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Water temperature modulation to prevent the South American rock mussel (*Perna perna*) from spawning during depuration

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ABSTRACT

Sewage pollution is an increasing problem for *Perna perna* mussel farmers in Brazil, and there is an urgent need to adopt post-harvest treatments, such as depuration, to reduce the associated microbiological risks. However, depuration of this species has been discouraged by experiences showing that the animals usually spawn during this procedure, bringing difficulties for water treatment and making them lighter, weakened, and undesirable to be traded as a live product. This study aimed at developing a protocol to prevent mussels from spawning by modulating the water temperature during depuration. For one year (from 2021-07-07 to 2022-06-29), market-size mussels harvested from the Brazilian southern coast were divided into groups of five and immersed during 50 hours in sets of experimental units (15 L acrylic aquariums) that emulated depuration tanks with different water temperatures (ranging from 8°C to 31°C). During this period, the experimental units were inspected nine times to check for spawning/spawned mussels. Analysis of the overall results showed that 21 (95.5%) out of the 22 assays resulted in mussels spawning and that this behavior was mostly recorded (80.3%) within the first 5:30 hours of the assays. The models developed (binary logistic regression) indicate that conditioning the depuration water to temperatures 5°C lower than those registered at the harvest site holds the potential to reduce the chances of spawning by more than 50%, and these chances drop to 8.2% when this difference reaches 10°C. Further studies are needed to show how reducing water temperature during depuration influences its efficiency in terms of pathogen reduction, aiming to define the best protocol to optimize microbial removal and minimize spawning.

Keywords: Condition index, Meat yield, Mollusks, Purification

Filter-feeding bivalve mollusks accumulate microorganisms, including bacteria and viruses pathogenic to humans, when grown in sewage-polluted waters, and can pose a significant health risk when consumed raw or lightly cooked (Lees, 2000; Butt et al., 2004). To reduce the risk of human illnesses, public health controls are implemented on the commercial production and/or wild gathering of bivalve mollusks (de Souza et al., 2018). Essentially, these controls consist of monitoring fecal indicator organisms in mollusks and/or water and classifying production areas based on the risks evidenced by this monitoring. Depending on the classification, post-harvest treatments (depuration, relaying, and heat treatment) are required before sale for human consumption

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(Food and Agriculture Organization and World Health Organization, 2018).

Depuration is one type of post-harvest treatment to reduce microbiological risks and consists of placing bivalve mollusks harvested from moderately polluted areas in tanks with clean seawater for a period of time, allowing them to cleanse or purge themselves of microbiological contamination by continuing their normal filter-feeding and digestive processes (Rees et al., 2010). This procedure is applied to different groups of mollusks traded as live products worldwide, including mussels. A survey carried out by Lee et al. (2008) revealed that there are more than 1,000 depuration plants in countries such as Japan and France, and Italy, Spain, and the UK hold more than 50 of these plants each. These plants depurate different species including the oyster species *Crassostrea gigas* and *Ostrea edulis*, the mussel species *Mytilus edulis* and *Mytilus galloprovincialis*, and other bivalve such as *Cerastoderma edule*, *Ruditapes decussatus*, and *Tapes philippinarum*. According to the same authors, Italy, Spain, and the UK hold more than 50 of these plants each.

Depuration under controlled conditions involves harvesting mollusks from marine farms or natural banks, transporting and immersing them in the tanks of a depuration center containing seawater with physicochemical characteristics that usually differ from those registered at the source site. Temperature variations and physical stress are known to trigger spawning in bivalve species (Seed et al., 1992), so there are reports of species spawning during depuration procedures. Spawning makes the mollusks significantly lighter (the sexual gonads make up most of the mussel's meat weight), weakened and, according to Lee et al., (2008), undesirable and, in many instances, impracticable to market as live animals. The gametes released into the water increase the water turbidity, which in turn reduces the efficiency of UV disinfection systems, commonly used in depuration units (Richards, 1988; Lees et al., 2010; Power and Collins, 1989). Modulating water temperature and avoiding physical stress have been suggested as strategies to prevent this behavior (Lee et al., 2008). For two of the most produced species in the world, the Mediterranean mussel *Mytilus galloprovincialis* and the blue mussel *Mytilus edulis*, it is recommended to keep the water temperature during depuration at 5°C to 15°C to ensure adequate mollusk filtration activity and to prevent spawning (Lee et al., 2008).

