



## Prevalence of parasitism by *Bucephalus* (Von Baer, 1827) in *Perna perna* (Linnaeus, 1758) from mariculture of the coastal lowlands, Rio de Janeiro, Brazil

[Prevalência parasitária de *Bucephalus* (Von Baer, 1827) em *Perna perna* (Linnaeus, 1758) oriundos de maricultura das baixadas litorâneas, Rio de Janeiro, Brasil]

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### ABSTRACT

The present study aimed to analyze the parasite prevalence of 40 *Perna perna* mussels (Linnaeus, 1758), to verify the presence of the parasite of the genus *Bucephalus*, originating from mariculture in the coastal lowlands, Rio de Janeiro, Brazil. Collections were made in the four seasons of the year (autumn, winter, spring, and summer), every three months, from March 2019 to February 2020. In microscopic analysis of soft tissue of *P. perna*, a prevalence rate of 22.5% parasite was found. Comparison of the two collection areas showed a higher prevalence in Praia do Forno, Arraial do Cabo, during autumn, and both male and female individuals affected.

Keywords: bucephalosis, malacoculture, mussel, parasite

### RESUMO

O objetivo deste estudo foi analisar a prevalência parasitária de 40 mexilhões *Perna perna* (Linnaeus, 1758), oriundos de maricultura das baixadas litorâneas, Rio de Janeiro, Brasil, a fim de se verificar a presença do parasito do gênero *Bucephalus*. As coletas foram realizadas nas quatro estações do ano (outono, inverno, primavera e verão), trimestralmente, de março de 2019 a fevereiro de 2020. Em análises microscópicas das partes moles de *P. perna*, foi encontrada uma prevalência de 22,5% do parasito em questão. Comparando-se Armação de Búzios e Arraial do Cabo observa-se maior prevalência na Praia do Forno, em Arraial do Cabo, no período do outono e em indivíduos machos e fêmeas os mais afetados.

Palavras-chave: bucefalose, malacocultura, mexilhão, parasita

### INTRODUCTION

Digenean trematodes (subclass Digenea) are a group of very numerous parasites characterized by a complex life cycle, with two reproductive phases and that usually require at least two hosts to reach adult stage (Resgalla Junior, 2008).

Diseases affect the health of marine bivalves in various parts of the world, with implications not only for crop production, but also for extractivism (Boehs *et al.*, 2012).

Bucephalosis, also known as “orange disease”, is caused by a trematode parasite of the genus *Bucephalus*, which generates inflammatory processes in *P. perna* mussels (non-zoonotic). The parasite lodges in the gonads, digestive gland, mantle, and gills. This disease is considered relevant as it can impact the animal's reproductive process and affect the communities that make a living from the production and extraction of this mollusk (Garcia and Magalhães, 2008; Cochoa and Magalhães, 2008; Lima *et al.*, 2001).

In addition to the damage of the gonad tissues by the bucephalid (Lauckner, 1983), other organs

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can be affected by the parasite larvae, such as the gills, the digestive gland, and the adductor muscle. The weakening of the latter can impair valve closure (Bower *et al.*, 1992; Perkins, 1993).

Infestation by this parasite occurs at times of milder temperatures (23° a 28°C) and in water with greater bacteriological contamination (Costa, 2007). According to Loureiro and Morais (2001), in his study in Guanabara Bay, an increase in parasite prevalence associated with increased pollution and environmental degradation was observed.

According to Andrade (2017), habitat, temperature, salinity, and host species have a specific influence on the occurrence and prevalence of parasites em mollusc bivalve.

Therefore, the present study aimed to compare the prevalence of the parasite *Bucephalus* sp. in the cultivation of *P. perna* mussels in the coastal lowlands of the state of Rio de Janeiro.

## MATERIAL AND METHODS

The study was carried out in the coastal lowlands of the state of Rio de Janeiro, at two *P. perna* mussel cultivation sites: at Praia Rasa in Armação dos Búzios (Figure 1), located in area 1 (22° 44' 20.1" S and 41° 56 ' 52.3" W) and Praia do Forno (Figure 2), located in area 2 (22° 58' 283" S and 42° 0'25. 40" W) in the Arraial do Cabo Marine Extractive Reserve.

