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Temperature-induced changes in reproductive variables in the teleost fish *Lophiosilurus alexandri*

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ABSTRACT

The present study evaluates whether increased water temperature induces reproduction by *Lophiosilurus alexandri* under controlled conditions, and investigates the effects of this procedure on sexual steroids, hematological profile and behavior. A 44-week experiment was performed with four wild males and 12 wild females that had been acclimatized to captive conditions. Water temperature was maintained at 24.4 ± 1.0 °C for weeks 1–22, and then at 29.0 ± 1.1 °C for weeks 22–44. Spawn weight, number of eggs/spawn and hatching rate were satisfactory and ranged 27.5–127.5 g, 1209–5183 and 83–89%, respectively. Hematocrit, leukocytes and glucose were not influenced by increased water temperature, while higher values for erythrocytes were observed for both sexes. The lowest value for plasma protein was for females maintained at 29.0 °C, while the lowest value for testosterone was obtained at the end of the study period at a temperature in 29.0 °C. Serum values of 17β-estradiol were higher in females than in males, however, there was no evidence of variation as a function of experimental temperature or interaction with sex. The reproductive behavior of *L. alexandri* in captivity is described for the first time. The present study demonstrates that adult individuals are able to maintain a stable hematological profile during an increase in mean water temperature from 24.4 °C to 29.0 °C, even during the reproductive period, and still produce good quality larvae. Nonetheless, whether spawning was associated with increased 17β-estradiol levels could not be determined.

1. Introduction

Among the environmental factors that regulate the reproductive cycle of teleost fish, temperature is most important for controlling physiological and hormonal changes during reproduction (Hermelink et al., 2013; Levy et al., 2011; Nowosad et al., 2014; Unuma et al., 2012). Reproductive dysfunctions occur at different levels of response under adverse temperatures, and include deleterious effects on vitellogenesis, ovulation, egg production and fertility (Pankhurst et al., 1996; King and Pankhurst, 1999; Davies and Bromage, 2002). This is due the fact that fish development is restricted to a particular temperature range (King et al., 2003), as observed in a study with *Prochilodus argenteus*, in which water at 23 °C reduced the number of ovulated females by 33%, compared to water at 26 °C, with 26% of female vitellogenic oocytes not completing or even beginning maturation (Arantes et al., 2011).

Low levels of 17β-estradiol and testosterone are known to be related to fewer females reaching the final stage of egg maturation and low larval and sperm viability after experiencing water temperatures outside the range of thermal comfort for various species (Arantes et al., 2010; Hermelink et al., 2011, 2013; Mazzeo et al., 2014; Melo et al., 2016; Nowosad et al., 2014). The hematological and plasma biochemistry of broodstock can also be influenced water temperature (Kavadias et al., 2004; Tavares-Dias et al., 2004; Valenzuela et al., 2008; Matsche and Gibbons, 2012). Increased glycemia during the reproductive period was found associated with increased energy demand at the beginning of

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reproductive activity induced by elevated water temperature for *Dicentrarchus labrax* (Kavadias et al., 2004) and *Acipenser brevirostrum* (Matsche and Gibbons, 2012). Plasma protein has also been found to change under the influence of temperature, such as the reduction found for *Cyprinus carpio* in a controlled environment (Tavares-Dias et al., 2004). Likewise, leukocytes of *Oncorhynchus mykiss* (Valenzuela et al., 2008) and erythrocytes of *D. labrax* (Kavadias et al., 2004), were found negatively affected by conditions outside the thermal breeding range of broodstock. Knowledge of the effects of altered water temperature in captive conditions has been essential for improving breeding protocols for various species of teleost fish, and has enabled spawning throughout the year (Hermelink et al., 2013; Kucharczyk et al., 2008; Mazzeo et al., 2014; Nowosad et al., 2014; Utoh et al., 2013). Therefore, to maximize reproductive potential, it is of paramount importance to understand the reproductive responses of understudied species, such as *Lophiosilurus alexandri,* to increased water temperatures under controlled conditions.

L. alexandri is a carnivorous siluriforme endemic to the Rio São Francisco Basin. The species has high market potential due lacking intramuscular spines and having a taste with great culinary appeal (Sant´Ana et al., 2017). Not only does the species have potential for pisciculture in Brazil (Campeche et al., 2011), it has also attracted the interest of aquarists due to its exotic appearance (Santos and Luz, 2009). *Lophiosilurus alexandri* is on the list of animals vulnerable to extinction (ICMBio, 2016), and so studies on its reproductive biology would make valuable contributions to the conservation of its natural stocks. Spawning of *L. alexandri* is batch (Barros et al., 2007; Melo et al., 2011), and the nest is constructed on sandy substrate, with the male providing parental care (Costa et al., 2015; Sato et al., 2003). In nature, the reproductive period occurs between November and February, which is the rainiest and hottest period for the Rio São Francisco Basin, and when the mean water temperature reaches 28 °C (Sato et al., 2003). Wild *L. alexandri*, captured and acclimatized to conditions of cultivation, can achieve reproduction 30-days after an increase in water temperature from 26 °C to 28 °C (Costa et al., 2015). Despite its importance, information on sexual steroids, hematological profile, and reproductive behavior of *L. alexandri* is still scarce in the literature. Thus, the present study evaluated whether increased water temperature induces reproduction in *L. alexandri* under controlled conditions, and investigated the effects of this procedure on sexual steroids, hematological profile and behavior.

