Temporal and longitudinal variation of Corbicula fluminea (Mollusca, Bivalvia) biomass in the Rosana Reservoir, Brazil.

BAGATINI, Y.M., HIGUTI, J. & BENEDITO, E.

¹ Universidade Estadual de Maringá, Nupélia, Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais. Avenida Colombo, 5790, Bloco H-90, Cep. 87020-900, Maringá, PR, e-mail: ymorettob@hotmail.com; higuti@nupelia.uem.br; eva@nupelia.uem.br.

ABSTRACT: Temporal and longitudinal variation of Corbicula fluminea (Mollusca, Bivalvia) biomass in the Rosana Reservoir, Brazil. The aim of this study was to analyze temporal and longitudinal variation of C. fluminea biomass in the Rosana Reservoir situated in the Paranapanema watershed between the States of São Paulo and Paraná, and environmental predictor variables. Samplings were carried out every three months in littoral and profundal zones of lotic, intermediate and lentic stations of the reservoir. The higher values of C. fluminea dry biomass were recorded in March and December (summer), independent of the spatial distribution, although the analysis of variance did not show significant differences among the months. On the other hand, the variation in biomass was significant among the sampling stations. The biomass values were higher in the littoral zone than in the profundal zone and, were related to the shallower waters, higher oxygenation of the water and type of sediment. Through a multiple linear regression analysis, depth and medium sand were the main predictors of C. fluminea biomass in the Rosana Reservoir. Thus, the longitudinal variation of C. fluminea biomass in the Rosana Reservoir was influenced by a combination of environmental factors, mainly the depth and sediment type. But, temporal biomass variability was probably influenced by the availability of bacterioplankton. A length-weight model was developed to calculate the C. fluminea biomass in Rosana reservoir. Studies about this exotic species become very important, since the real impact of C. fluminea on the aquatic environments cannot be forecast.

Key-words: Corbicula fluminea, Bivalvia, biomass, reservoir

RESUMO: Variação temporal e longitudinal da biomassa de Corbicula fluminea (Mollusca, Bivalvia) no reservatório de Rosana, Brasil. O objetivo deste trabalho foi analisar a variação temporal e longitudinal da biomassa de C. fluminea no reservatório de Rosana, situado na bacia do Paranapanema entre os Estados de São Paulo e Paraná, e variáveis ambientais preditoras. As coletas foram realizadas trimestralmente, nas zonas litoral e profunda das três estações (lótica, intermediária e lêntica) do reservatório. Os maiores valores de biomassa seca de C. fluminea foram registrados nos meses de março e dezembro (verão), independente da estação de amostragem analisada, apesar da análise de variância não indicar diferença significativa entre os meses. Por outro lado, a variação longitudinal da biomassa foi significativamente diferente entre as estações de amostragem. Na zona litoral, os valores de biomassa foram maiores, em relação à zona profunda e foram relacionados às menores profundidades, maior oxigenação da água e ao tipo de sedimento. Através da análise de regressão linear múltipla, profundidade e areia média foram as principais preditoras da biomassa de C. fluminea no reservatório de Rosana. Assim, a variação longitudinal da biomassa de C. fluminea no reservatório de Rosana foi influenciada por uma combinação de fatores ambientais, principalmente a profundidade e o tipo de sedimento. A variação temporal da biomassa provavelmente foi influenciada pela disponibilidade de bacterioplâncton. Foi também elaborado um modelo para calcular a biomassa de C. fluminea no reservatório de Rosana. Estudos sobre espécies exóticas tornam-se ainda mais importantes, uma vez que o real impacto desses organismos nos ambientes aquáticos por eles colonizados não podem ser previstos.

