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Excrement production by brown mussels *Perna perna* (L. 1758) under static conditions

FAUSTO SILVESTRI[1,2] & ALEXANDER TURRA[1]

[1] *University of São Paulo, Oceanographic Institute, Laboratório de Manejo, Ecologia e Conservação Marinha. Praça do Oceanográfico 191, 05508-900 São Paulo, SP, Brazil.*
[2]*Capes Foundation, Ministry of Education of Brazil, Caixa Postal 250, 70040-020 Brasília, DF, Brazil. E-mail:* silvestri@usp.br

Abstract. This study aimed to find the excrement production in static conditions by the brown mussel *Perna perna* collected in artisanal farms considering different size classes. Eleven experiments were conducted with mussels classified into three size classes (I: <40 mm; II: 40 to 70 mm; III: >70 mm of shell length) in experimental units with absence of food and controlled temperature and salinity. The results showed that the amount of excrements produced varied significantly as size classes and also through the experiments. There was a mean of 4.19 ±0.27, 6.37 ±0.29 e 9.86 ±0.40 mg/h of excrement production for mussels of classes I, II e III, respectively. The results can be employed to support hydrodynamic models of biodeposition in mussel farms, which is a fundamental information for the definition of monitoring strategies.

Key words: aquaculture, biodeposits, integrated coastal management, feces

Resumo. Produção de excrementos por mexilhões *Perna perna* em condições estáticas. Este estudo teve como objetivo verificar a produção de excrementos em condições estáticas por mexilhões *Perna perna* cultivados em fazendas marinhas considerando diferentes classes de tamanho. Foram realizados 11 experimentos com mexilhões divididos em três classes de tamanhos (I: <40 mm; II: 40 to 70 mm; III: >70 mm de comprimento) em unidades experimentais com ausência de alimento e temperatura e salinidade controlada. Os resultados indicaram que a quantidade de excrementos produzida variou significativamente conforme o tamanho dos organismos bem como os diferentes experimentos realizados. Foi verificada uma produção média de 4.19 ±0.27, 6.37 ±0.29 e 9.86 ±0.40 mg/h de excrementos para as classes I, II e III, respectivamente. Tais resultados podem fornecer subsídios para modelos hidrodinâmicos de biodeposição de partículas em fazendas marinhas, constituindo uma informação fundamental para a definição de estratégias de controle e monitoramento.

Palavras chave: aquicultura, biodepósitos, fezes, manejo costeiro integrado

Introduction

The deposit of excrements produced by shellfish farms is one of the main problems of mariculture in terms of environmental impacts. When deposited *in situ* feces and pseudofeces directly interact with the benthic system generating biodeposits (Hartstein & Rowden 2004).

In sites densely occupied by shellfish farms, the accumulation of biodeposits can dramatically affect the environmental carrying capacity, generating chemical and physical changes in the sediment that affect the benthic community structure (Dahlback & Gunnarsson 1981, Mattsson & Lindén 1983, Chamberlain *et al.* 2001, Beadman *et al.* 2004, Hartstein & Stevens 2005, Miron *et al.* 2005, Costa & Nalesso 2006, Giles & Pilditch 2006, Cranford *et al.* 2009). In these areas the increase of organic matter in the sediment could cause the decrease of oxygenated layer, leading to anoxia of the sediment and the overlying water and the accumulation of free sulphide. This situation causes decreases of abundance and diversity species in the influence areas of marine farms and also the dominance of opportunistic species, as deposit-feeding polychaets.

Endogenous and exogenous factors such as weight, size and reproductive stage of mussels, quantity and quality of food, temperature, salinity and water pollution can affect metabolic rates and consequently the production of excrements (feces and pseudofezes) by mussels (Resgalla Jr. 2008).

Experiments performed *in situ* are best suited to determine the excrement production, because reflect with good accuracy the physiological rates of organisms that are situated in their own environment. Although accurate, this approach represents the behavior of organisms on a particular situation and does not consider the variability of environmental parameters in time and/or space scale (Widdows 1985).

