DOI: 10.53660/CLM-1161-23D48

Ultrasonic Waves for the Control of Limnoperna Fortunei – The Golden Mussel

Ondas Ultrassônicas para o Controle do Limnoperna Fortunei – O Mexilhão Dourado

Received: 2023-03-12 | Accepted: 2023-04-20 | Published: 2023-04-29

Paulo Santos Assis
Universidade Federal de Ouro Preto, Brasil
E-mail: assis@ufop.edu.br
LATTES: http://lattes.cnpq.br/7360474381329605
Afonso Pelli
https://orcid.org/0000-0001-8279-2221
Universidade Federal do Triângulo Mineiro, Brasil
E-mail: afonso.pelli@uftm.edu.br
LATTES: http://lattes.cnpq.br/3020343973167468
Gabriela Araújo Gois
Universidade Federal de Ouro Preto, Brasil
E-mail: gabriela.gois@aluno.ufop.edu.br
LATTES: http://lattes.cnpq.br/1516799916565644
Ana Cristina de Carvalho
Universidade Federal de Ouro Preto, Brasil
E-mail: anecris23@yahoo.com.br
LATTES: http://lattes.cnpq.br/2479882630820278
Gislaine Soares Araujo
Universidade Federal de Ouro Preto, Brasil
E-mail: gislaine.araujo@aluno.ufop.edu.br
LATTES: http://lattes.cnpq.br/9109670189881887

ABSTRACT

In order to verify the effect of ultrasonic waves to control the invasion of the golden mussel, *Limnoperna fortunei*, three experiments were made with different numbers of individuals and for each experiment the sonicator device was using at 40kHz frequency. In addition, the tests varied in time and days of exposure of the mussels to ultrasonic waves. As a result, several numbers for mortality and decoupling of the analyzed samples were noted, with significant differences regarding the exposure time per experiment and days in which the samples were submitted. Thus, the use of ultrasound to descale and kill the golden mussels was efficient and may be an alternative to control the invasion of *L. fortunei*.

Keywords: Bivalve mollusks; Control; Fouling.

RESUMO

Com o objetivo de verificar o efeito das ondas ultrassônicas no controle da invasão do mexilhão dourado, *Limnoperna fortunei*, foram realizados três experimentos com diferentes números de indivíduos. Em cada experimento, o dispositivo sonificador foi utilizado em uma frequência de 40kHz. Além disso, os testes variaram em relação ao tempo e aos dias de exposição dos mexilhões às ondas ultrassônicas. Como resultado, foram observados vários números de mortalidade e descolamento das amostras analisadas, com diferenças significativas em relação ao tempo de exposição em cada experimento e aos dias nos quais as amostras foram submetidas. Portanto, o uso de ultrassom para remoção e controle do mexilhão dourado foi eficiente e pode ser uma alternativa para o controle da invasão de *L. fortunei*.

Palavras-chave: Moluscos bivalves; Controle; Incrustação.

INTRODUCTION

In 1991, *Limnoperna fortunei* (Dunker, 1857), also known as the golden mussel, was found at the mouth of the Rio de la Plata and has rapidly expanded along the Paraguay and Paraná rivers, mainly due to vessel traffic between Argentina and Brazil (DINIZ, 2010; RIBOLLI et al., 2021). This invasive species from Southeast Asia has spread to South America through ballast water of ships from China. The golden mussel's morphological characteristics and sessile nature contribute to its prolific proliferation (PAULA et al., 2021), which can cause environmental, social, and economic problems in non-native habitats (IBAMA, 2020).

The invasion of golden mussels has negative environmental consequences, impacting biomes, lakes, vegetation cover, and water quality. The release of organic material by golden mussels affects phytoplankton and zooplankton communities, leading to loss of habitat for some fish species and other organisms (IBAMA, 2020). Hydroelectric plants and net cages are particularly affected by golden mussel fouling (PAULA et al., 2021; COSTA et al., 2012). Control strategies, such as detecting and preventing larvae in ballast water, are crucial to mitigate further contamination and proliferation of the species (SANTOS; WÜRDIG; MANSUR; 2005).

The golden mussel has been categorized as an exotic and invasive species with social, economic, and environmental risks according to the National Strategy on Invasive Exotic Species and CONABIO Resolution nº 5 (MINISTÉRIO DO MEIO AMBIENTE, 2009). Control methods using ultrasonic waves in hydroelectric power plants are still being studied, but show promise in containing and controlling the proliferation of *L. fortunei* and mitigating its implications on the environment, society, and economy (PEREIRA, 2012).

This study aims to investigate the effect of ultrasonic waves on the control of golden mussels (Limnoperna fortunei). The specific objectives of the study include conducting and disseminating information about the use of ultrasonic technique for controlling golden mussels, reviewing the existing literature on the impacts of Limnoperna fortunei invasions on industries and environments, as well as the potential for control measures, and evaluating the influence of ultrasonic waves on the control of golden mussels at different time intervals.

ULTRASONIC WAVES

In Physics, the field of wave acoustics studies the characteristics of sound waves, such as intensity, frequency, and timbre, as well as amplitude (ALMEIDA & SILVA, 2005). Sound is defined as mechanical waves that propagate through matter, and it plays a vital role in the perception and adaptation of organisms to their environment (STEIN, 2017). Sound waves require a medium to propagate and do not travel through a vacuum (BISCEGLI, 2003). The frequency of a sound wave is measured in Hertz (Hz), representing vibrations per second, with the audible range for humans typically between 20 and 20,000 Hz (DADAM, 2019). Frequencies below 20 Hz are considered infrasound, while those above 20,000 Hz are ultrasound. Higher frequency sound waves tend to be louder (DADAM, 2019).

Ultrasound waves have high frequencies, starting at 20,000 Hertz (20kHz), and are mechanical waves that transmit energy through electrical pulses, with characteristics such as length, amplitude, period, and frequency, as stated by Bassoli (2001).

Those waves cause agitation in the molecules of the environment and produce oscillations in the medium they propagate through, including liquids, solids, or gases, resulting in "deep heat by the propagation of its mechanical waves, which are essentially the same as sound waves, but with a higher frequency" (BLUME et al., 2005).

