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## EFFECT OF TEMPERATURE ON HATCHING AND GROWTH OF CUBAN GAR (*Atractosteus tristoechus*) LARVAE

### Efecto de la temperatura en la eclosion y el crecimiento de las larvas de manjuarí (*Atractosteus tristoechus*)

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**ABSTRACT.** Hatching success, growth, development and survival of Cuban gar (*Atractosteus tristoechus*) larvae were examined at different temperatures (26, 28 and 30 °C) up to 18 days after hatching (DAH). The time to hatching was inversely related to the incubation temperature (87, 100 and 111 h). Larval survival at the time of hatching was significantly lower at 30 °C (50.3 %), coinciding with the highest larval mortality (30.2 %). Growth rates were 1.75 mm d<sup>-1</sup> - 10.4 % d<sup>-1</sup> at 26 °C, 1.30 mm d<sup>-1</sup> - 10.2 % d<sup>-1</sup> at 28 °C and 1.40 mm d<sup>-1</sup> - 10 % d<sup>-1</sup> at 30 °C. Three similar critical periods were identified: 0-6, 7-11 and 12-18 DAH. During the first period, a similar increase in weight and a significant increase in total length occurred, mainly at 30 °C, indicating a more efficient reconversion of the yolk reserves. Later, growth was equally slow, corresponding with the transitional period from endogenous to exogenous feeding, indicating a similar physiologic pattern regardless of incubation temperature. Weight and length increased during the last period, with the greatest increase at 26 °C in contrast with the lowest gain at 30 °C. The inflexion points of many morphometric characters and the developmental stages accelerated with the increasing temperature. Although it was impossible to determine the optimal temperature, it was evident that 26 °C favored hatching success and larval growth.

**Key words:** Fish larvae, incubation temperature, gar, hatching, allometry, development, growth.

**RESUMEN.** Se examinaron el éxito en la eclosión, crecimiento, desarrollo y supervivencia de larvas de manjuarí (*Atractosteus tristoechus*) a diferentes temperaturas (26, 28 y 30 °C) hasta los 18 d después de eclosionadas (DDE). El tiempo de eclosión fue inversamente proporcional a la temperatura de incubación (87, 100 y 111 h). La supervivencia larval en el momento de la eclosión fue significativamente menor a 30 °C (50.3 %), coincidiendo con la mayor mortalidad larval (30.2 %). Las tasas de crecimiento fueron 1.75 mm d<sup>-1</sup> - 10.4 % d<sup>-1</sup> a 26 °C, 1.30 mm d<sup>-1</sup> - 10.2 % d<sup>-1</sup> a 28 °C y 1.40 mm d<sup>-1</sup> - 10 % d<sup>-1</sup> a 30 °C. Se identificaron tres períodos críticos similares: 0-6, 7-11 y 12-18 DDE. En el primero ocurrió un incremento similar en peso y un incremento significativo en la longitud total, principalmente a 30 °C, lo cual reveló una reconversión más eficiente de las reservas vitelinas. Posteriormente, el crecimiento fue igualmente lento, correspondiéndose al período de transición de la alimentación endógena a exógena, indicando un patrón fisiológico similar e independiente de la temperatura de incubación. En el último período, tanto el peso como la longitud aumentaron, con el mayor incremento a 26 °C, contrario a la menor ganancia a 30 °C. Los puntos de inflexión de muchos de los caracteres morfométricos y las etapas de desarrollo se aceleraron con el incremento de la temperatura. Aunque fue imposible determinar la temperatura óptima, se hizo evidente el beneficio para el éxito de eclosión y el crecimiento larval a 26°C.

**Palabras clave:** Larvas de peces, temperatura de incubación, lepisosteidos, eclosión, alometría, desarrollo, crecimiento.

## INTRODUCTION

Water temperature has been shown to be one of the most important factors influencing growth, survival, feed efficiency and development of fish larvae in both laboratory and natural environments (Seikai *et al.* 1986, Kling *et al.* 2007, Le *et al.* 2011). It affects the metabolism, activity, structure and quality of the developing embryo and larvae (Saka *et al.* 2005, Ahmad *et al.* 2011, Frisk *et al.* 2012). Fish generally present a temperature optimum for growth and survival that can change with age and size (Jonassen *et al.* 1999), and normally varies among species (Rana 1990a). Each species has an optimal range for the best developmental success depending on its ecology and life history.