The South American rock mussel, *Perna perna*, is an important aquaculture resource in Brazil, with a production of 14,000 tons in 2020 and only recently some processing plants started to implement depuration facilities aiming to increase the quality of their product. The lack of specific information on how to prevent spawning during depuration of this species has become an important research issue in Brazil, since the results of field studies (de Souza et al., 2022a) and the official monitoring program indicate that mollusks from most aquaculture zones on the coast of the state of Santa Catarina should undergo a post-harvest treatment to control microbiological risks. The first attempts to depurate this mussel species on an experimental scale date back from 1998 and spawning was observed in all assays carried out at room temperature (water temperatures ranging from 18°C to 20°C) (Suplicy, 1999). From those attempts to date, little new information on the depuration of this species has been released, most of which related to the ability of this process to reduce coliform levels (Guimarães Filho et al., 2022).

The *Perna perna* mussel has different water temperature comfort levels and reproductive behavior from those of *M. galloprovincialis* (Zardi et al., 2007; Silvestri et al., 2018). In a monitoring study in Plettenberg Bay, South Africa, Zardi et al. (2007) evaluated the reproductive behavior of these coexisting species and observed that *M. galloprovincialis* spawning events always occurred at temperatures ranging from 16.4 to 19.5°C, whereas *P. perna* spawned at the highest and lowest temperatures recorded in the 18 months of the survey (~14.5 and ~24.2°C). Therefore, it is reasonable to expect that a different protocol is needed to control *P. perna* spawning during depuration. This study aimed to investigate the relationship between the spawning behavior of *P. perna* and factors such as the water temperature

in the depuration tanks, the difference between this temperature and the water temperature in the harvesting areas (Temperature Difference - TD), and the condition index of the animals, aiming at developing a protocol to prevent the spawning of this species during depuration.

For one year (from 2021-07-07 to 2022-06-29), one hundred market-size mussels (mean length of 8.26 ±0.59 cm) were harvested every two weeks at Epagri's Experimental Farm in Florianópolis (27°29'21.11"S, 48°32'18.13"W). The animals were manually harvested and declumped, had incrustations removed with a cleaver, and were washed with pressurized water. These procedures followed the common practices adopted in most shellfish farms in Santa Catarina. Currently, there are 443 small-scale marine farms in Santa Catarina, 80% of them covering areas smaller than two hectares, which are mostly family-run businesses, with incipient mechanization of shellfish harvesting (Suplicy, 2019). The mussels were transported to the Seafood Laboratory of the Federal University of Santa Catarina in a maximum transportation time of one hour. The seawater temperature of the harvest site was recorded continuously every hour with a temperature sensor/data logger (HOBO®, Onset Computer Corporation, Bourne, MA).

In the laboratory, 24 mussels had their shell length and live weight measured. After that, they were cooked for ten minutes in boiling water (100°C) and their meat yield was determined by dividing the meat weight by the individual live weight. The remaining mussels were divided into groups of five and immersed in the experimental units, which were 15 L acrylic aquariums filled with filtered seawater (1µm) and with constant aeration, which emulated depuration tanks. The experimental units were kept in an experimental room with a constant temperature of 8°C, organized in sets of three aquariums (triplicates) that were positioned inside larger polystyrene tanks filled with water that functioned as a water bath. Water heating equipment was used to maintain specific water temperatures in each water bath, so each set of the three aquariums was kept at the same water temperature (Figure 1). A total of 22 assays were carried out, the first 11 with four sets of experimental units, all within the experimental room. The last 11 assays were carried out using five sets, with the additional one acting as a control, kept at room temperature. The temperatures tested varied between the assays, ranging from 8°C to 31°C. The water in the tanks was not renewed during the assays, as preliminary tests showed that the maximal NH_3 concentration generated during the 50-hour assay was 2.3 mg L^{-1}, below the toxic levels for mussels (Reddy and Menon, 1979). The assays lasted for 49 hours, starting at 10:30 am. During this period, the experimental units were inspected nine times (day 1: 11:30h, 14:00h, and 17:00h, day 2: at 8:30h, at 11:30h, at 14:00h, and 17:00h, day 3: at 8:30h and at 11:30h) to check for spawning/spawned mussels. Spawning was characterized by the existence of orange gametes settled in the bottom of the tanks, indicating female spawn, or whitish water, caused by the release of spermatozoids by male mussels.

