

BIOMETRIC AND REPRODUCTIVE INDICATORS IN *Perna perna* (Linnaeus, 1758) MUSSELS FROM COASTAL MARICULTURE IN RIO DE JANEIRO, BRAZIL

Indicadores biométricos e reprodutivos em mexilhões *Perna perna* (Linnaeus, 1758) de mariculturas costeiras no Rio de Janeiro, Brasil

Carlos Eduardo de Freitas Guimarães Filho^{1*}; Flávia Aline Andrade Calixto²; Maria Carmela Kasnowski³; Eliana de Fátima Marques de Mesquita³

¹Universidade Federal Fluminense, Programa de Pós-graduação em Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal, e-mail: carlooseduardo.fiperj@gmail.com. ORCID: <https://orcid.org/0000-0002-7118-528X>

²Fundação Instituto de Pesca do Estado do Rio de Janeiro, e-mail: faacalixto@gmail.com. ORCID: <https://orcid.org/0000-0001-6851-7899>

³Universidade Federal Fluminense, Faculdade de Veterinária, Departamento de Tecnologia de Alimentos. e-mails: mcarmela@id.uff.br (ORCID: <https://orcid.org/0000-0001-9339-160X>); elianafmmpescado@gmail.com (ORCID: <https://orcid.org/0000-0003-4176-2522>).

ABSTRACT

The aim of this study was to analyze biometric and reproductive mussel indicators (*Perna perna*) from coastal maricultures in Rio de Janeiro, Brazil. The collections were carried out with a total of 480 animals in the four seasons of the year (autumn, winter, spring and summer), from March 2019 to February 2020. Two mussel samples were collected each season (n = 60) with average measurements of 23.9 mm for width, 33.8 mm for height, 69.9 mm for length, and 28.3 g for weight (shell + soft parts) in both coastal regions. Samples from Forno beach exhibited higher homogeneity in the winter for all evaluated parameters (width, height, length and weight). Mussel weight was the most variable parameter due to factors such as water flow, nutrients, and gonadal maturation stage. Regarding reproductive parameters, a predominance of females was observed at both beaches throughout all four seasons. Additionally, a predominance of both males and females at substage IIIA was observed during autumn and winter, while substage IIIC showed the highest presence in spring and summer.

Keywords: Bivalve Mollusk; Growth indicators; Marine Biology; Reproduction

RESUMO

O objetivo deste estudo foi analisar os indicadores biométricos e reprodutivos de mexilhões (Perna perna) de mariculturas costeiras no Rio de Janeiro, Brasil. As coletas foram realizadas com um total de 480 animais nas quatro estações do ano (outono, inverno, primavera e verão), de março de 2019 a fevereiro de 2020. Duas amostras de mexilhão foram coletadas em cada estação (n = 60), com medidas médias de 23,9 mm de largura, 33,8 mm de altura, 69,9 mm de comprimento e 28,3 g de peso (concha + partes moles) em ambas as regiões costeiras. As amostras da praia do Forno apresentaram maior homogeneidade no inverno para todos os parâmetros avaliados (largura, altura, comprimento e peso). O peso do mexilhão foi o parâmetro mais variável devido a fatores como fluxo de água, nutrientes e estágio de maturação gonadal. Em relação aos parâmetros reprodutivos, foi observada uma predominância de fêmeas em ambas as praias durante todas as quatro estações. Além disso, uma predominância de machos e fêmeas no subestágio IIIA foi observada durante outono e inverno, enquanto o subestágio IIIC mostrou a maior presença na primavera e verão.

Palavras-chave: *Molusco bivalve; Indicadores de crescimento; Biologia marinha; Reprodução*

INTRODUCTION

Along the Brazilian coast, the extraction and production of mussels has played an important social role in generating jobs and sources of income in coastal communities. (Fagundes *et al.*, 2004; Lage, Jablonki, 2008).

At the beginning of the 70s, the former Navy Admiral Paulo Moreira da Silva Research Institute in Brazil began experimenting with mussel cultivation, choosing locations with excellent environmental conditions, such as Arraial do Cabo (RJ), in the state of Rio de Janeiro, Southeastern Brazil. At that time, the first raft culture models were developed, and mariculture material samples used in other countries were utilized, followed by implementation by Brazilian industries (Marques, 1998).

In the 90's, mariculture spread positively throughout the Brazilian economy. However, one of the obstacles to this activity was seed procurement, extracted from natural stocks to begin cultivation activities (Henriques *et al.* 2001).