Since temperature is generally the most influential and variable environmental parameter, and is also the most controllable in hatcheries, it has been the most thoroughly researched factor influencing fish development (Wang *et al.* 1987). Furthermore, its effects on growth have been described for several fish species including cod (Imsland *et al.* 2007, Hanna *et al.* 2008), halibut (Steinarsson and Björnsson 1999), brown flounder (Huang *et al.* 2008), mackerel (Mendiola *et al.* 2007b), pinfish (Reber and Bennett 2007), trahira (Petry *et al.* 2007), sea bass (Georgakopoulou *et al.* 2007, Dülger *et al.* 2012), yellowtail kingfish (Abbink *et al.* 2012) and others.

However, the relationship among incubation temperature, embryonic development and larval growth of Cuban gar has not yet been described. Cuban gar (*Atractosteus tristoechus*) is an endemic freshwater fish that inhabits the western region of Cuba, primarily Ciénaga de Zapata. The species was listed as vulnerable in 1999 (Pérez *et al.* 1999) because, apart from restrictions on its habitat, other factors such as habitat loss and ecological alteration have contributed to a decline in the natural populations. For these reasons, interest in its cultivation has increased in order to preserve and restore natural populations through stocking of cultured individuals. This study was undertaken to provide information on the effect of three temperatures (26, 28 and 30 °C) on hatching performance,

growth and development of Cuban gar larvae.

## MATERIALS AND METHODS

### Experimental design, sampling and measurements

Cuban gar (*Atractosteus tristoechus*) eggs were obtained from the induced spawning of one female and three males kept in captivity at the Center for Native Ichthyofauna Reproduction located in the peninsular region of Zapata, Cuba. Breeding adults were placed in a 3 x 2.5 m concrete pond with water to a depth of 50 cm. In order to favor the spawning behavior of the lepisosteids (León *et al.* 1978, Simon and Wallus 1989), artificial branches were placed throughout the pond to provide spawning substrates. A first injection of luteinizing hormone-releasing hormone analog (LHRH-A; 25 µg ml<sup>-1</sup> - Argent Chemicals, USA) was given to the broodstock, and a second injection was given 16 h later. Courtship and spawning occurred 9 h after the second injection. Fifteen minutes after spawning, nine artificial branches with 100 adhesive eggs on each were selected. The branches were carefully removed and placed in nine 15 L circular fiberglass tanks, where the trials took place for up to 18 d after hatching (DAH). The experiment was carried out in triplicate at three different constant temperatures (26, 28 and 30 ±1°C). The eggs taken from the broodstock pond and transferred to the experimental tanks were adapted gradually (four hours) to each test temperature. Room temperature was controlled (24 ±1°C). Eggs and larvae were reared under an 08:00 to 20:00 h light regime, oxygen levels were maintained above 6 ppm and 50 % of the water was exchanged daily in each tank after cleaning the bottom. After hatching, the larvae were fed live *Moina* ad libitum three times a day (09:00, 14:00 and 19:00 h).

Hatching time H<sub>50</sub> (50 % larval hatching) was determined through hourly observations of each experimental unit. Embryonic, pre-hatching and post-hatching mortalities were recorded at H<sub>50</sub>. Larval survival was verified at that moment, as well as at the end of the experiment.

From hatching up to 18 DAH, two to three larvae per tank were selected randomly, anaesthetized with tricaine methanesulphonate (MS 222), individually weighed on an Ohaus scale ( $\pm 0.1$  mg) and preserved in a 70 % ethanol solution for later examination. Seventeen morphometric characters (total length (TL); standard length; snout length; head length (HL); predorsal and preanal length; trunk and tail length; pectoral and pelvic fin length; cephalic height; pectoral, preanal and postanal height; caudal peduncle height; snout and head width) were recorded for each specimen using an ocular micrometer and digital caliper ( $\pm 0.1$  mm), considering the criteria defined and illustrated by Simon and Wallus (1989) for lepisosteid larvae. The relationship between incubation temperature and developmental larval stage, proposed by Comabella et al. (2010), was also analyzed.

### Data analysis

Growth rate (GR,  $\text{mm d}^{-1}$ ) was calculated as  $[TL_2 - TL_1]/(t_2 - t_1)$ , where  $TL_2$  and  $TL_1$  are the total length at times  $t_2$  and  $t_1$ , respectively. The Specific Growth Rate (SGR,  $\% / \text{d}^{-1}$ ) was calculated as  $100 [\ln W_2 - \ln W_1] / (t_2 - t_1)$ , where  $W_2$  and  $W_1$  are the wet body weights at times  $t_2$  and  $t_1$ , respectively.