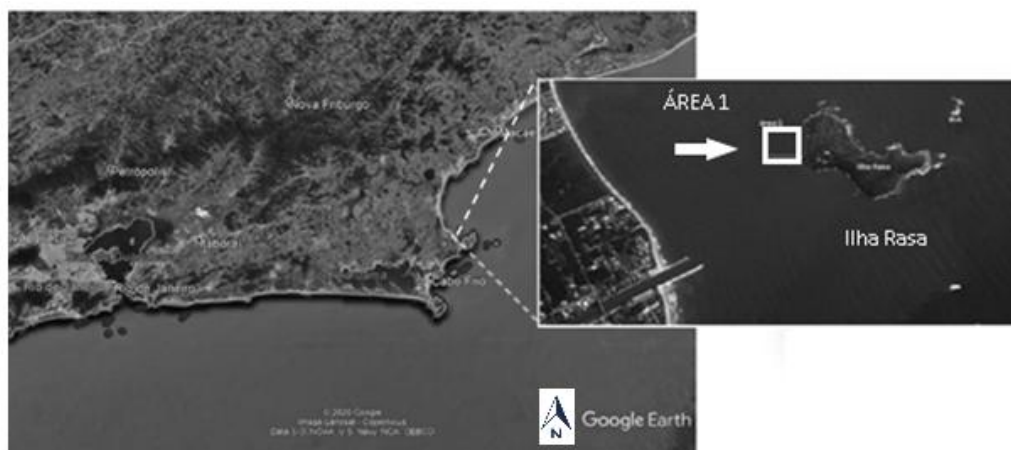


Figure 1. Location of marine farm in Praia Rasa close to Ilha Rasa in Armação de Búzios (cultivation area 1). Source: Prevalence, 2019.

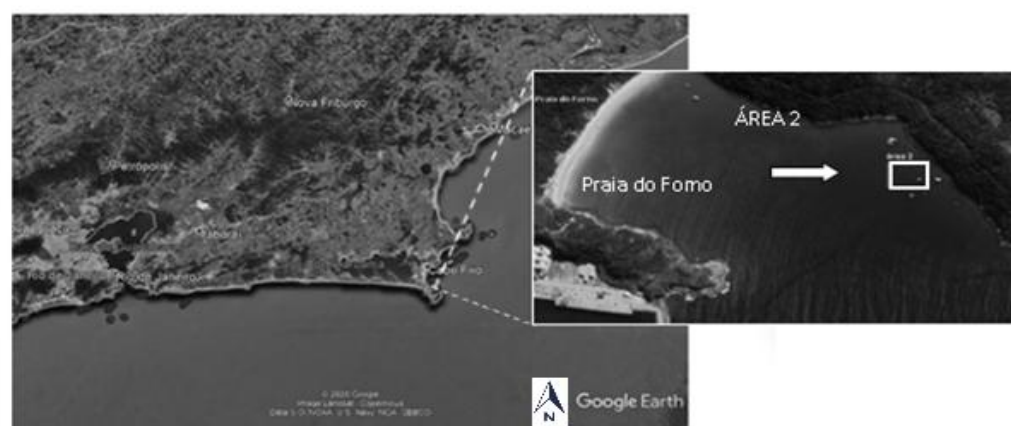


Figure 2. Location of the marine farm in Praia do Forno in the Arraial do Cabo Marine Extractive Reserve (cultivation area 2). Source: Prevalence, 2019.

### Prevalence of parasitism...

On the collection days, the temperature and salinity values of the seawater were measured at the two collection points (Armação de Búzios and Arraial do Cabo), using a refractometer and a thermometer, respectively.