The statistical analysis aimed to model the probability of detecting any spawning within the depuration tanks. Therefore, spawning was considered a boolean variable, meaning that we considered experimental units with spawning mussels as 1 and those without spawning as 0. The comparison of data for experimental units with and without spawning mussels was carried out using the Mann-Whitney U test, and binary logistic regression analysis was used to investigate the correlations between the explanatory variables and the occurrence of spawning and to model the probability of spawning.

Analysis of the overall results showed that 21 (95.5%) of the 22 assays resulted in mussel spawning, regardless of condition index and harvest season, and among all experimental units used in all assays combined, spawning was recorded in 34.3% of them. Most spawning events (80.3%) were recorded up to the third inspection, indicating that they tend to occur within the first 5:30 hours of depuration. The experimental units with spawning mussels were subjected to an overall higher depuration temperature than those without spawning mussels (Wilcoxon rank sum test $W = 4728.5$, p-value = 4.214×10^{-13}). The logistic regression model shows that the odds

of mussels spawning decrease as the temperature decreases ($p = 1.33 \times 10^{-10}$, log of the odds 0.21). According to the model, the probability of observing mussel spawning in the experimental units at 21°C (~mean sea temperature in the study area) is 45.5% and reduces to 22.2% if the depuration temperature decreases to 16°C (5°C lower), and to 8.8% at 11°C (10°C lower) (Figure 2-A). Similar findings were obtained when TD was considered as an explanatory variable. Higher TD values were observed in experimental units with spawning mussels (Wilcoxon rank sum test W = 16220, p-value $< 2.2 \times 10^{-16}$) and the logistic regression model showed that the chances of mussel spawning increased with higher TD values ($p = 1.46 \times 10^{-13}$, log of the odds 0.27). According to the model, if the water temperature during depuration is equal to that of the harvesting areas, the probability of mussel spawning is 58.4%. Reducing this temperature by 5°C reduces the probability of spawning to 26.2%, and decreasing the temperature by 10°C during depuration reduces the chances of spawning to 8.2%. No significant correlations were detected when the same analysis was carried out considering condition index (p-value = 0.82), live weight (p-value = 0.25), or cooked weight (p-value = 0.70) as explanatory variables.

We found no literature specifically addressing the effectiveness of modulating water temperature to prevent mussel *P. perna* from spawning during depuration. Our results corroborate previous studies (Suplicy, 1999) showing that South American rock mussel often spawn during depuration, as this behavior was recorded in 95.5% of the assays. Spawning occurred regardless of condition index and without seasonality. This finding is in line with studies on the reproductive behavior of *P. perna* in southern and southeastern Brazil, which show that this species spawns throughout the year, with reported peaks of reproductive activity that differ among studies (Luneta, 1969). Therefore, our results provide evidence that it is not possible to overcome spawning during the depuration of *P. perna* by avoiding harvesting mussels with a high condition index or at specific periods of the year, at least in the geographical area of this study.