2. Material and methods

The experiment was conducted at the Aquaculture Laboratory (Laqua) of the Veterinary School of the Federal University of Minas Gerais (UFMG). All procedures were approved by the Ethics Committee of UFMG (Protocol: 25/2010).

Four adult male and 12 adult female *L. alexandri* were captured in a natural environment and acclimatized to culture conditions for study (Costa et al., 2015). Individual sex was determined by cannulation of the genital papilla (Melillo Filho et al., 2016), which was followed by the implantation of a microchip. The weight of broodstock was measured using a scale with 0.5-g precision. The mean weight of males was 3.84 ± 1.2 kg and of females 3.50 ± 1.1 kg. The broodstock was kept in four 5-m^3 circular tanks with 2–2.5 m^3 of useful volume (one male and three females per tank), with a sand substrate, controlled temperature and a diffuser to maintain dissolved oxygen above 5 mg/L. Feeding was performed once a week with frozen eviscerated tilapia without the head, which was provided at 2% of biomass.

The experimental period lasted 44 weeks. During weeks 1–22, corresponding to the period of January to May, the water temperature was maintained at 24.4 ± 1.0 °C; from week 22 to 44, corresponding to the period between June and November, the water temperature was maintained at 29.0 ± 1.1 °C. The water was heated gradually over the course of one week using an 800-w electrical resistor.

2.1. Reproduction and egg and larval viability

After the increase in water temperature, the breeding tanks were assessed daily for the occurrence of spawning. When spawning was confirmed by the presence of a nest and parental care (Costa et al., 2015), the fish that was positioned on the eggs was identified using a microchip reader. The eggs were collected using a dip net and transferred to an incubation system. This system consisted of a box with a useful volume of 150 L, a 100-w heater with a thermostat, aeration and an internal biological filter with a volume of 5 L coupled to a submerged water pump with an average flow rate of 160 L/h. The eggs were incubated in a 25-cm diameter sieve (0.5 mm mesh) fixed to floats. The larvae remained in this system until seven days post-hatching.

During weeks 1–22, the temperature (mercury bulb thermometer), pH (Hanna HI98129 digital phagometer) and dissolved oxygen (Hanna HI9146 oximeter) averaged 24.4 ± 1.0 °C, 7.0 ± 0.72, 7.3 ± 0.4 mg/L, respectively, while during weeks 22–44, they averaged 29.0 ± 1.1 °C, 7.8 ± 0.6 and 6.6 ± 0.5. Temperature was measured daily for all tanks, while pH and dissolved oxygen were measured weekly. Temperature, pH, dissolved oxygen and total ammonia (NH_3 + NH_4) of the larval incubation and maintenance system were measured daily. Total ammonia was measured using an Alcon® commercial kit. The mean values for the water quality parameters in the incubation system were: temperature 27.8 ± 0.3 °C, pH 7.5 ± 0.9, dissolved oxygen 7.2 ± 0.3 mg/L and total ammonia 0.28 ± 0.1 mg/L.

The following measurements were made after spawning: spawn weight (SW), egg weight (EW), number of eggs (NE), hatching rate (HR), weight of newly hatched larvae (LW 0 dph), larval weight eight days post hatching (PL 8 dph) and larval survival eight days post hatching. The weight of eggs and larvae were estimated using n = 20. Due to the small size and fragility of eggs and larvae, EW and LW 0 dph were weighed for groups of five individuals and the mean calculated. As a function of the adhesiveness of the eggs, and the lack of a method to accurately estimate their number, the following formulas were adopted for calculating estimates:

NE = total number of larvae + number of eggs attacked by fungi

HR = (total number of larvae/total number of eggs)x100

2.2. Blood collection and hematological analyses

Blood was collected from broodstock at week 22 (May) and week 44 (November). The fish were restrained using a damp cloth while collection was performed by venipuncture in the caudal vertebral artery with ventral access. About 2 mL of blood was collected, from which 0.5 mL was stored in Eppendorf tubes, filled with sodium heparin (0.1–0.2% mg/mL of blood) as anticoagulant, intended for hematocrit, plasma protein, leukocyte, erythrocyte and glucose analyses. Each fish was considered a replicate.