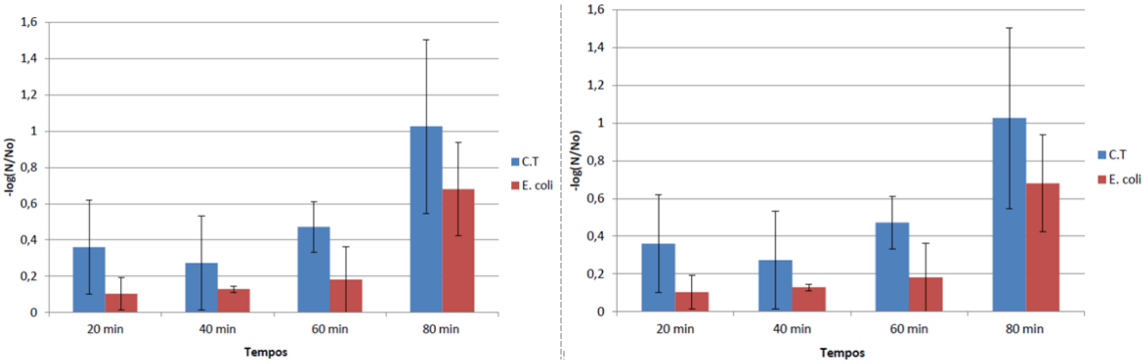
Ultrasonic techniques, known as sonolysis, induce changes in chemical structures and have various applications in environmental protection, such as the removal of chemical contamination (ROCHA, MAINIER, & ALVES, 2011). These techniques are based on the formation of high-frequency ultrasonic waves that cause cavitation, resulting from cycles of expansion and contraction of matter (DIAS et al., 2014). Sonochemistry, which utilizes ultrasonic bath devices, is another technique that operates at lower frequencies and accelerates precipitation reactions through the generation of high temperatures and pressures during the collapse of bubbles created by acoustic cavitation (TOMITÃO, 2018).

Acoustic cavitation is a significant effect of ultrasound, occurring at frequencies between 20kHz and 1000kHz, wherein bubbles form, grow, and collapse in the liquid medium. The collapse of these bubbles generates high energies, including pressures of hundreds of atmospheres and temperatures of thousands of degrees, both inside and in the vicinity of the bubble, resulting in chemical and mechanical effects on sonochemistry. The rise in temperature and pressure inside the bubbles, along with the shock wave produced by their collapse, create enormous shear forces in the surrounding liquid medium (RONCHI, 2014).

On the effect of cavitation, Pereira (2012) points out that this can cause the destruction of microorganisms in liquids through mechanical efforts. In the survey by Grando et al. (2016, p. 197), the authors understand that “the cavitation forces induced during bubble collapse are known to cause many physical and chemical effects, for example, the lysis of molecules, resulting in the formation of free radicals, increasing the rates of chemical reactions”.

Medeiros et al. (2019) studied the possibility of using ultrasound, specifically at frequencies of 25KHz and 40KHz, as an alternative disinfectant for sewage previously treated by an anaerobic process, with the intention of removing organic material – in the parameters of Chemical Oxygen Demand (COD). Then, they conclude that the longer exposure time to ultrasound has greater disinfectant power, as shown in Figure 1.

Figure 1 – Effect of the 25 KHz (left) and 40 KHz (right) frequency on the inactivation of total coliforms (C.T) and *Escherichia coli*.



Source: (Medeiros et al., 2019).

The different effects of ultrasound are influenced by numerous physical and biological parameters, including intensity, duration of exposure, spatial and temporal characteristics of the ultrasonic field, and physiological condition of the target tissue. The complex interplay of these variables makes it challenging to fully comprehend the precise mechanism of action of ultrasound in its interaction with biological tissues (BLUME *et al.*, 2005)

Grando *et al*. (2016) observed the use of ultrasonic waves in the clarification of dairy industry effluent, and perceived the coagulation/flocculation process associated with ultrasound as a promising technique. Statistical data from the study by authors Grando *et al*. (2016) pointed to a significant removal of turbidity and solids and reduction of organic matter in the samples, as well as an improvement in their appearance.

One of the methods of environmental ultrasonic treatment that has been studied is the use in ballast water of marine vessels. In this method, ultrasonic mechanical waves with frequencies ranging between 15 and 100 KHz are emitted into the water to eliminate the organisms present (PEREIRA, 2012).

Guimarães (2019) carried out research on the effect of ultrasound on the swimming behavior of meroplanktonic larvae in the nesting phase. Based on the results obtained, the author considers that ultrasound affects the behavior of *Cypris larvae* (infraclass *Cirripedia*, of the

Maxillopoda class), possibly causing a decrease in the settlement rate, reinforcing the possibility of using ultrasound as an antifouling method.

LIMNOPERNA FORTUNEI, THE GOLDEN MUSSEL

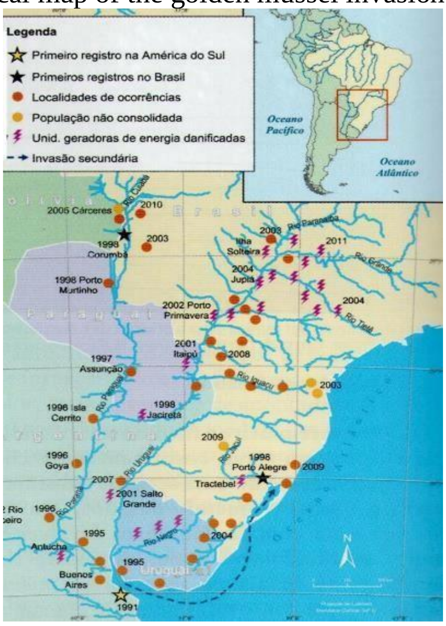
Known in Brazil as the golden mussel, the species called *Limnoperna Fortunei* (Dunker, 1857) is a freshwater mussel (Figure 2).

Figure 2 – Specimen of *L. fortunei*, collected in Rio Grande in April 2022.

Source: the authors (2022).

According to the taxonomy pointed out by Simas (2018), *L. fortunei* belongs to the *Animalia* kingdom and the *Mollusca phylum*. It falls under the class Bivalvia, order Mytilida, and superfamily Mytiloidea. Within the *Mytilida* superfamily, it belongs to the family *Mytilidae*. The genus name of the golden mussel is *Limnoperna*, and the species name is *fortunei*. Small in size, in its adult stage reaching sizes between 3 and 4 centimeters, this exotic species of bivalve mollusk originated in China and has expanded to other countries in Asia and South America (IBAMA, 2020). It feeds mainly on phytoplankton, zooplankton and suspended organic particles resulting from water filtration (CORDEIRO *et al*., 2016).

Figure 3 – Historical map of the golden mussel invasion in South America.



Source: Mansur *et al*. (2012).

