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Brief communication

Effect of adhesive coat elimination and temperature on hatching of eggs of striped catfish, *Pangasianodon hypophthalmus*

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ABSTRACT

Objective. This study aimed to assess the effect of tannic acid and protease on the elimination of adhesiveness in fertilized eggs of striped catfish, *P. hypophthalmus*, to improve the incubation conditions. **Materials and methods.** Tannic acid at 0.5 g L^{-1} for 5 min and protease at 5 mL L^{-1} for 8 min were used to eliminate the adhesive coat of fertilized eggs, and a control group was used without chemical addition. Then, the eggs from all treatments were incubated at 26, 28, and 30°C. **Results.** The eggs treated with protease did not hatch at any temperature, while in eggs treated with tannic acid and incubated at 26 and 28°C, hatching rates above 60% were observed, where those eggs incubated at 28°C (84.7±1.3%) had the highest hatching rates ($p<0.05$) among all the treatments. The eggs of the control group had significantly higher hatching percentages (14.2 ± 0.6%) when they were incubated at 28°C than at 26 or 30°C. **Conclusions.** The results showed a significant interaction between the type of degumming and the incubation temperature, indicating that tannic acid produces the best hatching percentage at 28°C.

Keywords: Adhesive coat; hatching rate; incubation temperature; protease; tannic acid (*Source: ICYT de Biología Animal*).

RESUMEN

Objetivo. El propósito del presente trabajo fue evaluar el efecto del ácido tánico y de las proteasas para eliminación de la capa adherente en huevos fecundados de bagre asiático, *P. hypophthalmus*, para mejorar las condiciones de incubación. **Materiales y métodos.** Se utilizó ácido tánico a 0.5 g L^{-1} por 5 min, proteasa a 5 mL L^{-1} por 8 min para la eliminación de la capa adherente de huevos fecundados y un grupo control, sin la adición de estos químicos. Todos los tratamientos fueron incubados a 26, 28 y 30°C, para determinar el porcentaje de eclosión. Todos los tratamientos fueron realizados por triplicado. **Resultados.** Los huevos tratados con proteasa no eclosionaron en ninguna temperatura de incubación, mientras que los tratados con ácido tánico e incubados a 26 y 28°C tuvieron porcentajes de eclosión mayor al 60%, siendo los porcentajes de eclosión más altos (84.7±1.3%) los que se obtuvieron a 28°C. Los huevos del grupo control presentaron los

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porcentajes de eclosión significativamente más altos (14.2±0.6%) cuando fueron incubados a 28°C que a 26 o 30°C. **Conclusiones.** Los resultados mostraron una significativa interacción entre el tipo de desgomado y de la temperatura de incubación, indicando que el ácido tánico produce el mejor porcentaje de eclosión a los 28°C.

Palabras clave: Ácido tánico; capa adherente; porcentaje de eclosión proteasa; temperatura de incubación (*Fuente: ICYT de Biología Animal*).

INTRODUCTION

The eggs of teleost fish are surrounded by an envelope composed of two layers: an internal zona radiata composed of protein with protective functions and an external zona radiata composed of mucopolysaccharides that, in some fish such as siluriforms, presents a gelatinous layer that serves to adhere the eggs to the substrates on which they are laid (1,2). In particular, the eggs of the striped catfish, *Pangasianodon hypophthalmus*, are pelagic, slightly oval, and adhere to each other or to some substrate, which prevents them from being washed away by currents (3). However, for aquaculture production, the formation of the adherent layer has negative consequences, such as low oxygenation and the development of pathogens, which can be reflected in low hatching rates and larval survival (4). However, despite the commercial importance of Asian catfish culture, little has been explored to improve the hatching rate.

