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Original Article

Effect of temperature on embryonic development and first exogenous feeding of goldfish *Carassius auratus* (Linnaeus, 1758)

Efeito da temperatura no desenvolvimento embrionário e na primeira alimentação exógena do goldfish *Carassius auratus* (Linnaeus, 1758)

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Abstract

Goldfish or Kinguio is a widely marketed species worldwide due to the ornamental market. There is some lack of acknowledgment of the production of the species under specific climatic conditions. To evaluate the effect of temperature on embryonic development and the first exogenous feeding of goldfish, an experiment was proposed. Fifteen incubators, organized in five treatments (18, 22, 26, 30, and 34 °C) with three replications each, were used to keep the fertilized goldfish eggs until the first exogenous feeding of the larvae. The main development events were observed to understand the possible effects of these temperatures on embryos and larvae of the species. Temperature influences embryo development and the time of first exogenous feeding of goldfish. The temperature of 34 °C was lethal to the species causing 100% of anomalies in the embryos and larvae. The experiment data allow us to conclude that the species presents a maximum thermal limit during embryogenesis, and these data are important to the aquaculture industry and to understand the effect of climate changes on goldfish. The data obtained in this experiment will assist in the management of invasive species and production of the species (aquaculture).

Keywords: aquaculture, ornamental fish, thermal tolerance, ontogeny, frying.

Resumo

O peixe dourado ou Kinguio é uma espécie amplamente comercializada em todo o mundo devido ao mercado ornamental. Existe alguma falta de conhecimento da produção da espécie em condições climáticas específicas. Para avaliar o efeito da temperatura no desenvolvimento embrionário e na primeira alimentação exógena do Kinguio, um experimento foi proposto. Quinze incubadoras, organizadas em cinco tratamentos (18, 22, 26, 30 e 34 °C) com três repetições cada, foram utilizadas para manter os ovos fertilizados de Kinguio até a primeira alimentação exógena das larvas. Os principais eventos do desenvolvimento foram observados para entender os possíveis efeitos dessas temperaturas em embriões e larvas da espécie. A temperatura influencia o desenvolvimento do embrião e o tempo da primeira alimentação exógena do Kinguio. A temperatura de 34°C foi letal para a espécie causando 100% de anomalias nos embriões e larvas. Os dados do experimento permitem concluir que a espécie apresenta um limite térmico máximo durante a embriogênese, sendo esses dados importantes para a indústria da aquicultura e para entender o efeito das mudanças climáticas no Kinguio. Os dados obtidos neste experimento auxiliarão no manejo de espécies invasoras e na produção da espécie (aquicultura).

Palavras-chave: aquicultura, peixe ornamental, tolerância térmica, ontogenia, alevinagem.

1. Introduction

Temperature concerns the measure of kinetic energy linked to the movement of particles. This variable is fundamental for thermodynamics, promoting free energy necessary for chemical reactions. Thus, it has already been stablished that temperature is the main abiotic factor that will influence the viability of the fish in the environment in which they live (Fry and Hart, 1948; Fry, 1958).

Furthermore, the consequences of the increase in temperature and its effects on an ectothermic organism range from the molecular impacting chemical reaction rate and the stability of weak chemical bonds, until macro level, influencing behavioral patterns, such as locomotion (Baldisserotto and Val, 2002; Schulte, 2011; Little et al., 2020).

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For fish, during embryonic stage, there is a temperature range that promotes better development and hatching rate, a characteristic that may vary among different species (Radael et al., 2016; Thépot and Jerry, 2015; Pereira et al., 2016). When the issue of the influence of temperature is considered by the fish production chain, the environment can be controlled to meet the demands of the species.

Therefore, it is extremely important to know the limits at which the species can be maintained and that do not affect production negatively (*e.g.* abnormalities; low hatching rate) (Arenzon et al., 2002; Tucker Junior et al., 2002). Within this context, water temperature controlling in farming is an interesting strategy to potentiate the production of a given species, like the Goldfish or Kinguio (*Carassius auratus*).

In this context, there is a lack of information about the embryonic development of *Carassius auratus* at high temperatures (> 18 °C), a fact that limits the production of the species in tropical areas. Being this fish mentioned above one of the most commercialized in the world branch of aquariophilia, the objective of this research was to present the ideal temperature for the embryonic development of *C. auratus* also ensure information about the time of the first exogenous feeding of the species at different temperatures, in order to improve productivity.