The *L. fortunei* has three main life periods: larval, juvenile, and adult stages, with larval stages being considered the main stage for its propagation due to its easy dispersion by running water and traffic of colonized vessels (PEREIRA et al., 2019; SILVA, 2021). Larvae of *L. fortunei* preferentially encrust themselves in shaded areas of rocks, tunnels, cracks, and other conspecific-colonized sites, indicating site selection driven by adaptive advantages (IWASAKI, 2015).

Reproductive capacity and tolerance to extreme environmental conditions also facilitate the invasion of new habitats by *L. fortunei* (GIGLIO, 2016). Furthermore, *L. fortunei* has high tolerance to low temperatures, which may contribute to its invasion success (XIA et al., 2021). Similar means of dispersion have been observed in other biological invasions, such as zebra mussels and invasive corals Tubastraea spp. on oil/gas platforms and vessel hulls (BATISTA, 2018; SILVA, 2014).

> Ballast water has become, for the globalized world, a concern related to environmental issues. [...] Despite its importance for the safe operation of the ship, ballast water is also recognized, along with the biofouling present on the ship's hull (living works), as one of the main vectors for the introduction of potentially harmful aquatic species. invasive (BATISTA, 2018, p. 175).

Silva et al. (2021) highlight the extensive spread of *L. fortunei* in Brazilian rivers facilitated by vessel traffic and fishing activities without control measures, leading to rapid encrustation on boat hulls, bait, and equipment. The lack of measures and the species' rapid reproduction contribute to its proliferation for 6 to 10 months of the year. Golden mussels form macroclusters in natural habitats, with high densities observed in both natural and man-made structures.

According to the National Plan for the Prevention, Control and Monitoring of the Golden Mussel in Brazil (IBAMA, 2020), global changes and globalization have facilitated species invasions, including *L. fortunei*, which is a non-selective freshwater mollusk capable of inhabiting various aquatic habitats. Recent studies by Paula et al. (2021) report the established presence of golden mussels in South and Southeast regions of Brazil, with recent observations at the border of Bahia and Pernambuco states, highlighting the need for effective control measures to mitigate the spread of this invasive species.

Figure 4 – Cluster of golden mussels in substrate, collected in June 2022 (approximate scale referring to the average size of individuals).

Source: the authors (2022).

Golden mussel encrustations are voluminous, compact, and resistant to currents due to overlapping individuals attached to each other and to substrates by byssus filaments. *L. fortunei* settles on various substrates, including bivalves, gastropods, and crustaceans, causing harm to biodiversity (SANTOS et al., 2012, p. 27).

These clusters of golden mussels also accumulate in man-made structures, leading to economic losses (SILVA et al., 2020). Invasive *L. fortunei* in Brazil causes diverse impacts, from environmental to economic, due to its morphological characteristics, sessile nature, and tolerance to various conditions (PAULA et al., 2021; EL HAJ, BOHN, & SOUZA, 2019; KARATAYEV et al., 2007).

Poor conservation of aquatic environments in Brazil facilitates the invasion of *L. fortunei*, causing changes in ecosystems, competing with native species, altering food chains, and affecting water clarity (FREIRE; MARAFON, 2018; SILVA et al., 2020). The presence of *L. fortunei* also increases cyanobacterial flowering, reducing prey availability and fish feeding activity, and affecting fish embryonic development and survival (PAOLUCCI et al., 2017).

In Lake Guaíba, the first cases of *L. fortunei* invasion in Brazil were registered in 1998, leading to significant damages to native fauna, vegetation, and economic infrastructure (SANTOS; WÜRDIG; MANSUR, 2005).

One of those affected by the encrustation of the golden mussel is the hydrogenerators. In addition, among other components, hydraulic turbines are significantly affected (FELIX, 2011).

According to Guimarães (2019, p. 1), biofouling causes different effects, and these “have consequences both in the economic, environmental and social spheres, strengthening the need to develop preventive measures to the effects of biofouling”. Furthermore, as pointed out by Bergmann et al. (2010, p. 22),

> [...] the species attaches to substrates by means of byssus filaments forming massive encrustations, differing from other native and invasive limnic bivalves that burrow in the sediment. The magnitude of the encrustations of the golden mussel is similar to the encrustations formed by the blue mussel (Mytilus edulis Linnaeus, 1758), which lives in marine and estuarine ecosystems.

Karatayev et al. (2007) identified *L. fortunei* as an ecosystem engineer species that can alter ecosystem structures and functions. The proliferation of golden mussels has significant impacts on aquatic environments, including loss of vegetation cover, formation of continuous mattresses on sandy substrates, changes in phytoplankton and zooplankton communities, and alterations in fish diets, such as the shift in Leporinus obtusidens (known as Piau Verdadeiro) from aquatic plants to *L. fortunei* as a primary food source (SIMAS, 2018).

In addition, the invasion of *L. fortunei* has negative effects on water quality, including increased ammonia, nitrate, and phosphate levels, changes in phosphorus/nitrogen ratio, increased water transparency due to adult mussels' filtering capacity, and decreased seston, phytoplankton, and primary productivity (IBAMA, 2020).

Besen and Marengoni (2019) highlight that fish farming in net tanks of hydroelectric power plant reservoirs is a major contributor to national aquiloca production, but it faces challenges from golden mussel incrustation on screens of these net cages.

The first cases of *L. fortunei* invasion in South America were reported in 1991 (SILVA et al., 2016). *L. fortunei* has been documented invading water treatment plants, irrigation systems, and fish farms, leading to negative impacts on infrastructure and survival of aquatic animals due to lack of oxygen (SARDIÑA; CATALDO; BOLTOVSKY, 2008).

Boltovyskoy and Cataldo (1999) stated that mussels can infiltrate industrial facilities in their early stages of development and their larvae can alter production processes by attaching to hard substrates, such as metal and plastic, quickly building up and causing blockages in pipes, turbines, and other equipment. Mansur et al. (2003) noted that *L. fortunei* fixation can decrease water passage and speed in pipes, block water collection systems, contaminate water with mass mortality, and cause blockages in pumps, filters, and cooling systems, resulting in significant financial losses for hydroelectric plants and increased need for maintenance and hiring of professionals.