However, laboratory experiments with mussels require a minimum of ten days of acclimatization in order to stabilize the respiration rate (Resgalla Jr. *et al.* 2006) and do not reproduce precisely the environmental conditions. An experiment carried out in field and laboratory conditions, where the mussels feed directly in the sea and release their excrements in a controlled environment with absence of food can provide a baseline values with good accuracy and feasible to be used in models to predict biodeposition impacts.

The mariculture is passing through a critical consolidation period in Brazil, requiring measures to ensure its sustainability, including zoning and the standardization and regulation of the farms. Extensive information is needed to support these actions.

Currently in Brazil the mussel culture represent 84.7% of total bivalve production (MPA 2010). Most of these farms does not present an environmental license despite the legislation requires that farms be licensed based on environmental-impact assessments, including the determination of their areas of direct and indirect influence.

The brown mussel *Perna perna* (Bivalvia, Mytilidae) occuring in Brazil,Venezuela, Uruguay, Argentina and South Africa (Rios 1984). *P. perna* is widely produced by suspend culture throughout the south and southeast coast of Brazil (Marques 1998). The collection of mussels on rocky shores is also widespread in the region. Besides the economic aspect, the species is widely used in ecotoxicological tests and used as bioindicator through the accumulation of heavy metals.

The determination of excrement production in mussels of different size classes may be useful information in experiments on strategic areas as energetic physiology, ecotoxicology, molluscs depuration and studies of carrying capacity of coastal environments. The estimates of feces in mussels of different size classes can provide more accurate data in these studies. Knowledge of excrement production can be useful in the prediction of environmental impacts, as well as in the creation of standards and guidelines aimed to environmentally sustainable activity. In this sense, this study aimed to determine the excrement production in static conditions of brown mussel *Perna perna* from mussel farms considering different size classes.

Materials and Methods

From February 2008 through January 2009, monthly samples of mussels were collected in commercial farms at Praia da Cocanha, southern of Brazil (Fig. 1). The study area is situated on the northern coast of Sao Paulo state, a region with an irregular coastline composed by many bays and coves. Oceanographically, this area is defined as an oligo-mesotrophic region (Castro-Filho & Miranda 1998) influenced by seasonal upwelling (Lorenzetti & Gaeta 1996, Gaeta & Brandini 2006).

The characterization of local environmental parameters included the monthly monitoring of the nine sites (Fig. 1). Temperature, salinity, water transparency, suspended particulate matter, chlorophyll *a*, and phaeopigments were measured monthly, at 1 m depth, preferably at low tide. Suspended particulate matter concentrations (total, organic and inorganic fractions) were obtained by the gravimetric method (APHA 2005). Chlorophyll *a* and phaeopigments were obtained by spectrophotometry from the equations described by Lorenzen (1967).

Monthly about 50 mussels from different long-lines were collected and immediately transported dry in a cooler to the "Clarimundo de Jesus" Oceanographic Institute Research Station. For avoid the feces release and promote the accuracy of the amounts of excrement estimates the total time of transport did not exceed 30 minutes. Mussels with open shells were eliminated of experiments. In the laboratory, the mussels were quickly scraped and brushed with seawater.

Ten mussels were randomly chosen for three classes of sizes (I: <40 mm; II: 40 to 70 mm; III: >70 mm of shell length), and were immediately placed in 500 mL experimental systems with aeration and controlled temperature and salinity. In these systems, seawater was previously filtered through Millipore AP40 glass fiber filters (0.7µm) used to guarantee the absence of food and release only the food ingested in the field. For better accuracy of the results the mussels were maintained for 2 hours in the experimental systems. The excrement production (mg /h) was obtained by the arithmetic average of the excrements produced in 2 hours period.