In coastal areas, the use of artificial seed collectors has proven to be a sustainable and efficient method of obtaining seeds for mariculture activities, reducing the risks and dangers of accidents on natural coasts (Ferreira *et al.* 2006).

The study of the reproductive cycle, the monitoring of water temperature and the identification of the highest frequency of emission of gametes in the different periods of the year, are indispensable information to define the best periods of release of seed collectors into the sea, with the purpose of ensure greater efficiency in the settlement of larvae in these collectors (Marques, 1998; Ferreira, Magalhães, 2004).

The southeastern and southern regions are the main bivalve mollusk producers in Brazil, while the southern region is considered the primary national producer, including the state of Santa Catarina, which, according to the Epagri mariculture and Fisheries Development Center (EPAGRI, 2018), produced 13,699 t of mussels, oysters and scallops in 2017, representing a 10.93% reduction compared to 2016 (15,381 t).

According to the annual Fishing Institute Foundation of the State of Rio de Janeiro report (2017), farmers from the Arraial do Cabo Fishermen's Association - APAC and the Aquaculture Workers Association - Armação dos Búzios work in the municipalities of Arraial do Cabo and Armação dos Búzios, in Rio de Janeiro. The region exhibits a potential to expand mariculture development, since its have geographical, environmental and water quality characteristics are favorable for the growth of bivalve mollusks.

According to Lunetta (1969) macroscopic and microscopic observations of *Perna Perna* mussel gonatic tissue indicate three stages, I, II and III. Stage III is also categorized into three sub-stades, namely IIIA, comprising a very thick mantle and follicles full of gametes, reacting easily to abiotic environmental factors, such as temperature and salinity, sub-stage IIIB, where the follicles are partially or totally empty and the mantle is not very thick and, IIIC, the gametogenesis phase, exhibiting restoration follicles.

As the regions of Arraial do Cabo and Armação de Búzios present different conditions of water temperature, turbidity (transparency), and the difficulty in acquiring *P. perna* mussel seeds, this study aimed to correlate biometric and reproductive data of mussels from marine farms located in the coastal Baixadas region of the state of Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Study area

This study was carried out in the coastal region of the state of Rio de Janeiro, at two locations: Rasa Beach, at Armação dos Búzios (Figure 1), located in area 1 (22°44'20.1"S and 41°56'52.3"O) and Forno Beach (Figure 2), located in area 2 (22°58,2'83"S and 42°0.25'40"O), in the Arraial do Cabo Marine Extractive Reserve.

Figure 1 - Location of the Rasa Beach mussel marine farm at Armação dos Búzios (cultivation area 1). Source: Google Earth (2019)