According to CEMIG (2014) data, the United States spent US$ 3.1 billion between 1993 and 1999 to combat golden mussels, indicating significant costs associated with this invasive species. In Brazil, Lopes et al. (2010) pointed out that most hydroelectric plants capture water upstream in open cooling systems without treating it, making them vulnerable to the diverse effects of biofouling and the natural entry of *L. fortunei*. Haubrock et al. (2022) presented a global panorama from 1980 to 2020, showing that the annual cost of damage from fouling by freshwater bivalves averaged over 1 trillion dollars per decade. Adelino et al. (2021) highlighted that in Brazil, costs of at least 105.3 billion dollars were reported between 1984 and 2019 due to the damage caused by invasive species. They also observed that the costs of losses.

TREATMENT AND CONTROL: POSSIBILITIES

Broadly speaking, it is possible to consider three types of population control for *L. fortunei*: physical control, chemical control and biological control (MINISTRY OF THE ENVIRONMENT – MMA; IBAMA, 2017). According to Rosa and Assis (2020, p. 226), physical methods, unlike chemical methods, “have less environmental impact, as they do not release toxic residues into aquatic environments”.

Mechanical scraping involves the use of robots or divers to physically remove the mussels, but it is costly, time-consuming, and can damage surfaces leading to corrosion. Filtration methods such as sand filters or self-cleaning mechanical filters are used in industrial plants to remove gold mussel larvae. Blasting, which uses high-pressure pumps, is a reactive treatment that effectively removes mussels adhered to equipment, metal surfaces, and concrete. Ultraviolet radiation is a proactive treatment that can inactivate *L. fortunei* larvae, but its effectiveness is limited by the high content of suspended solids in South American rivers. The use of a magnetic field is one of the most efficient methods to inhibit settlement and can cause mortality in a short period of time. It is widely used by water collection and treatment companies in Rio Grande do Sul. Electric current, at a voltage of 7 kV, can immobilize 80% of larvae under flow conditions, preventing settlement. Ultrasound is another proactive treatment method, where a dose of 44kWs/L at a frequency above 100kHz can cause 30% mortality of *L. fortunei*, while a frequency of 20kHz can cause 100% mortality. Turbulence, which indicates high turbulence flow, has shown success in preventing fouling problems in some watershed reservoirs, but there are no successful experiences reported in Brazil. Turbulence can cause mortality of up to 80% in some cases (MMA, IBAMA, 2017).

Pereira's thesis (2012) points out that physical control can be performed through the use of ultrasonic waves. The lowest ultrasonic frequency ranges, between 15 and 100 kHz, are more efficient in the destruction of microorganisms in liquids (PEREIRA, 2012). Still, according to the author, ultraviolet irradiation is one of the main existing technologies for the control of the golden mussel.

With regard to composite materials with antifouling action, Castro, Westphal and Fillmann (2011, p. 1029), conclude that, for the material to be ideal for its purpose, it must have:

> [...] broad spectrum of action, in order to efficiently prevent the establishment of the various species of organisms that initiate the formation of encrusting biofilm; low toxicity to mammals; low solubility in water; low potential for bioaccumulation and biomagnification; low or no environmental persistence; be compatible with the equipment and painting techniques currently in use and, costs and durability competitive with the products currently available on the market.

According to MMA, IBAMA (2017), other possible controls and chemicals for golden mussel management include pH adjustment, MXD-100, ozone, sodium dichlorocyanurate, copper sulfate, didecyl dimethyl ammonium chloride, Bayluscid, Bulab 6002, bioencapsulated, chloride ammonium, among others. However, there are no registered field experiences or products specifically for golden mussel control, with limited information from bench studies on microbial agents used for dipteran control.

Silva et al. (2020) suggest that predatory fish could potentially be "biocontrolling" agents for *L. fortunei* based on observations on fish feeding on golden mussels. However, further research is needed to fully understand and utilize this method for controlling the invasion.

Ultrasound, as a physical method, is considered more viable and environmentally friendly compared to chemical products, as it does not release toxic waste into the aquatic environment (ROSA; ASSIS, 2020).

The "National Plan for Prevention, Control and Monitoring of the Golden Mussel (*L. fortunei*) in Brazil," established by IBAMA in 2020, aimed to control invasive exotic species and reduce their impacts on Brazilian biodiversity. The Plan included an initial diagnosis of the golden mussel's biology, ecology, native distribution, invasion routes, prevention, eradication, monitoring, and population control methods. The Plan also recognized ultrasound as a relevant tool for combating exotic species in hydroelectric plants, with different frequencies causing varying levels of mortality in golden mussels (IBAMA, 2020).

METHODOLOGY

This study deals with an experimental and bibliographical research, starting from the collection of samples of golden mussels taken from their natural habitat (Rio Grande) with the performance of experiments using ultrasonic waves to descale these individuals.

Three experiments were carried out, at different times, with similar parameters for the first and last batch of samples (such as the number of individuals per sample, amount of ambient water and chlorinated water, temperature and time), with the intention of that there was observation of the results referring to similar conditions between the experiments. The second experiment was carried out with parameters quite different from those used in the other two, so that it was possible to observe results with more distant times and numbers of individuals per sample. In the following, all these procedures will be detailed.

Sample collection: Between April 24 and 28, 2022, the first samples of golden mussels were collected in the Rio Grande, in the Volta Grande Reservoir area. The Figure 5, below, shows a photograph of a cluster of mussels taken on site to be used in the first samples.

Figure 5 – Golden mussels collected in the Rio Grande, in April 2022 (approximate scale referring to the average size of individuals).

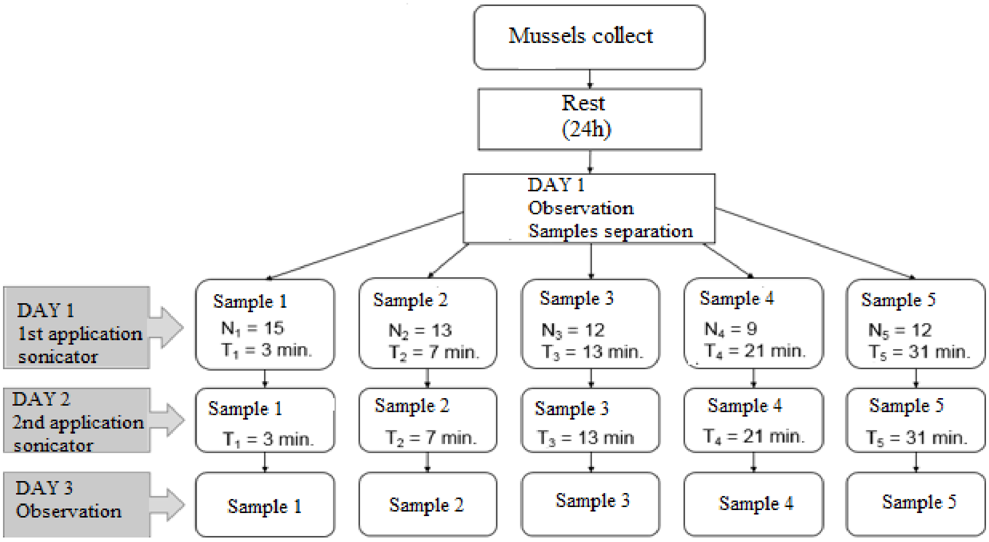
Source: the authors (2022).

