# Sodium chloride as reducing agent of stress induced in guppy *Poecillia reticulata* Peters, 1859

*Cloreto de Sódio como agente redutor de estresse induzido em Poecillia reticulata Peters, 1859*

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## Abstract

**Objective** – To evaluate the effects of sodium chloride to control induced stress in juvenile *Poecillia reticulata*. **Methods** – The animals were grown in a closed system, monitored by parameters: temperature, pH, conductivity, turbidity, dissolved oxygen and color. Thermal stress was induced by temperature variation using ice and hydric stress. Stress was induced by temperature variation, using ice and water, after standardization of mortality, NaCl was used as a potential agent for prevention of the harmful effects of stress in different concentrations of 1, 3 and 5g/L. **Results** – The results showed a beneficial effect of adding NaCl at concentrations between. Without the addition of salt, the mortality was 43% and after use of NaCl, there was a decrease in the mortality of 19 % (1g/L), 24 % (3g/L) and 28% (5g/L), of NaCl. **Conclusion** – The authors concluded that the addition of sodium chloride, and reduce stress, is a product easily available and inexpensive, which facilitates its use by developers.

**Descriptors**: Acclimation

## Resumo

**Objetivo** – Avaliar o efeito do cloreto de sódio (NaCl) para controlar o estresse induzido em juvenis de *Poecillia reticulata*. **Métodos** – Os animais foram cultivados em sistema fechado e monitorados pelos parâmetros: temperatura, pH, condutividade, turbidez, oxigénio dissolvido e cor. O estresse foi induzido por variação de temperatura, utilizando gelo e estresse hídrico, após a padronização da mortalidade, foi utilizado o NaCl como um possível agente de prevenção dos efeitos prejudiciais do estresse em diferentes concentrações de 1, 3 e 5g/l. **Resultados** – Os resultados mostraram um efeito benéfico da adição de NaCl a concentrações intermédias. Sem a adição do sal, a mortalidade foi de 43% e após a utilização de NaCl, houve uma redução da mortalidade para 19, 24 e 28% a respectivas concentrações de 1, 3 e 5g/l de NaCl. **Conclusão** – Os autores concluíram que a adição de cloreto de sódio, além de reduzir o estresse, é um produto de fácil obtenção e de baixo custo, o que favorece o seu uso por criadores.

**Descritores**: Aclimatação

## Introdução

Epicontinental aquatic environments are characterized by dynamic processes with variations in the concentrations of dissolved oxygen, pH, conductivity and other physical-chemical parameters[1]. Such changes are capable of causing stress and reduce the ability to maintain homeostasis of organisms. The stressful factor has been a major cause of decreased productivity in fish farms, reflecting in the animal's metabolism causing slow the growth and carcass yield[2].

The responses of stress are presented in three stages denominated in together as Adaptation Syndrome General constituting a series of biochemical changes and physiological. The first is alarm phase, consisting in physiological reactions that occur in order to compensate the disorder, the second stage is the resistance, which represents the physiological responses adjustment or compensatory aiming once again achieve homeostasis, and last one is the exhaustion that occurs when the duration or severity caused by exposure to agent promoter of stress exceeds the limits, may cause pathology or death[3A-3B-4-5].

Considering that antibiotics are expensive, and that inappropriate use of these can select pathogen resistant strains[6] and be considered a source of environmental pollution[7]. In this scenery, the use of prebiotics acquires great importance, maximizing animal welfare and some resistance modulation mechanisms of specific and nonspecific defense against opportunistic pathogens by increasing the absorption of food nutrients reducing physiological changes such as hyperglycemia and hypercortisolemy, providing alleviation stress[2] reducing costs and favoring the profitability, so supplements promising easy marketing and use[8].

The aim of this study it was to evaluate the effects of NaCl to control induced stress in juvenile *P. reticulata* and standardize mortality (LD50) to a fish widely distributed and easily accessible in order to minimize or prevent the deleterious effects induced by stress.

## Methods

Adult specimens of *Poecillia reticulata,* Peters, 1859 were grown in a closed system. Juveniles with approximately 0.8cm (± 0.2cm) of total length were used in the experiment conducted in aquariums with a capacity of 1000ml using the volume of 600ml. All specimens were fed with 55% crude protein in the first month of life and 36% protein in the diet of juveniles and adults.

To evaluation the total size has been adapted the anesthesia protocol used by[9]. The fish were transferred

the aquarium to another with 500ml of water and 3ml of the stock solution (Eugenol® 1ml, 0.5ml of absolute ethanol and 10mL of distilled water). The period of anesthesia was approximately 1 minutes and 40 seconds, which was already possible to perform the measurement of the animals with the aid of a caliper King Tools® 502,200 BL. Immediately after the evaluation, the fishes were placed in the home aquarium with aeration until complete recovery, that occurred about five minutes after anesthesia.