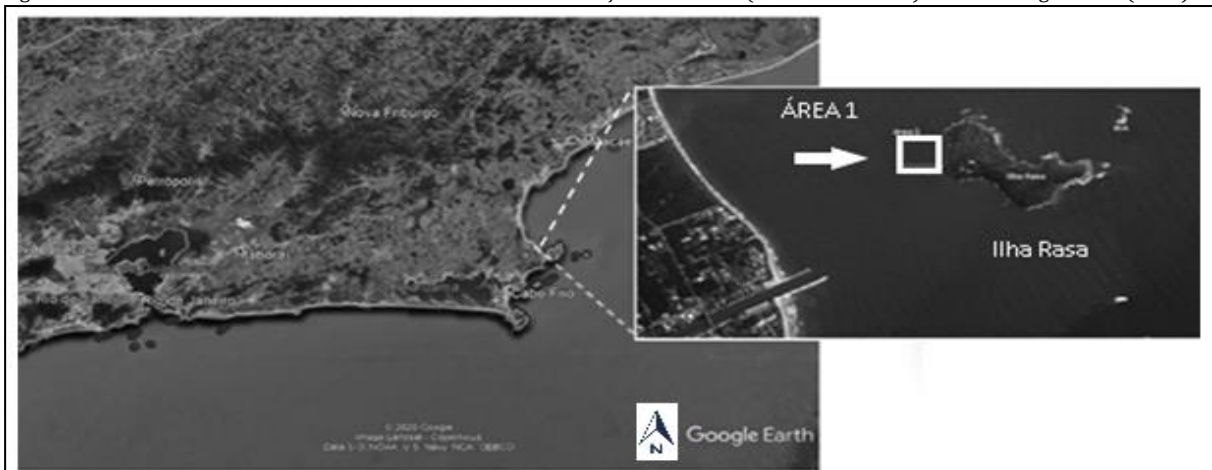
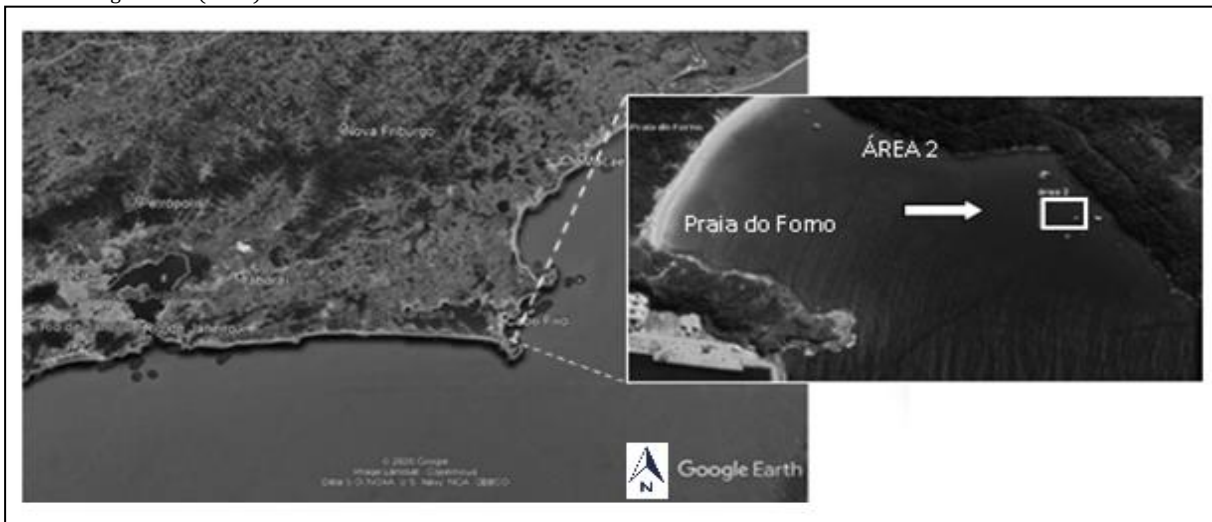


Figure 2 - Location of the Forno Beach mussel marine farm at the Arraial do Cabo Marine Extractive Reserve (cultivation area 2). Source: Google Earth (2019)



The municipality of Armação de Búzios is surrounded to the north, east and south by the Atlantic Ocean and to the west by the municipality of Cabo Frio. According to the Brazilian Institute of Geography and Statistics's last census, in 2010, its estimated population in 2019 will be of 40,532 inhabitants, with a demographic density of 392.16 inhabitants/km² (IBGE, 2010). The municipality of Arraial do Cabo is surrounded to the north by the municipality of Cabo Frio, to the east and south by the Atlantic Ocean and to the west, by the municipality of Araruama. According to the census, its estimated population in 2019 will be 30,349 inhabitants with a demographic density of 172.91 inhabitants/km² (IBGE, 2010).

Field Sample

Samples were collected every three months during each season (autumn, winter, spring and summer) from March 2019 to February 2020, totaling 480 animals from the two coastal regions of Búzios and Arraial do Cabo, directly from farming strings at different mussel cultivation points (*Perna perna*).

Each sampling was performed by region, where the 60 sampled organisms were transported in isothermal boxes to the Laboratory of Microbiological Control of Animal Origin Products (CMPOA), belonging to the Department of Food Technology in Veterinary Medicine, at the Federal Fluminense University (Niterói, RJ).

At the CMPOA laboratory, biometric and morphological analyzes of the reproductive parameters of the individuals collected were performed. The mollusks were cleaned with a brush under running water to remove encrusted sediments and organisms and left to dry on a previously disinfected plastic tray. Subsequently, valve thickness, width and lengths were measured with a caliper and total weight obtained using a precision scale (Biometrics) for all 480 animals.

After measuring, the valves of 240 of the sampled individuals were opened for morphological observations concerning reproductive parameters, macroscopic sex determination, visual gonad observation, according to the sexual maturation stages described by Lunetta (1969). Animals reach sexual maturity at stage III, where females and males display orange and creamy white gonads, respectively.

Statistical analysis

Valve width, height, length and total weight data were expressed in a table as the means of total values, 95% confidence interval of the means, 5% of the supported means and the respective standard deviations. The coefficient of variation of the samples was calculated to verify data variability in terms of their mean values.

