Revista ARACÊ
ISSN: 2358-2472

MORTALITY RATE OF MELANOIDES TUBERCULATA (O. F. MÜLLER, 1774), UNDER UNCONTROLLED LABORATORY CONDITIONS, IN FRONT OF DIFFERENT LAMPS

TAXA DE MORTALIDADE DE MELANOIDES TUBERCULATA (O. F. MÜLLER, 1774), EM CONDIÇÕES DE LABORATÓRIO NÃO CONTROLADAS, DIANTE DE DIFERENTES LÂMPADAS

TASA DE MORTALIDAD DE MELANOIDES TUBERCULATA (O. F. MÜLLER, 1774), EN CONDICIONES DE LABORATORIO NO CONTROLADAS, FRENTE A DIFERENTES LÁMPARAS

https://doi.org/10.56238/arev7n6-302

Submitted on: 05/26/2025 **Publication Date:** 06/26/2025

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Revista ARACÊ
ISSN: 2358-2472

ABSTRACT
Humanity has always had a close relationship with mollusks. They are an important source of protein in food, and are used for decoration, aquarium hobby and jewelry production. In addition to maintaining the balance of ecosystems, they can act as hosts for trematodes of medical and veterinary importance. Numerous fish find valuable sources of food and energy in these animals. Among this biodiversity is Melanoides tuberculata, frequently used as an object of study for numerous purposes. As with most organisms, light is an important factor that affects not only their behavior and biology. This study aimed to measure the possible effects of different types of lamps on M. tuberculata. The specimens were captured in Rio Grande, near Água Comprida/MG. In the vivarium, five animals were separated into oxygenated aquariums and the physicochemical parameters were measured. Six experimental groups were used, each subjected to a different type of light as a control. The treatments were an ultraviolet C lamp; an ultraviolet A lamp; an incandescent lamp; a cool white compact fluorescent lamp; and a warm white compact fluorescent lamp. The measured variables behaved as expected, with no statistically significant fluctuations. The survival of the animals fluctuated significantly, according to the treatment used. The parametric test indicated a “p” value of 0.000. Thus, it is interpreted that the variations were not random, but rather, attributed to the different treatments. The UVC lamp caused the death of all organisms. The treatment with the highest survival rate was the warm white compact fluorescent lamp.

Keywords: Tropical freshwater gastropoda. Malaysian trumpet snail. Invasive freshwater Thiaridae. Biodiversity. Invasive species control. Ecosystem resilience.

RESUMO
A humanidade sempre teve uma relação próxima com os moluscos. Eles são uma importante fonte de proteína na alimentação, sendo utilizados para decoração, aquarismo e produção de joias. Além de manterem o equilíbrio dos ecossistemas, podem atuar como hospedeiros para trematódeos de importância médica e veterinária. Inúmeros peixes encontram nesses animais valiosas fontes de alimento e energia. Dentre essa biodiversidade está Melanoides tuberculata, frequentemente utilizada como objeto de estudo para diversos fins. Como na maioria dos organismos, a luz é um fator importante que afeta não apenas seu comportamento e biologia. Este estudo teve como objetivo mensurar os possíveis efeitos de diferentes tipos de lâmpadas sobre M. tuberculata. Os espécimes foram capturados em Rio Grande, próximo a Água Comprida/MG. No biotério, cinco animais foram separados em aquários oxigenados e os parâmetros físico-químicos foram mensurados. Foram utilizados seis grupos experimentais, cada um submetido a um tipo diferente de luz como controle. Os tratamentos foram: lâmpada ultravioleta C; lâmpada ultravioleta A; lâmpada incandescente; lâmpada fluorescente compacta branca fria; e lâmpada fluorescente compacta branca quente. As variáveis medidas se comportaram conforme o esperado, sem flutuações estatisticamente significativas. A sobrevivência dos animais flutuou significativamente, de acordo com o tratamento utilizado. O teste paramétrico indicou um valor de "p" de 0,000. Assim, interpreta-se que as variações não foram aleatórias, mas sim atribuídas aos diferentes tratamentos. A lâmpada UVC causou a morte de todos os organismos. O tratamento com maior taxa de sobrevivência foi a lâmpada fluorescente compacta branca quente.