Palavras-chave: Corbicula fluminea, Bivalvia, biomassa, reservatório

Introduction

Bivalve mollusk populations, in addition to occurring in practically all freshwater

systems (McMahon, 2000), are very abundant, constituting a large portion of the benthic biomass (Henry & Simão, 1984). Corbicula fluminea (Müller, 1774) is a freshwater bivalve of Asiatic origin, which has spread to the majority of fluvial systems (Cataldo & Boltovskoy, 1999; Martins et al., 2006) and reservoirs (Isom, 1986; Layzer et al., 1993). Introduced into Brazil in 1970 (Veitenheimer-Mendes, 1981), C. fluminea has been successful in colonizing various Brazilian aquatic environments (Callil & Mansur, 2002; Bagatini et al., 2005), mainly due to its characteristics as r-strategist species, such as short lifecycle and high fertility, as well as rapid growth and dispersion rates (McMahon, 1983; Darrigran, 2002).

The biomass estimate has been used to study of invertebrate fauna in aquatic ecosystems as it provides information about the transfer of material and energy along the food chain (Benke et al., 1999; Bagatini et al., 2007). Some studies about bivalve biomass have been performed in different environments as marine (Sejr et al., 2002; Babarro et al., 2003) and freshwater (Henry & Simão, 1984; Hakenkamp & Palmer, 1999). Despite of its importance, few studies have been carried out with the aim of determining the biomass of mollusks in freshwater ecosystems (Vaughn et al., 2004; Ravera et al., 2007), where non-native bivalves, as Dreissena polymorpha (Burlakova et al., 2006) and C. fluminea (Vaughn & Spooner, 2006) has spread very quickly.

Another question that has to be considered in biomass studies, is the preservative method applied to conservation the aquatic macroinvertebrates. Of Researches carried out with some different methods of preservation verified that all of them cause significant changes in the invertebrate body mass after the preservation (Howmiller, 1972; Dumont et al., 1975; Smith & Lanfair, 1994). Martin (2001) verified that the freezing, of bivalve samples, had a negative effect (loss) on the body dry weight. Biomass losses also were found to ethanol (Howmiller, 1972; Smith & Lanfair, 1994), and formaldehyde (Howmiller, 1972; Dumont et al., 1975; Smith & Lanfair, 1992). Nevertheless, when compared to others preservative (freezing, ethanol), the formaldehyde is the most appropriate and used method, because it causes the lower weight loss (Howmiller, 1972; Smith & Lanfair, 1994; Benke et al., 1999; Sejr et al., 2002). In this way, we chose 4% buffered formaldehyde to preserve C. fluminea

specimens, considering the least dry weight alteration caused by this solution.

The aim of the present study was to investigate the temporal and longitudinal variation of C. fluminea biomass in the Rosana Reservoir, situated on the Paranapanema River, between São Paulo and Paraná States, and to identify its environmental controlling factors (biotic and abiotic).

Materials and methods

Study Area

The Paranapanema River has a cascading series of reservoirs, which starts at the Jurumirim reservoir and, in its last stretch, meets the Rosana Reservoir. The dam of the Rosana Hydroelectric Power Plant is located between the municipalities of Rosana (São Paulo State) and Diamante do Norte (Paraná State). The reservoir covers an area of 220 km², with an average depth of 25 meters. Three sampling stations were established along the longitudinal axis of the reservoir, which were denominated the (22°39'05"S 52°10'52"W), lotic and intermediate (22°36'57"S and 52°29'20"W) and the lentic stations (22°35'24"S and 52°49'51"W) (Fig. 1).

Data collection

Corbicula fluminea sampling were carried out every three months in the period from March to December 2002, in littoral profundal zones and of the lotic. intermediate and lentic stations in the Rosana Reservoir. At each station, three samples were obtained, using a modified Petersen grab (0.018 m²), for evaluate C. fluminea biomass, and one sample for granulometric analysis. C. fluminea specimens were separated using a system of sieves (mesh sizes of 2.0, 1.0 and 0.2 mm). The material caught in the 0.2 mm mesh was fixed with 4% formaldehvde buffered with calcium carbonate and later sorted under a stereoscopic microscope. The organisms caught in the field, on the larger mesh size, were also fixed with 4% buffered formaldehyde. Formaldehyde buffered with calcium carbonate or tetraborate (borax) neutralize the pH of this substance and avoid damage to the organisms (Wetzel et al., 2005; Blettler & Bonecker, 2006).