RESULTS

Regarding the physicochemical parameters of the waters used in the culture of bivalve molluscs, in the four seasons of the year (autumn, winter, spring and summer) (Table 1), the water temperature measured in Rasa beach ranged from 24.3 ± 2.3 °C and at Forno beach, from 23.5 ± 1.7 °C, which are characteristic of subtropical regions. Salinity ranged between 35 ± 0.5 g/L at Praia Rasa and between 36 ± 1.0 g/L at Praia do Forno; pH was 7.0 (neutral) on both beaches. Therefore, several studies report that seawater is a slightly alkaline solution, with a pH ranging on average between 7.5 to 8.4, and according to Conama Resolution No. 357 (CONAMA, 2005), which regulates the cultivation of bivalve molluscs intended for human consumption, pH varies between 6.5 and 8.5. However, most pathogenic bacteria in the environment grow and multiply in acidic environments (with a pH ranging between 0 and 6.5). However, there are families of bacteria that only live in a neutral environment and others in an alkaline environment. Turbidity

at Rasa beach ranged from 2.00 ± 0.5 m of visibility and at Forno beach it ranged from 5.40 ± 1.7 m of visibility, demonstrating that the region of Armação de Búzios had less transparency (amount of light reaching the water), greater amount of suspended matter, providing more food.

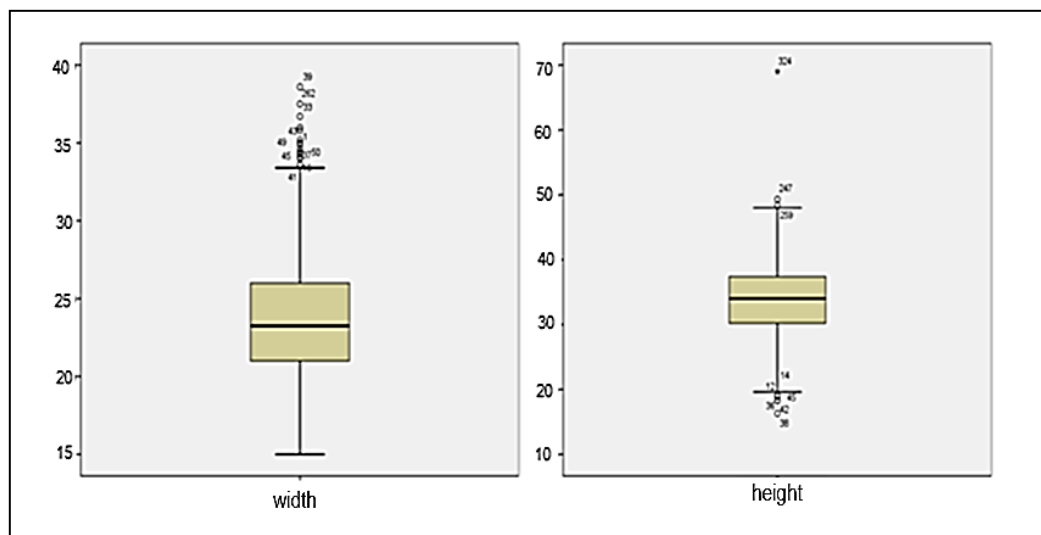
Table 1 - Physicochemical parameters of water from cultivation in coastal lowlands municipalities (Armação de Búzios and Arraial do Cabo), in the state of Rio de Janeiro

		Seasons of the year				
Beach	Parameters	October/2019	Winter/2019	Spring/2019	Summer/2020	Mean/SD
Rasa	Turbidity (m)	1.30	2.04	2.10	2.55	2.00 ± 0.5
	pH	7	7	7	7	7
	Salinity (mg/L)	34	35	35	35	35 ± 0.5
	Temperature (°C)	26	21	24	26	24.3 ± 2.3
Forno	Turbidity (m)	3.70	4.60	7.75	5.55	5.40 ± 1.7
	pH	7	7	7	7	7
	Salinity (mg/L)	37	36	37	35	36 ± 1.0
	Temperature (°C)	23	22	23	26	23.5 ± 1.7

During the study period, eight samplings were performed were done, totaling 12 months of analyses. Regarding the investigated biometric variables, the mean width of the mussels was 23.9 mm, with a minimum value of 15.0 mm observed in the summer and a maximum value of 38.6 mm recorded in autumn according to Figure 3.