Palavras-chave: Gastrópodes tropicais de água doce. Caracol trombeta da Malásia. Thiaridae invasor de água doce. Biodiversidade. Controle de espécies invasoras. Resiliência do ecossistema.

RESUMEN
La humanidad siempre ha tenido una estrecha relación con los moluscos. Son una fuente importante de proteínas y se utilizan para la decoración, la acuariofilia y la fabricación de joyas. Además de mantener el equilibrio de los ecosistemas, pueden actuar como hospedadores de trematodos de importancia médica y veterinaria. Numerosos peces encuentran en estos animales valiosas fuentes de alimento y energía. Entre esta biodiversidad se encuentra Melanoides tuberculata, objeto de estudio frecuente para diversos fines. Como en la mayoría de los organismos, la luz es un factor importante que afecta no solo su comportamiento y biología. Este estudio tuvo como objetivo medir los posibles efectos de diferentes tipos de lámparas en M. tuberculata. Los especímenes fueron capturados en Río Grande, cerca de Água Comprida/MG. En el vivero, cinco animales fueron separados en acuarios oxigenados y se midieron sus parámetros fisicoquímicos. Se utilizaron seis grupos experimentales, cada uno sometido a un tipo de luz diferente como control. Los tratamientos fueron una lámpara ultravioleta C; una lámpara ultravioleta A; una lámpara incandescente; una lámpara fluorescente compacta de luz blanca fría; y una lámpara fluorescente compacta de luz blanca cálida. Las variables medidas se comportaron como se esperaba, sin fluctuaciones estadísticamente significativas. La supervivencia de los animales fluctuó significativamente según el tratamiento utilizado. La prueba paramétrica indicó un valor de p de 0,000. Por lo tanto, se interpreta que las variaciones no fueron aleatorias, sino atribuidas a los diferentes tratamientos. La lámpara UVC causó la muerte de todos los organismos. El tratamiento con la mayor tasa de supervivencia fue la lámpara fluorescente compacta de luz blanca cálida.

Palabras clave: Gasterópodos de agua dulce tropicales. Caracol trompeta de Malasia. Thiaridae invasores de agua dulce. Biodiversidad. Control de especies invasoras. Resiliencia de los ecosistemas.

INTRODUCTION

The Phylum Mollusca has around 2,500 known species, being one of the richest phyla in living species (Pelli et al., 2022). Within this classification is the gastropod *Melanoides tuberculata* (O. F. Müller, 1774). Known as "asian snail" it is a strategic ovoviviparous species, which allows it to maintain high reproduction rates. It had an operculum, which protects it from environmental elements, allowing it to resist in periods of drought (Shuhaimi-Othman et al., 2012). According to Chagas, Barros & Bezerra (2018) these organisms can live in different habitats, occurring in perennial and temporary fresh and brackish water bodies. As it is not a native Brazilian species, these organisms are considered invasive (Camargo et al., 2022a; Camargo et al., 2022b; Camargo et al., 2022c).

Studies on invasive species are becoming increasingly frequent due to the negative effects that these species can have in the new environment. The introduction of invasive species can reduce or even eliminate native species, some of commercial importance, due to competition, character displacement or contamination by pathogenic agents, which can also cause public health problems (Rares, Brandimarte, 2014).

Such problems have been observed after the introduction of the invasive species *Melanoides tuberculata*, belonging to the Gastropod class and family Thiaridae, native to north and east Africa and southeast Asia. This species is present in several water bodies, both fresh and salt water (Vogler et al., 2012; Raw et al., 2016).

Studies indicate that this organism was introduced into the Americas around the second half of the 20th century, as a result of the aquarium trade (Lopes et al., 2021). The first records in Brazil were made in the city of Santos in 1967 (Chagas, Barros & Bezerra 2018). Since then, it has been found in several Brazilian states (Fernandez et al., 2003).

In the research carried out by Chagas, Barros & Bezerra (2018), the authors point out that this species does not cause harm to agriculture and fish farming but can cause damage to other native species of snails. On the other hand, *Melanoides tuberculata* organisms may have potential in studies of environmental bioindicators, due to their rapid adaptation and reproduction (Araújo et al., 2020).