2. Material and Methods

This research was funded by the Pescarte Environmental Education Project (PEA), which is a mitigation measure required by the Federal Environmental Licensing, conducted by IBAMA. Goldfish from the Universidade Estadual do Norte Fluminense Darcy Ribeiro aquaculture laboratory were used to obtain fish eggs by naturally induced spawning. Four adult goldfish were acclimatized in a glass tank with a useful volume of 180 L. A ratio of three males to one female was used. Fish reproductive behavior began during the early morning and, around 6 a.m., the fish were collected for extrusion. For this purpose, gametes of males and females were carefully mixed with the aid of a silicone spoon. For egg hydration, water from the breeder tank itself was used, which was at an approximate temperature of 26 °C.

The fish eggs were transferred individually to rectangular incubators with a useful volume of 20 L, where the temperatures were tested. Thirty eggs were placed in each incubator. Based on the lack of information and the need to know the effect of high temperatures (> 18° C) on the embryonic development of the species, five test temperatures (18, 22, 26, 30, and 34 °C) were selected for egg incubation and three replicates were performed for each tested temperature. Thus, 15 incubators were used during the experimental period.

To maintain the test temperatures, the experiment was conducted in a laboratory equipped with air conditioning that kept the air temperature close to 16 °C. Heaters with thermostats (OCEANTECH-WARMER X5 300 W) were installed inside the incubators in order to control the water temperature. The heater variation with the thermostat predicted by the manufacturer was ± 1 °C. The baseline temperature of all incubators was 26 °C, and the temperature was then gradually adjusted to reach the proposed value for each treatment. The temperature variation during the acclimatization phase was approximately 1 °C/20 min.

To ensure the correct water oxygenation and to minimize any possibility of thermal stratification, a porous stone was placed in each incubator. The porous stones were filled with atmospheric air and pressurized with an air compressor (4.0 $L.min^{-1}$). The amount of air in each incubator was manually controlled with the aid of an individual metal diffuser. The photoperiod was adjusted to 12 hours of light using a digital timer that activated the laboratory lamps at 6 a.m. and turned them off at 6 p.m.

To control water quality, some parameters were manually measured during the experimental period. The pH was measured daily (pHtek® PHS-3E, ± 0.02), always at 9 a.m. and temperature and oxygen were measured every hour (YSI® 550 A, ± 0.01). A researcher was responsible for environmental control.

The stages of embryonic development that were evaluated for the effect of temperature can be summarized as defined by Radael et al. (2014) and Fujimoto et al. (2004, 2006): cleavage, blastula, gastrula, and segmentation. Cleavage is defined as the period of division of the blastodisc cells until they reach the number of 64 blastomeres, the blastula is defined as the period during which the blastodisc has 128 to 1021 blastomeres, the gastrula is defined as the covering of the yolk by the process of epibolism of the blastoderm until the closing of the blastopore, and segmentation is defined as the phase where the emergence of tissues and organs occurs in the embryo.

For the observation of embryos and larvae, three optical microscopes with an attached camera (Nikon® Eclipse e200) were used. The events were observed every 30 minutes and when some change in fish development was detected, it was recorded along with the time of occurrence. At each observation time, 20% of the animals (n = 6) in each incubator were analyzed. It is important to mention that three experienced researchers were always working at the same time in order to speed up the egg observation process. The team was careful so that each microscope analysis time does not exceed 45 seconds.

The occurrence of the event was only reported when 50% of the eggs showed the same differentiated event, with the exception of the "first hatch" and "last hatch" events, which were reported when the first egg hatched and when the last egg hatched, respectively. The hatching rate in each incubator was calculated as the quotient of hatched egg number and total number of eggs. The hatching percentage was obtained by multiplying the hatching rate by 100.

After optical microscopy analysis, the eggs were returned to their respective incubators. An adapted Pasteur pipette and some histological slides were used for egg collection and larva observation by optical microscopy.

All newly hatched larvae were measured (Western® 6", 150 ± 0.01 mm) and weighed (Shimadzu® AUX, 220 ± 0.001 g) and then returned to their respective incubators.

To obtain the percentage of anomalous larvae per incubator, the ratio between the number of larvae with some anatomical anomaly and the total number of newly hatched larvae multiplied by 100 was calculated.