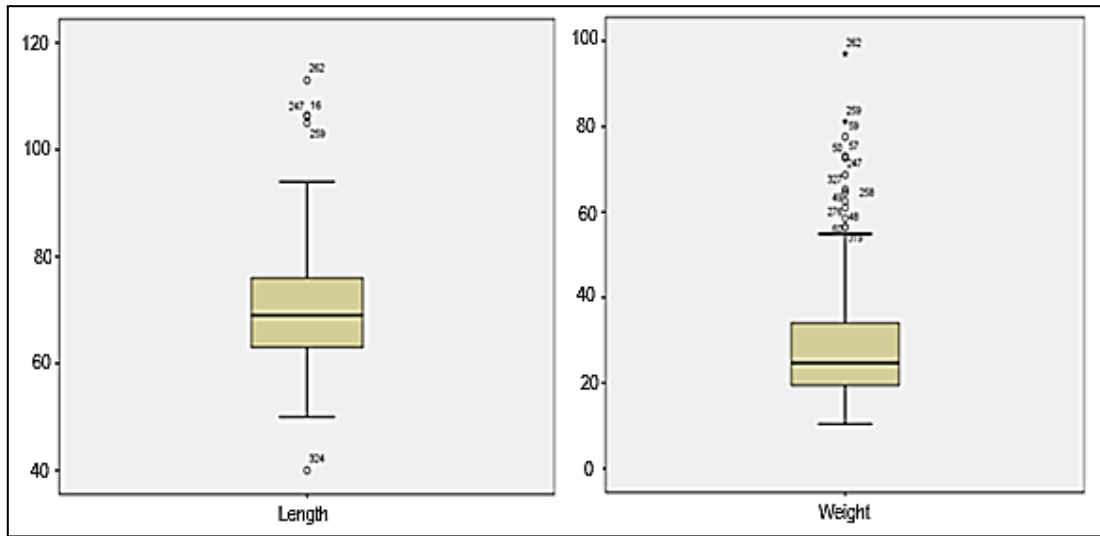
The mean mussel height (Figure 3) was 33.8 mm, with a minimum value of 16.3 mm observed in the autumn and a maximum value of 69.0 mm recorded in the summer.

Figure 3 - Box-plot graph representing *Perna perna* mussel width and height (minimum, maximum and means) at the two sampled beaches in coastal Rio de Janeiro, southeastern Brazil



Mean mussel length was 69.9 mm, with a minimum value of 40.0 mm observed in the summer and a maximum value of 113.0 mm recorded in the spring according to Figure 4. Mean mussel average weight of the mussels (Figure 4) was 28.3 g, with a minimum value of 10.4 g observed in the winter, and a maximum value of 97.0 g recorded in the spring.

Figure 4 - Box-plot graph representing *Perna perna* mussel representing length and weight (minimum, maximum and average) at the two sampled beaches in coastal Rio de Janeiro, southeastern Brazil



The lowest standard deviation for width displayed in Table 2 was of 2.24 millimeters in the spring, and while the lowest height (2.76 millimeters), length (5.44 millimeters) and weight (5.74 grams) standard deviations were observed in the winter.

Table 2 - Minimum, maximum, mean (\bar{X}), 95% confidence interval of the mean with upper and lower limits (CI), standard deviation (s), coefficient of variation (CV) of values related to the biometric width parameters (mm), width (mm), height (mm) and weight (g), found in Rasa beach in Armação de Búzios, Rio de Janeiro, Brazil

Season	Year					2019					2020									
	Fall		Winter			Spring			Summer											
	Min.	\bar{X}	IC (%)	s	C.V. (%)	Min.	\bar{X}	IC (%)	s	C.V. (%)	Min.	\bar{X}	IC (%)	s	C.V. (%)	Min.	\bar{X}	IC (%)	s	C.V. (%)
	Max.					Max.					Max.					Max.				
Width (mm)	19,4	29,5	27,6	5,16	17,4	18,0	21,3	20,5	2,28	10,7	17,3	22,7	22,2	2,24	9,86	15,0	21,7	20,9	3,20	14,7
	38,6		31,4			26,0		22,2			29,4		23,3			29,0		22,5		
Height (mm)	16,3	23,0	21,7	3,59	15,6	23,0	31,9	30,9	2,76	8,65	22,8	33,5	32,6	3,14	9,37	23,0	30,2	29,1	4,19	13,8
	29,9		24,4			38,0		33,0			41,0		34,3			44,0		31,3		
Length (mm)	55,0	68,5	65,8	7,38	10,7	56,8	64,1	62,0	5,44	8,48	56,5	70,0	68,4	6,10	8,71	50,0	63,0	60,9	8,02	12,7
	85,2		71,3			81,0		66,1			84,0		71,5			84,0		65,1		
Weight (g)	18,4	28,6	25,6	8,09	28,2	10,4	18,2	16,1	5,74	31,5	14,5	25,4	23,4	7,47	29,4	10,7	21,8	19,4	9,25	42,4
	46,0		31,6			37,5		20,4			52,4		27,3			52,4		24,2		