One of the limiting factors for organisms is light. When crossing the layers of air and water, attributes are changed, with consequences for living beings (Camargo, Pelli, 2023). Light not only changes the behavior of animals and plants but also influences their

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reproductive period and distribution. In addition to the photo period, that is, the time of exposure to light, intensity and spectrum also affect it (Pelli et al., 2022).

Light is a type of electromagnetic radiation. It propagates in the form of waves, be it ultraviolet radiation types A, B or C, visible radiation (to human eyes) or infrared radiation. In order of decreasing wavelength or increasing frequency, waves are divided into: radio waves (low energy), microwaves, infrared, visible light, ultraviolet, X-rays and gamma rays (high energy) (Oliveira, Santos, Souza, 2022; Relyea, Ricklefs, 2021).

The exposure of organisms to light can be natural, with primary or secondary sources, or to artificial lights. The primary light sources are the sun, scattered landscape light, stars, and bioluminescent organisms. Naturally, fire, auroras and lightning still exist. However, these sources do not determine the patterns of distribution and abundance of organisms in time and space. The main secondary light source is the moon. In artificial sources, the most common are incandescent or fluorescent lamps, in different wavelengths (Oliveira, Santos, Souza, 2022; Pelli et al., 2022).

The exposure of organisms is also linked to the color temperature of the lamp, according to the ranges of light that each lamp will emit. Color temperature refers to the color appearance of the light emitted by the lamp, or other lighting source. The higher the color temperature on the Kelvin scale, the bluer the light; and the lower the temperature, the more yellowish the light is in relation to daylight (Figures 01 and 02).

FIGURE 1 - Relationship between color and temperature of lamps, according to NBR ISO 8995-1 (ABNT, 2013).

Color Appearance	Temperature
Hot	Below 3.300 K
Intermediate	3.300 K a 5.300 K
Cold	Acima de 5.300 K

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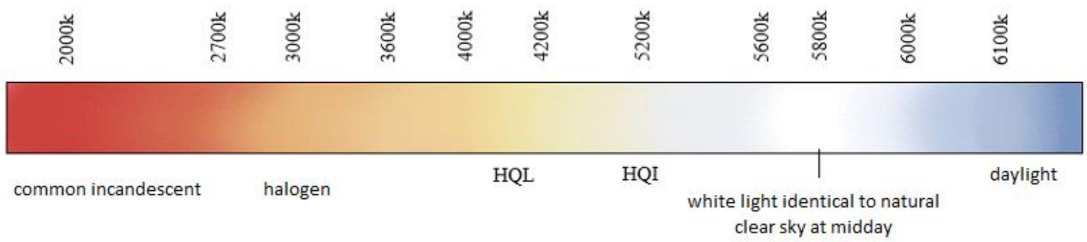
FIGURE 2 - Light bands emitted by commercial lamps, according to criteria adopted by NBR ISO 8995-1 1 (ABNT, 2013), in Brazil.



HQL: An Energy-efficient Mercury Vapor Lamp, offers lighting with high luminous flux; HQI: a Metallic Vapor Lamp is efficient in emitting light in the blue spectrum, with high intensity and penetrating power; ideal for plant growth.

The introduction of invasive species is directly and indirectly related to the biotic and abiotic factors of an ecosystem. One of these factors may be the incidence of different types of light waves. According to Pelli et al (2022), the light factors that interfere with an organism can be intensity, spectrum and duration.

In this sense, this research aimed to observe the effect caused to *Melanoides tuberculata* by different types of lamps.

MATERIALS AND METHODS

Specimens of *Melanoides tuberculata* (O. F. Müller, 1774) were sampled in Rio Grande, in the Municipality of Água Comprida - MG, with coordinates 20º05'27.7” S and 48º06'33.8” W. In this stretch, the Rio Grande is the border between the states of Minas Gerais and São Paulo (Fig. 03).

The mollusks were collected manually, placing the animals in plastic bags with water from the collection site. They were later transported to the UFTM Ecology & Evolution laboratory, approximately 50 kilometers away. Ninety-four individuals were collected along the bank.