To eliminate the adherent layer of the eggs in those species of fish that present this characteristic, various methods have been used, including mechanical treatments such as shaking the eggs to separate them, as used for walleye, *Stizostedion vitreum* (5); the use of natural products such as sand, clay, powdered milk, and pineapple juice to degum eggs of tench, *Tinca tinca* (6), pike perch, *Sander lucioperca* (7) and bullseye puffer, *Sphoeroides annulatus* (8,9); and the application of chemical treatments such as urea, sodium sulfite, proteolytic enzymes, and tannic acid to degum eggs of pike perch, *S. lucioperca* (10) ballan wrasse, *Labrus bergylta* (11), African catfish, *Clarias gariepinus* (12), rainbow trout, *Oncorhynchus mykiss* (13), tench, *T. tinca* (14), walleye, *S. lucioperca* (15), and rainbow smelt, *Osmerus mordax* (16).

On the other hand, temperature management is essential for the successful incubation of eggs because it is one of the factors that most affects various aspects of embryonic and larval development, growth, fertilization, and hatching rates and efficiency in the use of metabolic energy (17, 18).

Therefore, the objective of this paper was to evaluate the effect of tannic acid and protease on eliminating the adhesiveness of eggs of striped catfish, *P. hypophthalmus*, as well as the effect of different incubation temperatures on the hatching rate.

MATERIALS AND METHODS

Fish management: This study was performed at the laboratory of "Biotecnologia Acuicola Sustentable" belonging to Facultad de Ciencias del Mar, Universidad Autonoma de Sinaloa. From brooders maintained under natural conditions of photoperiod and temperature (23°12'31.7" N and -106°25'28.18" W) and fed at 3% biomass on a commercial diet (35% protein, Purina Nutripec®), two females were selected (total length of 61 and 61.5 cm; weight of 2248 and 2538 g) with a modal diameter of oocytes ≥1.0 mm, and one male (total length of 60.5 cm, and weight of 2190 g) with fluent sperm. Spawning was induced by injections of human chorionic gonadotropin (HCG, Chorulon®). Two doses were applied to females: the first dose was 500 IU kg^{-1} body weight, and the second dose was 2000 IU kg^{-1} body weight, eight hours after the first dose. One dose of HCG at 2000 IU kg^{-1} body weight was applied to males at the same time as the second dose for females. After twelve hours, gametes were obtained, and oocytes were fertilized by the dry method (19).

Experimental design: Spawning from both females was mixed and divided into three portions. Each portion of spawning was submitted to the following treatments for the elimination of adhesiveness: 1) eggs without degumming (as a control), 2) eggs treated with 0.5 gL^{-1} tannic acid for 5 min, and 3) eggs treated with 5 gL^{-1} proteolytic enzyme from *Aspergillus oryzae*, ≥500 Ug^{-1} (Sigma-Aldrich® P6110), for 8 min.

After treatment application, eggs from all treatments were washed with flowing water. From each treatment, 850 fertilized eggs were placed in 1 L flasks with constant aeration and incubated at 26, 28, and 30°C. Incubation was performed in triplicate for each degumming treatment and temperature. After 24 hours, the hatching rate was estimated by the following formula:

Hatching rate (%) = (number of larvae)/(initial number of eggs) x 100

Statistical analysis: To evaluate the effects of the degumming treatments, the temperature of incubation, and their interaction on the hatching rate of fertilized eggs, a two-way ANOVA with the Holm-Sidak test was performed to verify significant differences between the treatments. Previously, percentage data were transformed by the square root arch-sin method. The statistical analysis was performed with a confidence level of α=0.05.

Ethical aspects. The procedures performed in this study were carried out under Mexican legislation in the "Ley General de Bienestar Animal", Title 5, Chapter III, published on February 11, 2016.

RESULTS

The results obtained in this study are shown in Figure 1, where each factor (degumming treatments and temperature of incubation) and their interaction resulted in highly significant effects (p<0.001) on the hatching rate of eggs of striped catfish.

No hatching was obtained in eggs in the protease treatment at any temperature of incubation. Hatching rates up to 60% were observed in eggs treated with tannic acid and incubated at 26 and 28°C; however, the eggs incubated at 28°C reached the highest hatching rates (84.7±1.3%), while the lowest hatching rates were observed in eggs incubated at 30°C (14.2±0.6%). In the control treatment, hatching rates below 20% were observed at all incubation temperatures; nevertheless, the highest hatching rates were obtained in eggs incubated at 28°C (14.2±0.6%) in this treatment (Figure 1).