Hematocrit values were determined from capillary tubes filled with approximately 2/3 of previously homogenized and centrifuged (10 min at 10,000 rpm) blood. The reading was performed on the appropriate card, matching the plasma meniscus with the top line of the ruler (line 100) and equating the lower end of the erythrocyte portion with the bottom line of the ruler (line 0), so that the result indicated the value of the line based on the microhematocrit technique validated by Goldenfarb et al. (1971). A Goldenberg optical refractometer was used to measure plasma protein. For erythrocyte and leukocyte counts, blood (10 μL) was diluted in Dacie solution (1000 μL) and the cells counted manually in a Neubauer chamber within 12-h after blood collection. Plasmatic glucose was determined using a commercial COBAS.

An aliquot of about 1500 μL of blood was collected without anticoagulant, centrifuged for 5 min (1000 rpm for 1 min and 3000 rpm for 4 min) for serum separation and then frozen at − 80 °C for further analysis of steroids. Analyses of 17β-estradiol and testosterone were

performed by chemiluminescence using a DXI 800 Immunoassay System manufactured by Beckman Coulter.

2.3. Exposure tests with larvae

Two exposure tests were performed for each spawn following the methodology proposed by Luz et al. (2012); the first test with the newly hatched larvae and the second test eight days post-hatching. Ten larvae per experimental unit were used with five replicates for each spawn for a total of 50 larvae. The larvae were collected from the incubator, transferred to a sieve (mesh diameter of 0.5 mm) and exposed to air. Blotting paper was used to remove excess moisture. After 30 min of air exposure, the larvae were transferred to a beaker with a useful volume of 500 mL and constant aeration, and maintained in a thermostat-controlled bath at 28 °C for another 24 h. Survival was then determined by direct counts of the animals, and the rate of stress resistance determined.

2.4. Reproductive behavior

The frequency and duration of reproductive behaviors were recorded and evaluated during the last experimental week. Filming took place with lighted conditions for 24-h using an internal circuit system (cameras and DVR). The filmed behaviors were coded through Solomon Coder Beta® software using the methods of focal sampling and continuous recording. During video analysis, males and females were identified by external characteristics such as color and body size. Only one of the combinations (tanks) experienced spawning during the study period. The duration of the reproductive behaviors of this male and female during the day of spawn were compared with the average durations of the behaviors of males and females in the other three tanks where no spawning occurred.

2.5. Statistical analysis

A split-plot (2 × 2) design was used. The main plot comprised sex (male and female) and subplot by mean water temperature (24.4 and 29.0 °C). Reproductive parameters were compared between tanks and for the unfolding of the interactions, using the Tukey test ($P < 0.05$). Stress resistance of larvae was compared among different ages using Student's *t*-test ($P < 0.05$). All data were tested for normality (Kolmogorov-Smirnov) and homogeneity of variances. Because data for duration and frequency of reproductive behavior were not normally distributed, comparisons were made using Kruskal-Wallis and Dunn tests ($P < 0.05$). Statistical analyses were performed using the software Assistat 7.7 (2017).

3. Results

3.1. Reproduction and egg and larval viability

The first spawn occurred in July in tank 1, 26 days after raising the mean water temperature to 29.0 °C (Table 1). The first spawns in tanks 3 and 4 occurred 32 and 34 days after the temperature increase, respectively. The mean intervals between spawns were 38, 29 and 16 days for tanks 1, 3, and 4, respectively. The shortest spawning interval was 12 days for tank 4 while the greatest was 43 days for tank 1. The month with the highest occurrence of spawning was August. Because reproduction occurred at night, and there was more than one female in each tank, it was not possible to determine if the recorded spawns were from the same female. Tank 2 experienced no reproduction during the entire experiment. Males on nests were observed to voluntary fast on the day that spawning occurred.

Mean values of spawn weight, egg weight, larval weight 0 dph, larval weight 8 dph, number of eggs/spawn, hatching rate and survival 8 dph were similar ($P > 0.01$) among tanks (Table 2). Spawn weight ranged from 27.5 to 127.5 g. The minimum and maximum number of eggs was 1209 and 5183, respectively. Hatching rate varied between 83% and 89%. Larval survival was equal to or greater than 95%. Larval stress resistance was higher ($P < 0.05$) for 8 dph than for newly hatched larvae; however, stress resistance was similar ($P > 0.05$) among spawns of the different tanks.

Table 1
Frequency of *L. alexandri* spawns in four tanks during the period with a mean water temperature of 29.0 °C.