At the Nico Nieser Vivarium, of the Ecology & Evolution Discipline, under the responsibility of biologist Afonso Pelli, samples from five animals were separated in each aquarium with an approximate volume of 800 ml. Each group of three small aquariums were placed inside six larger containers with a volume of approximately 19 liters. Thus, in each experimental group there was a large aquarium, with three smaller ones inside, closed and isolated from each other, but with the same circulating water.

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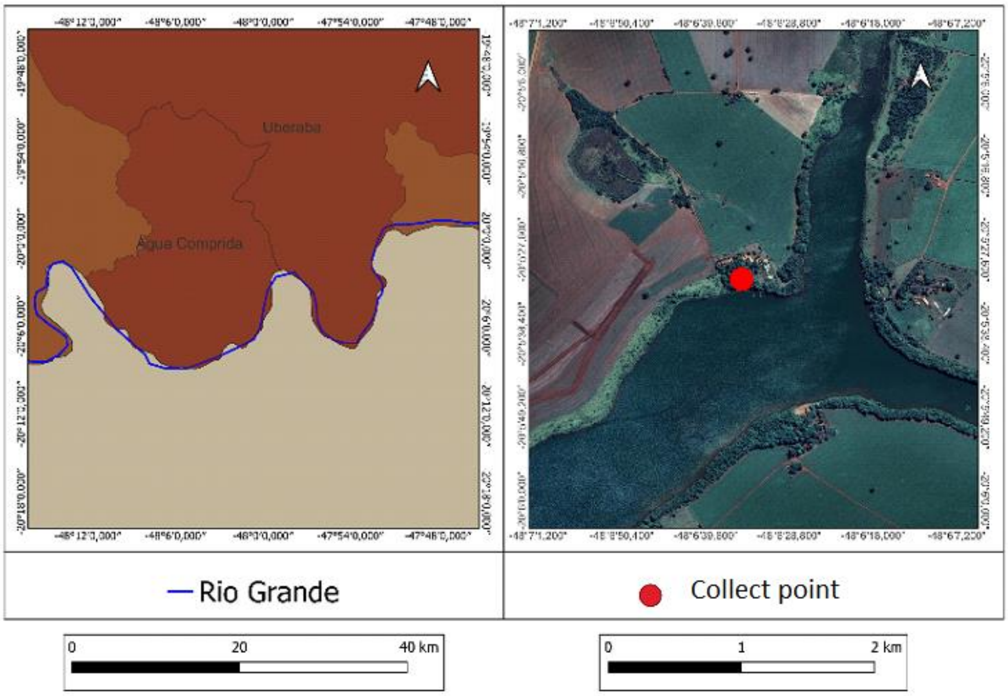
Figure 3 - Collection site for specimens of *Melanoides tuberculata* (O. F. Müller, 1774), sampled in Rio Grande, in the Municipality of Água Comprida/MG, in the State of Minas Gerais.



Water taken from the river was used to fill the containers, in order to avoid thermal and osmotic shock, partially maintaining the physical-chemical characteristics close to the organisms' natural habitat. The volume of the aquariums was filled with dechlorinated drinking water, left to rest for 4 days, with forced mechanical aeration.

The aquariums were equipped with a BOYU 110v motor pump, with a capacity of 300 liters per hour, with an attached mechanical and biological filter; and an oil-free blower with porous stone to force oxygenation. Thus, throughout the experimental period the water remained oxygenated close to 100%. The physical-chemical parameters of the water were measured with the aid of a Horiba U50® multi-parameter probe.

Each of the six larger aquariums was subjected to a different type of light, one of which was designated as control, without any lamp, just with ambient lighting. The lamps described in Table 1 were also used; a 12-hour light/dark photo period was used during the experiment.

The luminescence measurement described in Table 1 was carried out using the free application LUX Light Meter FREE from Nipakul Buttua®, measuring 8.1 MB and compatible with Apple operating systems. The equipment used was an Iphone 11®.

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The larger aquariums were covered with brown paper so that ambient light did not invade the interior of the aquariums. The incidence of the lamps was controlled using an analog timer programmed for 12 hours on/off. The One-Way ANOVA parametric test was used, an analysis of variance, which compares the means of two or more independent groups; determining whether the means are statistically different or not. When the data did not allow the presumption of normality, Pearson's Chi-Square Test ($\chi 2$) was used.