Figure 1: Location of the Rosana Reservoir stations at Paranapanema River.

In both of the zones, the concentration of dissolved oxygen and water temperature (digital portable YSI), pH and electrical conductivity (digital portable potentiometer) were measured close to the sediment. Concentrations of total nitrogen (Zagatto et al., 1981), total phosphorous and chlorophyll a (Golterman et al., 1978) and turbidity (portable turbidimeter LaMotte) were only measured at the surface in the central region of each station.

Granulometric composition was determined using the pipette method, described in Suguio (1973), using the Wentworth Scale (Wentworth, 1922).

Biomass determination

samples The for the biomass determination were storage for approximately one year. Smith & Lanfair (1999) observed that the body mass loss, in the formaldehyde, was stabilized until the 163 days of preservation and in the 385 days this body mass loss was only 0.5%. For the dry biomass determination, all the soft tissue was removed from the shell (Willians & McMahon, 1989) and kept in distilled water, for approximately 1 hour, to take off the formaldehyde excess. After that, the water excess in the soft tissue was removed in a filter paper and the soft part dried in oven at 60°C for 24 hours. Similarly, the shell was rinsed in distilled water and dried in oven. The dry weight was measured using Sartorius micro-analytic balance, and the total Corbicula fluminea biomass (shell and soft tissue) was expressed in milligrams of dry weight per square meter (mg.m⁻²).

Before the dry weight, approximately a total of seven hundred specimens, sized between 0.1 mm and 28 mm, were measured using a stereoscopic microscope or a pachymeter. The shell-length dry mass regression model was used to predict the individual C. fluminea biomass in the Rosana reservoir.

Data analysis

In order to obtain dry weight values corresponding to the shell length values, a non linear regression model was calculated using the relation between the length (L) and body total dry weight (DW_{total}) of each specimen of Corbicula fluminea. The exponential equation, $W = a (L^b)$ was used to calculate the model, where, W is the total dry weight (mg), constant a is the regression intercept, constant b is the regression angular coefficient and L is the total length of the shell (mm).

A Principal Components Analysis (PCA) of environmental variables (water temperature, pH, electrical conductivity, dissolved oxygen and sediment types) was carried out to examine for the distribution pattern of these variables in the different stations of the reservoir. All of the data had previously been transformed into log (except for pH) and the axes of the PCA were selected according to the Broken-Stick criterion. This analysis was performed in the PC-ORD program (McCune & Mefford, 1999).

The relationship between the abiotic variables and the C. fluminea biomass was verified through a multiple linear regression analysis. This regression is defined by the equation Y = a+b(X), where Y is the dependent variable (C. fluminea biomass), a (intercept) and b (coefficient) are constants and X are the independent variables (abiotic values). The assumptions of linearity, homocedasticity normality, and independence were tested, and the adjustment of the model was carried out by a residual analysis. A non-parametric ANOVA (Kruskal-Wallis) was used to test the differences in the biomass values, among the months and the stations, in both littoral and profundal zones. Both analyzes were performed by the program Statistic 7.1 (StatiSoft, Inc, 2005).

Results

The mean values of water temperature and electrical conductivity had little variation, varying between 23.9°C to 26.2°C 58.6 **m**6.cm⁻¹ to 61.6 **m**6.cm⁻¹, and respectively. The mean dissolved oxygen concentration was high in all of the stations however; higher values were recorded for the littoral zone. On the other hand, higher depths were observed in the profundal zone. The pH values were neutral in all the stations of the Rosana reservoir (Tab. I). In general, the Rosana Reservoir presented reduced concentrations of nutrients, as well as low mean values of turbidity and chlorophyll a in all the sampling stations (Tab. I).

