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Research Article

Effect of temperature on the predation rate of the pearl cichlid (*Geophagus brasiliensis*) on the channeled applesnail (*Pomacea canaliculata*)

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ABSTRACT. An experiment was conducted to evaluate the effect of temperature on the intensity of predation upon snails. The experiment was run for 60 days using 15 cement tanks with water temperatures of 18, 22, 26, 30 and 34°C in a completely randomized design with three replications. The pearl cichlids (n = 4 per tank) were distributed in tanks with heaters, which increased the water temperature by 1°C h^{-1}, in a room held at 17°C. The snails were selected (up to 3 mm) and distributed among the tanks (n = 32 per tank). The period of exposure to predation was 18 h, and the index of predation was significantly lower at 18°C. Temperatures between 26 and 34 did not result in significant differences in predation. It was conclude that the pearl cichlids prey efficiently on snails at temperatures of 26°C and above.

Keywords: *Geophagus brasiliensis*, *Pomacea canaliculata*, flooded rice pests, biological control, fish.

Efecto de la temperatura en la tasa de depredación de la castañeta (*Geophagus brasiliensis*) sobre el caracol manzana (*Pomacea canaliculata*)

RESUMEN. Se realizó un experimento para evaluar el efecto de la temperatura sobre la intensidad de depredación de caracoles. Las pruebas experimentales se realizaron durante 60 días, dentro de 15 estanques de cemento llenas con agua a diferentes temperaturas, 18, 22, 26, 30 y 34°C, en un diseño de distribución completamente aleatorio, con tres réplicas. Las castañetas (n = 4 por estanque) fueron distribuidas en estanques con calentadores, lo que aumentó la temperatura del agua en 1°C h^{-1}, en una habitación a 17°C. También, se seleccionaron los caracoles (hasta 3 mm) y fueron distribuidos en los estanques (n = 32 por estanque). El período de exposición a la depredación fue de 18 h, observándose que, el índice de depredación fue significativamente inferior a una temperatura de 18°C, mientras que a temperaturas entre 26 y 34°C no se encontraron diferencias significativas. Se concluye que las castañetas se alimentan de manera eficiente de los caracoles a temperaturas de 26°C o más.

Palabras clave: *Geophagus brasiliensis*, *Pomacea canaliculata*, arroz irrigado, plagas, control biológico.

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INTRODUCTION

Rice cultivation is one of the main economic activities of Rio Grande do Sul State, which is the largest producer and exporter of this grain, being responsible for 67% of Brazilian rice production (approximately 7.3 million ton in 2011/12), from an area of more than 1.17 million ha (IBGE, 2013). It is estimated that this production corresponds to R$5 billion (approximately US$2.5 billion) annually.

Rice cultivation in southern Brazil is conducted through flooding of the crop, and many rice farmers adopt the technique of seeding pre-germinated rice. This type of cultivation increases the occurrence of pests and consequently requires chemical control (Martins *et al.*, 2005).

The principal pests found in pre-germinated rice fields include *Pomacea* sp. snails (Magalhães Jr. *et al.*, 2005). The snail *Pomacea canaliculata* (Lamarck, 1822) (Gastropoda, Ampullariidae), known as "channeled applesnail" presents a large shell, which is rounded and light brown in color with brown stripes, and exhibits high reproducibility rate. In southern Brazil (known as "aruá"), oviposition occurs from August through May (Martins *et al.*, 2005).

During the first few days after hatching, the snails feed on micro-algae as well as waste and residues of animal and plant origin. After one week, small snails feed on higher plants (Yusa & Wada, 1999).

The damage to rice seedlings caused by snails with shells measuring more than 25 mm can reach 100% (Sin, 2003). Total devastation occurs mainly on pre-germinated rice system containing snails with shells measuring more than 10 mm (Hickel *et al*., 2012). Snails have become pests in rice, and their natural predator, the snail hawk *Rosthramus sociabilis*, does not control their populations (Magalhães Jr. *et al*., 2005). In rice-growing areas, the habitat of this hawk is damaged by the removal of all woody vegetation in which the bird could land to prepare its attack on *Pomacea*. In addition, the height of rice plants makes it difficult to locate these snails and hinders their predation by the bird (Silva *et al*., 2005).

The most effective methods to snails control are the application of molluscicides or snail repellents (Yusa & Wada, 1999), but they cause risk to human health and can contaminate the water on rice fields and springs around. Water resources act as integrators of all biogeochemical processes. Thus, surfaces or underground, are the main destinations of chemicals when applied in agriculture. The more sustainable agriculture development, without disorders in human health and in the environment, depends of the farm workers training (Ribas & Matsumura, 2009).