Data for  $H_{50}$ , final survival and growth rates were analyzed with a one-way analysis of variance (ANOVA) to detect differences ( $p < 0.05$ ). Significant ANOVAs were followed by a post hoc multiple comparison test (Tukey test). Prior to the ANOVA analyses, data expressed as percentages were arcsine square-root-transformed. A regression analysis was used to describe larval growth. The analyses were carried out with the Statistica program for Windows (Stat-Soft, Tulsa, Okla.).

Allometric growth was calculated as a power function of X ( $X = \text{TL}$  (or HL for widths)) using non-transformed data:  $y = aX^b$ , where y was the measured character, a the intercept and b the growth coefficient (Fuiman 1983). The equations were established from regressions carried out on log-transformed data, using TL or HL as the independent variable (Gisbert 1999; Gisbert et al. 2002). When growth is isometric, the growth coefficient is

$b=1$  for length, height or width and is  $b = 3$  for weight when compared with X (Osse & Boogart 2004). Allometric growth was positive when b was  $> 1$  or 3 and negative when  $< 1$  or 3. Diphasic growth may be described with two different growth curves. The X value, where the slope changes, is called the inflexion point. Inflexion points were determined using iteration procedures according to Snik et al. (1997), Gisbert (1999) and Gisbert et al. (2002). The xy data set was sorted according to an increasing X. Regression lines were calculated for  $X_{min}$  until  $X_{intermediate}$ , and for  $X_{intermediate}$  until  $X_{max}$ , where  $X_{intermediate}$  varied iteratively from  $X_{min} + 2$  to  $X_{max} - 2$ . Also, t tests were applied to check whether the growth coefficients for  $X_{min}$   $X_{intermediate}$  and  $X_{intermediate}$   $X_{max}$  differed significantly. The  $X_{intermediate}$  value that resulted in the largest t was defined as the inflexion point. Growth coefficients were compared statistically with a t-test. The accepted significance level was  $p < 0.05$ .

## RESULTS

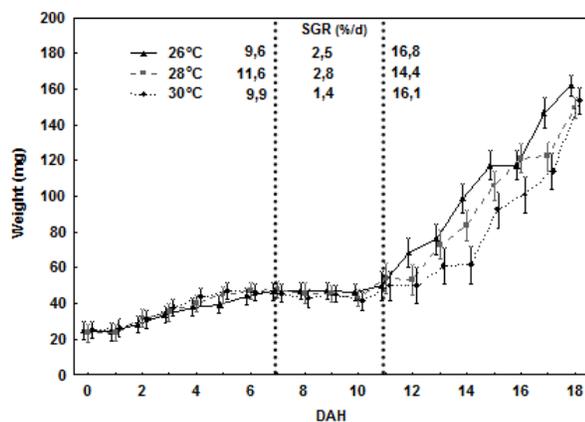
### Survival and hatching success at various incubation temperatures

The time required to reach  $H_{50}$ , the mortalities and the larval survival at this moment and at various constant temperatures are presented in Table 1. The time to hatching ( $H_{50}$ ) was inversely related to the incubation temperature. Larval survival at the time of hatching was significantly lower at 30 °C (50.3 %), and coincided with the highest post-hatching larval mortality (30.2 %).

### Incubation temperature effect on larval growth

Although final larval survival was high ( $> 90$  %) and no significant differences were found for the tested temperatures (Table 1), larval growth varied among the treatments during the first 18 d of Cuban gar life. Figure 1 shows the weight growth of the *A. tristoechus* larvae reared at the tested temperatures, with a 6 - 6.5 fold increase in weight from 0 to 18 DAH. The increase in total weight was described by the equations:  $TW_{(26^\circ\text{C})} = 22,593 \times \exp(0,1006 \times \text{DAH})$ ,  $TW_{(28^\circ\text{C})} = 23,867 \times \exp(0,0913 \times \text{DAH})$

and  $TW_{(30^{\circ}\text{C})} = 25,736 \times \exp(0,0783 \times \text{DAH})$ . The specific growth rate averaged  $10.4\% \text{ d}^{-1}$  ( $26^{\circ}\text{C}$ ),  $10.2\% \text{ d}^{-1}$  ( $28^{\circ}\text{C}$ ) and  $10\% \text{ d}^{-1}$  ( $30^{\circ}\text{C}$ ) during the experiment. It is possible to distinguish three similar periods of larval growth for each temperature: 0-6 DAH, 7-11 DAH and 12-18 DAH. This rate increased during the first and third periods. However, during the 7-11 DAH period, the increase in body weight was slow in all the treatments. The greatest specific growth rates were recorded for  $28^{\circ}\text{C}$  during the first and second periods and for  $26^{\circ}\text{C}$  during the third.