However, the lowest standard deviation noted in Table 3 occurred in the winter for width, height, length and weight, where these parameters ranged between 2.49 mm, 3.08 mm, 7.62 mm and 7.30 g, respectively.

Using the standard deviation data presented in both tables, the coefficient of variation (C.V.) can be calculated as $C.V. = 100 \cdot (\text{without the means}) (\%)$, to arrive at an independent dispersion measure concerning the investigated parameters. The width, height and length C.V.s at both beaches were uniform according to season.

With regard to mussel weight, the C.V.s were higher in all seasons, ranging from 28 to 48%. This is due to the fact that weight depends on several different factors, such as water flow, nutrients and gonadal maturation stage, which will determine higher or lower weights for specimens of similar sizes (GALVÃO *et al.*, 2006).

Table 3 - Minimum, maximum, mean (\bar{X}), 95% confidence interval of the mean with upper and lower limits (CI), standard deviation (s), coefficient of variation (CV) of values related to the biometric width parameters (mm), height (mm), length (mm) and weight (g), found in Forno beach em Arraial do Cabo, Rio de Janeiro, Brazil

Season	Year										2020									
	Fall					Winter					Spring					Summer				
	Min. Max.	\bar{X}	IC (%)	s	C.V. (%)	Min. Max.	\bar{X}	IC (%)	s	C.V. (%)	Min. Max.	\bar{X}	IC (%)	s	C.V. (%)	Min. Max.	\bar{X}	IC (%)	s	C.V. (%)
Width (mm)	16,3 35,0	26,3	24,4 28,1	4,90	18,6	16,0 27,0	20,8	19,9 21,7	2,49	11,9	16,6 37,5	25,5	24,4 26,6	4,15	16,2	18,0 31,0	24,3	23,5 25,1	3,22	13,2
Height (mm)	28,0 47,4	38,4	36,4 40,3	5,13	13,3	27,0 42,0	34,0	32,8 35,1	3,08	9,05	29,0 49,3	38,5	37,4 39,6	4,27	11,0	28,0 69,0	37,2	35,8 38,7	5,63	15,1
Length (mm)	57,3 106,4	78,8	74,4 83,1	11,6	14,7	53,0 85,0	67,0	64,1 69,8	7,62	11,3	53,0 113	76,2	73,2 79,2	11,7	15,3	40,0 92,0	71,4	69,0 73,8	9,32	13,0
Weight (g)	11,6 77,6	38,0	31,1 45,0	18,5	48,6	12,6 43,6	23,9	21,2 26,6	7,30	30,5	11,0 97,0	33,9	29,8 38,0	15,9	46,9	15,8 65,3	34,2	31,4 36,9	10,6	30,9

A predominance of females was noted (Figure 5), representing a total of 69 individuals (57.5%) at Rasa Beach and 62 individuals (51.7%) at Forno Beach.

However, when considering the four sampled seasons of the year (Figure 6), a predominance of females was noted in autumn, winter and spring, while the summer presented a higher number of male specimens.

Figure 5 - *Perna perna* mussel sex ratio from Armação dos Búzios and Arraial do Cabo sampled from October 2019 to February 2020