Collections were carried out seasonally (autumn, winter, spring, and summer) from March 2019 to February 2020, in 40 animals from two regions of the coastal lowlands (20 animals from each region - Búzios and Arraial do Cabo), directly from the production ropes and at different points of mussel (*P. perna*) cultivation in isothermal boxes for the Fish and Aquatic Health Laboratory of the Food Technology Department of the Veterinary School of the Fluminense Federal University (Niterói, RJ).

In the laboratory, the mollusks were cleaned with the aid of a brush and under running water for removal of sediments and encrusting organisms, and parasitological analysis was then carried out.

The valves were opened, and the soft parts removed, for naked-eye inspection of the entire surface of the mollusk (gills, gonads, mantle, digestive gland, and foot) and, with the help of a

stereoscopic microscope, the sanitary conditions and the presence or absence of parasites were checked. Subsequently, then transferred to 10% formalin, cleaved, and cut transversely to inspect the gills, gonads, mantle, digestive gland, and foot.

Microscopic analysis was performed on 40 samples for the paraffin embedding technique, with 5µm thick sections and using a hematoxylin and eosin staining procedure for the tissue samples (Behmer *et al.*, 1976).

The coefficient of variation for temperature and salinity was calculated to verify the variability of data in relation to its average value. To further detail the prevalence of *Bucephalus*, a comparison test was carried out between its presence and other variables, such as place of origin, season, and sex.

### RESULTS

During the study period, at Praia Rasa (Armação de Búzios), salinity ranged from 34 to 35 mg/L ( $35 \pm 0.5$ ) and temperature ranged from 21 to 26°C ( $24.3 \pm 2.3$ ), as can be seen in Figure 3.

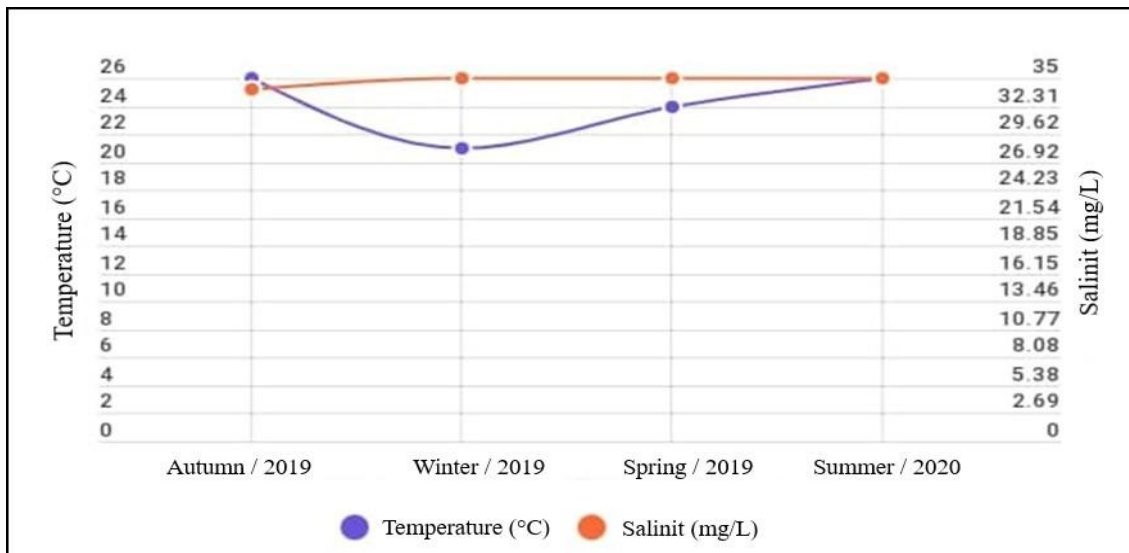


Figure 3. Temperature and Salinity of seawater from the marine farm in Praia Rasa near Ilha Rasa in Armação de Búzios (cultivation area 1).

On the other hand, on Praia do Forno (Arraial do Cabo) shown in Figure 4, salinity ranged from 35

to 37mg/L ( $36 \pm 1.0$ ) and temperature ranged from 22 to 26°C ( $23.5 \pm 1.7$ ).