Month	Tank			
	1	2	3	4
June	–	–	–	–
July	1	–	1	1
August	3	–	1	3
September	1	–	–	–
October	2	–	–	3
November	–	–	–	1
Total	7	0	2	8

3.2. Hematological parameters and plasma glucose levels

Hematocrit, leukocytes and glucose (Table 3) did not vary according to sex, water temperature, or the interaction between these two factors ($P > 0.05$). The highest value for erythrocytes was obtained at the end of the period with the mean water temperature of 29.0 °C ($P < 0.01$), but there was no effect of sex or interaction ($P > 0.05$) between sex and temperature. Plasma protein was affected by the interaction between temperature and sex ($P < 0.01$). Lower plasma protein was recorded for females maintained at 29.0 °C than for females maintained at 24.4 °C (Table 4). There was no difference in plasma protein between males and females, or between males in the two temperature ranges.

3.3. Reproductive steroids

Increased water temperature influenced serum testosterone levels ($P < 0.01$), but without an effect of sex or interaction (Table 5). The lowest value for testosterone was obtained after the period of 22 weeks with the mean water temperature at 29.0 °C. Serum values for 17β-estradiol were higher in females than in males ($P < 0.05$), however, there was no evidence ($P > 0.05$) of variation as a function of water temperature or the interaction between factors.

3.4. Reproductive behavior

The recorded behaviors were grouped into four behavioral categories: inactivity, locomotion, courtship behavior and reproductive behavior. The description of these behavioral categories and what they entail are provided in Table 6. In the tanks where there was no spawning (during filming), the fish spent most of their time sitting on the bottom of the tank or buried in the sand (Fig. 1); two individuals remained buried in the sand for up to seven hours. This burying behavior was not observed for the spawning pair on the day of spawn. The behaviors “pursuit without bite”, “pursuit with bite”, “lateral side position”, “posture and fertilization of eggs” were observed only for the spawning male and female on the day of spawning. “Parental care” was performed only by the male of the spawning pair. “Swim to the bottom” and “overlay” behaviors were exhibited by animals of both sexes, including the non-spawning animals, but with a longer duration for females than males ($P < 0.05$).

The manifestation of reproductive behavior began with an increase in active swimming at the bottom of the tank by both the male and female, at around 16:00 h. The female and male then started exhibiting the behaviors of “lateral side position” and “posture and fertilization of

Table 2
Means (± SD) for spawn weight, egg weight, larval weight 0 days post hatching (dph), larval weight 8 dph, number of eggs/spawn, hatching rate, survival 8 dph, and stress resistance (Re) for newly hatched larvae and larvae 8 days dph of *L. alexandri*.

Parameters	Tank			
	1	2	3	4
Spawn weight (g)^{ns}	81.3 ± 37.8a	–	90.9 ± 21.5a	81.1 ± 18.4a
Egg weight (mg)^{ns}	18.2 ± 1 .7a	–	20.2 ± 3.3a	17.6 ± 6.1a
Larval weight 0 dph (mg)^{ns}	8.5 ± 0.3a	–	8.0 ± 0.7a	8.4 ± 0.14a
Larval weight 8 dph (mg) ^{ns}	20.6 ± 2.0a	–	20.4 ± 3.2a	19.8 ± 0.3a
No. eggs/spawn^{ns}	3021.2 ± 1580.9a	–	3399.0 ± 587.9a	2492.2 ± 196a
Hatching rate (%)^{ns}	83.1 ± 12.4a	–	89.4 ± 17.5a	87.1 ± 2.7a
Survival 8 dph (%)^{ns}	98.51.6a	–	95.3 ± 1.4a	96.4 ± 1.4a
Re 0 dph (%)	42.0 ± 19.8Ba	–	50.0 ± 32.0Ba	41.0 ± 36.1Ba
Re 8 dph (%)	67.0 ± 31.5Aa	–	83.0 ± 10.6Aa	57.5 ± 29.7Aa

Means in the same row followed by different lowercase differ significantly according to the Tukey test (*P < 0.05). ns = not significant.
Means in the same column followed by different capital letters differ significantly according to the Student's *t*-test (P < 0.05).

Table 3
F-values and means (± SD) for hematocrit, plasma protein, leukocytes, erythrocytes and glucose.