TABLE 1 - Description of lamps used in experiments in a partially controlled environment, exposing specimens of *Melanoides tuberculata* (O. F. Müller, 1774) to treatments: control (Contr); Ultraviolet C (UVC) lamp; Ultraviolet A (UVA) lamp; Incandescent lamp (Inca); Cool White Compact Fluorescent Lamp (LFBF) and Warm White Compact Fluorescent Lamp (LFBM).

Light bulb	Luminescence (LUX)	Power (W)	Temperature (K)
Contr	315	-	-
UVC	185	17	-
UVA	22	20	-
Inca	6.471	25	Warm light until 3.000K
LFBM	100.200	20	2.700
LFBF	115.052	20	6.400

Morphometric measurements were taken with a King Tools 502.150BL® digital caliper. The volume of the animals was calculated by the area of the cone, considering its extension and maximum diameter.

RESULTS AND DISCUSSIONS

The water temperature values of the experimental units were very close. However, the experimental unit with an incandescent lamp presented slightly higher values, 3.6 °C above the control and 1.65 °C above the experimental unit with a UVC lamp. The incandescent lamp, in the present experiment, had a slightly higher power than the UVC, but not enough to produce the observed differences. It is known that the incandescent lamp also emits light in the infrared wavelength, and has lower efficiency, dissipating part of the

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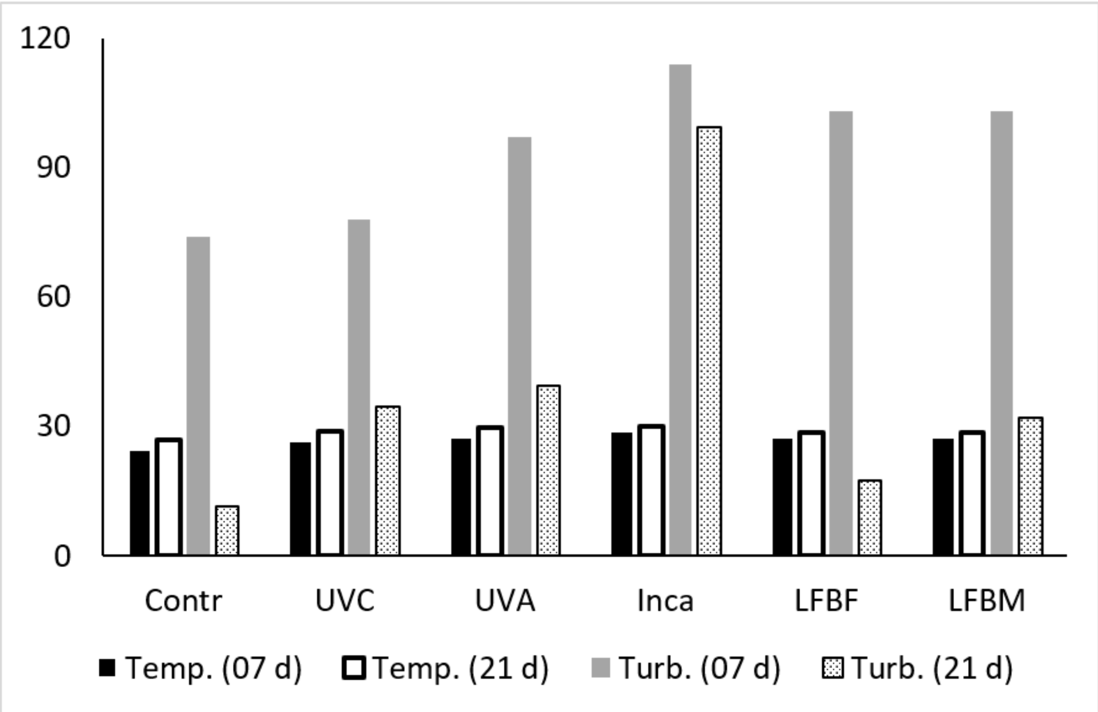
energy in the form of heat. Perhaps for this reason the temperature values were higher in the experimental units with incandescent lamps (Fig. 04).