The environment was monitored by measuring the physical and chemical parameters: temperature, pH, conductivity, turbidity, color and dissolved oxygen. The measurements were performed by electrometric methods: HandLab OX1 O2 meter Schott®; meter pH meter-Tec 2® Tecnical; Conductivity of Tecnical 4MP-Tec®; Turbidity AP 2000 Policontrol®, Colorimeter Aquacolor of Policontrol® and HandLab OX1 O2 meter Schott®, respectively.

The aquariums were kept at room temperature (25 ± 2°C). The thermal stress occurred with a significant decrease in the temperature with addition of ice (made with water from the aquarium). Sometime later the fishes were subjected to water stress, being kept on paper towels for 90 seconds, where a mortality rate was expected near 50% of the sample LD50 (Lethal Dose for 50% of subjects tested). Was used as a standard protocol to induce stress and thus there would be a treatment aiming at prevention of mortality caused by stress. Otherwise, there would increase the amount of ice and / or time of water until it was obtained a value close to the desired mortality.

The combinations between time out of water and/ or application of ice both are important factors and recurrent stress for fishes[2], here we performed[13] experiments types: Control Group; Experiment 1) 60 seconds out of water; Exp. 2) 90 sec. out of water; Exp. 3) 180 sec. out of water; Exp. 4) Added 20 ml of ice; Exp. 5) Added 20 ml of ice and ± 30 sec. out of water; Exp. 6) Added 20 ml of ice and ± 60 sec. out of water; Exp. 7) Added 20 ml of ice and ± 90 sec. out of water; Exp. 8) Added 40 ml of ice and ± 90 sec. out of water. Exp. 9) Added 60 ml of ice and ± 90 sec. out of water; Exp. 10) Added 80 ml of ice and ± 90 sec. out of water; Exp. 11) Added 80 ml of ice and ± 90 sec out of water witch 1g NaCl; Exp. 12) Added 80ml of ice and ± 90 sec out of water witch 3g NaCl; Exp. 13) Added 80 ml of ice and ± 90 sec. out of water witch 5g NaCl.

For the experiment which utilized NaCl as a preventive agent deleterious effect of stress, aquariums were used at the concentrations of 1 (Exp. 11), 3 (Exp. 12) and 5 (Exp. 13) grams NaCl per liter. After adding sodium chloride to the acclimatization period was set at three days. We performed a total of 26 repetitions with each of the methods proposed up to the LD50 was reached, and 520 used a total of 20 individuals per experiment. On the fourth day the experiment for stress induction was performed.

The morphometric analysis data were done using the program Minitab® and the analysis of variance were performed to compare the total length of dead and survivors individuals. The results were analyzed through the survival rate obtained for each treatment after the induction of stress with and without sodium chloride as an agent for preventing stress.

This study it was submitted and approved by Ethics Committee local (protocol number 198).

## Results and discussion

The creation of anesthesia protocol was efficient for the species and from that measurement of all individuals used in the experiments. Statistic tests showed that the variation the size does not represented an survival factor for individuals, but random events, linked to intra-population variation and plasticity of the population, 10 show significant differences in the different wild populations of *Poecillia reticulata*, when phenotypic and genotypic variables are in question. For individuals who survived, the average total length was 7,988 ± 1,361mm, 2,556 mm versus 8,304 ± of those who died, with p-value equal to 0.475. Within the range of variation studied, the size did not appear as a factor of influence on the survival of *P. reticulata* when subjected to stressors.

When analyzing the values of water temperature in the control group and the experiments in which there was no addition of ice, it was observed average temperature of 25.04 ± 0.61°C. All experiments until the tenth no significant changes in the values of electrical conductivity, turbidity, color and dissolved oxygen percentage of aquariums before and after the experiment. The same way to mortality rate has not reached proposed by this work.

In all experiments performed the pH change was not statistically significant with mean values of 6.1 (± 0.5) before the experiment and 5.9 (± 0.4) after the experiment. Even though this parameter scale is logarithmic and that the pH 5 is 10 times more acid that your pH 6, the variation of 0.2 units has little biological significance, and the animals are capable of acclimation, that is to shape the environment showing variation, this capability being genetically determined[11-13] found that during transport of tambaqui (*Colossoma macropomum*) the physico-chemical parameters measured also remained with low variation before and after treatment.

The experiment 10 neared the LD50 being used as standard with 43% mortality in an "n" of 37 individuals, the temperature variation was approximately 9 + 1°C the other variables showed no significant change during this experiment.

It sets out the 11, 12 and 13 using NaCl, had initial average temperature of 25.1°C (± 0.4) and the final mean temperatures respectively of 17.1°C (± 1) 18.1°C (± 1) and 18.5°C (± 1).