Statistics	F-value				
	Hematocrit (%)	Plasma protein g/dL	Leukocytes x10^5/μL	Erythrocytes x10^5/μL	Glucose mg/dL
Sex	0.10 ^{ns}	0.00 ^{ns}	0.50 ^{ns}	0.14 ^{ns}	0.25 ^{ns}
Temp.	0.51 ^{ns}	2.75 ^{ns}	0.92 ^{ns}	17.61**	3.33 ^{ns}
Sex X Temp.	0.81 ^{ns}	18.75**	1.89 ^{ns}	0.41 ^{ns}	3.14 ^{ns}
	Means of the Sexes				
Females	20.73 ± 3.93a	6.51 ± 0.47a	5.54 ± 2.67a	5.71 ± 2.12a	4.53 ± 3.29a
Males	19.88 ± 4.12a	6.48 ± 0.89a	7.41 ± 4.70a	5.46 ± 1.79a	3.47 ± 4.40a
	Means of the Temperature Period				
24.4 °C	20.79 ± 3.97a	6.55 ± 0.73a	7.82 ± 7.06a	4.17 ± 0.84b	2.67 ± 3.30a
29.0 °C	19.81 ± 4.12a	6.44 ± 0.69a	5.12 ± 3.30a	7.00 ± 1.57a	5.33 ± 3.97a

Means in the same column followed by different letters differ significantly (**P < 0.01). ns = not significant.

Table 4
Deviation of the interactions (± SD) for plasma protein (g/dL).

Sex	Temperature Period	
	24.4 °C	29.0 °C
Females	6.73 ± 0.60 aA	6.29 ± 0.18aB
Males	6.37 ± 0.46 aA	6.58 ± 0.28 aA

Means in the same column followed by different lowercase letters, and means in the same row followed by different upper case letters, differ significantly.

Table 5
F-values and means (± SD) for testosterone and 17β-estradiol.

Statistic	F-value	
	Testosterone (ng/dL)	17β-estradiol (pg/mL)
Sex	0.20 ^{ns}	6.36*
Temp.	38.55**	1.95 ^{ns}
Sex X Temp.	2.38 ^{ns}	0.96 ^{ns}
	Mean of the Sexes	
Females	269.46 ± 121.91a	685.87 ± 236.78a
Males	291.50 ± 146.21a	299.62 ± 386.52b
	Means of the Temperature Period	
24.4 °C	381.17 ± 83.39a	577.58 ± 373.20a
29.0 °C	179.79 ± 79.92b	407.92 ± 367.39a

Means in the columns followed by different letters differ significantly.
ns = not significant.
* P < 0.05.
** P < 0.01).

eggs" (Fig. 2A), the duration of which increased gradually until the end of spawning, which occurred ten hours later. "Overlay" behavior was exhibited by both the male and female during the nine hours prior to spawning (from 16:30 h to 01:30 h). "Overlay" was initiated by, and had a longer duration for, the female (P < 0.05). The peak duration of "overlay" for the female occurred during the last three hours prior to spawning. Increase in the duration of "overlay" was associated with the peak of spawning; it subsequently decreased at the end of egg deposition. No "overlay" between individuals was observed after spawning.

Oocytes were deposited and fertilized on the bottom of the tank in a depression in the sand. The choice of spawning site occurred at 17:00 h, as indicated by the male and female staying in a specific area of the tank. The formation of the nest was initiated after 23:00 h by circular movements of the male and female on the sand. After spawning, the female moved away from the male and remained sitting on the sand more than three body-lengths away from him. The male remained on the eggs for the next eleven hours, constantly moving its pectoral fins and rotating its body on its axis (Fig. 2B).

The greatest frequency of bites was performed by the male prior to spawning (P < 0.05) (Fig. 3), and were directed at the spawning female. Non-spawning individuals and the spawning female did not exhibit post-spawn biting behavior. All bites performed by the male after spawning occurred on individuals other than the spawned female.

4. Discussion

The present study found that increasing the water temperature from 24.4 °C to 29.0 °C stimulated reproduction of *L. alexandri* under controlled conditions with a satisfactory response of larval viability. Fish started exhibiting reproductive behaviors late in the afternoon, with spawning occurring at night. There was no evidence of variation in serum values of 17β-estradiol as a function of increased water temperature, but there was a decrease in testosterone. Furthermore, adult *L. alexandri* were able to maintain stable hematological levels and

Table 6
Description of the behaviors observed for *L. alexandri*.