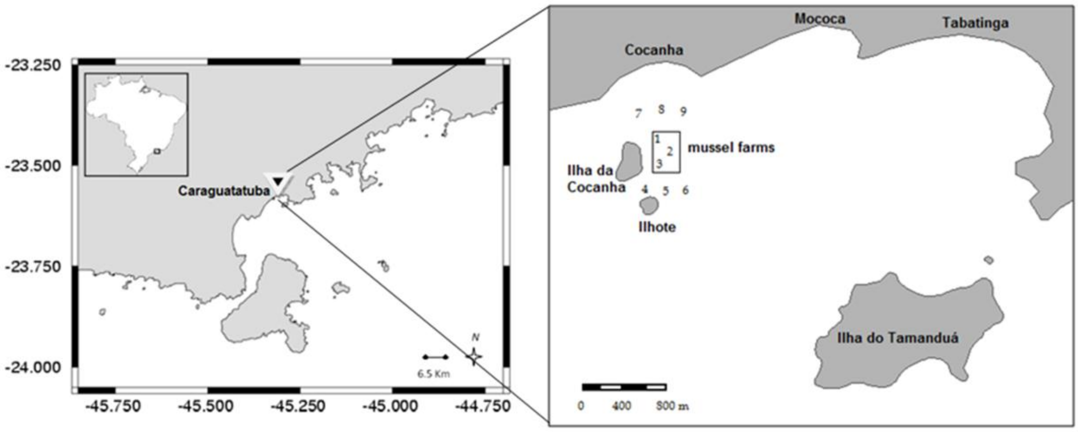

Figure 1. Left square: Caraguatatuba city, northern coast of São Paulo State, southeastern Brazil (NOAA/MGGD, 1:250,000). Right square: Cocanha beach and adjacent beaches and islands. The total area of mussel farms and the nine sampling sites for environmental parameters are highlighted.

After this period, the mussels were removed and the water containing the excrements was filtered through 0.7 µm fiber glass filters. The filters containing excrements were subsequently washed with distillated water for remove the salts, dried at 60°C for 24 hours and weighed on an analytical balance to determine the amount of excrement. The weight of white filters was subtracted of total weight (APHA 2005).

The variability of excrement production was tested with Two-Way ANOVA type I (Underwood 1997) comparing the mean values among size classes (fixed factor, 3 levels) and experiments nested in size classes (random factor, 11 levels). Subsequently, the Tukey HSD Post-hoc test was used to test for pairwise differences between groups and experiments. The multiple regression analysis between excrement production performed independently for the three size classes and all environmental parameters evaluated were performed singly and in combinations.

Results

The results indicated that the excrement production ranged from 1.17 to 22.23 mg/h (Table I). Large mussels produced in average of 9.86 ±0.40 mg excrement per hour, representing more than 57.5% of excrements production of class I and 35.4% of excrements produced by mussels at class II. The excrements produced in the experimental systems were basically fecal pellets and the production of pseudofeces was not observed.

Table I. Mean, standard error, confidence interval, maximum and minimum values of excrements (mg/h) produced by *Perna perna* among the 11 experiments in the 3 size classes (I <40 mm; II 40 to 70 mm; III >70 mm of shell length).

Classes of size	Valid N	Mean	Standard error	CI*	Minimum	Maximum
I	99	4.19	0.27	0.53	1.17	14.33
II	95	6.37	0.29	0.56	2.11	15.85
III	95	9.86	0.40	0.78	2.69	22.23

* α=0.05

Size classes and the interaction of experiment with size classes showed significant differences in the study (Table II). In general larger mussels (class III >70 mm) produced more excrement than smaller ones (Tukey HSD; III> II> I) (Fig. 2). There were no significant differences in excrement production among the experiments regarding mussels from classes II. For small mussels (class I) equal tendency was observed except in May where was observed higher values of excrement with mussels Class I produced more feces of Class II. The larger mussels (Class III) have significant lower feces production in the June and August experiments (Fig. 3).

Table II. Two-way ANOVA of excrements produced by *Perna perna* among different experiments and size classes.