Along with the samples, 7.5 liters of water from the golden mussel habitat were collected. Another 7.5 liters of dechlorinated water was added to an aquarium, as the natural environment water was not enough to fill the entire tank. The mussels were acclimated in the aquarium for 24 hours before starting the experiment, which took place in a rectangular tank with dimensions of 60 cm x 30 cm x 30 cm. The tank was modified with artificial lighting, natural substrate from Rio Grande, and aeration by oxygen rock pump. The properties of the water used in all experiments were: initial temperature of 22.5°C, pH of 6.62, conductivity of 162 µS/cm, turbidity of 0 NTU, dissolved solids of 105mg/L, and dissolved oxygen greater than 5.0 mg/L.

Sonicator device: The device used is the Ultrasonic Washer 2840 DA Biodont®. The product has an external steel coating and an internal stainless-steel tank, (ODONTOBRAS, 2022). Its strong ultrasonic cleaning system is mainly based on the transformation of electrical energy into mechanical energy, causing a high-frequency mechanical vibration, performing the so-called "ultrasonic cavitation". It can work at a frequency of 40kHz, mainly for clinical uses (medical and dental), and also at 28kHz, preferably for industrial uses (LSO Export & Import, s/d). For the experiment, the frequency of 40kHz was used, an option provided by the device.

First experiment: The experiment was conducted from April 24 to 28, 2022. Golden mussels were collected along with 7.5 liters of water from their habitat, and were kept at rest for 24 hours before the experiment. The experiment involved five samples of mussels subjected to different times of ultrasonication, performed in descending order from sample 5, as specified in the flowchart (Figure 6), without using rocky substrate. The process was repeated the next day, and on the third day, mortality and decoupling were analyzed.

Figure 6 – Flowchart of the procedures of the first experiment.



Source: the authors (2022).

Second experiment: A second test was carried out between the 20th and 25th of June, in the same location and collection environment. However, the second experiment had a larger number of individuals per sample, with 4 samples, containing 112, 104, 40 and 60, and time of 30, 40, 10, and 20 minutes for the samples #1, #2, #3 and 4#, respectively

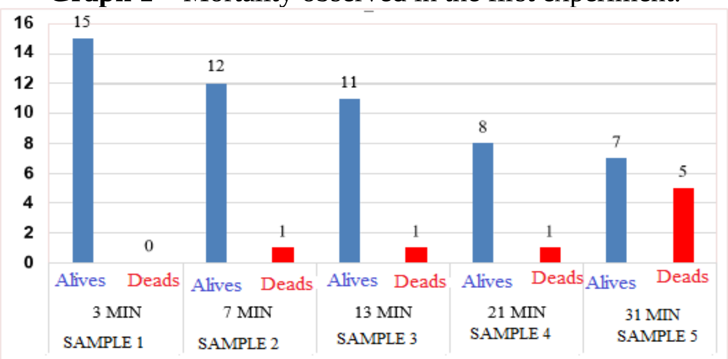
Third experiment: In August, the third collection was conducted at the same location as previous collections. The same number of individuals and samples from the first test were selected for a counterproof. Water from the natural habitat was collected and mixed with chlorinated water. The experiment started on the collection day and lasted four days, ending on August 12. The samples were subjected to the same time period as the first experiment. However, there were some differences: two tests were conducted, one with rocky substrate and one without, with mussels embedded in plants and roots. Additionally, in this experiment, the mussels were treated daily for all four days.

RESULTS AND DISCUSSION

The criteria for evaluating the results were initially focused on surveying the mortality rates per sample that were observed one day after the experiment. Other factors were also observed, such as the cavitation and decoupling process (descaling).

First experiment: In the first test, after the mussels rested in the aquarium for 24 hours, the first day of observation and experiments began. It was observed that there was no death or descaling after resting. Then, the application of the sonicator device was carried out, respecting the times and amounts per samples already specified. There were no deaths or decoupling of the golden mussels that day. In each application, the physical process of cavitation caused by the ultrasonic waves was noted, corroborating the authors Pereira (2012), Dias et al. (2014) and Ronchi (2014).

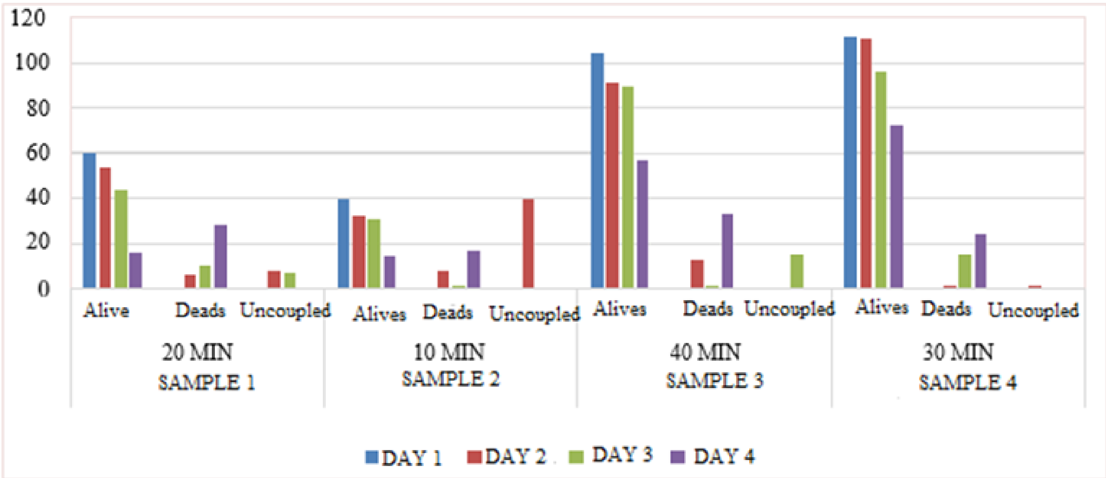
Graph 1 – Mortality observed in the first experiment.



