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Scientific Note

Size comparison of quadrats in sample of non-native bivalve *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae)

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Abstract. We aimed to designate which is the best quadrat area to be used in sampling of *C. fluminea* for determination of their population parameters. The quadrat of 0.0625 m^2 showed the best cost efficiency and a lowest sampling effort, being recommended for study of *C. fluminea* in lentic environments.

Key words: invasive bivalve, quadrat sampling, lentic environments, Sapucaí river

Resumo. Comparação de tamanhos de quadrats na amostragem do bivalve não-nativo *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae). Nós indicamos qual é a melhor área de quadrat a ser utilizada na amostragem de *C. fluminea* para determinação de seus parâmetros populacionais. O quadrat de 0,0625 m^2 apresentou o melhor custo-benefício e um menor esforço amostral, sendo recomendado para estudo de *C. fluminea* em ambientes lênticos.

Palavras chave: bivalve não-nativo, amostragem por quadrats, ambientes lênticos, rio Sapucaí

One of the most relevant exotic species of the phylum Mollusca is the bivalve *Corbicula fluminea* (Müller, 1774), due to its presence in several countries as a result of human actions (e.g. Darrigran & Pastorino 1993, Darrigran & Damborenea 2005, Ayres 2008, Vianna & Avelar 2010). This mollusc is restricted to freshwater environments (salinity ≤ 5%) (Darrigran 1992), presenting a robust shell and a length that varies between 20 and 60 mm (i.e. shell opening length) (Darrigran & Damborenea 2005). Distribution of these bivalves in the aquatic ecosystem invaded is aggregated and they consequently present high densities and biomass (Santos *et al.* 2012). According to Sousa *et al.* (2009), this species is considered invasive and transformer (i.e. bio-engineer) (Figure 1).

Darrigran & Damborenea (2005) studied the distribution history of genre *Corbicula* Megerle von Mühlfeld, 1811 in the Neotropical region, verifying that the environment of origin is the south of China. They state that Ituarte (1981) first reported the presence of *C. fluminea* and *C. largillierti* (Philippi, 1844) for South America in the River Plate, Argentina, establishing the introductory date of this species – from the southeast of Asia to this region – as being at the end of the 1960s and beginning of the 1970s. Darrigran (2002) suggested that man is the introduction vector of this species by means of boats and ballast water. Darrigran & Damborenea (2005) also suggested that a possible method of introduction of this species in these locations is the release of living specimens brought from the place of origin as food on-board the vessels. At around this time, Veitenheimer-Mendes (1981) published the genre in Brazil for the first time and estimated introduction as being at the start of the 1970s. Martínez (1987) recorded its occurrence in Caripe and San Juan rivers (Venezuela) at the beginning of the 1980s. In

1979, Veitenheimer-Mendes & Olazarri (1983) recorded *Corbicula* sp. in Uruguay. In the south of the Neotropical region, specimens of *C. fluminea* were recorded in the sandy coastline of River Colorado (39º01´S - 64º01'W), northern limit of the Argentinean Patagonia (Cazzaniga 1997). In 2001, Beasley *et al.* (2003) indicated the presence of *C. fluminea* for the first time in the lower basin of the Brazilian Amazon, where individuals were found in parts of Amazonas and Tocantins rivers, and estimated that the arrival of this species occurred between 1997 and 1998. Santos *et al.* (2012) also confirmed the presence of this species in upper Parana and lower Amazonas (e.g. Araguaia).

Figure 1. Furnas reservoir sampling site with great accumulation of *Corbicula fluminea* dead shells, indicating high density of this specie. (Photo: V. M. Azevedo-Santos). Bar: 4 cm.

The first reference to *C. fluminea* in lentic South American ecosystems occurred in areas of artificial environments of Argentina ("canteras de conchilla" or shell quarries), that correspond to the geomorphologic profile of a costal dyke (i.e. impermeable floor with shell deposits). The presence of *C. fluminea* was detected in these environments both in the coastline and in areas with a depth of two metres (Darrigran 1999). Santos *et al.* (2012) state that representatives of *C. fluminea* in Brazilian regions prefer lotic environments – as observed in most cases – but that when they occupy lentic environments (e.g. lakes and reservoirs), they usually occupy well oxygenated marginal areas with a slight slope and a preferentially sandy substrate. The latter characteristics were also recorded for the study area (i.e. Furnas reservoir). In 2007, Paschoal *et al.* (2013) recorded the occurrence of *C. fluminea* for the first time in the UHE Furnas reservoir, in areas with depths that varied between 9 and 27 metres and average density of 295 ind./m^2 – ranging from 19 to 1.350 ind./m^2 throughout the year.