Effect	***df***	***MS***	***F***	***p***
Source of variation	1	12368.8	421.6	<0.001
Size Classes	2	742.9	25.3	<0.001
Experiment (Size Classes)	30	30.5	4.1	<0.001

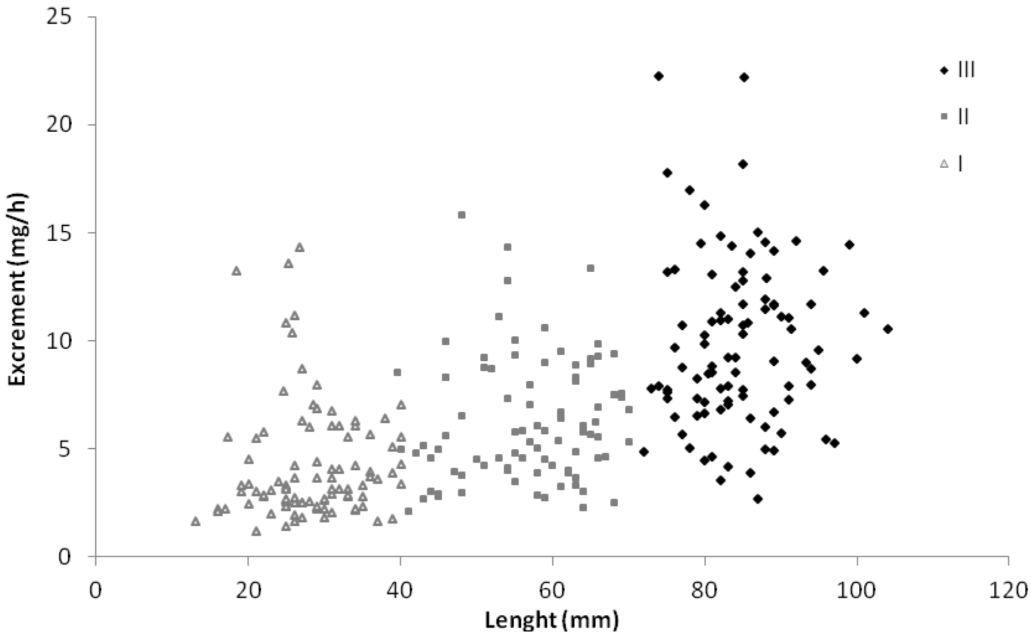

Figure 2. Scatterplot of excrements production (mg/h) by 3 classes of *Perna perna*: class I <40 mm; class II: 40 to 70 mm; class III: >70 mm of shell length.

Comparisons among different methodologies applied to determination of excrement production by bivalve molluscs are described in Table III. The approach used in this study present some practical advantages although this estimate have generated underestimated values of feces.

The synthesis of environmental parameters of mussel farms is represented in Table IV. The water temperature showed no obvious temporal pattern, but was highest during summer and early autumn periods. Salinity showed slight fluctuations during the study period and transparency showed significant differences among periods (p<0.001). The lowest values were possibly influenced by river discharge and sediment resuspension by waves.

The mean concentration of total particulate matter (TPM) at Cocanha Beach showed significant variation (p<0.001) among months. Inorganic matter (PIM) predominated throughout the year, but higher concentrations of organic matter (POM) were observed in some samples. The concentrations of chlorophyll *a* varied significantly over the year (p<0.001). In general, phaeopigment concentrations were lower than those of chlorophyll a, but with no evident temporal pattern through the samples.

The multiple regression analysis between excrement production performed independently for the three size classes and all environmental parameters evaluated showed no influence of any of the variables tested, both singly and in combinations.