Higher the variation in temperature was greater mortality rate within the population, the fish has an optimal temperature range and tolerance is variable between species, stage of development and acclimation period in which they were submitted[14]. These attributes are

themselves known phenotypic plasticity may be adaptive adjustment which increases the half of the individuals[15]. Various environments subject to changes like time and space, such as aquarium fish that were tested represent a micro-environment that was subject to variations in physic-chemical parameters constantly whether by experiments or by the normal fluctuation of these parameters by seasonality, the theory of "habitat template"[17] proposes that temporal and spatial variability of habitat influences the evolution of behavior, physiology and life history characteristics of the species[18]. As the test subjects are all the same species, were at the same stage of development (juvenile) and were previously acclimated to laboratory conditions. Thus, one cannot attribute mortality to these external factors.

In nature animals are free to escape these adverse conditions that represent stress, like in fish farm animals are confined, the EMBRAPA[19] recommends that the ideal temperature for a good production quality is maintained between 26 and 28ºC, rapid changes in temperature by water dynamics and temporal factors are increased stress to the fish, which decrease productivity. The temperature variation caused exceeded the limits of optimal temperature, thus verifying high mortality rates. Evolutionary pressures shape the ecological strategies of a population where the middle is the model of those changes. In contrast, the phylogenetic characteristics limit some specific characteristics, regardless of habitat[20].

Fish subjected to low temperatures led certain period of time so that there was a compensatory response to heat stress which were undergoing. According to 21 fish thermoregulation is activated second or minutes after the heat stress, but adaptation can take hours, days or weeks. This Fact that observed during the experiments in which a portion of 60% of the surviving population continued to be tested, resisting better to the induction of stress than fish that replaced the dead.

It was observed that there was a decrease in the mortality rate and reducing the deleterious effects induced by stress, by adding sodium chloride to the corresponding low concentrations of 1 and 3 g/l NaCl (Figure 1). All experiments were performed out in triplicates, and tested a total of 60 individuals of P. reticulata for the control group and 60 for the other test groups of each experiment.
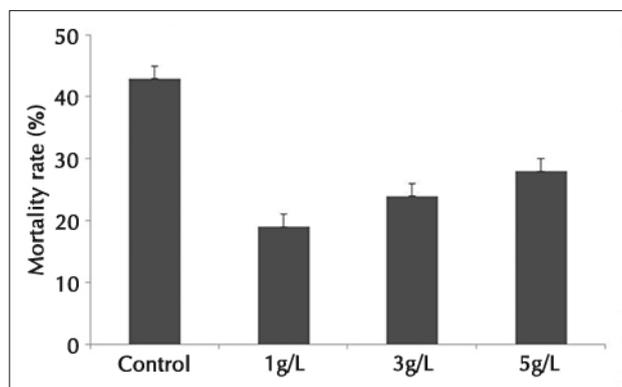


**Figure 1. Mortality rate in the control group and the various concentrations of sodium chloride during the experiment.**

The experiment was based on NaCl table proposed by[22], where 0.5 to 0.8% salt concentrations already have beneficial effects for the maintenance of osmoregulation in freshwater fish and stress relief resulting from handling and transportation of these animals. The presence of sodium ions ($Na+$) in water favors the mechanism of active transport of ammonium in the blood of fish to water. In active transport occurs at the entrance of a sodium ion and an ammonium ion output, favoring the elimination of ammonia even negative gradient of ammonia concentration. It promotes good results will aquaculture minimizing stress-related problems and health issues of the activity.

The number of experiments 11, 12 and 13 corresponding concentrations of 1, 3 and 5g/l NaCl showed positive results in reducing mortality stress, accordingly, there was a further fall to a lower concentration (19 ± 2%) and intermediate (24 ± 4%) [23]obtained similar results for matrinxã – Brycon cephalus. After four hours of transport density of 150kg/m³, using 6g of salt / L, with a suppression of stress responses. Sodium chloride at the highest concentration tested (8g/L water) was efficient to remove most of the physiological responses to stress tambaqui – Colossoma macropomum[24]. The use of sodium chloride as a reducing agent of stress is widely spread in aquaculture to approximate the osmotic pressure between the middle and the plasma inside the freshwater fish are hypertonic in relation to the external environment (concentration of dissolved salts in freshwater is about 5 g/L) so that there is a reduction in diffusion of ions into water[24]. The salt also stimulates the secretion of mucus on the gills epithelium, hindering the passage of ions through the cell membranes[25]. Besides reducing stress, salt also has prophylactic effect easy to obtain and inexpensive, with proven efficacy in several species[26-28].

## Conclusion

With the obtained results we can conclude that the anesthesia protocol was efficient for P. reticulata.

The initial temperature does not interfere in the mortality rate, but this variation by external factors, that the temperature variation is a factor highly stressful and lethal for P. reticulata, but should take into account other physical and chemical parameters in the context in which the fish live, because despite low significance and/or little variation of this parameters in this study are also stress factors and lethality.

And that the reduction of deleterious effects caused by stress can be reduced preferentially with the addition of NaCl in the concentration of 1 and 3g/L.

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