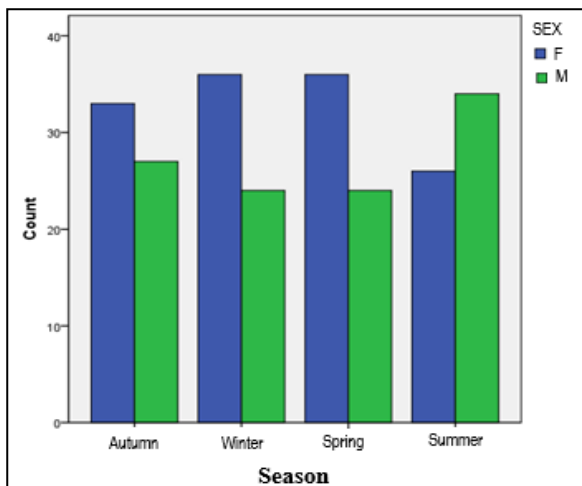
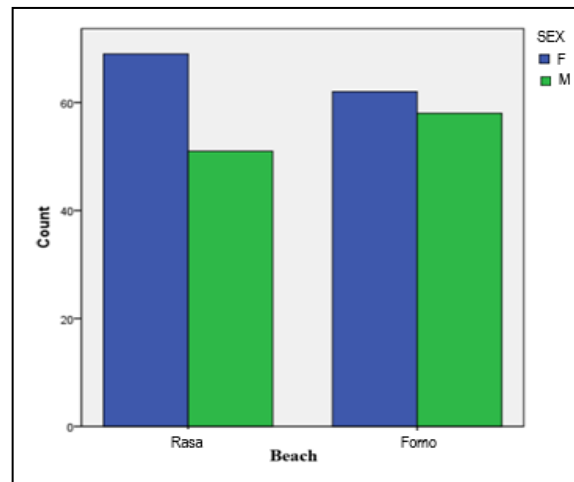


Figure 6. *Perna perna* mussel sex ratio from Armação dos Búzios and Arraial do Cabo during the four sampled seasons (autumn, winter, spring and summer)

The reproductive cycle was in general, mostly observed as stage III for both assessed beaches, at substage IIIA, with a quite thick mantle and with follicles full of gametes (Figure 7).

However, when considering the four sampling seasons (Figure 8) at both beaches, was a predominance of substage IIIA was noted in the autumn and winter and a predominance of substage IIIC in spring and summer. Therefore, mussels reproduced almost throughout the entire year, and from spring onwards a greater number of animals was observed in the gametogenesis phase, with follicle restoration.

Reproductive activities and an increase in the number of individuals with empty gonads (substage IIIB) were observed at both beaches (Figure 8) in the autumn and winter. When entering spring and summer, however, several individuals were observed in the follicle restoration stage (substage IIIC), this characteristic can be due to the good availability of food, the small temperature variation as shown in table 1 and the minimum stress on these farm animals.

Figure 7 - *Perna perna* mussel sexual maturation substages at the Rasa and Forno beaches from October 2019 to February 2020

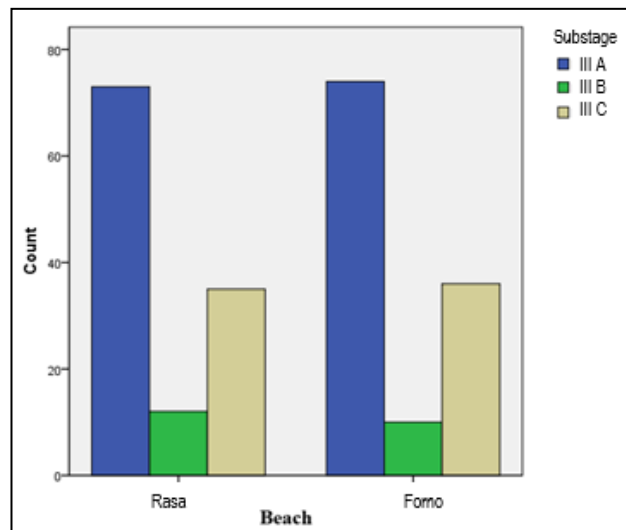
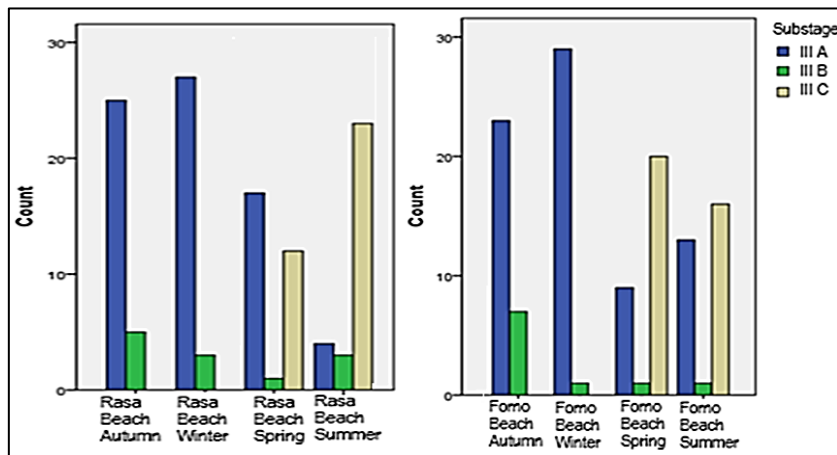


Figure 8 - *Perna perna* mussel sexual maturation substages at the Rasa and Forno beaches during the four sampled seasons (autumn, winter, spring and summer).