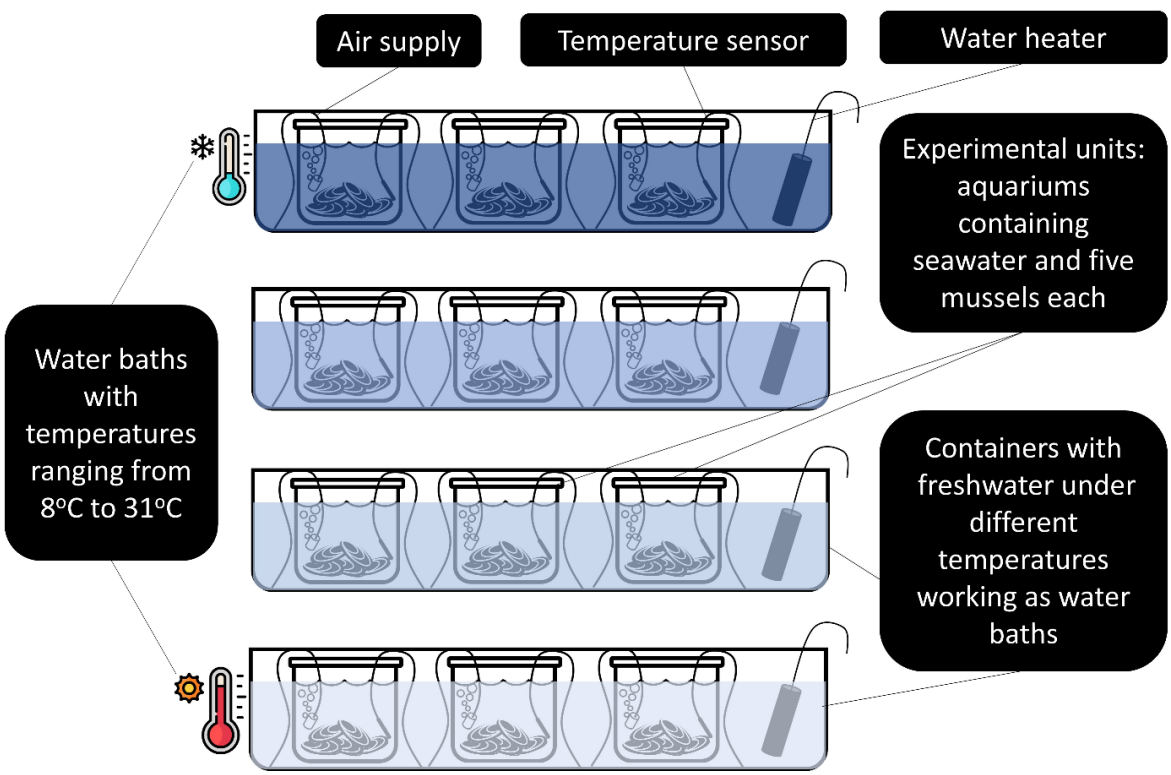

Figure 1. Scheme demonstrating the experimental design.