In sample 5 (31 minutes) and sample 4 (21 minutes), no decoupling occurred, but the water temperature increased by 4°C (22.5°C to 26.5°C), consistent with findings by Bassoli (2001) and Ronchi (2014). Sample 3 (13 minutes), sample 2, and sample 1 showed no decoupling or temperature increase. After returning the samples to the aquarium for another day, on the third day, no significant effect on decoupling was observed with different exposure times. The mortality rate varied with exposure time, with the highest rate of 41.66% in sample 5 (31 minutes) and zero mortality in sample 3 minutes. The first experiment showed a trend of higher mortality with longer exposure times, but further experiments with larger sample sizes are needed for a more conclusive analysis.

Second experiment: The second wave of tests with the sonicator was carried out as explained in item 4.4. Graph 2 shows the results achieved with each sample over the four days of the experiment, considering the number of live, dead and uncoupled golden mussels.

Graph 2 – Results of the four days of the second experiment.



In this experiment, the results draw attention, initially due to the time in which the samples were submitted. In the two shortest times, the highest mortality rate was observed, considering the proportion between the total number of dead at the end of the experiment over the total number of individuals who started the experiment. At the end of the fourth day of the experiment, deaths occurred in 73.3% of the total number of sample #1 (20 minutes), and 65% of total deaths for sample #2 (10 minutes). While for longer times, the proportional mortality for samples #3 (40 minutes) and #4 (30 minutes) were lower, 45.19% and 35%, respectively. It is worth emphasizing that in the samples of longer times, the amount of golden mussels was much higher, which may possibly influence the ratios obtained. In relation to the uncoupling, the results vary a lot, and in sample nº 2 there was the uncoupling of 100% of the individuals, only on the second day of the test. Samples nº 1 and nº 3 presented total uncoupling of 15 individuals each, while sample nº 4 presented only 1 uncoupling.

Third experiment: Finally, the third wave of samples was performed with the same exposure times in minutes per test and number of individuals in the samples of the first wave, one experiment with substrate and the other without substrate.

With substrate: The experiment, which was initially supposed to take place over four days, was ended on the third day, due to the occurrence of 100% mortality in all samples. Table 1 presents the quantitative results of the samples with substrate, counting the observations of individuals per sample, both during and after the experiment. This experiment was observed for 24 hours after its completion.

Table 1 – Results of the third experiment, with rocky substrate.

	SAMPLE 1 3 MIN | N = 15			SAMPLE 2 7 MIN | N = 13		
	Alives	Deads	Uncoupled	Alives	Deads	Uncoupled
DAY 1	7	8	0	11	2	0
DAY 2	2	5	1	1	10	2
DAY 3	0	2	0	0	1	0
DAY 4	-	-	-	-	-	-
TOTAL	**0**	**15**	**1**	**0**	**13**	**2**

	SAMPLE 3 13 MIN | N = 12			SAMPLE 4 21 MIN | N = 9		
	Alives	Deads	Uncoupled	Alives	Deads	Uncoupled
DAY 1	5	7	0	5	4	5
DAY 2	1	4	1	0	5	0
DAY 3	0	1	1	-	-	-
DAY 4	-	-	-	-	-	-
TOTAL	**0**	**12**	**2**	**0**	**9**	**5**

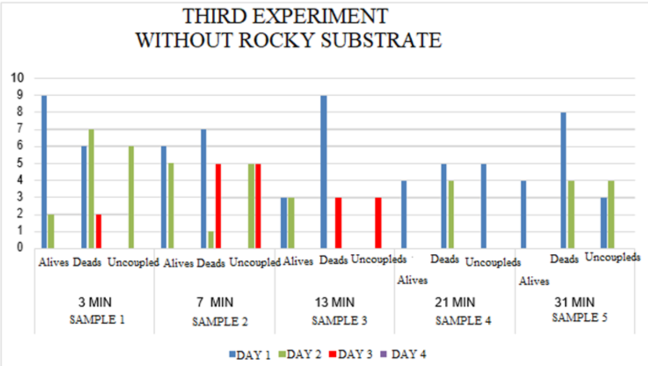
	SAMPLE 5 31 MIN | N = 12		
	Alives	Deads	Uncoupled
DAY 1	4	8	1
DAY 2	1	3	0
DAY 3	0	1	1
DAY 4	-	-	-
TOTAL	**0**	**12**	**2**

Sample #1: 15 individuals underwent 3 minutes of sonication. First day mortality was 53.33%, second day mortality was 75%, and one individual uncoupled (8.33%). The last individual died on the third day. Sample #2: 7 minutes of sonication for 13 individuals. First day mortality was 15.4%, but increased to 90.9% on the second day, leaving only one individual alive. Two uncouplings were observed (15.8%). The last individual died on the third day. Sample #3: 13 minutes of sonication. First day mortality was 58.3%, and second day mortality was 80%, with only one individual surviving until the next day when it died. Sample #4: 21 minutes of sonication. First day mortality was 44.4%, and all 5 remaining individuals died on the second day, resulting in 100% mortality within two days. Two uncouplings were observed.

Sample #5: 31 minutes of sonication. Highest mortality observed with 66.7% on the first day and 75% on the second day. The last individual died on the third day. Two uncouplings were observed. Overall, it is evident that mussels are susceptible to mortality, with higher mortality rates observed with longer sonication times.

No substrate: The quantitative data referring to the experiment carried out without the substrate are presented below, with the mussels exposed to the apparatus coupled to plants and roots in their natural habitat. Graph 3 allows a broad view of the results observed on each of the days:

Graph 3 – Quantitative results of the third experiment, without rocky substrate.



Results show minimal differences between samples with and without rocky substrate, except for sample nº 2 where substrate led to higher mortality on the second day (90.9%) compared to without substrate (16.6%). Sample 3 without substrate had higher mortality on the first day (16.7% difference) and no mortality on the second day. Uncoupling was more common in samples with roots and aquatic plants (31 uncouplings) compared to rocky substrate (12 uncouplings), possibly due to differences in material where the golden mussel is embedded.

In general, it is noticeable that the longer the time exposed to ultrasonic waves, in addition to the higher mortality rate, the mussels die more quickly.

CONCLUSIONS

Despite bringing several consequences to industries with systems already infested by *L. fortunei*, given the research carried out in recent years, this problem can be solved, since many methods have become efficient in combating mussels, such as the use of sonicator devices.