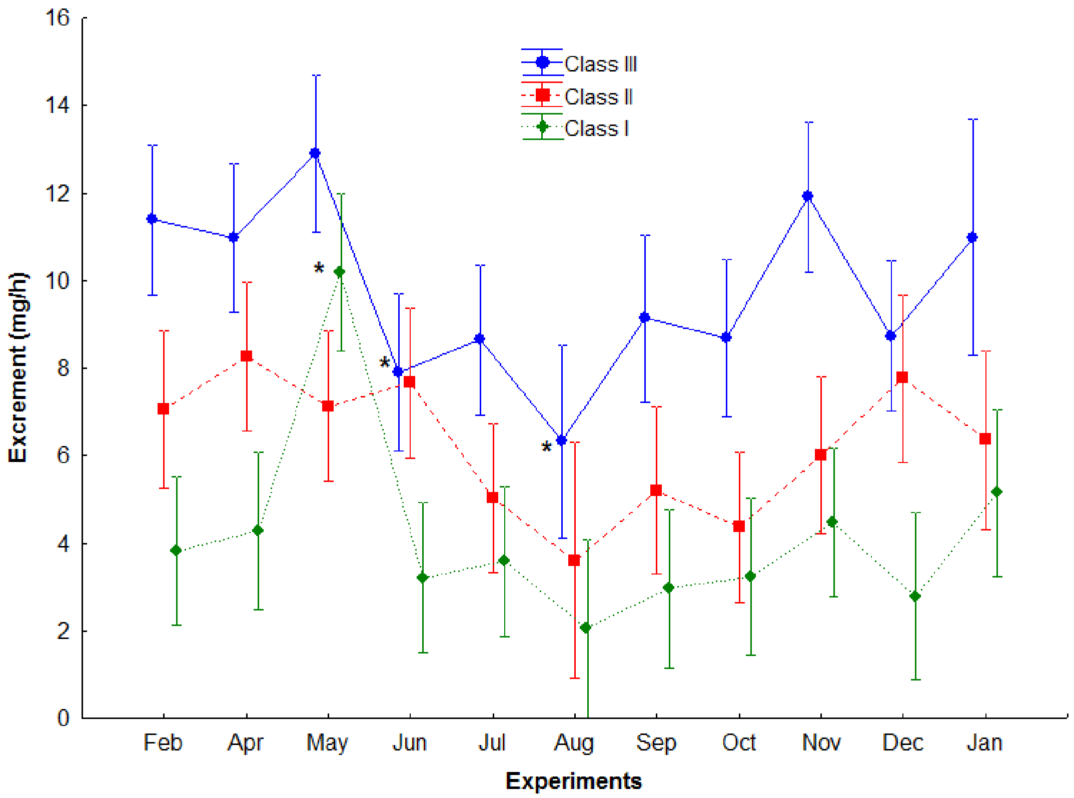

Figure 3. Averages and Standard errors of excrement (mg/h) produced for individual mussel (*Perna perna*) at different size classes (class I <40 mm; class II: 40 to 70 mm; class III: >70 mm of shell length) along 11 experiments. (*) represent statistical differences by Tukey's Test (α=0.05).

Table III. Synthesis of methodologies applied to estimate excrement production by different bivalve molluscs.

Methodology	Advantages	Disadvantages
Sediment traps	*in situ* measurement, easy and practical, evaluates feces and pseudofeces, molluscs feeding on natural seston	large number of traps, low accuracy, expensive, non-selective
Biodeposition approach	*in situ* measurement, highly accuracy, evaluates feces and pseudofeces, molluscs feeding on natural seston, applied in ecofisiologicical models	very laborious, expensive, relative slow-time responses
Laboratory static conditions with food	high accuracy, evaluates feces and pseudofeces	very laborious, expensive, feeding on artificial diets, long period of acclimation in laboratory conditions, slow-time responses
Laboratory Static conditions without food	easy, practical, universal, low coast, feeding on natural seston, short-time responses	evaluates only feces, sub estimative values of excrements production, low accuracy

Table IV. Environmental parameters evaluated around the Cocanha mussel farms (see Fig. 1) from February 2008 through January 2009. Mean value followed by * differ significantly at 5% among samples.