Behavior	Description
Inactivity	
Sitting on the sand	The fish in a stationary position with the ventral region resting on the sand, while moving the operculum or mouth.
Buried in the sand	The fish is partially or totally buried.
Locomotion	
Burying movement	The fish moves back and forth in a small area removing sand by moving the ventral region and caudal fin, ultimately covering its body with sand by moving the pectoral fins.
Short locomotion	The fish moves from one point to another for a period of less than three seconds, moving its body by a distance of less than one-half a body-length.
Swim to the bottom	The fish moves from one point to another for more than three seconds, through movements of the caudal fin; the ventral region remains within ¼ of a body-length from the bottom of the tank. The fish may pass over another fish that is sitting on the sand.
Swim at the side of the tank	The fish moves along the side of the tank, at up to ¼ of a body-length away from it, through movements of the caudal fin, which can reach the region of the water-air interface with the body oriented horizontally.
Swim vertically	The fish moves upward along the side of the tank at up to one-half a body-length away from it, through movements of the caudal fin, reaching the region of the water-air interface and moving to the right or left, but with the body oriented vertically.
Courtship behavior	
Pursuit without bite	The fish moves toward another fish, which is also in motion, chasing it by moving the caudal fin for more than four seconds. There is no physical contact.
Pursuit with bite	The fish moves toward another fish, which is also in motion, chasing it by moving the caudal fin for more than four seconds, and biting any region of the body of the other fish.
Bite	The fish bites another fish without moving.
Confrontation	The fish remains positioned in front of another fish, half of a body-length away, biting and moving the caudal fin.
Flight	The fish moves away in response to a chase by another fish by moving its caudal fin.
Overlay	The fish positions itself over another fish and remains there for more than two seconds.
Reproductive behavior	
Lateral side position	The fish remains sitting in lateral physical contact with another fish while facing the opposite position (heads pointed in opposite directions).
Posture and fertilization of eggs	Male and female remain on the nest, with head and fins directed in opposite directions while moving in a circular manner.
Parental care	The fish remains alone on the eggs, constantly moving the pectoral fins and moving in a circle turning its body on its axis.

glucose responses in this temperature range even during the reproductive period.

In this experiment, the *L. alexandri* broodstock reproduced in a period that did not coincide with the natural breeding period of this species. Reproduction for the species in nature occurs during the hottest and rainiest months of the year (November to February, mean = 28 °C) (Sato et al., 2003). The advance of the reproductive period of *L. alexandri* by four months in controlled conditions was made possible by increasing the water temperature; the first spawn occurred 26 days after the increase. These data confirm the hypothesis that temperature is one of the main factors controlling the reproductive cycle of *L. alexandri*. Costa et al. (2015) found natural reproduction of *L. alexandri* maintained for 20 weeks at 26 °C, to be possible 30 days after increasing the water temperature to 28 °C. The most satisfactory results obtained for female *Sander lucioperca* at final maturation was when they were habituated at 12 °C for 12 weeks, followed by an increase and maintenance of the temperature at 14 °C for eight weeks (Hermelink et al., 2013). Maintaining a water temperature of 6 °C was found to be necessary for stimulating initial gonadal maintenance in *Conger myriaster*, with an increase in oocyte diameter from 300 µm to 600 µm; but oocytes with a diameter of 995 µm were obtained only 41 days after the water temperature was raised to 10 °C (Utoh et al., 2013). Final maturation was obtained for *A. anguilla* that were kept for six weeks at 10 °C, followed by an increase to 15 °C (Mazzeo et al., 2014). These studies indicate that the ideal temperature for breeding is species-specific.

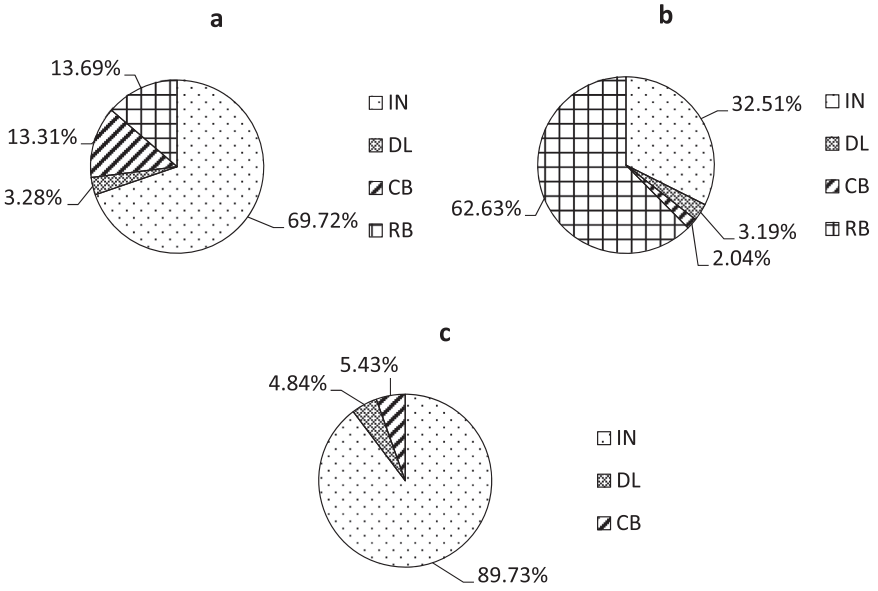

Fig. 1. Proportion of behaviors — Inactivity (IN), Locomotion (DL), Courting Behavior (CB) and Reproductive Behavior (RB) — exhibited by the breeding female (a) and male (b) on the night of spawning, and by the non-spawning individuals (c) of *Lophiosilurus alexandri* during the 24 h of observation.

