-hydrated) (Fenerich-Verani *et al*., 1984), *Brycon insignis* (1175 µm non-hydrated) (Andrade Talmelli *et al*., 2002) and *Brycon cephalus* (1001.6 µm non-hydrated) (Romagosa *et al*., 2001). However, *P. mangurus* oocytes were smaller than those observed for others Neotropical Siluriformes, such as Zungaro Jahú (1600 µm non-hydrated and 2400 µm hydrated) (Nogueira *et al*., 2012), *R. quelen* (1470 µm non-hydrated and 2640 µm hydrated) and *P. charus* (1660 µm non-hydrated and 2670 µm hydrated) (Vieira Sampaio & Yoshimi, 2006).

The presented data suggest that the moderate temperature of 26°C is more suitable for embryo development in the five studied species, as observed similarly in previous work with the yellowtail tetra *Astyanax altiparanae* (Dos Santos *et al*., 2016). At 30°C, decreased hatching rates and a high number of abnormal embryos were observed. Surprisingly for the marbled catfish, all embryos died when incubated at 30°C. Most Neotropical fish species spawn during the rainy season, during which time the water temperature commonly reached a lethal limit. Therefore, this may explain the spawning behaviour of migratory fish in which rain is a main trigger to induce spawning in the reproductive season. After upstream migration, most Neotropical species wait for rain, and such behaviour synchronizes spawning of several species. Then, it is expected that rain triggers reproduction and improves egg hatchability with better water quality within an optimum range for incubation. In addition, synchronized spawning may improve survival for all fish species, including for carnivore species that have better food availability.

Only 4°C separates the optimal incubation temperature (26°C) from the temperature at which all *P. mangurus* embryos die (30°C) and this situation shows that the successful reproduction of fish species can be hampered by global warming (Ficke *et al*., 2007), leading to a decline in the populations of several species. Such evidence was observed in our study due to the increased embryo mortality and larval abnormality in *P. maculatus* when incubated at 30°C. Similar results was also evidenced for others Neotropical fish such as *A. altiparanae* (Dos Santos *et al*., 2016), *Brycon amazonicus* (da Silva *et al*., 2017) and *R. quelen* (Rodrigues-Galdino *et al*., 2010).

The establishment of incubation temperatures is interesting for application in studies involving germ cell transplantation. For blastomere transplantation, which involves embryo-to-embryo transplantation, both donor and host species must be at the blastula stage (Yamaha *et al*., 1998). However, development of donor and host embryos may identify a specific temperature for incubation and a specific velocity of development. Although synchronizing both embryos to the blastula stage is challenging, temperature may be easily employed to manipulate embryo development. The marbled catfish *P. mangurus* and the spotted catfish *P. maculatus* are an interesting model for blastomere transplantation. However, based in these results, the blastula period is very short in both species, limiting the transplantation period to a few minutes. For instance, hatching in both Neotropical catfishes in this study took place within 11 to 28 h post-fertilization, although other transplanted species reported in the literature have longer hatching periods, e.g. medaka *Oryzias latipes* (10 days post-fertilization) (Shinomiya *et al*., 2003; Iwamatsu, 2004), loach *Misgurnus anguillicaudatus* (48 h) (Fujimoto *et al*., 2006), zebrafish (48 h) (Kimmel *et al*., 1995; Lin *et al*., 1992) and also in salmonids in which the embryonic development takes place after several weeks (Velsen *et al*., 1980; Takeuchi *et al*., 2001; Takeuchi *et al*., 2003; Winckler-Sosinski *et al*., 2005; Okutsu *et al*., 2007). As seen above, most germ cell transplantations have been performed in cold water species, in which the transplantation period is longer. In Neotropical species, the presented data suggest that the transplantation strategy should be different than that for cold water species, due to a shorter transplantation period. Thus, a lower number of transplants per each egg batch will be produced in Neotropical species, suggesting that an increased number of egg batches is then required to produce an adequate number of transplanted embryos.