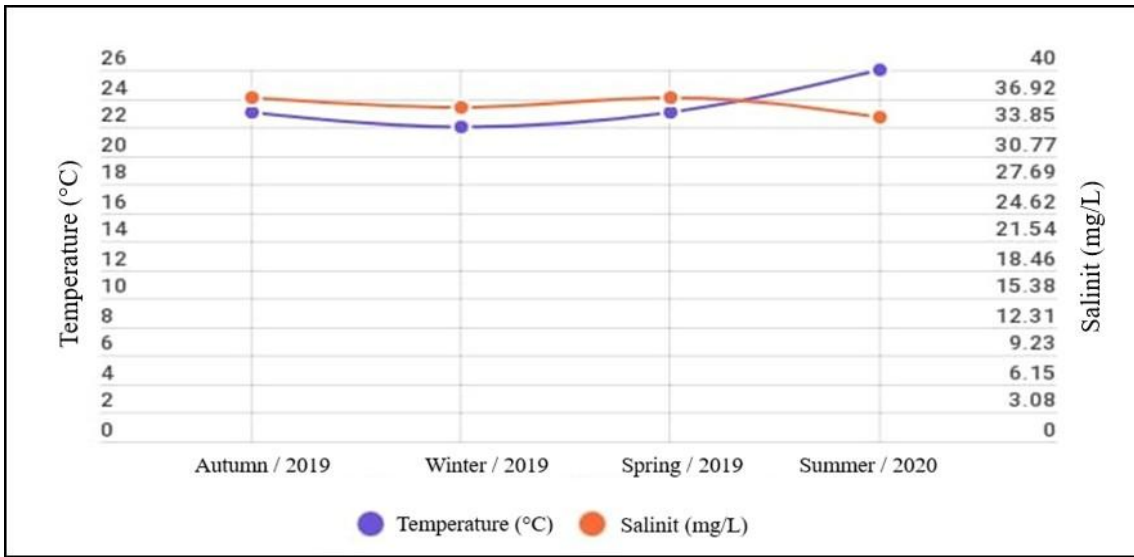


Figure 4. Temperature and Salinity of seawater from the marine farm in Praia do Forno in the Arraial do Cabo Marine Extractive Reserve (cultivation area 2).

Histopathological analyzes showed the presence of *Bucephalus* sp. in mussels from two regions of the coastal lowlands of the state of Rio de Janeiro (Praia Rasa -Armação de Búzios and

Praia do Forno – Arraial do Cabo). Forty animals (total sample) were analyzed, of which 77.5% were healthy and 22.5% had bucephalosis, as shown in Figure 5.