This study was able to prove the effectiveness of ultrasound for the decoupling and death of golden mussels, which enables their control based on this physical treatment. Therefore, this study becomes extremely relevant, above all, given the need to reduce the economic and environmental impacts caused by the golden mussel which, as mentioned in this research, can range from the obstruction of pumps in hydroelectric plants to impacts on the quality of water and biomes, among other problems.

Finally, it is possible to carry out future research that explores other parameters for the experiments, such as the use of other ultrasound bath devices, as well as different exposure times. Other delimitations that may also contribute to this line of research can address how ultrasonic waves can be used in loco, in order to establish a control method in the place where incrustations cause damage to companies and other environments that need control, thus being able to bring advances to the solutions for controlling the golden mussel through ultrasonic waves.

ACKNOWLEDGMENTS

The authors would like to thank CAPES, FUNDAÇÃO GORCEIX and Cemig/ANEEL, project GT-0604, for supporting and encouraging this research.

REFERENCES

ADELINO, José et al. The economic costs of biological invasions in Brazil: a first assessment. **NeoBiota**, v. 67, p. 349-374, 2021.

ALMEIDA, Manuela G.; SILVA, Sandra M. **Acústica**. Portugal: Universidade do Minho – Escola de Engenharia, 2005.

BASSOLI, Dyjalma Antônio. **Avaliação dos efeitos do ultra-som pulsado de baixa intensidade na regeneração de músculos esqueléticos com vistas à aplicabilidade em clínica fisioterapêutica**. Dissertação (Mestrado) - Escola de Engenharia de São Carlos/Faculdade de Medicina de Ribeirão Preto/Instituto de Química de São Paulo - Universidade de São Paulo, 2001.

BATISTA, William Romão. Água de Lastro–problema e soluções. **Revista Marítima Brasileira**, v. 138, n. 01/03, p. 174-174, 2018.

BERGMANN, C. P. et al. Seleção de materiais e revestimentos para o controle de incrustações do mexilhão dourado na Usina Hidrelétrica de Ibitinga (SP, Brasil). **Matéria (Rio de Janeiro)**, v. 15, p. 21-30, 2010.

BESEN, Mateus Antonio; MARENGONI, Nilton Garcia. Incrustação de mexilhão- dourado (Limnoperna fortunei) nas telas de tanques-rede. **Acta Iguazu**, v. 8, n. 5, p. 60-68, 2019.

BISCEGLI, Clovis Isberto. **Conceitos da Física do Ultra-som**. Embrapa Instrumentação-Documentos (INFOTECA-E), 2003.

BLUME, K. et al. Dosimetria proposta para o tratamento por ultra-som – uma revisão de literatura. **Fisioterapia em Movimento (Physical Therapy in Movement)**, v. 18, n. 3, 2017.

BOLTOVSKY, D.; CATALDO, D. **Population dynamics of Limnoperna fortunei na invase fouling mollusk in the lower Paraná River *Argentina.** Biofouling, v. 14, p. 255-263, 1999.

CASTRO, Ítalo B.; WESTPHAL, Eliete; FILLMANN, Gilberto. Tintas anti-incrustantes de terceira geração: novos biocidas no ambiente aquático. **Química Nova**, v. 34, p. 1021-1031, 2011.

CORDEIRO, Nelmara IS et al. Effect of starvation and subsequent feeding on glycogen concentration, behavior and mortality in the golden mussel Limnoperna fortunei (Dunker, 1857)

(Bivalvia: Mytilidae). **Journal of Limnology**, v. 75, n. 3, p. 618-625, 2016.

COSTA, Juliana Mara et al. Incrustação de mexilhão dourado Limnoperna fortunei em tanques-rede. **Revista Cultivando o Saber**, v. 5, n. 2, p. 37-46, 2012.

CEMIG, Companhia Energética de Minas Gerais. **O mexilhão Dourado, uma ameaça às águas e hidrelétricas brasileiras.** Belo Horizonte, Ed. CEMIG, p. 24, 2004.

DADAM, Allan Pedro. **Analisador de Frequência e Espectro Sonoro para Alinhamento de Sistemas de Áudio**. Trabalho de Conclusão de Curso - Instituto Federal de Santa Catarina. Campus Joinville. Graduação. Curso Superior de Tecnologia em Mecatrônica Industrial, Joinville, 2019.

DIAS, A. L. B. et al. Extração de Pimenta Malagueta (Capsicum Frutescens L.) a Baixa Pressão Assistida por Ultrassom. **Blucher Chemical Engineering Proceedings**, v. 1, n. 2, p. 4752-4759, 2015.

DINIZ, D.M.A. Verificação da influência do mexilhão dourado nas grades de tomadas d'água. **Universidade Federal de Minas Gerais**, Programa de pós-graduação em saneamento e recursos hídricos, Belo Horizonte, 2010.

EL HAJ, Yasmin; BOHN, Sofia; SOUZA, Marta Marques. Tolerance of native and invasive bivalves under herbicide and metal contamination: an ex vivo approach. **Environmental Science and Pollution Research**, v. 26, n. 30, p. 31198- 31206, 2019.

FREIRE, Cassio Geremia; MARAFON, Andrea Tozzo. Espécies de moluscos invasores nos ecossistemas aquáticos brasileiros e seu impacto no meio ambiente. **InterfacEHS**, v. 13, n. 1, 2018.

GIGLIO, Matías L. et al. Reproductive pattern of the aggressive invader Limnoperna fortunei (Bivalvia, Mytilidae) in South America. **Invertebrate Reproduction & Development**, v. 60, n. 3, p. 175-184, 2016.

GRANDO, Adriana et al. Associação de sulfato de ferro e ultrassom na clarificação de efluente de indústria láctea. **Engevista**, v. 18, n. 1, p. 189-206, 2016.

Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. **Instrução Normativa nº 17, de 21 de outubro de 2015**. Diário Oficial da União, seção 1, p. 126. Brasília, DF, 2015.

Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. **Plano nacional de prevenção, controle e monitoramento do mexilhão-dourado (Limoneperna fortunei) no Brasil**. Diretoria de Uso Sustentável da Biodiversidade e Florestas. – Brasília, DF: IBAMA, 2020.

IWASAKI, Keiji. Behavior and taxis of young and adult Limnoperna fortunei. In: **Limnoperna fortunei**. Springer, Cham, 2015. p. 249-260.

KARATAYEV, Alexander Y. et al. The invasive bivalves Dreissena polymorpha and Limnoperna fortunei: parallels, contrasts, potential spread and invasion impacts. **Journal of Shellfish Research**, v. 26, n. 1, p. 205-213, 2007.