Turbidity values showed fluctuations between experimental units. The highest values were observed in the Incandescent lamp (Inca) treatments; Cool white compact fluorescent lamp (LFBF) and Warm white compact fluorescent lamp (LFBM) (Fig. 04). In Inca treatments; LFBF and LFBM, were compared to the control, the values were higher 64; 17.55 and 24.85 NTU, respectively (Fig. 04).

The Control experimental unit was not covered, and was exposed to natural lighting, inside the laboratory and a few meters away from the window. Again, the incandescent lamp presented higher average turbidity values. In this case, the highest values are probably attributed to plankton production, favored by the attributes of the lamp that interfere with vegetable production: intensity, duration and spectrum (Pelli et al., 2022; Relyea, Ricklefs, 2021). Therefore, if the premise is correct, the lamps closest to natural conditions would be, in descending order, Inca; LFBM and LFBF. On the other hand, lamps within the ultraviolet range would be further away from natural conditions.

Figure 4 - Temperature (°C) and turbidity (NTU) values in the control experimental units (Contr); Ultraviolet C (UVC) lamp; Ultraviolet A (UVA) lamp; Incandescent lamp (Inca); Cool White Compact Fluorescent Lamp (LFBF) and Warm White Compact Fluorescent Lamp (LFBM); on experimental days 07 and 21 for *Melanoides tuberculata*.



For electrical conductivity values, close values were observed between treatments, except for the incandescent lamp treatment, which presented much higher values, with an average greater than twice that of the other treatments (Fig. 05).

A similar pattern was observed for total dissolved solids (mg/L), when a value almost 50% higher than the other treatments was observed.

For variations in the oxidation-reduction potential (POR), very close values were observed for all treatments, not indicating that the lamps could, in any way, interfere with this variable, at least for the period that the experiment lasted.

Hydrogen potential is measured on a logarithmic scale, so small variations present, or may present a biological significance. The values were slightly higher in the Inca and LFBM treatments (Fig. 06). The authors attribute this small difference to the primary production carried out by phytoplankton. During primary production, the medium tends to become more alkaline, as pointed out by Pelli and collaborators (2022).

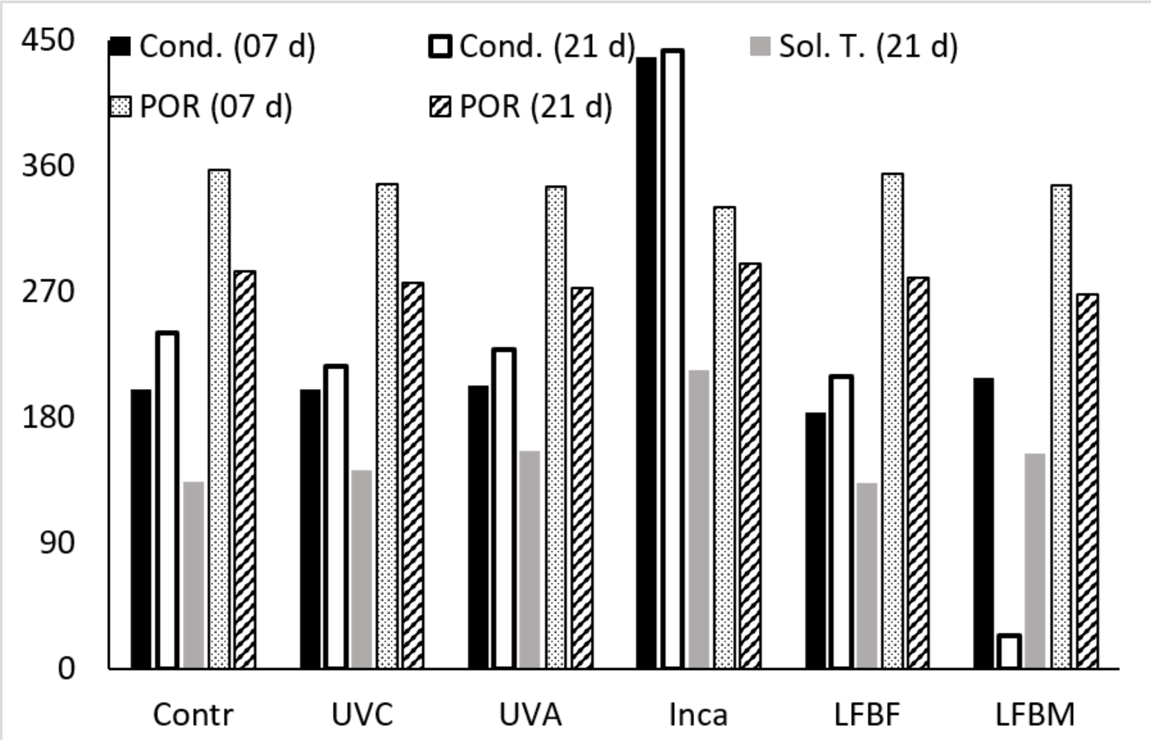
The ultraviolet rays emitted by the sun are responsible for causing part of the photo cutaneous changes (FLOR et al. 2006). Damage caused to the skin occurs due to the formation of free radicals, as a consequence of the interaction between UV radiation and reactive oxygen species.