Table I: Mean values between sampling periods and variation amplitude (between parentheses) of the biotic and abiotic variables in the lotic, intermediate and lentic stations of the Rosana Reservoir (DE = depth, WT = water temperature, DO = dissolved oxygen, EC = electrical conductivity, C a = chlorophyll-a, TU = Turbidity, TN = total nitrogen, TP = total phosphorus, Cf = Corbicula fluminea, P = profundal, L = littoral and nd = no defined, n = number of C. fluminea specimens. To all the other variables n = 4).

Variables	Lotic		Intermediate		Lentic	
	P	L	Р	L	Р	L
DE (m)	12.0	2.3	13.8	1.1	24.5	1.5
	(12-12.5)	(1.5-5)	(12-21)	(1-1.5)	(24-31)	(1-2)
WT (°C)	24.1	24.3	23.9	26.0	24.3	26.2
	(21.7-26.8)	(21.7-26.9).	(22.1 - 26.9)	(22-28.5)	(21.7 - 27.4)	(22-28.3)
DO (mg.L ⁻¹)	6.7	7.4	5.6	7.9	5.6	8.2
	(5.5 - 7.7)	(7.3 - 7.7)	(O. 7 -7.6)	(7.2-8.3)	(O.3-7.9)	(7.8 - 7.9)
рН	7.3	7.1	7.4	7.3	7.4	7.4
	(7.2 - 7.5)	(6.9-7.2)	(7.2-7.5)	(7.2 - 7.4)	(7.1-7.5)	(7.1-7.5)
EC (ns .cm ⁻¹)	61.6	59.7	60.5	59.4	59.4	58.6
	(56.7-63.3)	(55-59.7)	(56.3-60.6)	(57-63.1)	(55.9-60.3)	(56.5-57.9)
$C a (mg.L^{-1})$	0.5	nd	0.6	nd	0.5	nd
	(O.5-1)		(O.1-1.6)		(O.1-1.3)	
TU (NTU)	13.9	nd	3.1	nd	5.4	nd
	(1.6-47.2)		(1.3-6)		(1.6-14.4)	
TN (ng .L ⁻¹)	385.9	nd	401.4	nd	405.4	nd
	(285.8-503.7)		(337.1-514.4)		(334.5-490.1)	
TP (mg.L ⁻¹)	20.4	nd	12.8	nd	13.0	nd
	(10.1-43.4)		(9.2-18.4)		(8-21.5)	
Cf (mg.m ⁻²)	17.6	532.3	0	1357.3	11.4	898.7
	(15.7-18.8)	(236.8-831.3)		(1168-2276.9)	(22-23.7)	(427-1304)
	n=116	n=73		n=458	n=4	n=371

Axes 1 and 2 of the PCA explained 56% of the physical and chemical variables of the water and sediment, emphasizing granulometric composition as the main variable in the distinction of both the zones (littoral and profundal) of the Rosana Reservoir. In axis 1, the littoral of the reservoir was composed mainly of medium and fine sands, while in the profundal zone the predominant sediment was silt and clay. In axis 2, the profundal zone of the intermediate and lentic stations were distinguished mainly by the presence of very fine sediments (silts) and deeper sites, while in the lotic station the sediment was composed of coarse particles (Fig. 2).



Figure 2: Ordination of the abiotic variables in the two first axis of the Principal Components Analysis in the Rosana Reservoir (Fsi - fine silt; VFsi - very fine silt; Msi - mediun silt; Csi - coarse silt; CL - clay; VFS - very fine sand; FS - fine sand; MS - mediun sand; CS - coarse sand; VCS - very coarse sand; GR - gravel; DE - depht; EC - electrical conductivity).

Depth and medium sand were selected as the main predictors of Corbicula fluminea biomass in the Rosana Reservoir by the multiple linear regression, whose determination coefficient was 84%. Thus, the biomass values in relation to depth (DE) and medium sand (MS) were expressed by the equation: y = -0.76 (DE) + 0.33 (MS) + 6.59. A positive correlation was observed for medium sand, which favored an increase of C. fluminea biomass. On the other hand, a negative correlation was found for depth, i.e., in deeper sites were found low biomass values (Fig. 3). It was observed, through the studentized residual analysis, that the majority of the data was well distributed in the \pm 1 interval, indicating that the equation and the model were well adjusted.