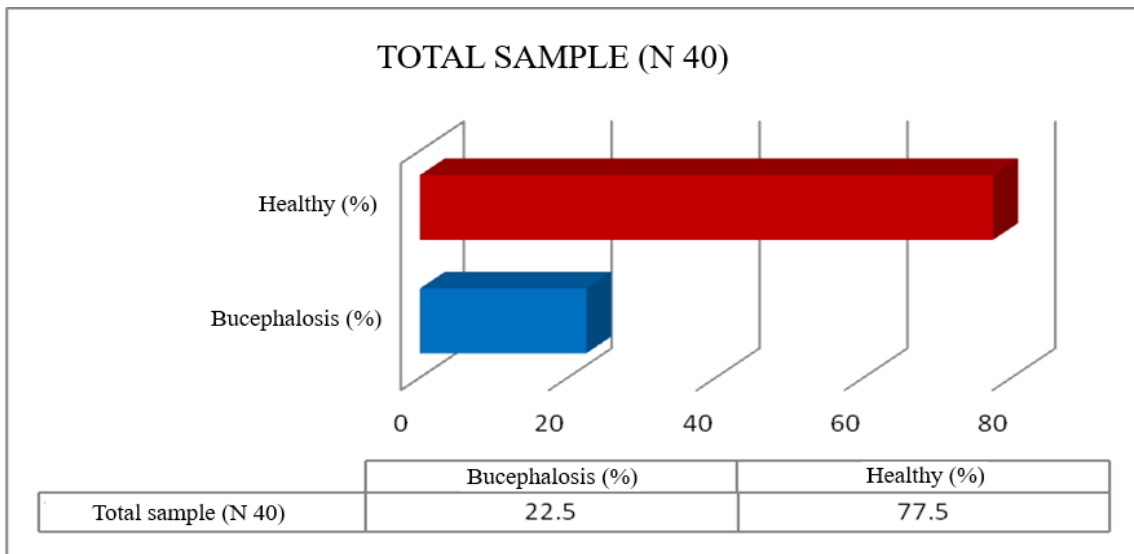


Figure 5. Total number of healthy mussels and those with bucephalosis in the two regions (marine farm in Praia Rasa – Armação de Búzios and the marine farm in Praia do Forno – Arraial do Cabo).

Regarding the seasons (autumn, winter, spring, and summer) from March 2019 to February 2020 (Figure 6), there was a higher prevalence of bucephalosis (60%) in autumn, and 40% in winter and spring, which coincides with the drop

in seawater temperature values in the region during the period (Figures 3 and 4). The lowest prevalence of bucephalosis was in summer (20%).

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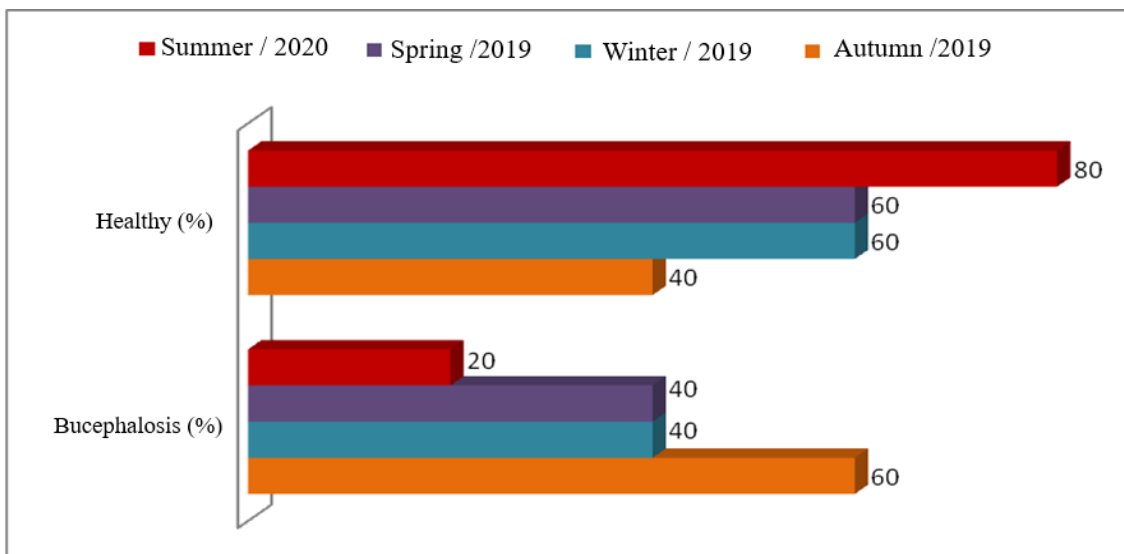


Figure 6. Percentage of prevalence of healthy mussels and those with bucephalosis in the two regions (marine farm in Praia Rasa – Armação de Búzios and in the marine farm in Praia do Forno – Arraial do Cabo).

For each region, 20 animals (a total of 40 animals) were analyzed and it was found that in Praia do Forno (Arraial do Cabo) there was a 40% prevalence of bucephalosis and in Praia

Rasa (Armação de Búzios) only a 5% prevalence of the disease, that is, the differences between the regions were significant, as shown in the Figure below.