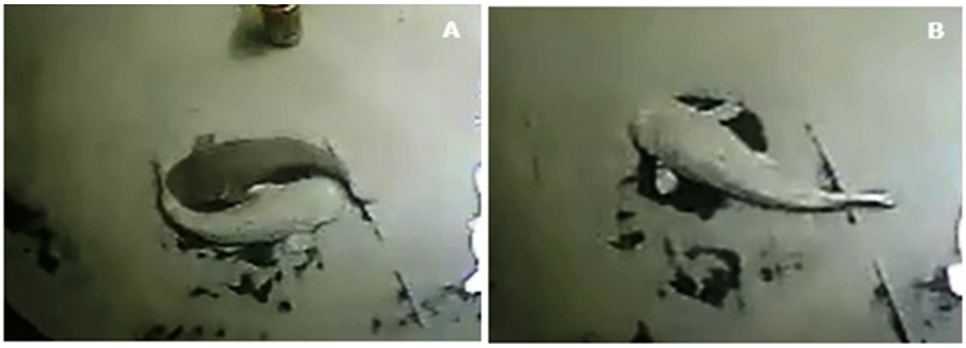

Fig. 2. (A) Male (bottom) and female (top) on the nest prior to spawning. Note their lateral opposite position. (B) Male on eggs in the nest during parental care.

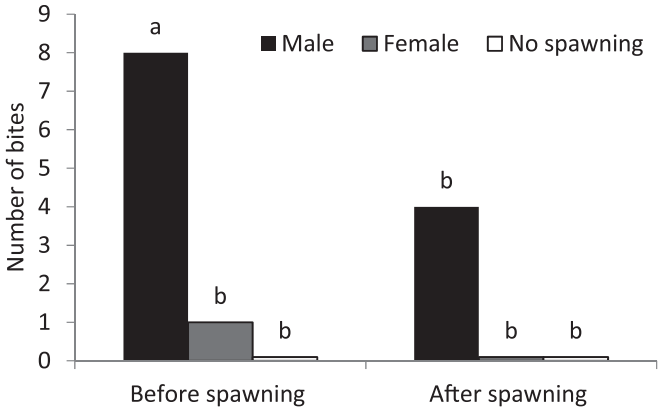

Fig. 3. Frequency of biting behavior pre- and post-spawning for the female and male spawning fish and the non-spawning fish of *Lophiosilurus alexandri* during the 24 h of observation. Bars with different letters represent significant differences ($P < 0.05$).

The lowest value for testosterone in the present study was obtained after the period of 22 weeks with the mean water temperature at 29.0 °C. This was probably related to it being at the end of the reproductive period, since the largest numbers of spawns were recorded in the months of August and October. Studies on the reproduction of other fish species found the highest values for this steroid during the maturation period, with reductions during the period of ovulation, and a post-ovulatory return to basal levels (Ammar et al., 2015; Arantes et al., 2011, 2010; Honji et al., 2013; Mazzeo et al., 2014; Mori et al., 2003; Pankhurst et al., 1996; Utoh et al., 2013). For example, testosterone levels of female *P. argenteus* varied markedly throughout the reproductive cycle, with the highest values (300 ng/dL) being recorded in the maturation period, when the temperature was 25 °C (Arantes et al., 2010). A modulating effect of temperature on steroid production during gonadal maturation was also reported for *A. anguilla,* where a constant temperature of 18 °C inhibited testosterone synthesis, whereas the highest value (800 ng/dL) was recorded for fish kept for six weeks at 10 °C followed by an increase to 15 °C (Mazzeo et al., 2014).

In females, testosterone acts primarily as a precursor of 17β-estradiol (Hermelink et al., 2011; Mazzeo et al., 2014), which is produced in follicular tissue under the control of hypophyseal gonadotropins, and stimulates the production of vitellogenin by the liver and, consequently, the deposition of vitellin granules in the oocyte (Ammar et al., 2015; Kazeto et al., 2011; Mori et al., 2003). Temperature influences the concentration of 17β-estradiol in broodstock of tropical species, such as *P. argenteus* (Arantes et al., 2011, 2010), as well as temperate species, such as *S. lucioperca* (Hermelink et al., 2011, 2013), *C. myriaster* (Utoh et al., 2013) and *A. anguilla* (Mazzeo et al., 2014). Although temperature determined the reproductive period of *L. alexandri* in the present study, there was no evidence that spawning was associated with increased 17β-estradiol levels since these levels were the same even after the temperature was increased. This seemingly divergent result is probably due to the asynchronous gonadal maturation of *L. alexandri* (Barros et al., 2007; Melo et al., 2011), which is in contrast to the synchronous development of the previously mentioned fish species.