An alternative method to biological control of snails is rice-fish culture. The common carp *Cypinus carpio* is a fish commonly used as a snail predator (Ichinose *et al.*, 2002). However, the trophic opportunism of *Geophagus brasiliensis* (Quoy & Gaimard, 1824), which is indicated to be omnivorous, suggests that pearl cichlids can also be used as snail predators. The diet of this fish consists mainly of fruits, seeds, debris, sediment, aquatic invertebrates and fish scales. It consumes a wide variety of foods at the bottom, including snails, which are crushed in its mouth in a protractile manner (Abelha & Goulart, 2004). This is an important factor in the success of this species in colonizing disturbed environments, such as those characteristically associated with rice crops.

The variation of body temperature in fish influences numerous physiological functions, such as oxygen consumption, feeding and digestibility (Evans & Claiborne, 2006), and affects functional aspects such as growth and reproductive fitness (Ham *et al*., 2003; Imholt *et al.*, 2011; Ostrowski *et al.*, 2011; Santos *et al.*, 2013).

Different species of fish display different preferable temperature ranges (Baldisserotto, 2002; Bicego *et al.*, 2007). In the case of *G. brasiliensis* (Perciformes, Cichlidae), the minimum and maximum lethal temperature is 9°C and 36°C, respectively (Rantin, 1980). In southern Brazil, the climate is subtropical, and a wide range of temperatures is observed, including in the water. The temperature can reach 32°C in summer and fall below 9°C in some cities in the winter (Sartori, 2003). *G. brasiliensis* inhabits lakes and shallow waters of South America, from the Amazon Basin to northern Argentina and Uruguay (Rantin & Petersen, 1985), being found under different thermal regimes and showing increased movement in the presence of light (Kadry & Barreto, 2010).

Data regarding water temperatures collected in several cities in Rio Grande do Sul from 1996 to 2004 show that the temperature ranged from 16 to 28°C in summer, 17-25°C in autumn, 14-17°C (up to 9°C in the coldest months) in winter and 14-21°C in spring (Garcia *et al*., 2008).

This study aimed to evaluate which water temperature, established in laboratory, serves as one greater stimulus to predation of *P. canaliculata* by *G. brasiliensis*. This study objective yet is to get information on the potential use of predators as agent's alternative for pest's control, which can be obtained by means of the rice-fish consortium.

MATERIALS AND METHODS

Location

The experiment was carried out in the Laboratório de Aquacultura Continental (LAC), Instituto de Oceanografia, Universidade Federal do Rio Grande (FURG), near Lagoa dos Patos, in Rio Grande, RS (32°01'40"S, 52°05'40"W), Brazil. The site belongs to the State Foundation for Agricultural Research of Rio Grande do Sul (FEPAGRO), ceded to FURG for research in aquaculture.

Organisms

Fishes

The pearl cichlids (*G. brasiliensis*; known as "cará" or "acará-diadema" in Brazil) were captured from the sandbanks of Patos Lagoon, in Rio Grande, RS, Brazil (31°59.235'S, 052°14.634'W), using cast nets, dip nets and trawls, under a license from the System for Authorization and Information Biodiversity-SISBIO, No. 35308-1. In this environment, a salinity of 6, water temperature of 14°C and dissolved oxygen content of 10.1 mg L^{-1} were recorded. Using a shipping box equipped with oxygen cylinder, the fish were transported to the LAC and stored in polyethylene tanks (200 L). The fishes had an average weight of 49.54 ± 6.72 g and an initial length of 11.70 ± 0.39 cm.

At the LAC, the fish were maintained at a starting temperature of 20°C under constant aeration in circular tanks (250 L). The total biomass in the four tanks was 4 kg m^{-3}, and the water was diluted slowly from a salinity of 6 to 0 (zero). The tanks were cleaned daily via siphoning, and the same volume of water was replaced at the same temperature as the withdrawn water. Renewal of water was performed twice a week at a ratio of 1/3 of the volume of the tank.

The fishes were fed with Guabi® (Campinas, São Paulo, Brazil) pelleted rations (between 2.5 and 3.2 mm in diameter) containing 40% crude protein (CP), following the recommendations of Amaral Jr. *et al*. (2011), at a rate of 5% of the fishes' biomass.

Snails

Nests of *P. canaliculata* collected from localities with rice paddies were transported to the laboratory and distributed among 6 tanks (45 L). The water was maintained at a depth of 10 cm, avoiding direct contact of the water with the spawning snails by depositing them on rock structures obtained from local creeks. The environment was maintained at 20°C with constant aeration until larval hatching. The newly hatched larvae were stored in tanks at a density of 30 larvae L^{-1}, and the water depth was increased gradually to reach a volume of 45 L. The tanks holding *P. canaliculata* were cleaned via siphoning, and the same volume of water was replaced at the same temperature as the removed water.