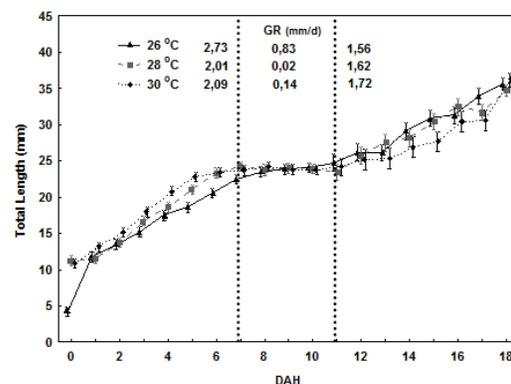
**Figure 1.** Changes in Cuban gar larvae mean wet weight (0-18 days after hatching-DAH) in relation to the three constant temperatures tested. Means are shown with a SE. The vertical lines define the three periods (0-6 DAH, 7-11 DAH, 12-18 DAH) of larval growth and the specific growth rates are indicated (SGR-%/d<sup>-1</sup>).

**Figure 1.** Cambios en la media del peso húmedo de las larvas de manjarí (0-18 días después de eclosionadas-DAH) con respecto a las tres temperaturas constantes probadas. Se ofrecen las medias con el error estándar (SE). Las líneas verticales define los tres períodos (0-6 DAH, 7-11 DAH, 12-18 DAH) del crecimiento larval y se muestran las tasas de crecimiento específicas (SGR-%/d<sup>-1</sup>).

Regarding the total length growth (Figure 2), the increase was 3 and 3.3 fold for  $28^{\circ}\text{C}$  and  $30^{\circ}\text{C}$  respectively, and 8.4 fold for  $26^{\circ}\text{C}$  due to a smaller larval size at the time of hatching at this temperature. The equations for the relationship between the days after hatching and the total length were:  $TL_{(26^{\circ}\text{C})} = 10,337 + 1,3862 \times \text{DAH}$ ,  $TL_{(28^{\circ}\text{C})} = 12,995 + 1,1673 \times \text{DAH}$  and  $TL_{(30^{\circ}\text{C})} = 14,338 + 1,0165 \times \text{DAH}$ . During the early larval development, the growth rate averaged  $1.75 \text{ mm d}^{-1}$  ( $26^{\circ}\text{C}$ ),  $1.30 \text{ mm/d}^{-1}$  ( $28^{\circ}\text{C}$ ) and  $1.40 \text{ mm d}^{-1}$

( $30^{\circ}\text{C}$ ). A three-period pattern similar to that mentioned above was observed, with the greatest growth rates during the first and second periods at  $26^{\circ}\text{C}$ , as well as during the third at  $30^{\circ}\text{C}$ . Also evident was the slow increase in body length during the 7-11 DAH period in all the treatments. A significant interaction was recorded between temperature and larval age for the total length and the wet weight.

The relationships between daily growth rate and specific growth rate vs temperature are presented in Figures 3 and 4 respectively. The temperature had a significant effect on both growth rates during the larval period, with the highest values recorded for the  $26^{\circ}\text{C}$  treatment. However, the optimal temperature for Cuban gar larvae growth was impossible to determine.



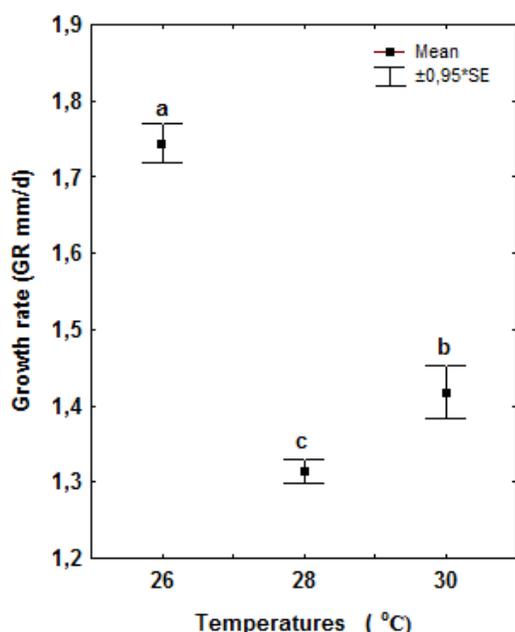
**Figura 2.** Crecimiento en largo total de larvas de manjarí cultivadas a diferentes temperaturas desde la eclosión hasta los 18 días después de eclosionas (DAH). Cada punto representa la media de nueve mediciones + SE. Las líneas verticales define los tres períodos (0-6 DAH, 7-11 DAH, 12-18 DAH) del crecimiento larval y se muestran las tasas de crecimiento (GR- mm/d<sup>-1</sup>).