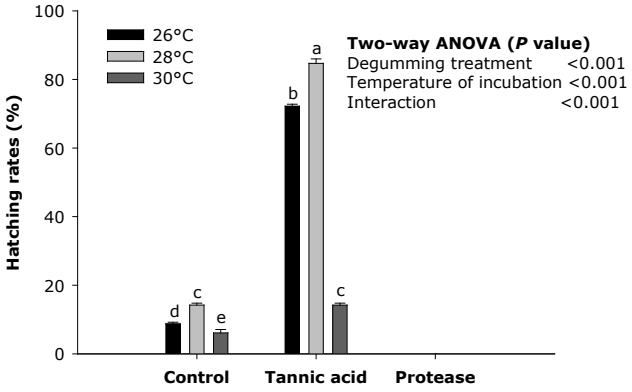

Figure 1. Hatching rates in eggs of *P. hypophthalmus* (mean ± standard deviation) treated with tannic acid (n = 850 eggs), protease (n = 850 eggs), and control (n = 850 eggs) and incubated at different temperatures. Different letters on top of the bars indicate significant differences (p<0.05).

DISCUSSION

The elimination of the adherent coat in eggs of striped catfish, *P. hypophthalmus*, with tannic acid and incubation at 30°C significantly improved the hatching rates (84.7±1.3%). Similar hatching rates, 80-85%, were reported for eggs of beluga, *Huso huso*, when tannic acid was used at 0.5 g L^{-1} with an egg exposure time of 1.5 min (20). Additionally, hatching rates up to 80% have been obtained in pike perch, *Sander lucioperca*, when tannic acid was employed at 0.75 g L^{-1} with between one and two min of exposure (10). However, hatching rates below 5% were obtained in eggs of African catfish, *Clarias gariepinus*, with tannic acid at the same concentration used in this work but with an exposure time of 0.5 min (12). Therefore, for the most efficient use of tannic acid for degumming eggs of catfish species, it may be necessary to have a longer exposure time, as in this work.

In this study, no hatching was obtained in eggs treated with protease at 5 g L^{-1} for 8 min at any temperature of incubation. Such results are different from those obtained for bullseye puffer, *Sphoeroides annulatus*, where a hatching rate of 93.03±3.01% was observed when this enzyme was used at the same concentration and exposure time employed in the present work (9). In eggs of pike perch, *Sander lucioperca*, a hatching rate of 85.4% was obtained with alkaline protease at 1.5 mL L^{-1} for 2 min (15). Likewise, in eggs

of tench, *Tinca tinca*, hatching rates exceeding 95% were obtained with alkaline protease at 8 mL L^{-1} for 1 minute (14). Therefore, according to these data and the results obtained in this work, lower concentrations of protease enzyme or shorter times of exposure are suggested to reach high hatching rates in eggs of striped catfish, *P. hypophthalmus*, because the concentrations used in this study were extremely high or the exposure time was exceptionally long, resulting in some injuries into the structure of the eggs and thereby affecting embryonic development.

On the other hand, for several species of teleost fishes, when the temperature rises into optimal ranges, the incubation time is accelerated; thus, the time to reach hatching is shorter. Such an optimal range depends on the biological and ecological characteristics of each species. Therefore, temperatures of incubation outside of such an optimal range have negative effects on the hatching rates of the eggs and larval survival (21). In a report where eggs of common carp, *Cyprinus carpio*, were incubated at the same temperatures used in this study, the highest hatching rates were reached at 26°C (98.79±1.23%) (22), which were higher values than those obtained here (72.2±0.6%). However, hatching rates of 76±3.0% were observed in *Pangasius sutchi* at an average temperature of 28.5°C (23), while in this work, values of 84.7±1.3% were observed at 28°C, representing slightly higher rates than those reported by Chad et al (23).

In conclusion, for the striped catfish, *P. hypophthalmus*, the treatment of fertilized eggs with tannic acid at 0.5 g L^{-1}, an exposure time of five min and incubation temperature of 28°C significantly increased the hatching rate.

Conflicts of interest

The authors declare having no conflicts of interest.

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