Plant material (2:1 lettuce: spinach) was liquefied together with 5 mL of molasses and 250 mL of water. After the larval stage, the provided food was no longer liquefied. The same proportions in the initial diet were maintained until the snails reached 3-5 mm. In the growth phase, the snails were fed until to reach the satiety and confined in density of 20 juveniles L^{-1}. The snails received aeration in the environment, enriched with minerals from broken shells of marine mollusks, until they reach the desired size for the experiment.

Abiotic variables

The pH (Solar®, SL110), dissolved oxygen (Lutron®, DO-5519), temperature (Lutron®, DO-5519) and salinity (Alfakit®) of the water in the tanks holding fish and snails were measured daily. In each tank containing snails, 5 g of lime (limestone 0.1 g L^{-1}) was added. Water samples were collected twice a week for analysis of nitrogen (ammonia and nitrite) via the colorimetric method (spectrophotometer UV Biospectro® SP-22).

Experimental design

Fifteen cement tanks (45 L), in which the water was heated to 18, 22, 26, 30 or 34°C, were used in the experiment under a completely randomized design with three replications. The room temperature was maintained at 17°C (one degree below the lowest temperature tested).

Sixty *G. brasiliensis* (n = 4 per experimental unit) were randomly allocated to the experimental treatments from an initial temperature of 20°C. The fishes were starved for 24 h before receiving the snails. Initially, the fishes were weighed on an electronic scale with a precision of 0.01 g (Bioprecisa ®, JH2102) and measured using a fish meter, registering their weight and total length. The biomass of the fish in each tank was 3.46 kg m^{-3}.

Individual heaters coupled to thermostats increased the water temperature in each experimental unit gradually (at 1°C h^{-1}). Soon after the temperature stabilized at the desired level, juvenile *P. canaliculata* snails were selected based on their size (up to 3 mm) and distributed (32 per tank), totaling 480 snails distributed among the 15 experimental units.

After 18 h of exposure of *P. canaliculata* snails to predation by pearl cichlids, *G. brasiliensis*, at the experimental temperatures under a 14:10 h (light/dark) photoperiod, according to Moraes *et al*. (2004), all of the water in the tank was passed through a sieve (mesh = 1 mm) to count the remaining snails. The number of consumed snails was calculated based on the difference from the initial numbers, and the rate of predation (PI) was determined using the following formula:

PI = (number of snails predated x 100) / number of snails offered

Proximal analysis

Surplus snails reared under the same conditions were transported to the Laboratory of Food Technology, FURG, after cooling, to analyze the chemical composition of the organisms ingested by pearl cichlids.

Two treatments were applied in triplicate in the proximal analysis. In the first treatment, the snails were liquefied with the remaining waste in samples collected from the cultivation environment (T1). In the second treatment, the snails were washed (T2) with 0 (zero) salinity water. The results were expressed on a dry matter (DM) basis. All determinations were performed in triplicate, following AOAC International (2002).

The analysis of lipid content was based on the cold fat extraction method using a mixture of three solvents: chloroform, methanol and water, in proportions at which they form a homogeneous solution.

Ash analyses were performed via incineration in a muffle furnace, in which all organic matter was burned. Each sample was placed in a porcelain crucible with a weight that had previously been registered and held in the oven (550 ± 5°C) for 4 h.

Protein analyses were performed using the Kjeldahl method, which determines the nitrogen content of organic origin, subjecting a 0.3 g sample to digestion. The procedure was based on the digestion of the sample with a sulfuric acid catalyst mixture containing copper sulfate and potassium sulfate. Moisture was determined by drying in an oven (105 ± 5°C) based on the removal of water by heating. The carbohydrate content was determined based on the difference.

Statistical analysis

The predation index was analyzed via one-way ANOVA, and the means were subsequently compared using the Tukey test, at the 95% confidence level. Data were tested through regression. The quadratic regression was calculated, and the temperature at which maximum predation occurred was estimated by deriving the regression equation. Proximal composition data were analyzed with the same statistic test.

RESULTS

In tanks used for the storage of fish after reaching salinity equal to zero, they were maintained throughout the experimental period. The dissolved oxygen content was 5.38 ± 0.9 mg L^{-1}, and the pH was 6.5 ± 0.6. The salinity in the tanks holding the snails remained equal to zero throughout the experiment. The dissolved oxygen content in the tanks was 6 ± 0.5 mg L^{-1}, and a pH of 6.7 ± 0.4. The temperature was maintained at 25°C. The temperature of 18°C resulted in the lowest rate of predation (Fig. 1).