The success of transplantation and subsequent production of germline chimera for surrogate propagation depends on the phylogenetic relationship between donor and host species, in which related species may increase the success of germline transmission (Yamaha *et al*., 2007). For the spotted catfish *P. maculatus*, this is interesting because several species of catfish are considered endangered in the Neotropical region (Machado *et al*., 2008). Siluriformes are considered to be the largest group in the Neotropical region, suggesting

that this species may become an interesting model fish for surrogate propagation with subsequent utilization in both academic and aquaculture purposes.

In conclusion, the present study verified the effect of temperature in the embryonic development of two Neotropical catfishes. This exercise is useful for embryo transplantation in Siluriformes because endangered catfish species can be used as donors of PGCs, and a common catfish species can be used as a host. This is strategic for this species and also applicable for other endangered or aquacultured catfish from the Neotropical region.

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Ethical standards

The experiments were conducted in accordance with the Ethics Committee for the Use of Laboratory Animals of the National Center for Research Conservation of Aquatic Biodiversity (CEUA/CEPTA; #010/2015). The fish were collected in natural environments using the sampling licence according to Brazilian law (Sisbio #55725-1).

References

Andrade Talmelli, E., Kavamoto, E. & Narahara, M. (2002). Fenerich-Verani, Reprodução induzida da piabanha, *Brycon insignis*, mantida em cativeiro. *Rev. Brás. Zootec.* **31**, 803–11.

Arenzon, A., Lemos, C. & Bohrer, M. (2002). The influence of temperature on the embryonic development of the annual fish *Cynopoecilus melanotaenia* (Cyprinodontiformes, Rivulidae). *Brazil. J. Biol.* **62**, 743–7.

Brasil (2016). Sumário executivo do Livro vermelho da fauna brasileira ameaçada de extinção. Brasília, 75 pp.

Bressan, P.M., Kierulff, M.C.M. & Sugieda, A.M. (2009). Fauna ameaçada de extinção no Estado de São Paulo. São Paulo: Fundação Parque Zoológico de São Paulo, Secretaria do Meio Ambiente.

Comabella, Y., Hurtado, A., Canabal, J. & García-Galano, T. (2014). Effect of temperature on hatching and growth of Cuban gar (*Atractosteus tristoechus*) larvae. *Ecosistemas y Recursos Agropecuarios* **1**, 19–32.

da Silva, R.C., dos Santos, M.P., Senhorini, J.A., Paes, M.d.C.F., Valentin, F.N., Fujimoto, T., do Nascimento, N.F., Yasui, G.S. & Nakaghi, L.S.O. (2017). The effect of temperature on the initial development of *Brycon amazonicus* Spix & Agassiz, 1829 as tool for micromanipulation of embryos. *Zygote* **25**, 637–51.

Davidsen, M. (2012). The effect of incubation temperature on embryonic development and muscle growth in yolk-sac larvae of the European eel (*Anguilla anguilla* L., 1758). Master thesis: Institutt for biologi. https://brage.bibsys.no/xmlui/handle/11250/245075

do Nascimento, N.F., Pereira-Santos, M., Piva, L.H., Manzini, B., Fujimoto, T., Senhorini, J.A., Yasui, G.S. & Nakaghi, L.S.O. (2017). Growth, fatty acid composition, and reproductive parameters of diploid and triploid yellowtail tetra *Astyanax altiparanae*. *Aquaculture* **471**, 163–71.

Dos Santos, M.P, Yasui, G.S., Xavier, P.L., de Macedo Adamov, N.S., do Nascimento, N.F., Fujimoto, T., Senhorini, J.A. & Nakaghi, L.S. (2016). Morphology of gametes, post-fertilization events and the effect of temperature on the embryonic development of *Astyanax altiparanae* (Teleostei, Characidae). *Zygote* **24**, 795–807.