Once the fry was able to open its mouth, nauplii of newly hatched *Artemia saline* were offered for observation of the first exogenous feeding. The method described by Motta et al. (2019) was used for the hatching of artemia cysts. The presence of food in the gastrointestinal tract was evaluated by examining the larvae under an optical microscope. As soon as the presence of food was detected, the larvae were removed from the experiment.

For the variables time of occurrence of each event, egg size, larva length, larva weight, hatching percentage, and defective larva percentage, a mixed linear model was applied using the MIXED procedure of the Statistical Analysis System (SAS System, Inc., Cary, NC, USA). The repeated command was used to model the variance-covariance matrix as independent variance components (VC), autoregressive (AR1), compound symmetry (CS), and unrestricted variance-covariance (UN) structures (Littell et al., 2006). The likelihood of the different variance-covariance structures was assessed by computing Akaike information criteria as suggested by Vieira et al. (2012). When a significant difference was detected, the Tukey test was applied at 0.05 probability. For the hatching rate, a function was estimated to calculate the optimum concentration point.

The functions relating to the characteristics of hatching rate was adjusted in relation to the treatment by using the REG procedure of the SAS statistical software (university edition, SAS System Inc.,Cary, NC, USA).

3. Results

Table 1 shows the mean and standard error of water quality parameters obtained during the experimental period. It is worth mentioning that the variation in dissolved oxygen values is normal since the treatments predicted different temperatures and the concentration of dissolved oxygen in water is directly related to water temperature (Mackay and Fleming, 1969). Nevertheless, it should be pointed out that the minimum oxygen value observed for a treatment in the present experiment was still higher than the minimum value (4.0 $mg.L^{-1}$) proposed for growing fish in warm waters (Wedemeyer et al., 1999).

Goldfish have adhesive spherical, yellowish eggs that are denser than water, and whose nucleus and cytoplasm form a small globule over a huge amount of yolk. The embryo develops according to the following pattern: cleavage, blastula, gastrula, organogenesis, and pre-hatching (see Figure 1A-1F).

Table 1. Water quality parameters during the experimental period.

Treatment	D.O ($mg.L^{-1}$)	Temperature (°C)	pH
18	9.59 ± 1.03	17.8 ± 0.8	6.91 ± 0.07
22	8.26 ± 0.55	22.2 ± 0.6	6.94 ± 0.06
26	7.28 ± 0.33	25.9 ± 0.4	6.92 ± 0.06
30	6.56 ± 0.51	30.1 ± 0.3	6.89 ± 0.05
34	5.33 ± 0.56	34.1 ± 0.2	6.89 ± 0.04

D.O: dissolved oxygen.

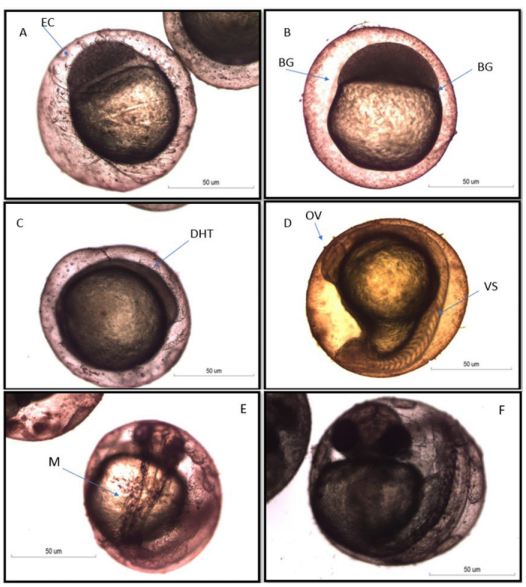

Figure 1. Initial development of goldfish exposed to different temperatures. A: (EC) end cleavage; B: (BG) Beginning of gastrula; C: (DHT) differentiation of head and tail; D: (OV) Optic vesicle, (VS) somites in "V"; E: (M) melanophore; F: pre-hatch period.

At the temperature of 34 °C some organogenesis stages were not clearly observed (as shown in Table 2). The time elapsed until the occurrence of the main events of the initial ontogeny of goldfish specimens was significantly affected by the water temperature of the incubators ($P < 0.05$) (see Table 2).