At 22ºC, the index was increased to 61.18% and reached 95.8% at 26ºC, both of which represented a significant difference. The PI observed at 26°C indicated thermal comfort for *G. brasiliensis*, which exerted predation on *P. canaliculata*. At 30°C and 34°C, the PI (nearly 100%) was slightly greater, though this difference was not significant relative to a temperature of 26°C. The PI's obtained at temperatures from 26 to 34°C were significantly greater than the PI observed at 18 and 22°C. The following equation describes the distribution of the PI (Predation Index) adjusted for a quadratic model, and, R² values:

$$Y = -0.2724x^2 + 17.839x - 190.36 \qquad R^2 = 0.9461$$

The distribution of the PI data was adjusted for a quadratic model ($Y = - 0.2724 x^2 + 17{,}839 x - 190.36$, $R^2 = 0.95$), where Y = PI, and x = T°C, and the curve generated for PI was more appropriate to show the influence of temperatures over the predation activity.

The pearl cichlids *G. brasiliensis* preyed efficiently on the snails at temperatures of 26°C and above. However, by deriving the regression equation, it was estimated that the highest efficiency of predation by the pearl cichlid on the snail *P. canaliculata* would be obtained at 32.8°C. It was observed that temperatures between 18 and 22°C resulted in proportionately significant differences in the PI ($P < 0.05$), whereas at 18°C, this index was less than 50% (45.9%). Conversely, the PI showed no significant differences ($P > 0.05$) for the temperatures of 26, 30 and 34°C, where it remained above 95% (minimum, 95.83%). There is a significant difference between the PI at 22ºC and the PI above 26ºC.

The *P. canaliculata* offered as food to pearl cichlid displayed an average protein content of 17.85%, both with and without washing, resulted in no significant difference (Table 1).

Instead, a high content of ash was observed (mean 68.74%), which was significantly higher in the T2 snails. The fat content was significantly higher in the T1 snails.

The data obtained in this study reveal that the predation index at temperatures of 26, 30 and 34°C is close to 100% for snails with a size of 3-5 mm. These temperatures are commonly observed and are very close to the records of García *et al*. (2008) in the hottest period of the year in some cities of Rio Grande do Sul. Thus, it can be inferred that the snails on the rice crop will be preyed upon by pearl cichlids when their shells measure 3-5 mm, *i.e.*, before reaching a size at which they are able to promote greater devastation in cultivated rice, which only occurs when the snails have measured more than 25 mm (Sin, 2003).

While the fish primarily feed to meet their energy needs (Pereira-Da-Silva *et al*., 2004), the voluntary intake of food can be influenced by the amount of energy in the diet. Lipids are good energy source for

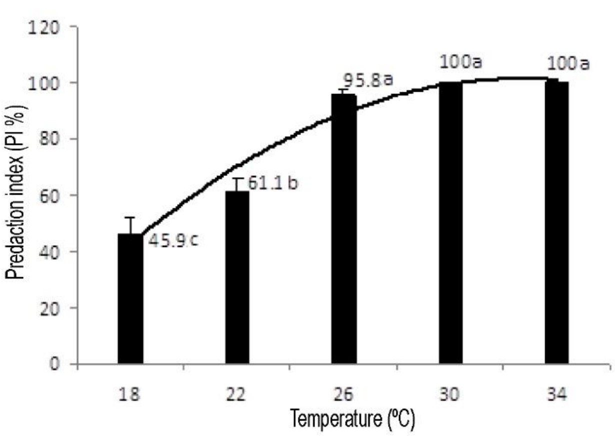

Figure 1. Predation index (PI) of pearl cichlid *Geophagus brasiliensis* on the snail *Pomacea canaliculata* at different temperatures (°C). The small vertical bars represent the standard deviations, and the values at the top of the black bars are the average PI values. The letters indicate the differences according to the Tukey test ($P < 0.05$).

Table 1. Proximal composition (dry matter) of the *P. canaliculata* snails, analyzing protein, fat, ashes and nitrogen free extract (NFE). Different letters beside the averages in the same column indicate significant differences according to the Tukey test. (*) = T2 with prewash; = T1 without washing, with the biofilm attached.