Fenerich-Verani, N., Godinho, H.M. & Narahara, M.Y. (1984). The size composition of the eggs of curimbatá, *Prochilodus scrofa* Steindachner 1881, induced to spawn with human chorionic gonadotropin (HCG). *Aquaculture* **42**, 37–41.

Ferraro, S.P. (1980). Embryonic development of Atlantic menhaden, *Brevoortia tyrannus*, and a fish embryo age estimation method. *Fish. Bull.* **77**, 943–9.

Ficke, A.D., Myrick, C.A. & Hansen, L.J. (2007). Potential impacts of global climate change on freshwater fisheries. *Rev. Fish Biol. Fish.* **17**, 581–613.

Froese, R. & Pauly, D. (2015). FishBase. World Wide Web Electronic Publication. www.fishbase.org

Fujimoto, T., Kataoka, T., Sakao, S., Saito, T., Yamaha, E. & Arai, K. (2006). Developmental stages and germ cell lineage of the loach (*Misgurnus anguillicaudatus*). *Zool. Sci.* **23**, 977–89.

Galdino, A.M.R. (2013). Plasticidade do desenvolvimento muscular e da expressão temporal de fatores reguladores miogênicos durante os estádios iniciais de *Rhamdia quelen* incubados em diferentes temperaturas. https://educapes.capes.gov.br/handle/1884/30598

Galves, W., Shibatta, O.A. & Jerep, F.C. (2009). Estudos sobre diversidade de peixes da bacia do alto rio Paraná: uma revisão histórica. *Semina: Ciências Biológicas e da Saúde* **30**, 141–54.

Godinho, H.P. & Godinho, A.L. (2003*). Águas, peixes e pescadores do São Francisco das Minas Gerais*, PUC Minas, Belo Horizonte.

Hu, F., Pan, L., Gao, F., Jian, Y., Wang, X., Li, L., Zhang, S. & Guo, W. (2015). Effect of temperature on incubation period and hatching success of fat greenling (*Hexagrammos otakii* Jordan & Starks) eggs. *Aquacult. Res.* **48**, DOI: 10.1111/are.12853

Iwamatsu, T. (2004). Stages of normal development in the medaka *Oryzias latipes*. *Mech. Dev.* **121**, 605–18.

Jordaan, A. (2002). The effect of temperature on the development, growth and survival of Atlantic cod (*Gadus morhua*) during early life-histories. Master Thesis: The University of Maine. https://digitalcommons.library.umaine.edu/etd/141/

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B. & Schilling, T.F. (1995). Stages of embryonic-development of the zebrafish. *Dev. Dynam.* **203**, 253–310.

Korwin-Kossakowski, M. (2008). The influence of temperature during the embryonic period on larval growth and development in carp, *Cyprinus carpio* L., and grass carp, *Ctenopharyngodon idella* (Val.): theoretical and practical aspects. *Arch. Polish Fish*. **16**, 231–314.

Lahnsteiner, F., Kletzl, M. & Weismann, T. (2012). The effect of temperature on embryonic and yolk-sac larval development in the burbot *Lota. J. Fish Biol.* **81**, 977–86.

Laurence, G.C. & Howell, W.H. (1981). Embryology and influence of temperature and salinity on early development and survival of yellowtail flounder *Limanda ferruginea*. *Mar. Ecol. Prog. Ser.* **6**, 11–8.

Lin, S., Long, W., Chen, J. & Hopkins, N. (1992). Production of germ-line chimeras in zebrafish by cell transplants from genetically pigmented to albino embryos. *Proc. Natl. Acad. Sci. USA* **89**, 4519–23.

Machado, A.B. M., Drummond, G.M. & Paglia, A.P. (2008). Livro vermelho da fauna brasileira ameaçada de extinção, MMA; Fundação Biodiversitas.