Wet weight growth was negatively allometric, biphasic and had the same inflexion point (14 DAH) in the three temperatures (Table 2). Of the 17 morphometric characters measured, only the standard length presented isometric growth as a function of total length during the early stages of development in all the tested temperatures. In addition, isometric growth was observed in another five characters (predorsal and preanal lengths, preanal and postanal heights, and tail length) at  $28^{\circ}\text{C}$  and  $30^{\circ}\text{C}$ . In contrast, the other body proportions and growth coeffi-

**Table 1.** Effect of temperature on time of hatching ( $H_{50}$ ) and larval survival during the first 18 d after hatching (DAH) (Mean  $\pm$  SE). Different superscripts (letters) indicate significant differences ( $p < 0.05$ ).

**Tabla 1.** Efecto de la temperatura en el momento de eclosión ( $H_{50}$ ) y en la supervivencia larval durante los primeros 18 d después de eclosionadas (DAH) (Media  $\pm$  SE). Letras desiguales indican diferencias significativas ( $p < 0.05$ ).

		26°C	28°C	28°C
Hatching time (hour)		111	100	87
$H_{50}$	Embryonic mortality (%)	5.5 $\pm$ 0.92	5.6 $\pm$ 0.81	11 $\pm$ 3.09
	Pre-hatching larval mortality (%)	8.9 $\pm$ 0.70	9.6 $\pm$ 2.24	8.6 $\pm$ 3.32
	Post-hatching larval mortality (%)	6.5 $\pm$ 2.40 <sup>b</sup>	6.5 $\pm$ 1.79 <sup>b</sup>	30.2 $\pm$ 3.14 <sup>a</sup>
	Larval survival (%)	79.1 $\pm$ 1.41 <sup>a</sup>	78.3 $\pm$ 4.25 <sup>b</sup>	50.3 $\pm$ 2.99 <sup>b</sup>
1-18 DHA	Larval survival (%)	94.4 $\pm$ 2.85	96.1 $\pm$ 0.92	91.3 $\pm$ 1.76



**Figure 3.** Growth rate (GR) vs temperature for Cuban gar larvae from 0 to 18 DAH. Different superscripts (letters) indicate significant differences ( $p < 0.05$ ).

**Figura 3.** Tasa de crecimiento (GR) vs temperatura en larvas de manjuarí desde 0 hasta 18 DAH. Letras desiguales indican diferencias significativas ( $p < 0.05$ ).

cients changed considerably during this period in all the treatments, showing biphasic growth patterns.

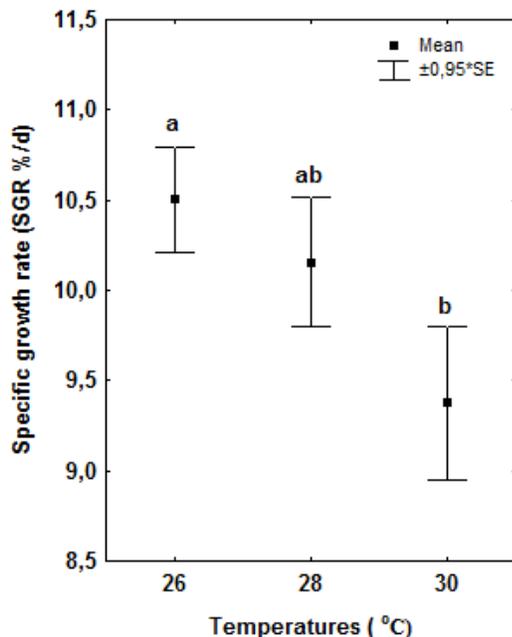
The head and snout lengths had a positive allometric growth during larval development with inflexion points at 6 DAH (26 °C and 28 °C) and 3 DAH (30 °C). The growth in head height and in head and snout widths was negatively allometric, with a remarkable anticipation of the inflexion point for head height at 30 °C. The trunk length growth was negatively allometric as well, with inflexion points that advanced from 3 DAH at 26 °C to 1

DAH at 30 °C. A similar pattern was found for the pectoral height, but the anticipation of the inflexion point was from 5 DAH at 26 °C to 3 DAH at 30 °C. The preanal-postanal heights and predorsal-preanal lengths showed a negative allometry only in the larvae reared at 26 °C. The pectoral and pelvic fins increased in length from hatching to 18 DAH with a positive allometric growth (except for the pectoral fins at 26 °C), and the same anticipation of the inflexion points was evident with the increase in incubation temperature. Finally, the tail length growth in the early larvae showed the same isometric trend as the other length characters presented in Table 2, except for 26 °C where a positive allometric growth appeared with an early inflexion point. The peduncle height showed a negative allometric growth throughout the 18 days of the experiment in all the treatments.