In order to obtain additional knowledge and an understanding of the environment and processes that occur in populations of non-native bivalves in nature, researchers have developed a wide range of sampling methods. In a recent revision related to monitoring and evaluation of the biology of non-native and invasive species in Brazil, Pereira *et al.* (2012) detected the use of eight different sampling methods, with their advantages and disadvantages. Area delimitation by means of quadrats was one of these methods. This method consists of analysing, measuring and counting organisms and their individual aspects within quadrat frame structures. Consequently, each quadrat provides different efficiencies according to the location and objective of each study. Moreover, the size and form of the quadrat can influence the obtained result. Elliott (1983) and Dowing & Anderson (1985) stated that the size and number of quadrats should be determined prior to sampling. In this way, biomass, density and other estimated population parameters are closer to reality and variances between samples are reduced.

This method is considered the most widely adopted in literature for sampling and estimation of structural parameters of *C. fluminea* (Lois 2010, Vianna & Avelar 2010, Sousa *et al.* 2012, Pereira *et al.* 2012, and others). However, there is no consensus in literature on the best size (area) that could present the best sampling effort. Some examples of this form of sampling are the studies of Lois (2010), Vianna & Avelar (2010) and Sousa *et al.* (2012). In the former study, the author used quadrats of 0.0625 m^2 to estimate the density of *C. fluminea*. In the second study, the authors adopted quadrats of 1 m^2 to estimate population aspects of the same species. Finally, in the third study, the authors preferred area quadrats of 0.25 m^2 to establish mortality in terms of density and biomass of several bivalves, including *C. fluminea*. Consequently, the aim of this study is to indicate the best area quadrat for sampling *C. fluminea* and determine its population parameters in lentic environments.

The Furnas reservoir (20°40'09"S, 46°19'08"W) is located in the southeast of the state of Minas Gerais. It was dammed in 1962 and is formed by Grande and Sapucaí rivers (of the upper Parana watershed) and other small tributaries. It is also one of the largest and most important reservoirs in the country (Azevedo-Santos *et al.* 2011). This study was conducted at the margins of a stretch of Sapucaí river, inserted in the Furnas reservoir (Figure 2). Characteristics of the area include high fluctuations of the water column level, sandy clay sediment, pebbles and small stones at the margins and a predominance of *Brachiaria* sp. (Trin.) Griseb. near the margin and *Eichornia azurea* (Kunth) ten metres from the margin (Paschoal personal observation).

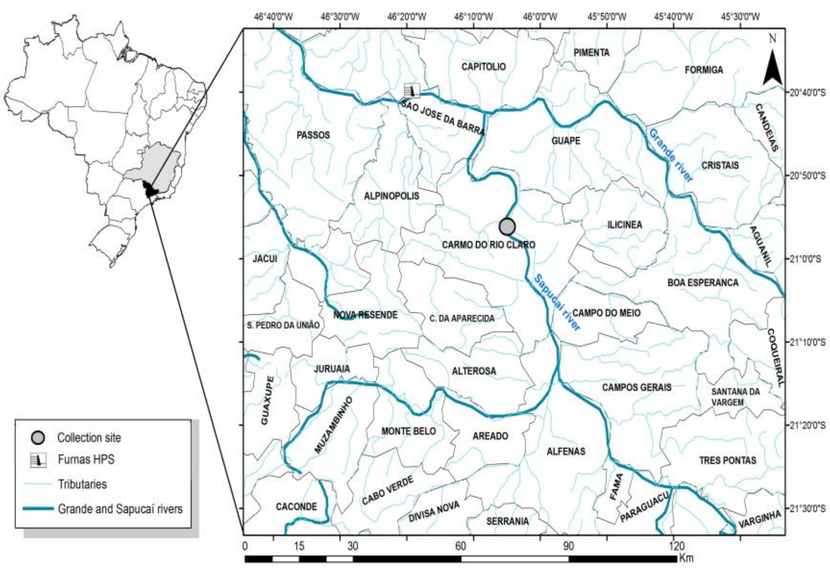

Figure 2. Map showing sampling site location of *Corbicula fluminea.*

Two samples were taken (October 2011 and August 2012) and grouped for statistical analysis. I*n situ* measurements of *C. fluminea* dead shells were taken between at 8 a.m. and 3 p.m. To determine which quadrat size (area) would present the best sampling effort, 90 quadrats were analysed in three horizontal transects of 50 metres along the left margin of Sapucaí river. Thirty quadrats (15 sampled in each year) of 0.01 m^2, 30 of 0.0625 m^2 and 30 of 0.25 m^2 areas were analysed. Means ($\overline{X}$) and standard deviations (sd) of dead shell frequencies, coefficient of variation (CV), mean time spent in sampling (Min./Q - minutes spent counting and measuring total number of individuals found in each quadrat) and total number of individuals recorded for 30 grouped quadrats of each area (Σ_{30}) were compared. Total sampling effort (TSE) for each area was calculated using the formula: $((\mathbf{\Sigma_{30}}/\overline{X}).\text{Min./Q}))/60$; thus it was possible to determine the amplitude of time spent (hours) for counting and measuring all individuals found in 30 quadrats. A Kruskal-Wallis test was applied to evaluate possible differences between the three sizes (areas) of the studied quadrats, subsequently complemented with *a posteriori* Tukey test to evaluate individual comparisons.