Goldfish larvae have closed mouths and an uninflated swimming bladder at the time of hatching. Mouth opening and swimming ability were observed after hatching. When the larvae were fed *Artemia* nauplii, their abdomen was orange in color. Although hatching occurred in eggs submitted to a temperature of 34 °C during the experimental period, none of the newly hatched larvae fed themselves and they all died hours after hatching. Time of death varied greatly, with some larvae dying a few hours after hatching, while others lasted a few days (2-3 days).

The percentage of hatching of goldfish eggs is presented in Table 3. The number of anomalous larvae is shown in the same table, and some of the anomalies can be observed in Figure 2A-D. It is important to mention that, at the temperature of 34 °C, it was possible to observe embryos with anatomical anomalies even before hatching.

Table 2. Time of occurrence of the main initial events of goldfish development at different incubation temperatures.

Event/stage	Time to event occurrence (hours)					Standard error
	18° C	22° C	26° C	30° C	34° C	
End of cleavage	11.96^{D}	5.93^{C}	4.90^{B}	4.82^{AB}	4.61^{A}	0.01
Beginning of gastrula	11.96 ^{D}	5.93 ^{C}	4.90 ^{B}	4.82 ^{AB}	4.61 ^{A}	0.01
Blastopore closure	20.90^{E}	11.27^{D}	8.83^{C}	7.83^{B}	4.61^{A}	0.01
Differentiation of head and tail	24.74^{C}	11.29^{B}	8.83^{A}	8.87^{A}	8.84^{A}	0.01
Optic primoridum	26.90^{C}	15.83^{B}	9.93^{A}	9.96^{A}	*	0.01
Somites	29.88^{D}	17.86^{C}	13.80^{B}	10.86^{A}	10.92^{A}	0.01
Optic vesicle	35.80^{D}	19.94^{C}	14.85^{B}	12.85^{A}	14.86^{B}	0.01
Muscle contraction	45.93^{D}	29.93^{C}	22.86^{B}	16.89^{A}	16.95^{A}	0.01
V size somites	46.82^{D}	22.75^{C}	17.94^{B}	13.90^{A}	*	0.01
Release of tail	48.86^{D}	37.83^{C}	26.89^{B}	26.95^{B}	17.92^{A}	0.01
Heartbeat	50.88^{D}	30.83^{C}	22.86^{B}	16.89^{A}	16.94^{A}	0.01
Circulation	59.77^{D}	38.31^{C}	26.90^{B}	19.89^{A}	19.90^{A}	0.01
Melanophores	64.76^{E}	46.93^{D}	27.86^{C}	22.93^{B}	17.95^{A}	0.01
Optic vesicle and otoliths	67.87^{D}	37.83^{C}	33.76^{B}	19.87^{A}	*	0.01
Ocular pigment	67.90^{D}	42.90^{C}	20.85^{B}	16.61^{A}	19.91^{B}	0.01
Melanophores on yolk	67.87^{E}	46.90^{D}	26.75^{C}	22.89^{B}	17.92^{A}	0.01
Caudal fin	73.85^{D}	63.91^{C}	28.89^{B}	24.57^{A}	*	0.01
Pectoral fin	89.96^{E}	73.96^{D}	49.54^{C}	35.85^{A}	38.91^{B}	0.01
First hatch	104.87^{E}	74.82^{D}	50.93^{C}	36.81^{A}	39.96^{B}	0.01
Last hatch	155.85^{D}	102.93^{C}	64.95^{B}	51.93^{A}	64.89^{B}	0.01
First exogenous feeding	216.00^{D}	140.88^{C}	125.93^{B}	111.82^{A}	*	0.01

The presence of different letters in the same line means a significant difference between treatments ($p < 0.05$). *Event not observed.

Table 3. Effect of temperature on the percentage of hatching and anomalous larvae of goldfish (*C. auratus*).

Treatment	Hatch (%)	Confidence interval (%)	
		Lower	Upper
18	81.1^{A}	70.2	88.7
22	96.7^{A}	88.7	99.1
26	93.3^{A}	84.5	97.3
30	82.2^{A}	71.5	89.5
34	10.0^{B}	4.8	19.6
Treatment	Anomalous larvae (%)	Confidence interval (%)	
		Lower	Upper
18	12.3^{A}	6.0	23.7
22	6.9^{A}	2.8	16.0
26	4.8^{A}	1.6	13.5
30	5.4^{A}	1.8	15.2
34	100.0^{B}	0.0	100.0

The presence of different letters in the same column means a significant difference between treatments ($p < 0.05$).