### Incubation temperature effect on larval development

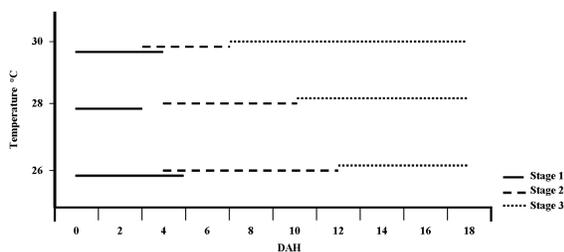
Incubation temperatures also influence the morphological development of larvae. According to Comabella *et al.* (2010), three stages of Cuban gar larval development were established: Stage 1- Attached, Stage 2- Transitional and Stage 3- Free Swimming. Figure 5 presents the relationship between larval development stage and age at different temperatures. Larval development accelerated at 30 °C as, from 3 DAH onwards, the larvae were in the second stage, whereas this occurred one day later at the other two temperatures. This difference was even more marked in the tran-

sition to the third stage which was reached on 7 DAH at this temperature and on 10 DAH (28 °C) and 12 DAH (26 °C) in the other treatments.



**Figure 4.** Specific growth rate (SGR) vs temperature for Cuban gar larvae from 0 to 18 DAH. Different superscripts (letters) indicate significant differences ( $p < 0.05$ ).

**Figura 4.** Tasa de crecimiento específica (SGR) vs temperatura en larvas de manjuarí desde 0 hasta 18 DAH. Letras desiguales indican diferencias significativas ( $p < 0.05$ ).



**Figure 5.** Age-developmental stage relationship of Cuban gar larvae reared at different temperatures. (Stage 1- Attached; Stage 2- Transitional and Stage 3- Free swimming according to Comabella et al. (2010))

**Figura 5.** Relación entre etapas de desarrollo-edad en larvas de manjuarí mantenidas a diferentes temperaturas (Etapa 1-Adherida; Etapa 2- Transición y Etapa 3- Libre nadadora de acuerdo con Comabella et al. (2010))

## DISCUSSION

Broodstock spawning, embryo development, survival and growth of fish authors considered that these physiological occur within a narrow range of

water temperatures that may differ among species, ages and body sizes, and may reflect their temporal and spatial distribution in the field (Imstrand et al. 1996, Saka et al. 2005, Teletchea et al. 2009). Incubation temperature has a direct effect on the timing of embryonic development and determines hatching efficiency, as was observed in Cuban gar larvae. The first differences were observed in the time of hatching ( $H_{50}$ ), which was inversely related to the incubation temperatures, as has been reported for other species (Rana 1990a, Kamler et al. 1994). In comparison with other lepisosteids, Márquez (1998) found that 75-80 % of *Atractosteus tropicus* larval hatching occurred between 21 and 80 hours post fertilization at 25, 30 and 35 °C. In the case of *A. spatula*, this process took place from 50 to 57.5 hours post fertilization at 27-28 °C (Morales 1999, Mendoza et al. 2002). In the present study, the time required to reach  $H_{50}$  varied from 87 to 111 h post fertilization at our temperatures (26, 28 and 30 °C), indicating a larger embryologic period for *A. tristoechus*. During this critical moment in the life cycle of the fish, significant differences were observed in post-hatching larval mortality. The greatest percentage at 30 °C coinciding with the lowest larval survival shows the harmful effect of this temperature on hatching success. However, the embryonic and pre-hatching mortalities were not statistically different at the tested temperatures, and no deformities in larvae at the time of hatching were observed for the highest temperature. Sometimes, when incubation temperatures are outside the optimal range, development appears to be inhibited at the early stages, generally near the time of gastrulation (Wang et al. 1987). This results either in embryo death or in the development of abnormalities that may increase mortality (Hart Purser 1995). This pattern has been observed in white sturgeon as well as in other species (Wang et al. 1987). Our data indicate that a temperature of 30 °C increases mortality at the time of hatching, but it cannot be associated with a supra-optimal temperature that may cause drastic effects in embryo development, such as spinal and jaw deformities and a smaller fish size at the time of hatching, as has been described for other species (Georgakopoulou et al. 2010).

Also, larval survival in all treatments of our experiment was high and similar to that reported for other *Atractosteus* species of 80-90 % (Márquez 1998, Morales 1999, Mendoza *et al.* 2002).