Nogueira, L.B., Azevedo, P.G., Canelhas, M.R., Bedore, A.G., Lopes, J.M. & Godinho, H.P. (2012). Induced spawning and early ontogeny in hatchery-reared catfish *Zungaro jahu* (Siluriformes: Pimelodidae). *Neotrop. Ichthyol.* **10**, 89–98.

Okutsu, T., Shikina, S., Kanno, M., Takeuchi, Y. & Yoshizaki, G. (2007). Production of trout offspring from triploid salmon parents. *Science* **317**, 1517.

Pepin, P. (1991). Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can. J. Fish. Aqua. Sci.* **48**, 503–18.

Piferrer, F., Beaumont, A., Falguiere, J.-C., Flajshans, M., Haffray, P. & Colombo, L. (2009). Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* **293**, 125–56.

Price, J.W. (1940). Time-temperature relations in the incubation of the whitefish, *Coregonus clupeaformis* (Mitchill). *J. Gen. Physiol.* **23**, 449–68.

Rodrigues-Galdino, A.M., Maiolino, C.V., Forgati, M., Donatti, L., Mikos, J.D., Carneiro, P.C.F. & Rios, F.S.A. (2010). Development of the neotropical catfish *Rhamdia quelen* (Siluriformes, Heptapteridae) incubated in different temperature regimes. *Zygote* **18**, 131.

Romagosa, E., Narahara, M. Y., Borella, M.I. & Fenerich-Verani, N. (2001). Seleção e caracterização de fêmeas de matrinxã, *Brycon cephalus*, induzidas a reprodução. *Boletim do Instituto de Pesca* **27**, 139–47.

São Paulo (2014). Decreto No. 60.133, De 7 De Fevereiro De 2014.

Shinomiya, A., Shibata, N., Sakaizumi, M. & Hamaguchi, S. (2003). Sex reversal of genetic females (XX) induced by the transplantation of XY somatic cells in the medaka, *Oryzias latipes*. *Int. J. Dev. Biol.* **46**, 711–7.

Takeuchi, Y., Yoshizaki, G. & Takeuchi, T. (2001). Production of germ-line chimeras in rainbow trout by blastomere transplantation. *Mol. Reprod. Dev.* **59**, 380–9.

Takeuchi, Y., Yoshizaki, G. & Takeuchi, T. (2003). Generation of live fry from intraperitoneally transplanted primordial germ cells in rainbow trout. *Biol. Reprod.* **69**, 1142–9.

Velsen, F.J., Bernard, F., McKinnell, S. & Jamieson, G. (1980). *Embryonic Development in Eggs of Sockeye Salmon*, Oncorhynchus nerka. Canadian Department of Fisheries and Oceans.

Vieira Sampaio, E. & Yoshimi, S. (2006). Biologia reprodutiva e desova induzida de duas espécies de bagres (Osteichthyes: Siluriformes) da bacia do rio São Francisco. *Acta Scientiarum. Biol. Sci.* **28**, DOI: 10.4025/actascibiolsci.v28i3.227

Winckler-Sosinski, L., Schwarzbold, A. & Schulz, U. (2005). Survival of rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Salmoniformes–Salmonidae) eggs in an altitude stream in southern Brazil. *Acta Limnol. Bras.* **17**, 465–72.

Yamaha, E., Mizuno, T., Hasebe, Y., Takeda, H. & Yamazaki, F. (1998). Dorsal specification in blastoderm at the blastula stage in the goldfish, *Carassius auratus*. *Dev. Growth Diff.* **40**, 267–75.

Yamaha, E., Saito, T., Goto-Kazeto, R. & Arai, K. (2007). Developmental biotechnology for aquaculture, with special reference to surrogate production in teleost fishes. *J. Sea Res.* **58**, 8–22.

Yasui, G., Fujimoto, T., Sakao, S., Yamaha, E. & Arai, K. (2011). Production of loach (*Misgurnus anguillicaudatus*) germ-line chimera using transplantation of primordial germ cells isolated from cryopreserved blastomeres. *J. Anim. Sci.* **89**, 2380–8.