Table 4. Effect of temperature on egg size and, weight and length of newly hatched goldfish *(C. auratus)* larvae.

Treatment	Size of eggs (mm)	Larvae weight (mg)	Larvae length (mm)
18	1.48 ± 0.1 ^{A}	0.78 ± 0.01 ^{A}	4.31 ± 0.07 ^{A}
22	1.47 ± 0.1 ^{A}	0.80 ± 0.01 ^{A}	4.41 ± 0.07 ^{A}
26	1.49 ± 0.1 ^{A}	0.78 ± 0.01 ^{A}	4.37 ± 0.07 ^{A}
30	1.48 ± 0.1 ^{A}	0.79 ± 0.01 ^{A}	4.39 ± 0.07 ^{A}
34	1.49 ± 0.1 ^{A}	0.79 ± 0.01 ^{A}	3.88 ± 0.11 ^{B}

The presence of different letters in the same column means a significant difference between treatments ($p < 0.05$).

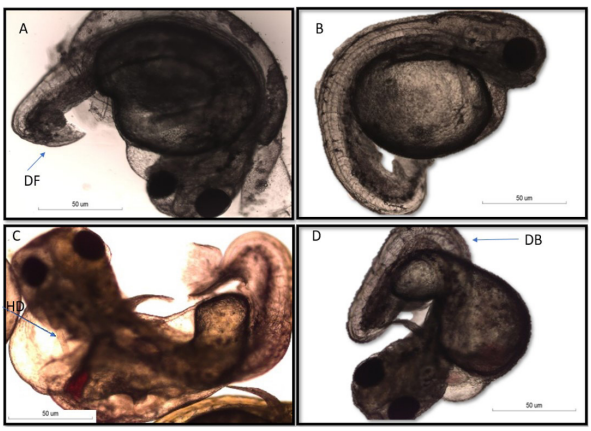

Figure 2. Effect of temperature on the appearance of deformities in goldfish submitted to incubation at a temperature of 34 °C. A: (DF) deformed fin; B: deformed newly hatched larva; C: (HD) heart with deformity; D: (DB) deformed body.

Figure 3 shows the quadratic regression line obtained as the result of the effect of temperature on the hatching of goldfish eggs. The estimated optimum temperature for hatching goldfish eggs is 23.9 °C (it was the "*x*" in the maximum point of quadratic regression). The parable shown in Figure 3 indicates the resilience of goldfish during embryogenesis.

Table 4 shows the size of goldfish eggs and the weight and length of newly hatched larvae submitted to different hatching temperatures.

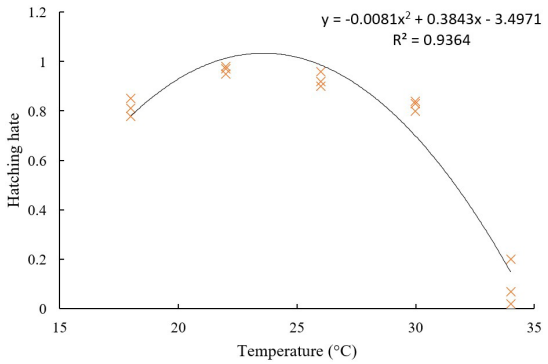

Figure 3. Effect of temperature on the hatching of goldfish eggs (*C. auratus*).

4. Discussion

The embryogenesis steps observed for goldfish in the present experiment are similar to those reported by other authors (Battle, 1940; Tsai et al., 2013) and are also similar to those described for other freshwater teleost species (Radael et al., 2014; Mattos et al., 2015).

As was the case in the present experiment, an acceleration of the time of occurrence of events related to temperature increases has been reported for other freshwater teleost species (Radael et al., 2016; Pereira et al., 2016). The delay in the occurrence of events during embryogenesis correlated with a temperature decrease may be due to the effect of temperature on cell division through the mitotic cycle. This fact is explicit in the initial events (e.g., cleavage) when cell division is most evident (Radael et al., 2016). The effect of temperature on cell division has been extensively studied (Begasse et al., 2015).