Temperature has been proven to affect almost every aspect of the early development of fish: hatching, initial feeding time (Huang *et al.* 2008), yolk conversion efficiency, and size and body condition at first feeding (Ojanguren *et al.* 1999). These authors considered that these physiological responses reveal the controlling effect of temperature on metabolic processes through thermal dependence on enzymatic activity. As poikilotherms, fish cannot maintain a body temperature different from the surrounding water; therefore, temperature affects their biochemical and physiological activities. Many studies have reported species-specific metabolic and behavior responses to thermal acclimation, indicating changes in hematological pattern (Das *et al.* 2009, Martins *et al.* 2011), excretion (Smatresk Cameron 1982b, Nerici *et al.* 2012b), mobility and swimming (Hanna *et al.* 2008, Martin *et al.* 2011), feeding (Bailey Alanärä 2006), bone ontogeny (Loffler *et al.* 2008, Georgakopoulou *et al.* 2010), muscle cellularity (Carey *et al.* 2008), rate of oxygen consumption (Barnes *et al.* 2011, Nerici *et al.* 2012a), and even the air-breathing frequency in facultative air breathers such as lepisosteid fish (Rahn *et al.* 1971, Smatresk Cameron 1982a,b).

In the present study, three similar critical periods for larval weight and length growth were identified for the three tested temperatures. The first period (0-6 DAH) was characterized by a similar increase in weight at all temperatures and a significant increase in the total length mean, with the highest growth rates at this time in relation to all the days of the experiment. Specifically in the case of total length, larvae at the time of hatching seemed to be significantly smaller at lower temperatures than at higher ones. The size of newly-hatched larvae of several fish species has been shown to be positively and negatively influenced by temperature (Mendiola *et al.* 2007b). At the time of hatching, larval size may decrease as incubation temperature increases (Kamler *et al.* 1994). Larvae could convert more

of their yolk to body tissue at higher temperatures (Rana 1990b). Others could reach a maximum body size at intermediate temperatures (Sun *et al.* 2006, Mendiola *et al.* 2007b). In our case, this difference was found only at the time of hatching, as the total larval length in all the treatments was similar after 24 h.

During the first days, larvae are in a lecithotrophic phase, remain vertically adhered to vegetation and feed exclusively off the yolk sac (Comabella *et al.* 2010). At the beginning, larvae may increase in length due only to the use of the yolk sac elements, while weight remains almost stable as there is no uptake of external nutrients. This was confirmed in a previous study (Comabella *et al.* 2006) that observed a significant increase in larval protein concentration starting on 9 DAH, together with an input of new protein sources from exogenous feeding. Although no differences in weight were recorded in the three tested temperatures during this first period, the gain in total length was significantly higher with the increase in temperature. This could reveal a more efficient use and reconversion of the yolk reserves into an increase in body size at the highest temperature. Similar results have been reported for white sturgeon (Wang *et al.* 1987) and Atlantic salmon (Ojanguren *et al.* 1999). These findings indicate that larvae have the capacity to absorb endogenous nutrients differently under different water temperature conditions, and this means that the rate of absorption of the endogenous nutrients can be controlled by water temperature. However, further study is needed to determine which other environment parameters affect the larval absorption of reserves under rearing conditions.

After this period, from 7 to 11 DAH the body weight and total length increased slowly in the three treatments, corresponding to a lecithoexotrophic - exotrophic stage. The process of obtaining food begins at this moment, though the yolk reserves are still used. This is the transitional feeding period that is defined as an interval in which feeding ability develops and feeding starts, with some reserves still present to meet the energetic demands of prey capture (Moteki *et al.* 2001, Williams *et al.* 2004). This period is considered to be a critical and vul-

nerable time in the initial ontogeny of fish due to predation and competition for food (Balon 1985; Coughlin 1991, Makrakis *et al.* 2005). It has also been considered as the period of greatest change in larval appearance, and in organ and structure development (Williams *et al.* 2004), and when a decrease in growth could occur (Gisbert *et al.* 2002, Geerinckx *et al.* 2008). Our results indicate that this change from endogenous to exogenous feeding, which ends with the exhaustion of the yolk reserve, follows a similar physiologic pattern regardless of incubation temperature.