UV radiation can be divided into three parts, according to the region of the wave spectrum in which it is found. The first of these is UVA (400-320 nm), which contains almost all natural UV rays, being responsible for inducing skin pigmentation and may also induce skin cancer.

Next, there is UVB (320-280 nm), which causes premature aging of cells, which can damage DNA and suppress the skin's immune response, in addition to causing sunburn. UVA radiation affects the Earth's surface more intensely than UVB (Oliveira, Santos, Souza, 2022).

Finally, UVC (280-100 nm), which, as it has more energy, becomes extremely harmful to living beings. But in the stratosphere, it is absorbed by oxygen and ozone, so almost no UVC radiation reaches the Earth's surface (Khalil et al., 2020).

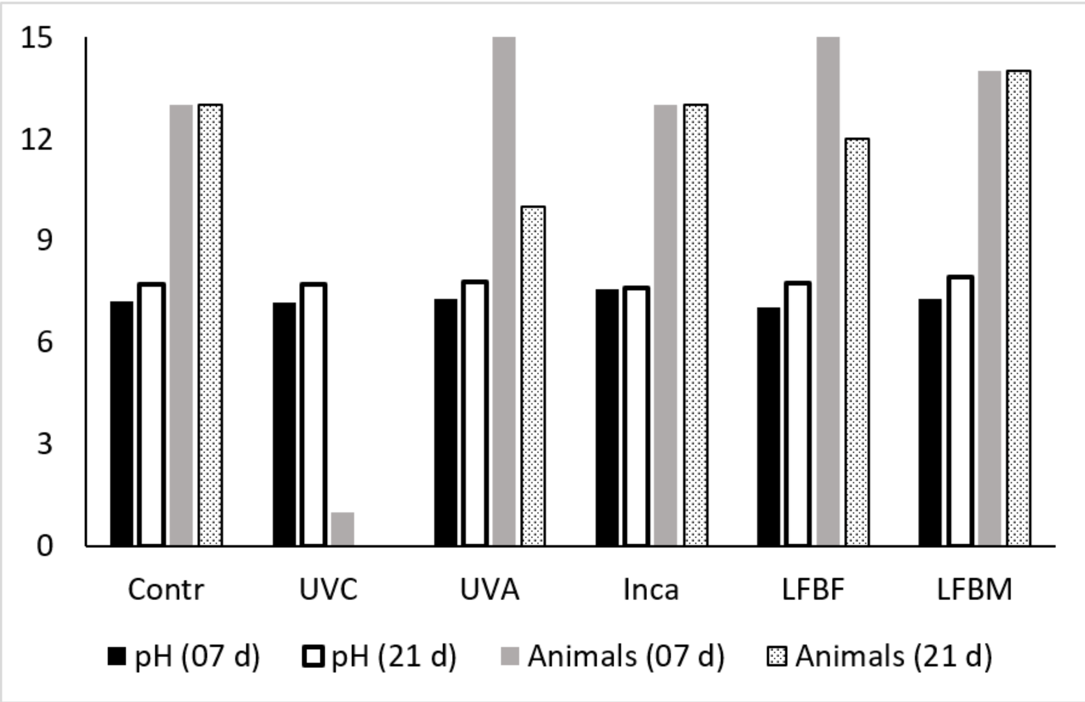
Figure 5 - Electrical conductivity values (µS/Cm); total dissolved solids (mg/L) and redox potential (POR) in the control experimental units (Contr); Ultraviolet C (UVC) lamp; Ultraviolet A (UVA) lamp; Incandescent lamp (Inca); Cool White Compact Fluorescent Lamp (LFBF) and Warm White Compact Fluorescent Lamp (LFBM); on experimental days 07 and 21 for *Melanoides tuberculata*.