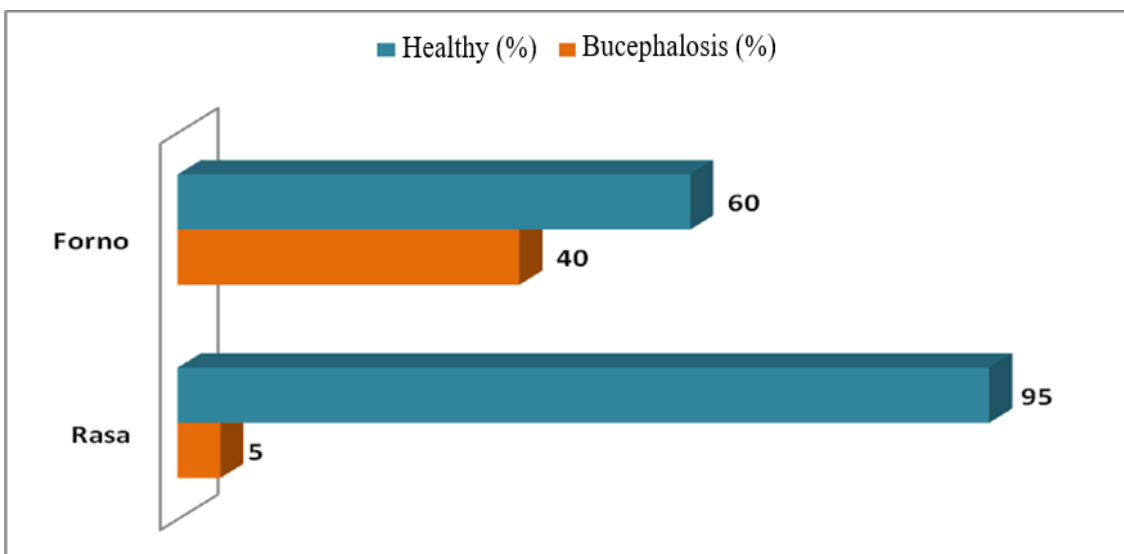


Figure 7. Percentage of prevalence of healthy mussels and those with bucephalosis in the two regions (marine farm in Praia Rasa – Armação de Búzios and in marine farm in Praia do Forno – Arraial do Cabo).

Analysis of the sexual aspect of the mussels revealed that females and males located at the marine farm at Praia do Forno had a higher

prevalence of bucephalosis (Figure 8), while at the marine farm at Praia Rasa, there was a 5% prevalence only in females (Figure 9).