After this critical transition period, larvae are exotrophic and are able to detect food items and prey efficiently in the water column. This period is characterized by a significant increase in daily weight and total length due to an effective assimilation of external nutrients. However, this pattern was inverted in the case of our temperatures, as the greatest increase in weight and total length was obtained at 26 °C and the lowest at 30 °C. Since yolk represents the main source of energy and materials for sustaining growth in early embryos and pre-feeding larvae, the effect of temperature on growth and development processes is well seen in this phase. At this time, conflicting demands and constraints by other organic functions appear (e.g. locomotion, feeding, social interaction), and metabolic processes and physiological activities may be affected. This could suggest that long-term exposure of Cuban gar larvae to 30 °C may induce some type of stress. This thermic stress could impede the appropriate obtaining and/or assimilation of nutrients, resulting in a decreased growth. It is also possible that the composition, mobility or survival of live prey could be affected by this higher temperature. However, no significant changes in behavior, survival, health or oxygen demand were observed at this higher temperature, compared with the other treatments. In the present study, larval growth was examined only in terms of length and weight. Further detailed analyses should be carried out to monitor daily food and oxygen consumption, and other parameters that may explain the reason for this larval growth pattern once the yolk reserves are finished.

An uncommon statistically significant interac-

tion between temperature and larval age in Cuban gar was also evident. Previous studies with Japanese flounder (Seikai *et al.* 1986), halibut (Jonassen *et al.* 1999), pollack (Ruyet *et al.* 2006), nase (Keckeis *et al.* 2001) and Atlantic mackerel (Mendiola *et al.* 2007a) have shown there is no interaction between larval weight or length and incubation temperature during early development. It could be interesting to evaluate this phenomenon in other lepisosteid larvae to determine whether it is a specific family pattern or exclusive of the Cuban gar.

Every fish species has an optimum temperature for growth (Kooka *et al.* 2007) that differs even at each developmental stage (Jonassen *et al.* 1999, Saka *et al.* 2005). The typical bell-shaped curve described by Jobling (1997) shows that maximum growth rates are achieved at some middle temperature, with decreasing growth rates at lower and higher temperatures (Kling *et al.* 2007, Rushworth *et al.* 2011). Our results suggest that a lack of data, particularly for the lower temperatures, has consequences in establishing the optimum temperature for growth of the Cuban gar larvae. Prior knowledge of the growth-temperature relationship indicates that this species has better growth rates at 26 °C during the first 18 days after hatching (larval period). However, attempts will be made to determine its optimum temperature in order to optimize the culture conditions for this stage. Studies on *A. spatula* and *A. tropicus* showed the best growth at 30 °C (Márquez 1998, Aguilera *et al.* 2002), in contrast with our results.

Comabella *et al.* (2013b) provided a detailed explanation of the allometric growth of this species using larvae reared at 28 °C. The present analysis revealed that some body proportions changed considerably during larval development depending on the incubation temperature. In relation to larval weight growth, it was interesting to find the same negatively allometric growth, with a similar growth coefficient and identical inflexion points for the three incubation temperatures. However, many morphological characters such as the head, snout, paired fins, trunk (lengths), head and pectorals (heights), showed a remarkable anticipation of the inflexion point with an increase in incubation temperature.



Many fish species exhibit allometric growth during the larval period, from the absorption of the yolk sac to the onset of metamorphosis (Mello et al. 2006, Geerinckx et al. 2008). These patterns reflect morpho-anatomical growth priorities according to their importance for primary living functions (Sala et al. 2005, Choo Liew 2006) that guarantee an appropriate survival. As mentioned above, increasing temperatures accelerated not only many inflexion points but also the morphological development stages. Yolk depletion varied from 13 to 8 d when water temperature increased from 26 to 30°C, indicating that the rate of yolk use was directly proportional to the temperature. However, the significant change in larval wet weight occurred at 14 DAH for the three temperatures. Although the final survival rate under different temperature conditions was similar, the least gain in length and weight in the exotrophic phase was obtained at 30 °C. These results indicate that a faster larval development at high temperatures seems to be disadvantageous as an early strategy. The early life stages of Cuban gar are considered to have survival strategies characterized by piscivorous feeding habits, precocious digestive systems (Comabella et

al. 2006, Comabella et al. 2013a) and fast growth rates (Comabella et al. 2010). However, the absorption of the endogenous nutrition within a few days when at 30 °C left the larvae with only a short period to change from endogenous to exogenous nutrition, a process that involves various complex morphological and physiological changes.

The results of the present laboratory study constitute the first available data on the development, growth and survival of Cuban gar larvae reared at different incubation temperatures. Although it was not possible to establish their optimal temperature, the best hatching success and larval growth was recorded at 26 °C. Also, a temperature of 30 °C shortened the incubation period and caused an earlier onset of exogenous feeding, it affected larval survival at the time of hatching and reduced weight and length gain during the exotrophic stage.

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