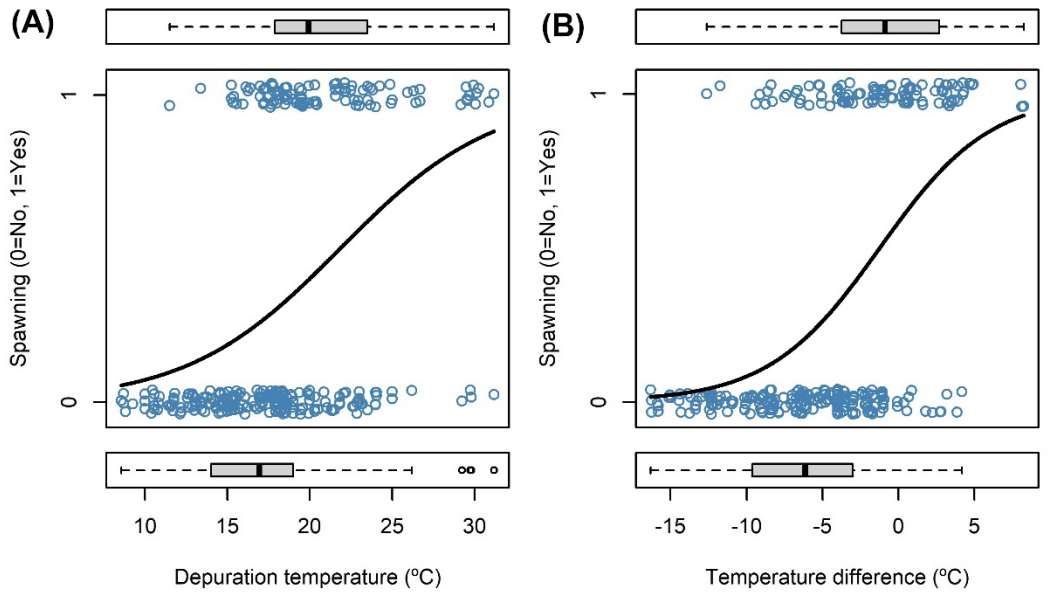

Figure 2. Logistic regression models describing the odds of mussel spawning according to the water temperature during depuration (left) and the water temperature difference between the marine farms and the experimental units (right). The boxplots describe the data distribution in the experimental units where spawning was observed (above the scatter plots) and in those without spawning (below the scatter plots).

Our results also show that spawning tends to occur during the first hours of depuration. A wide variety of depuration periods are used around the world, and international regulations vary in this aspect. A minimum of 42 hours is specified in the UK and 44 hours in the US National Shellfish Sanitation Program, whereas periods of 18–24 hours are commonly used in Italy (Lee et al., 2008). There is also consensus that the use of shorter periods decreases the ability to eliminate pathogens, such as viral pathogens and vibrios (de Souza et al., 2022b). Therefore, adopting shorter depuration cycles would not prevent *P. perna* mussels from spawning during depuration and would minimize the microbial reduction effect.

On the other hand, the results show that *P. perna* spawning during depuration is a problem that can be overcome by modulating the temperature, confirming our hypothesis. The models developed indicate that conditioning the depuration water to temperatures 5°C lower than those recorded at the harvest site holds the potential of reducing the chances of spawning by more than 50%, and these chances drop to 8.2% when this difference reaches 10°C. We did not find any article that specifically addressed the effects of temperature modulation on the spawning behavior of other mussel species during depuration, for comparison. Anyway, our findings are in line with international guidelines that recommend lower depuration temperatures to prevent spawning in other cultivated species worldwide (Lee et al., 2008).

The filtration rates of mollusks are known to be influenced by temperature (Zippay and Helmuth, 2012). Considering that the effective bivalve depuration depends on their filtration behavior, the temperatures during this treatment must be within a range in which the mussels are actively filtering. The minimum depuration temperature tested in our study was 8°C. As a reference, the sea temperature in the geographical area where the study was carried out ranges around 16°C and the low lethal temperature for *P. perna* is 3°C, as reported by de Bravo et al. (1998). Although this was not the main goal of our article, the production of feces during the assays, even in the experimental units subjected to the coldest

temperatures, suggests that the animals were physiologically active. It is important to bear in mind that even when physiological activity is maintained, cooling the depuration water can significantly reduce the efficiency of microbial removal, especially that of viruses (Lee et al., 2008). For this reason, different countries have defined temperature ranges for the depuration of mollusks, including mussels. For instance, UK standards determine 5°C as the minimum temperature for the depuration of the blue mussel, *Mytilus edulis* (UK Food Standards Agency, 2016). Future studies aimed at investigating the efficiency of *P. perna* depuration in reducing pathogen levels may benefit from our findings to establish the best protocol to optimize microbial removal and minimize spawning.

Finally, it is worth mentioning the urgency of implementing strategies to minimize the microbiological risks of mollusks produced in Brazil. Shellfish sanitation programs classify production areas to determine their potential risk of contamination by pathogens. The results of the official monitoring and classification program carried out since 2012 in Santa Catarina show that, of the 28 zones currently monitored, only two are permitted for harvest aimed at direct trade. Mussels from 20 zones have their harvest conditioned to post-harvest treatments, whereas harvesting is forbidden in the remaining areas (CIDASC, 2022). International shellfish safety regulations in general (including the Brazilian one) stipulate that mollusk from moderately polluted areas intended to be traded live must undergo depuration. In Brazil, *P. perna* mussels are traditionally sold and consumed cooked and the products traded by the industry are subjected to heat treatment as the only post-harvest treatment. To date, there is no specific protocol that ensures the effectiveness of heat treatment for minimizing the microbiological risks of *P. perna* mussels. A group of international experts discussed this issue in 2013 and pointed out the need to develop a protocol for this species (de Souza, 2014). Furthermore, different studies provide evidence that the light cooking practices (steaming, searing) usually adopted by final consumers and restaurants do not necessarily provide the temperature/time combination required for the efficient inactivation of pathogens in bivalves (de Souza et al. 2022b). Therefore, we expect our findings to encourage the use of depuration as post-harvest treatment in Brazil and in other *P. perna* producing countries.