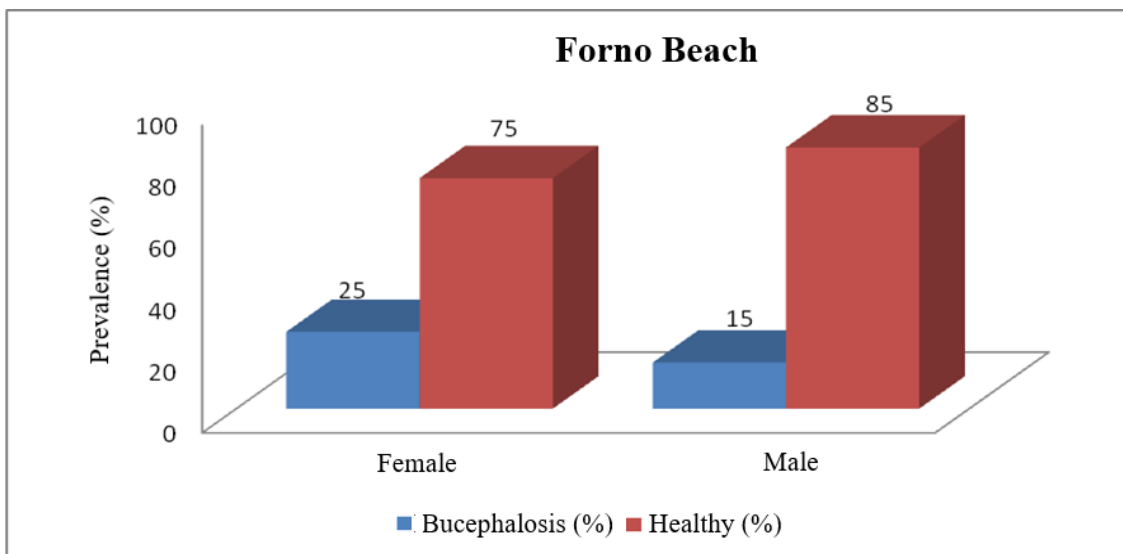


Figure 8. Percentage of prevalence of healthy female and male mussels and those with bucephalosis at marine farm of Praia do Forno – Arraial do Cabo.

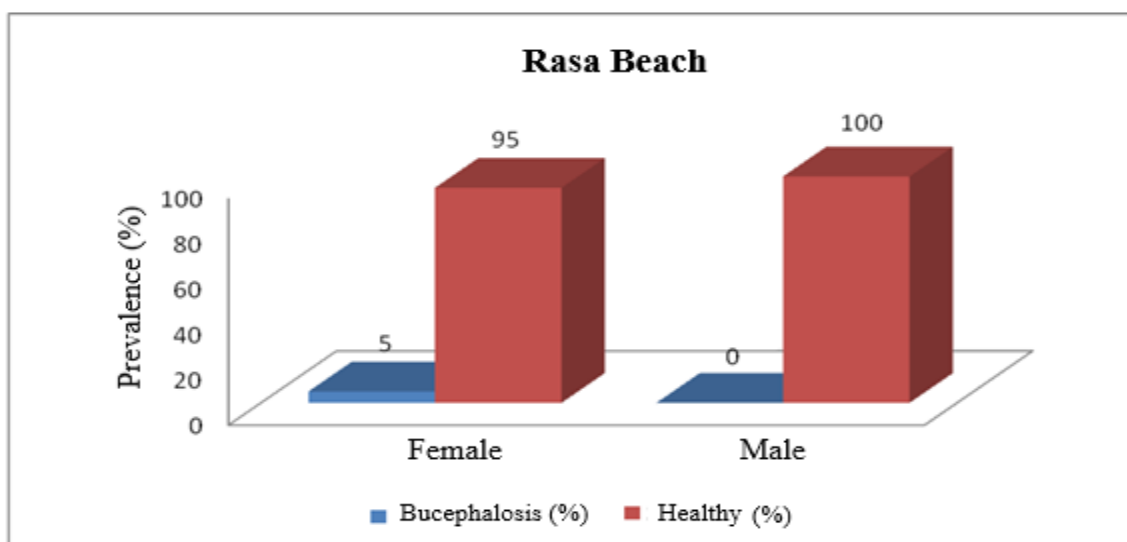


Figure 9. Percentage of prevalence of healthy female and male mussels and those with bucephalosis in the marine farm of Praia Rasa – Armação de Búzios.

In each location, parasitism rates were compared (Figures 8 and 9), to determine whether the differences were statistically significant.

During the research, it was found that the mussels did not show signs of bucephalosis in

macroscopic analysis. The parasite was observed under an optical microscope (Olympus CX31) with 10x and 40x objectives (Figure 10), for histopathological analysis.