LOPES, A. D. A et al. **Avaliação de sobrevivência de organismos transportados por água de lastro de navios mercantes**. Arraial do Cabo: Instituto de Estudos do Mar Almirante Paulo Moreira Departamento de Oceanografia, cap. 1, p.1-11, 2010.

MACEDO, N. D. **Iniciação à pesquisa bibliográfica**: guia do estudante para a fundamentação do trabalho de pesquisa. São Paulo, SP: Edições Loyola,1994.

MANSUR, M. C. D. et al. Primeiros dados quali-quantitativos do mexilhão dourado, Limnoperna fortunei (Dunker), no delta do jacuí, no lago guaíba e na laguna dos patos, Rio Grande do Sul, Brasil e alguns aspectos de sua invasão no novo ambiente. **Revista Brasileira de Zoologia**, nº 20. p. 75-84, 2003.

MEDEIROS, Raphael Corrêa; Lazarotto, Joseane Sarmento; VOLPATTO, Fernanda. Desinfecção de esgoto sanitário proveniente de tanque séptico com uso do ultrassom. **In: Congresso Nacional de Saneamento e Meio Ambiente XXIX**, São Paulo, 2019.

MINISTÉRIO DO MEIO AMBIENTE. **Resolução CONABIO nº 5 de 21 de outubro de 2009**. Secretaria de Biodiversidade e Florestas – Comissão Nacional de Biodiversidade. Brasília/DF, 2009.

MINISTÉRIO DO MEIO AMBIENTE; IBAMA. **Diagnóstico sobre a invasão do mexilhão-dourado (Limnoperna Fortunei) no Brasil**. 2017.

PAOLUCCI, Esteban M. et al. Veligers of the invasive bivalve Limnoperna fortunei in the diet of indigenous fish larvae in a eutrophic subtropical reservoir. **Austral Ecology**, v. 42, n. 7, p. 759-771, 2017.

PAULA, Rayan Silva et al. A Case for the Continued Study of the Golden Mussel Invasion of Brazil: Efficient Detection and Containment of Limnoperna fortunei (Dunker, 1857) Dispersion Involves Multiple Approaches and Different Actors.
Oceanogr Fish Open Access, v. 13, n. 4, p. 1-4, 2021.

PEREIRA, N. N. **Alternativas de tratamento da água de lastro em portos exportadores de minério de ferro**. Tese (Doutorado) – Escola Politécnica da Universidade de São Paulo. 2012.

PEREIRA, S. M.; COLARES, E. P.; VIEIRA, J. P. How far can the Golden mussel (Limnoperna fortunei–Mytilidae) go with its own foot. **International Journal of Zoology and Animal Biology**, v. 2, n. 5, p. 1-8, 2019.

RIBOLLI, Josiane et al. Optimized and validated protocol to the detection of the invasive bivalve Limnoperna fortunei from eDNA plankton samples. **Acta Limnologica Brasiliensia**, v. 33, 2021.

ROCHA, A. C. M; MAINIER, F. B.; ALVES, C. H. F. Processo de Remoção de Contaminantes Industriais por Ultrassom. **In: VIII Simpósio de Excelência em Gestão e Tecnologia**.
Resende/RJ, 2011.

RONCHI, Renata Peterle; SÃO MATEUS, E. S. **Avaliação da eficiência do ultrassom no processo de separação de fases em água produzida e em emulsões sintéticas do tipo O/A**. Dissertação (Mestrado em Energia) – Universidade Federal do Espírito Santo, Centro Universitário Norte do Espírito Santo, São Mateus, 2014.

ROSA, Daniel de Melo; ASSIS, Paulo Santos. Limnoperna Fortunei no Brasil: Histórico de Dispersão, Biologia Populacional, Impactos e Controle. In: HAYASHI, Carmino; SARDINHA, Diego de Souza; PAMPLIN, Paulo Augusto Zaitune (Org). **Ciências Ambientais**: Recursos

Hídricos. 1. ed., p. 219-235, Ribeirão Preto, SP: Carmino Hayashi, 2020.

SANTOS, Cíntia P. WÜRDIG, Norma L. MANSUR, Maria. C. D. Fases Larvais do mexilhão dourado *Limnoperna fortunei* na bacia do Guaíba, rio Grande do Sul, Brasil. **Revista Brasileira de Zoologia**, v.22, n.3, p. 702-708, 2005.

SANTOS, Sonia Barbosa et al. Espécies de moluscos límnicos invasores no Brasil. **Moluscos límnicos invasores no Brasil: biologia, prevenção e controle**, p. 25-49, 2012.

SARDIÑA, P; CATALDO DH, BOLTOVSKY, D. The effects of the invasive mussel, Limnoperna fortunei, on associated fauna in South American freshwaters: Importance of physical structure and food supply. **Fundamental e Apply Limnology**, nº 173, p. 135-144, 2008.

SILVA, Amanda Guilherme. **Vivendo com o inimigo**: **competição entre os corais invasores Tubastraea spp. e a esponja Desmapsamma anchorata na Baía de Ilha Grande, RJ**. Dissertação (Mestrado em Ecologia e Evolução) – Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2014.

SILVA, Fabiano A. et al. Mexilhão-dourado no Brasil: detecção de um perigoso invasor. **Ciência Hoje**, v. 57, 2016.

SILVA, Ivana et al. Fish vs. Aliens: predatory fish regulate populations of Limnoperna fortunei mitigating impacts on native macroinvertebrate communities. **Hydrobiologia**, v. 848, n. 9, p. 2281-2301, 2021.

SILVA, Samara Hermes et al. Limnoperna fortunei **-** Updating the geographic distribution in the Brazilian watersheds and mapping the regional occurrence in the Upper Uruguay River basin. **Biota Neotropica**, v. 21, 2021.

SIMAS, Sunshine de Ávila. **Interações e recursos alimentares para a comunidade de peixes: a invasão do mexilhão-dourado no alto rio Uruguai**. Tese (Doutorado em Aquicultura) – Universidade Federal de Santa Catarina, Florianópolis, 2018.

TOMITÃO, Isabela de Macedo. **Síntese rápida para obtenção de óxido de estanho utilizando agitação mecânica associada a sonoquímica**. Dissertação (Mestrado em Química) – Universidade Federal da Grande Dourados, Dourados, 2014.

XIA, Zhiqiang et al. Golden mussel (Limnoperna fortunei) survival during winter at the northern invasion front implies a potential high-latitude distribution. **Diversity and Distributions**, v. 27, n. 8, p. 1422-1434, 2021.