DISCUSSION

The predominance of females (Figures 5 and 6) in this study corroborates the study carried out Silvestri *et al.* (2018) at Ilha Grande, in Angra dos Reis, Rio de Janeiro. In contrast, studies carried out at the Itaipu Lagoon, in Niterói, in the state of Rio de Janeiro (MESQUITA *et al.*, 2001), and in São Paulo, in the cities of Urubueçaba and Guaraú (GALVÃO *et al.*, 2006), reported a predominance of male individuals. However, no records were found in the literature indicating the predominance of females in studies on *P. perna*.

According to Oper (2019), low standard deviations indicate more homogeneous samples. The lowest standard deviation for width displayed in Table 2 was of 2.24 millimeters in the spring, and while the lowest height (2.76 millimeters), length (5.44 millimeters) and weight (5.74 grams) standard deviations were observed in the winter.

According to Galvão *et al.*, (2006) the presence of mature animals (substage IIIA) was noted during all months from October 2000 to September 2001. Silvestri *et al.*, (2018), on the other hand, observed three gamete emission periods, from early May to mid-June (autumn) and from early July to late October (winter to spring) until December (spring to summer).

Evaluations of the mussels' reproductive parameters indicate a predominance of females on both beaches sampled and during the four seasons, with a predominance of both males and females in substages IIIA in the fall and winter and in substages IIIC in the spring and summer. These results corroborate Silvestri *et al.* (2018), showing that *P. perna* mussels reproduce practically all year round.

This study shows that on both beaches, there was a predominance of substages IIIA, IIIB (empty gonads) from fall to winter and a predominance of substage IIIC in spring and summer. However, Silvestri *et al.* (2018) found reproductive activity in early July and late October to December (winter, spring and summer), with an increase in the percentage of individuals with empty gonads in substages III-B. In both periods, the sexually mature mussels were found to be in substages III-A.

Magalhães *et al.* (1987) found that the elimination of *P. perna* gametes occurred throughout the year on the coast of Florianópolis, Santa Catarina, with greater intensity in the summer and then in the spring. However, Araújo (1994) recorded the release of gametes throughout the year, with peaks in April and December.

Therefore, according to Loureiro (2001), no specific reproductive period for *Perna perna* mussels is noted, indicating reproduction with variable peaks throughout the year and almost throughout the entire year (GALVÃO *et al.*, 2006).

The findings reported herein assessed at two coastal municipalities (Armação dos Búzios and Arraial do Cabo) in Rio de Janeiro indicate that the Forno Beach samples exhibited higher homogeneity in the winter for all assessed morphometric parameters (width, height, length and weight).

And the most variable parameter was mussel weight, as indicated by the high coefficient of variation in all investigated seasons, due to factors such as water flow, nutrients, and gonadal maturation stage.

The average total weight of the mussels in this study was 28.3 grams, with a minimum of 10.4 grams in the winter season and a maximum of 97.0 grams in the spring season. Counteracting the study by Silvestri *et al.* (2018) what ranged from a minimum of 20.8 grams in winter to a maximum of 30.7 grams in early summer.

Therefore Silvestri *et al.* (2018) obtained total mussel lengths ranging from 66.1 millimeters in autumn to 73.6 millimeters in spring. However, the overall average length of the mussels in this study was 69.9 millimeters, with a minimum value of 40.0 millimeters observed in the summer season, to 113.0 millimeters, the maximum value recorded in the spring season.

CONCLUSIONS

It is concluded that evaluations of the mussels' reproductive parameters indicate a predominance of females on both beaches sampled and during the four seasons sampled, with a predominance of males and females in substage IIIA in the fall and winter and substage IIIC in the spring and summer. And the Forno Beach samples exhibited higher homogeneity in the winter for all assessed morphometric parameters (width, height, length and weight), samples from Rasa beach, however, exhibited higher homogeneity in the spring regarding width.

To understand the reproductive cycle of *P. perna* in the two regions, it is necessary to continuity of this study, as well as investigating the influence of environmental parameters on their reproductive activity.

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Ethical - approval It is not required to get an “Approval of Animal use Protocol” when using invertebrates as experimental animal in Brazil.

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