The number of individuals recorded for the three quadrat areas presented significant differences. Mean values, standard deviations and CV's of all quadrats for each of the three areas, mean time spent on sampling and sampling effort are summarized in Table I. In general, there was a significant difference between the sizes of studied quadrats (H = 15.16, *p*: < 0.001). However, only the quadrat of 0.0625 m^2 did not differ from the rest (Table II). This size was chosen to establish population variable in lentic environments because it provided representative mean value and standard deviation and CV, mean time and effort spent on sampling that were considered acceptable in comparison with the other sizes.

Table I. *Corbicula fluminea.* Mean ($\overline{X}$), Standard deviation (sd), Coeficient of variation (CV), mean time spent in sampling (Min./Q), total number of individuals recorded (Σ_{30}) and amplitude of total sampling effort (TSE) for the three quadrat areas used in this study.

Area (m^2)	$\overline{X}$	**Sd**	**CV**	**Min./Q**	Σ_{30}	**TSE**
0.01	5	3.53	72.34	0.3–2	177	11 min. – 1 h 30 min.
0.0625	20	11.77	57.76	3–5	598	1 h 40 min. – 2h 30 min.
0.25	41	26.87	58.82	6–8	1,465	2h 35 min. – 4 h 45 min.

From the standpoint of scientific research, the need for samples that are representative and precise leads to a series of queries in relation to the obtained results and adopted sampling methods. Results, discussion and conclusion of a study are based on sampling but they are inferred for the entire population or community. Sample delimitation is therefore essential to minimize possible sampling errors and provide more robust results (Gotelli & Elison 2011). Spinetti *et al.* (1992) proposed a sample effectiveness comparison for *C. fluminea* in lotic environments based on three methods – drag net, "D" net and manual collection – and they observed variations in biometric standards using different sampling modalities. Darrigran (1998-1999) applied the sampling method of perpendicular transects and a cylindrical extractor (0.07 m^2) to study malacofauna of the coastline of the River Plate, mainly comprising *C. fluminea* and *C. largillierti*. Pereira *et al.* (2012) stated that the sampling method using quadrats is effective to estimate population aspects of *C. fluminea*, especially in relation to density. These same authors emphasize the success of this method when monitoring invasive molluscs and that the use of quadrats requires the observance of depth and substrate type prior to application of this methodology.

In this study, the quadrat of 0.0625 m^2 presented the best results to determine population variables of *C. fluminea*. Downing & Downing (1992) explained that the use of smaller quadrats with a greater number of replicas does not only reduce sampling efforts (e.g. time spent on sampling), but may also reduce the variance of certain population parameters for molluscs (e.g. biomass and density).

Table II. *Corbicula fluminea.* Results of Tukey tests for all quadrat areas used in this study. * statistically significant at $p \leq 0.05$.

Area (m^2)	**0.01**	**0.0625**	**0.25**
0.01	-	0.15	0.0003*
0.0625	0.15	-	0.15
0.25	0.0003*	0.15	-

Downing & Downing (1992) state that even when an environment presents large numbers of bivalves and these bivalves are easy to collect due to the type of substrate and the presence of quadrats with a high number of individuals – characteristics found in this study – more than ten replicas are needed to obtain precise estimates. These authors also state that 85% of estimates of average density and total number of benthonic macrofauna published before the 90s were based on small samples (i.e. 3 or less). This precaution was observed in this study, where 30 replicas were used for each quadrat area, being that the area quadrat of 0.0625 m^2 presented the best results due to the acceptable mean sampling time and, consequently, lesser amount of money spent on each sample.

In spite of the significant differences registered between the three studied quadrat areas (Table II), the quadrat of 0.0625 m^2 was the only one that did not showed significant differences from the others, thus this area is recommended to evaluate population parameters of *Corbicula fluminea* in lentic environments. Even the quadrat area of 0.01 m^2 exhibited lower mean time spent to counting and measuring *C. fluminea* dead shells in field and a smaller sampling effort, this area showed a high CV value and a standard deviation very close to their mean (see Table I). Thus, using this quadrat area would be inappropriate, inducing multiple biases in statistical analysis and underestimating the population parameters (e.g. biomass, density and mean shell size). In this study, it was possible to determine that area quadrat 0.0625 m^2 presented the best cost efficiency in terms of time and money spent on reliable samplings, which is essential for the success of research and scientific projects. This quadrat also presented an acceptable sampling effort and greatest field precision when compared with the other quadrats, due the lesser CV registered. Results of this study partially fill the gap represented by the lack of information related to sampling of *C. fluminea* and its standardization, creating a foundation for future studies on the management and control of this non-native species that is responsible for serious environmental and financial problems in reservoirs and hydroelectric plants in Brazil and in the other countries of the Neotropical region.

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