Animal survival varied between treatments (Fig. 06). The treatment with a Warm White Compact Fluorescent Lamp (LFBM) showed a higher survival rate (average of 4.67 live animals) than the Control, with an average of 4.33. Apparently, this system produced greater welfare for the animals. The third best treatment, when considering survival, was LFBF, with an average of 4 survivors, followed by UVA and Incandescent treatments with an average of 3.33. In the UVC treatment, survival was zero at the end of the experiment. Similar patterns were reported by Pelli et al. (2022) for the gastropod mollusk *Physa acuta*. However, *Physa acuta* does not have an operculum as in the case of *Melanoides tuberculata*. The authors consider that the operculum could provide protection for the animals, but in the present study this possible benefit was not observed.

The number of individuals alive at the end of the experiment differed significantly. When using the parametric test for analysis of variance, comparing the means of the treatments; a “p” value equal to 0.000 was obtained, indicating a probability of less than one millionth that the differences are random.

Figure 6 - pH values and number of *Melanoides tuberculata* specimens in the control experimental units (Contr); Ultraviolet C (UVC) lamp; Ultraviolet A (UVA) lamp; Incandescent lamp (Inca); Cool White Compact Fluorescent Lamp (LFBF) and Warm White Compact Fluorescent Lamp (LFBM); on experimental days 07 and 21.



Considering that a “p” value equal to or less than 5% is statistically significant, when the treatments are analyzed two by two, it appears that the UVC Treatment was statistically different from the other treatments, except LFBF, as the numbers of individuals were equal; that is, without variance, the analysis cannot be carried out. However, in this case, it could be inferred that the 3 sets of 4 animals surviving are different from three sets of zero animals. Biologically, it can be said that the treatments produced discrepant results (Table 02). In this particular case, considering the variance as zero in both groups, the Pearson's Chi-Square Test ($\chi 2$) was performed. A probability of less than 5% that the differences were due to chance was indicated, with the difference between treatments being accepted as statistically significant.

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ISSN: 2358-2472

TABLE 2 - Comparison of means between treatments with *Melanoides tuberculata*, exposed to treatments: control (Contr); Ultraviolet C (UVC) lamp; Ultraviolet A (UVA) lamp; Incandescent lamp (Inca); Cool White Compact Fluorescent Lamp (LFBF) and Warm White Compact Fluorescent Lamp (LFBM); at the end of the experiment.

	Contr	UVC	UVA	Inca	LFBF
UVC	**0,000**				
UVA	0.101	**0.001**			
Inca	0.349	**0.019**	1.000		
LFBF	0.374	**5% >**	0.116	0.492	
LFBM	0.519	**0.000**	**0.047**	0.230	0.116

Numbers in bold indicate statistical significance for “p” values.

The possibility that UVC light not only causes irreversible damage to mollusks, but also to all aquatic biota present in the experimental units, cannot be ruled out either. In this way, the death of mollusks could be caused by inadequate food. However, the short time span of the experiment would not be enough to determine the death of the animals due to lack of food.

CONCLUSIONS

Water quality did not differ significantly between treatments. The Incandescent Lamp seems to have favored primary production.

The survival rate varied significantly between treatments. Treatment with the UVC Lamp resulted in the death of all animals. On the other hand, the treatment with the highest survival rate was the treatment with a warm white compact fluorescent lamp. This type of lamp could be the best, if the objective was to grow *Melanoides tuberculata* in the laboratory. However, the UVC lamp proved to be effective in any system that sought to control these animals.

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