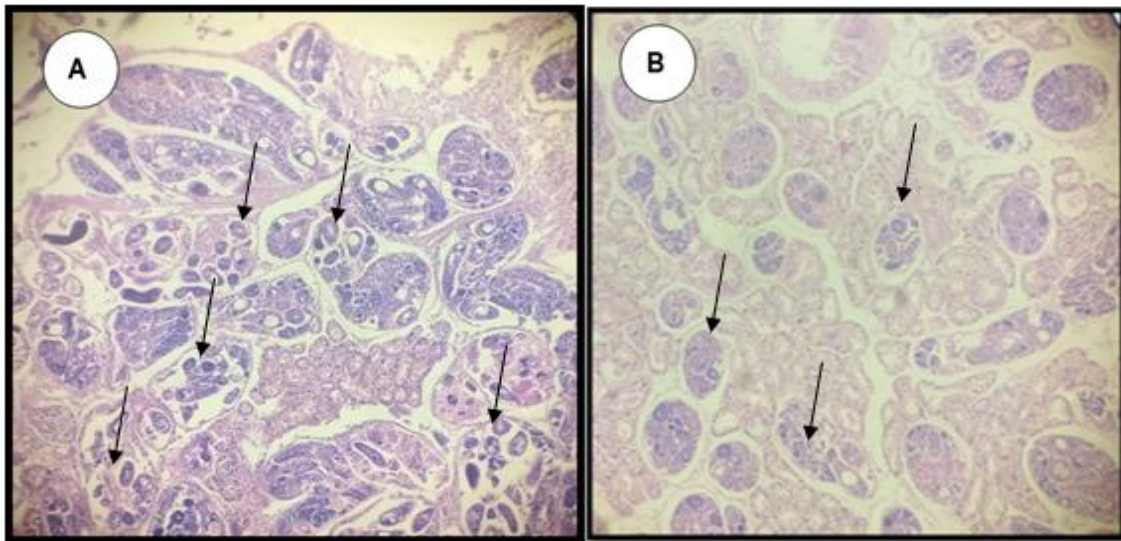


Figure 10. Photomicrographs (40x and 10x) of the digestive gland, A and B – Digestive gland parasitized by *Bucephalus* sp.; sporocysts containing different forms of parasite development (arrows).

#### DISCUSSION

In a study carried out in Baixada Santista, SP, Henriques (2004) analyzed 480 animals and noted a prevalence of 11.67%, a value lower than that found in the present study (22.5%). In contrast, Lima *et al.* (2001), when investigating the prevalence of mussels infected by *Bucephalus* sp. in Lagoa de Itaipu, RJ, noted a prevalence of 2.5% in the analysis of 120 animals, and Galvão *et al.* (2006), in a study carried out on Ilha Urubuqueçaba and Praia de Guaraú, SP, analyzed 960 animals and found a prevalence of 5.83%.

The fact that the prevalence of *Bucephalus* sp. was higher at Praia do Forno (Arraial do Cabo) can be attributed to the higher level of pollution, compared to Praia Rasa (Armação de Búzios). This happens because Praia do Forno is located in a city with infrastructure problems related to the discharge of the rainwater drainage network caused by excessive rainfall, sewage and a flow of tourists that increase the local population. Therefore, the water is expected to be more polluted than that of Praia Rasa (Armação de Búzios). The influence of pollution, with its impact on increasing parasite prevalence, has been investigated by several authors, such as Henriques (2004), Galvão *et al.* (2006), Costa (2007) and Loureiro and Morais (2001). This study did not, however, aim to verify the influence of pollution on the development of

*Bucephalus* in *Perna perna*. Therefore, further research is needed.

In a study carried out in the state of São Paulo, Galvão *et al.* (2006) reported that histological analysis corroborated previous macroscopic observations and allowed the identification of the sex of some specimens infested by *Bucephalus* sp. They also reported that parasite prevalence varied on a seasonal basis. The highest values were observed in autumn, winter, and spring for mussels from Urubuqueçaba Island, in Santos Bay/SP and, in autumn and winter, for those from Praia de Guaraú, which were seasons of milder temperatures and less rainfall.

The highest prevalence rates of bucephalosis occurred in the autumn months, corroborating a study carried out by Costa (2007) and Galvão *et al.* (2006), who reported the highest infestation peaks in periods of milder temperatures. On the other hand, in studies conducted by Cochôa and Magalhães (2008) and by Silva *et al.* (2002), the highest prevalence of this parasite occurred in winter.

There are few records of historical data on parasitism in mussels, which makes diagnosing and mapping infestations over the years difficult. It is suggested that similar studies are carried out to preventively monitor one of the aspects of mussel health in the coastal lowlands of Rio de

Janeiro, including the main natural stocks of this mollusk.

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