Snails*	Humidity	Proximal composition on a dry basis			
		Protein	Fat	Ashes	NFE
T1	87.96^a	17.33 ± 2.36^a	0.97 ± 0.68^a	71.07 ±0.11^a	10.66 ±2.44^a
T2	91.50^a	18.37 ± 1.04^a	3.17 ± 0.10^b	66.41±0.16^b	12.05 ±2.32^a

fish, containing 2.25 times more energy than carbohydrates. Using the presented equation, a curve was obtained showing probable meeting points between the temperatures and the calculated PIs. The generated R^2 (0.95) demonstrates the strong influence of temperature on the consumption of *P. canaliculata* by *G. brasiliensis*.

DISCUSSION

The range of thermal tolerance in waters inhabited by fish is from 4 to 25°C, which is greater than the range observed for fish in a tropical climate (25-28°C) or cold waters (4-15°C) (Frascá-Scorvo *et al*., 2001). *G. brasiliensis* is a species that shows good adaptability to the variety of climates found in Brazil. However, it was evident that the snail *P. canaliculata* was subject to greater predation at higher temperatures. García *et al.* (2008) reported that the water temperature in the period from 1996-2004 ranged from 16 to 28°C in summer (below 9°C in the coldest months in winter and between 14 and 21°C in spring).

Predation of *P. canaliculata* by *G. brasiliensis* was stimulated by elevated temperatures, and a satisfactory rate of predation was achieved in the treatment with a temperature of 26°C. The low external temperature maintained during the course of this experiment had no effect because the tests were performed in an air-conditioned environment and because the water temperature did not change during the 18 h of observation. However, at a treatment temperature of 18°C, the interest of the pearl cichlid on the provided food (snail) did not appear to be stimulated, thus resulting in a low rate of predation. The interest of the fish in predation increased slightly at a temperature of 22°C, though remaining significantly lower than that observed above 26°C, where the PI approached 100%. Based on tests conducted at the LAC, the activity of *G. brasiliensis* as a biological control agent preying on *P. canaliculata* rice pest might be effective because the rice-planting period coincides with temperatures that are conducive to the consumption of snails by fish, or to temperatures of 14 to 28°C, as reported by García *et al*. (2008).

Despite increasing levels of productivity of rice in Rio Grande South, the planting season is one of the practices which play a role featured in obtaining high and stable levels of productivity, because increasing the chances that the critical stages of plant escape the adverse and/or coincide with favorable climatic conditions (SOSBAI, 2007).

The development of rice growing, early-stage irrigation until early forming the panicle is affected more by water temperature that the air because of the yolks responsible for the development of leaves, tillers and panicles remain under water. Higher temperatures will cause damage to seedling with pre-germinated seeds, seedlings as not complete the germination process at elevated temperatures (>36°C). In this case, it is recommended the application of small water depths, more frequently, to reduce the temperature of the soil and stagnant water. Low temperatures (<20°C) impair early seedling development and the development of culture in the vegetative and reproductive stages (SOSBAI, 2010).

The data obtained in this study reveals that the predation index at temperatures of 26, 30 and 34°C is close to 100% for snails with a size of 3-5 mm. These temperatures are commonly observed and are very close to the records of García *et al*. (2008) in the hottest period of the year in some cities of Rio Grande do Sul. Thus, it can be inferred that the snails on the rice crop will be preyed upon by pearl cichlids when their shells measure 3-5 mm, *i.e.*, before reaching a size at which they are able to promote greater devastation in cultivated rice, which only occurs when the snails have measured more than 25 mm (Sin, 2003).

While the fish primarily feed to meet their energy needs (Pereira-Da-Silva *et al*., 2004), the voluntary intake of food can be influenced by the amount of energy in the diet. Lipids are good energy source for fish, containing 2.25 times more energy than carbohydrates. However, the snails used in the experiment constitute a food with low lipid content in relation to the quantity required for omnivores, as demonstrated in studies on trout, salmon and catfish. In nature, the amount of fat in the diets of these fish varies from 2 to 20% (Santos, 2003). In the proximate analysis, it was found that *P. canaliculata* contains 0.97 to 3.17% lipids (Table 1) via the method of cold fat extraction using solvents (AOAC International, 2002). It can be inferred that the observed responses of *G. brasiliensis* regarding its rate of predation were conditioned by the stimulatory effects of the temperatures at which the fish were held, rather than a potentially greater need to consume energy.

CONCLUSION

To decrease the incidence of *P. canaliculata* snails as pests in rice plantations, it is recommended the use of *G. brasiliensis* as a biological predator in the period when temperatures are above 26°C (rate = 95.8% predation on snails measuring 3-5 mm); which coincides with the temperatures recorded at the time of planting rice in southern Brazil. The predation rate decreases at lower temperatures, as occurs at 22°C and 18°C.

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