	Valid N	Mean	Std Err	Maximum	Minimum
Temperature (°C)	108	23.4*	0.2	27.0	19.0
Salinity	108	35.2*	0.1	36.0	31.0
Transparency (m)	108	3.9*	0.2	8.2	1.7
TPM (mg/l)	273	66.2*	1.6	174.8	7.5
POM (mg/l)	273	28.2*	1.3	157.5	0.4
PIM (mg/l)	273	37.9*	0.9	65.7	3.7
Chlorophyll a (µg/l)	342	1.06*	0.05	6.01	0.00
Phaeopigments (µg/l)	342	0.59*	0.04	4.27	0.00

Discussion

Preliminary observations of excrement production over a long period (10 hours) in same experimental systems indicated that the release of excrement was concentrated in the first 2 h (Silvestri, unpublished data). After this period the absence of excrement in the experimental systems was associated with frequent spawning events, the latter can compromised the analysis. Berry & Schleyer (1983) and Suplicy *et al.* (2003), in experiments with *P. perna,* also found that a 2 h period was sufficient for the complete passage of food through the digestive tract and the release of all excrement.

The method used for excrement determination proved to be effective and generated values compatible with other studies (Masilamoni *et al*. 2001; Callier *et al.* 2006; Weise *et al.* 2009). The advantage of the method employed in this study is that the mussels produced excrement from the food filtered only in their natural environment, without the addition of microalgae diets, which are unlikely to accurately represent the food available in the environment (Iglesias *et al.* 1998). Furthermore the method proposed here is simple, practical, and economical and can be replicated in other areas. Another advantage of this method is that it provides a response relatively quickly compared to others methodologies.

In general there is wide variability in biodeposition rates among different studies of mussels (Jaramillo *et al.* 1992, Hatcher *et al.* 1994, Crawford *et al.* 2003, Hartstein & Stevens 2005, Callier *et al.* 2006, Weise *et al.* 2009, Alonso-Pérez *et al.* 2010). Although different techniques were employed in these studies part of this variability can be explained by genetic and/or oceanographic differences among sites (Pérez Camacho *et al.* 1995). In order to settle these differences we chose to use seawater filtered without food, generating basal values of excrement production.

In this study, excrement production by mussels varied over the experiments and was directly proportional to the size of mussels, as also observed in the green mussel *Perna viridis* (Masilamoni *et al.* 2001). Resgalla Jr. *et al.* (2006) also observed variation in the excretion rates of different-sized individuals of *P. perna*. Part of the variability of excrements produced by mussels along the 11 experiments could be explained by seasonality of samples and your different environmental characteristics. In field all seawater parameters analyzed showed significant differences along the experiments period and may have influenced the physiological energetic of mussels. As the experiments were performed in different months with distinct oceanographic conditions, variability was observed in the excrement production by mussels within the same size classes. However there was no correlation between the environmental parameters and the excrements produced in laboratory. Seasonality changes in water temperature, salinity, suspended matter and chlorophyll concentrations in the water column influenced the filtration, rejection, ingestion, excretion and assimilation of nutrients process which contributed to the variability of excrement values (Bayne & Newell 1983).

The seston composition affects the physiology of *Perna perna* (Resgalla Jr & Brasil 2007) . The proportion of rejected material varies depending on the availability of seston and on its organic content, with increased filtration rates in organic-matter-poor diets (Bayne *et al.* 1993). In environments such as Cocanha Beach, where seston concentrations are high, a higher rate of rejection by mussels is expected. However as there was no food in the experimental systems there was no particles rejection and consequently only feces production.

The absence of food in the experimental systems probably influenced the feeding physiology of mussels and may have altered the normal rates of filtration, rejection and excretion (Robson *et al.* 2010; Resgalla Jr. *et al.* 2007). Since there was no food available for molluscs, smaller amounts of excrements may have been produced, but this was not assessed in this study. However, it should be noted that the values obtained in this study are underestimates and do not fully reflect the physiological behavior of the mussels in the natural environment.

The approach used in the present study can be employed to support future studies of carrying capacity of shellfish farms areas and hydrodynamic models of dispersion and deposition of organic particles.

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