The low survival rate, anomalous embryos and the percentage of anomalies detected in embryos and larvae when submitted to a temperature of 34 °C indicate problems in cell division, cell membrane permeability, and tissue formation in this species under these conditions. Thus, this temperature has deleterious effects on the species in this developmental phase. On the subject, an interesting theory that should be considered is the concept of oxygen- and capacity-dependent thermal tolerance in aquatic ectotherms. According to Pörtner (2010), the rising temperatures causes the rate of aerobic metabolism to increase, as well as the demand for O_2, which will only be achieved through increases in the activities of the respiratory and cardiovascular systems.

Based on the data reported by Ford and Beitinger (2005), in the present experiment, the minimum and maximum thermal limits were not exceeded and, therefore, the species should present mechanisms to resist the proposed temperatures. But it is necessary to point out that the present experiment analyzed the effect of temperature on fish embryos, and that there is a lack of information about the oxygen- and capacity-dependent thermal tolerance in these stages of development.

As was the case in the present experiment, Lopes et al. (2018) reported that extreme climatic conditions (increase in temperature and concentration of CO_2) led to anomalies in the skeletal structure of tambaqui larvae (*Colossoma macropomum*). Also, Urushibata et al. (2019) described more than half (> 50.0%) abnormal embryos of *C. auratus* when incubation water temperature was higher than 26 °C. These values of embryos abnormalities were higher than those observed in the present study.

Urushibata et al. (2019) suggest that embryo abnormalities may be related to cytoplasmic structure, since it consists of microtubules and/or microfilaments (Beams et al., 1985). Microtubules are formed by the polymerization of the tubulin protein and have special importance in the constitution of the mitotic spindle, promoting the separation of sister chromatids. High temperatures can cause depolymerization of tubulin filaments, resulting in abnormalities (Yamaha et al., 2002).

One point to be discussed is the influence of the water temperature of the breeder tank at the time of spawning. It is essential to remember that fish have the ability to acclimatize to different temperatures (Wiegand et al., 1989; Pérez et al., 2003; Pype et al., 2015) and that the acclimatization process performed in the present experiment was gradual. According to the results reported by Ford and Beitinger (2005), the acclimatization to the temperatures proposed for each treatment in the present study was within the thermal variation tolerated by the species. Interestingly, Wiegand et al. (1989), working with goldfish embryos, demonstrated that exceeding the thermal limit tolerated by the species can lead to deleterious processes, while sudden variations in water temperature do not affect embryo survival. Based on the results of the present experiment, it is unlikely that the variation of water temperature in the breeder tank using the temperatures tested in the incubators significantly affected embryo development. This statement is supported by the fact that fish development occurred consistently at most of the temperatures tested, with problems in embryo development being detected only at 34 °C. It is possible that, at this stage of the life cycle of goldfish, the temperature of 34 °C exceeded the maximum thermal limit for the correct process of cell division and tissue formation.

Despite the issues mentioned above, the results of hatching and mainly the anomalous fish observed at the temperature of 34 °C demonstrate that there is a deleterious temperature for goldfish embryogenesis. For *Abramis brama*, another Cyprinid, the results obtained by Kucharczyk et al. (1997) demonstrated that the species is not as resistant as goldfish and that temperatures above 21.1 °C considerably reduce successful egg hatching. Pype et al. (2015), working with embryos of zebrafish (*D. rerio*), another Cyprinid, also reported that increases in water temperature above 32.5 °C cause embryo malformation and that a temperature of 36.5 °C causes a low hatching rate.

Like Cyprinidae, other fish families also have a maximum tolerance limit for increasing water temperature. Rana (1990), working with Nile tilapia (*Oreochromis niloticus*), a cichlid notoriously resistant to environmental fluctuations, demonstrated the existence of a maximum deleterious temperature at which embryo survival is less than 50%. Linares-Casenave et al. (2013), studying *Acipenser medirostris* larvae from the family Acipenseridae, observed that at some point the temperature increase reaches a maximum thermal limit for the species, compromising hatching.

On the other hand, in the present study, no minimum deleterious temperature was observed, whereas Rana (1990) reported that the survival of *O. niloticus* embryos was below 50% at the temperature of 20 °C. This was probably due to the fact that goldfish are originally from a subtropical climate and therefore are more adapted to lower temperatures than Nile tilapia, which is a natural species from a tropical climate. Wiegand et al. (1989) demonstrated that the incubation of goldfish eggs at a temperature of 13 °C affects embryo formation, causing fish anomalies. In the present experiment, this minimum thermal limit was not considered since this information already exists in the literature.