CONCLUSIONS

The South American rock mussel spawns very frequently under simulated depuration conditions. Spawning tends to occur during the first 5:30 hours and this behavior is recorded regardless of the condition index or seasonality. Therefore, it is not possible to overcome this issue by avoiding harvesting mussels with a high condition index or at specific periods of the year, at least in the geographical area studied (Southern Brazil). Neither is it possible to overcome this issue through the adoption of shorter depuration periods. *P. perna* spawning during depuration is a problem that can be overcome by temperature modulation, since the chances of spawning can be significantly reduced by conditioning the depuration water to lower temperatures than those recorded at the harvest site. Future studies should investigate how reducing the water temperature during depuration influences its efficiency in terms of pathogen reduction, aimed at defining the best protocol to optimize microbial removal and minimize spawning.

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AUTHOR CONTRIBUTIONS

F.M.S.: Conceptualization; Investigation; Writing – Original draft; Writing – Review & Editing.

R.V.de S.: Methodology; Software; Formal Analysis; Writing – Review & Editing.

G.T.: Resources; Writing – Review & Editing.

REFERENCES

Butt, A. A., Aldridge, K. E. & Sanders, C. V. 2004. Infections related to the ingestion of seafood Part I: Viral and bacterial infections. *The Lancet. Infectious Diseases*, 4, 201-212. DOI: https://doi.org/10.1016/S1473-3099(04)00969-7.

CIDASC. (Defesa Sanitária Animal). 2022, 4 April. *Resultados das Análises Microbiológicas em Moluscos Bivalves Mapa de Situação Microbiológica*. Available at: http://www.cidasc.sc.gov.br/defesasanitariaanimal/resultado-de-analise-microbiologica/. Access date: 26 April 2022.

De Bravo, M. I. S., Chung, K. S. & Pérez, J. E. 1998. Salinity and temperature tolerances of the green and brown mussels, *Perna viridis* and *Perna perna* (Bivalvia: Mytilidae). *Revista de Biologia Tropical*, 46, 121-125.

De Souza, R. V., Rupp, G. S., De Campos, C. J. A. & Lee, R. 2014. *Moluscos bivalves: medidas de controle microbiológico para atender às exigências da União Europeia*. Florianópolis, Epagri.

De Souza, R. V., De Campos, C. J. A., Garbossa, L. H. P., Vianna, L. F. De N., Vanz, A., Rupp, G. S. & Seiffert, W. 2018. A critical analysis of the international legal framework regulating the microbiological classification of bivalve shellfish production areas. *Reviews in Aquaculture*, 10, 1025-1033. DOI: https://doi.org/10.1111/raq.12222.

De Souza, R. V., Moresco, V., Miotto, M., Souza, D. S. M. & De Campos, C. J. A. 2022a. Prevalence, distribution and environmental effects on faecal indicator bacteria and pathogens of concern in commercial shellfish production areas in a subtropical region of a developing country (Santa Catarina, Brazil). *Environmental Monitoring and Assessment*, 194, 286. DOI: https://doi.org/10.1007/s10661-022-09950-5.

De Souza, R. V., Moresco, V., Miotto, M., Souza, D. S. M., Campos, C. & Suplicy, F. M. 2022b. Depuração e tratamento térmico para redução dos níveis de patógenos em moluscos bivalves produzidos em Santa Catarina, Brasil. *Agropecuária Catarinense*, *35*, 78-82. DOI: https://doi.org/10.52945/rac.v35i2.1351

FAO (Food and Agriculture Organization of United Nations) & WHO (World Health Organization). 2018. *Technical guidance for the development of the growing area aspects of bivalve mollusc sanitation programmes*. Rome, FAO: WHO.