The results of the present study indicate that within the temperature range of 24.4–29 °C, adult individuals of *L. alexandri* are able to maintain stable levels of hematocrit and glucose even during the reproductive period. Likewise, juveniles of the same species were found to exhibit no difference in these parameters within the temperature range of 23–29 °C (Costa et al., 2016). In contrast, higher erythrocyte values were observed for *L. alexandri* broodstock after the water temperature was increased. A reproductive function is the likely reason for this difference, since changes in hematological profile as a function of gonadal maturation have been previously reported (Fiszbein et al., 2010; Kavadias et al., 2004; Matsche and Gibbons, 2012; Pradhan et al., 2012; Sarameh et al., 2013; Valenzuela et al., 2006, 2008). On the other hand, the number of leukocytes was not affected by the reproductive period in *L. alexandri*. As with other vertebrate groups, leukocytes are responsible for the nonspecific immune response in teleosts (Cerezuela et al., 2009; Clerton et al., 2001; Menezes et al., 2006; Shahkar et al., 2015). The lower value for plasma protein recorded for females maintained at 29.0 °C, compared to females maintained at 24.4 °C, suggests a possible deposition of essential proteins necessary for the formation of gametes in the period preceding reproduction, as reported by Tavares-Dias et al. (2004). In addition, plasma protein is an important indicator of the osmotic balance between extracellular and intracellular compartments (Machado and Duncan, 2017), and can be influenced by, among other factors, water temperature (Costa et al., 2016), mainly during the different reproductive phases throughout the year (Matsche and Gibbons, 2012). Males of *L. alexandri* were less sensitive, exhibiting less variation in this parameter as a function of temperature.

The reproductive success of the fish in tanks with spawning was satisfactory. For example, spawn weight, number of eggs/spawn and hatching rate ranged 27.5–127.5 g; 1209–5183; and 83–89%, respectively, which are close to the values of 49–191 g; 240–4600; and 95% recorded for this same species for natural breeding in captivity (Costa et al., 2015). These results show that increased temperature not only enables reproduction, but also the viable production of eggs and larvae of this species kept in the laboratory. This information is highly relevant to reproduction since variation in water temperature outside the comfort range can cause thermal stress during gonadal maturation, which can result in disturbances to the reproductive cycle, poor quality eggs (Nowosad et al., 2014; Targońska et al., 2011, 2014; Unuma et al., 2012) and even follicular atresia (Pankhurst et al., 1996; Santos et al., 2008; Thomé et al., 2012). In addition, high mortality rates of embryos have also been reported at such temperatures (Kucharczyk et al., 2008; Sanches et al., 2011).

L. alexandri started exhibiting reproductive behaviors late in the afternoon, with spawning occurring at night. These results are in agreement with previously observations by Costa et al. (2016) and Sato et al. (2003). This nocturnal reproductive behavior — which makes

direct observation difficult — is probably why it has remained undescribed until now (Costa et al., 2016). The observations of the present study were made possible by keeping lights on at all times while recording the event, which did not affect breeding. The great amount of swimming, biting and overlaying observed for the breeding pair in the hours prior to spawning suggests that these behaviors are part of the species' courtship repertoire. The high frequency of male bites recorded suggests that this behavior may act as further stimulus for the female to spawn, and may also be important for female selection of a male (Alonso et al., 2012; Araújo et al., 2014; Chellappa et al., 2014; Keller-Costa et al., 2014). Furthermore, the observed post-spawning biting of non-spawned females by the male may be related to parental care, as documented for *Pterophyllum scalare* (Cacho et al., 2007) and *Crenicichla menezesi* (Araújo et al., 2014). Parental care can increase offspring survival, especially for fish with low fecundity due to split spawning (Araújo et al., 2014; Cacho et al., 2007; Mattos et al., 2016; Selz et al., 2014). This first description of *L. alexandri* reproductive behavior, however, should be considered preliminary because it was based on one reproductive event. Nonetheless, it is relevant for captive reproduction since the behaviors described may serve as indicators of the moment of spawning, and as a guide to the behavioral needs for *L. alexandri* reproduction.

5. Conclusion

An increase of mean water temperature from 24.4 °C to 29.0 °C made it possible to advance the reproductive period of *L. alexandri* four months ahead of its natural period, with a satisfactory response for larval viability. We found no evidence that spawning was associated with increased 17β-estradiol levels. In addition, hematological and glucose responses indicate that, for this temperature range, adult *L. alexandri* are able to maintain stable levels even during the reproductive period. A great amount of reproductive behavior for *L. alexandri* was documented, some for first time, including courtship and parental care.

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