The amplitude between the maximum thermal limit observed in the present experiment and the minimum thermal limit reported by Wiegand et al. (1989) demonstrates the resilience of the species during embryogenesis. Ford and Beitinger (2005) had already reported the ability of goldfish to withstand variations in temperature during the juvenile phase. Such information is fundamental for the improvement of indoor goldfish production and for the understanding of the propagation of specimens in nature.

Thépot and Jerry (2015), working with *Lates calcarifer* embryos, reported that temperature influences the time of occurrence of embryonic developmental events and hatching rate, as also observed in the present experiment. According to these authors, 26 and 36 °C are temperatures that exceed the minimum and maximum thermal limit, respectively, tolerated by *L. calcarifer* during embryogenesis. Furthermore, the hatching rate was below 50% at the temperature of 34 °C. Thus, although *L. calcarifer* tolerates a wide temperature range (14 - 40 °C) during more advanced development phases (e.g., juvenile), the species seems to be less resilient during embryogenesis (Tucker Junior et al., 2002; Thépot and Jerry, 2015). Similar results were reported by Kucharczyk et al. (1997) who suggested incubation of *Abramis brama* eggs at temperatures close to 21.1 °C, with increased egg mortality at higher temperatures. However, according to these authors, larvae of this species should be grown at temperatures of 27.9 °C, showing that *A. brama* larvae are more resistant to high temperatures than embryos.

As was the case in the present study, Pype et al. (2015), working with zebrafish (*D. rerio*), also observed a decrease in hatching time with increasing temperature until the maximum tolerated limit was reached. Pereira et al. (2016) and Radael et al. (2016) also reported a decrease in hatching time with increasing temperature in *Trichogaster leeri* and *Melanotaenia boesemani*, respectively; however, in contrast to the present experiment, they did not determine the maximum limit tolerated by these species.

The data on goldfish egg size obtained in the present study agree with those reported in the literature for the species (Battle, 1940). Kikko et al. (2014) stated that there is a correlation between water temperature variation and the egg size of *Gnathopogon caerulescens*. This is probably a temperature effect on the maturation of fish gonads, although it may not be the only variable that influences this process. In the present experiment, the temperature of the water in the breeder tank and of the water used to hydrate the eggs was the same, and only one female was used, but little can be discussed about the effect of temperature on egg size. Within this context, the data of the present experiment only permit to state that, after fertilization, the temperature does not affect egg size. Pereira et al. (2016) also did not observe any difference in the diameter of *T. leeri* eggs incubated at different temperatures.

The significant effect of temperature on the length of larvae submitted to the 34 °C treatment led to anomalies that caused curvatures in the larvae, decreasing their length. Corroborating this hypothesis, no difference was observed for larva weight. Although the length was different, body weight was the same for the larvae of all treatments. The length values of newly hatched larvae submitted to temperatures of 18 to 30 °C obtained in the present experiment were similar to those reported by Battle (1940) for goldfish.

As observed for goldfish in the present study, Mattos et al. (2015) reported that newly hatched *Symphysodon aequifasciatus* larvae have closed mouths. This evolutionary strategy demonstrates that the larvae of these species, when hatching, do not have exogenous feeding capacity and need to remain longer in an endogenous feeding period, known as the free embryo period. A different strategy is used by *T. leeri* and *M. boesemani* larvae, which have an open mouth soon after hatching and are thus able to start exogenous feeding, as reported by Pereira et al. (2016) and Radael et al. (2016).

In the present experiment, the influence of temperature on the onset of exogenous feeding was the same as observed for all events during embryogenesis. Pereira et al. (2016) reported a similar result for *T. leeri*. It is interesting to note that goldfish larvae incubated at 34 °C did not feed, probably owing to malformation problems induced by the deleterious effect of the high temperature to which they were exposed. On the basis of the present data, it is difficult to specify if these anomalous larvae did not have the capacity to ingest and digest food or if they could not move and reach the food.

Goldfish is a species resistant to different incubation temperatures. Thus, embryonic development of this species can occur at temperatures ranging from 18 to 30 °C without a significant loss in hatching rate. To minimize embryo mortality and the possibility of anomalous larvae, a temperature of 34 °C or higher should be avoided. For indoor production of goldfish, larvae should receive exogenous feeding between 111 and 216 hours after fertilization, depending on the water temperature of the incubators.

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