Guimarães Filho, C. E. F., Calixto, F. A. A., Kasnowski, M. C. & Mesquita, E. F. M. 2022. Analysis of microbiological contaminants in mussel *Perna perna* (Linnaeus, 1758), before and after depuration, from mariculture of the lowland coast, Rio de Janeiro, Brazil. *Food Science and Technology*, 42. DOI: https://doi.org/10.1590/fst.64121.

Lees, D., Younger, A. & Doré, B. Depuration and relaying. 2010. *In*: Rees, G., Pond, K., Kay, D., Bartram, J. & Santo Domingo, J. (eds.). *Safe Management of Shellfish and Harvest Waters*. Geneva: World Health Organization; London: IWA Publishing.

Lee, R., Lovatelli, A. & Ababouch, L. 2008. *Bivalve depuration: fundamental and practice aspects* (FAO Fisheries Technical Paper, No. 511). Rome, FAO.

Lees, D. 2000. Viruses and bivalve shellfish. *International Journal of Food Microbiology*, *59*, 81-116. DOI: https://doi.org/10.1016/S0168-1605(00)00248-8.

Luneta, J. E. 1969. Fisiologia da reprodução de mexilhões *Mytilus perna* L. (Mollusca: Lamellibranchia). *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo. Zoologia e Biologia Marinha*, *26*, 33-111.

Power, U. F. & Collins, J. K. 1989. Differential depuration of Poliovirus, *Escherichia coli*, and a coliphage by the common mussel, *Mytilus edulis*. *Applied and Environmental Microbiology*, *55*, 1386-1390.

Rees, G., Pond, K., Kay, D., Bartram, J. & Domingo, J. S. (eds.). 2010. *Safe Management of Shellfish and Harvest Waters*. London, World Health Organization.

Reddy, N. A. & Menon, N. R. 1979. Effects of ammonia and ammonium on tolerance and byssogenesis in *Perna viridis*. *Marine Ecology Progress Series*, *1*, 315-321.

Richards, G. P. 1988. Microbial purification of shellfish: A review of depuration and relaying. *Journal of Food Protection*, *51*, 218-251.

Seed, R. & Suchanek, T. 1992. Population and community ecology of *Mytilus*. *In*: Gosling, E. (ed.) *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture*. San Diego: Elsevier.

Silvestri, F., Cordeiro, G. B. & Costa, P. M. S. 2018. Parâmetros reprodutivos do mexilhão *Perna perna* (L. 1758) em fazendas marinhas na Ilha Grande (RJ). *Acta of Fisheries and Aquatic Resources*, *6*, 43-49.

Suplicy, F. M. 1999. Depuração do mexilhão *Perna perna* (L. 1758). *In*: *Oceanos: Fonte de Alimentos. Prêmio Jovem Cientista 1997*. Brasília, DF: CNPq, Fundação Roberto Marinho, Grupo Gerdau.

Suplicy, F. M. (Org.). 2019. *Plano Estratégico para o Desenvolvimento Sustentável da Maricultura Catarinense (2018-2028)*. Florianópolis, Epagri.

UK Food Standards Agency, 2016. *Guidance for Local Authority Authorized Officers on the Inspection of Purification Systems for Live Bivalve Mollusks in England Wales and Northern Ireland.* London: UK Food Standard Agency.

Zardi, G. I., Mcquaid, C. D., Teske, P. R. & Barker, N. P. 2007. Unexpected genetic structure of mussel populations in South Africa: Indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, 337, 135-144. DOI: https://doi.org/10.3354/meps337135.

Zippay, M. L. & Helmuth, B. 2012. Effects of temperature change on mussel, *Mytilus*. *Integrative Zoology*, 7, 312-327. DOI: https://doi.org/10.1111/j.1749K4877.2012.00310.x