The length-weight relationship model to the C. fluminea specimens in the Rosana reservoir, was defined by the equation $W = 2.9210^{-4}xL^{2.79}$, whose determination coefficient was 92% (Fig. 4). The biomass for the non-dried specimens of C. fluminea was estimated from this equation.

The higher mean biomass values for C. fluminea were recorded in the littoral zone of the reservoir, especially at the intermediate station (1,357.3 mg.m⁻²), followed by the lentic (898.7 mg.m⁻²) and lotic (532.3 mg.m⁻²) stations. In contrast, in the profundal zone, the biomass values were very low, with 17.6 mg.m⁻² being recorded at the lotic station, 11.4 mg.m⁻² at the lentic station and a complete absence

of C. fluminea in the intermediate station (Tab. I; Figs. 5a, c).

The analysis of variance revealed that the differences in the biomass values among the station were significant, both in the littoral zones (p < 0.001 and H = 14.12) and the profundal zones (p < 0.0001 and H= 25.00). C. fluminea biomass of the intermediate station was significantly different to the lotic station in the littoral zone; and in the profundal zone, the biomass of the lotic station was significantly different to the intermediate and the lentic stations (Figs. 5a, c). An increase in biomass values occurred through the year, with higher values recorded in the summer in both of the zones (Figs. 5b, d). However, there were no significant differences among the months during the period of study, either in the littoral (p = 0.2662 and H = 3.96) or the profundal (p = 0.5967 and H = 1.88) zones.



Figure 3: Multiple linear regression between C. fluminea biomass and depth (DE) and medium sand (MS).



Figure 4: Length-weight relationship of C. fluminea in the Rosana Reservoir during March to December 2001.



Figure 5: Box-whisker plots, median values, deciles (10-90%, bar) and quartiles (25-75%, rectangle) of C. fluminea dry biomass (mg.m² DW) during March to December 2001, in the three stations of Rosana Reservoir. (a, b) littoral zone and (c, d) profundal zone. Different codes (i and ii) show significant differences (p<0.05). (Lo = lotic, In = intermediate, Le = lentic, M = March, J = June, S = September, D = December).</p>

Discussion

Variation of Corbicula fluminea biomass was confirmed in the different stations of the Rosana Reservoir, with higher biomass values found mainly in the summer, although the analysis of variance did not show temporal significant differences. This result could be explained by the different sizes of C. fluminea specimens observed during the sampling period, which contributed to the high variability in the data. Furthermore, Bagatini et al. (2007), analyzing the energy content of C. fluminea, in the Rosana Reservoir observed differences in the caloric values of the bivalve, due mainly to the allocation of energy for reproduction in the summer.

The type of substrate, pH, hardness and nutrient concentration of the water can affect the biomass of mollusk species in ecosystems (Henry & Simão, 1984). In the sampling stations of the present study, shallow littoral zone and higher percentages of medium sand were found

to be the most favorable conditions to establishment and higher C. fluminea biomass (Fig. 3). A study carried out by Mansur et al. (1994) also confirmed higher densities of this species in shallow habitats with a higher percentage of sand in the substrate. In shallower sites, the sediments are more oxygenated and the water temperature is higher, which are factors that are compatible with the ecological requirements of the bivalve, as has been reported by various researchers (McMahon, 1983; Mansur et al., 2004). Therefore, the higher biomass values in littoral zone of all the stations (lotic, intermediate and lentic) were influenced by higher dissolved oxygen concentrations, shallow waters and sandy sediment (Tab. I).

On the other hand, the profundal zone of the three stations were characterized by higher depth and higher percentages of clay sediments, except for the lotic station, where fine sand predominated and many young specimens were found. Sickel & Burbank (1974) found that Corbicula, in its juvenile phase, prefer substrate constituted by fine sand and mud, as it was found in the profundal zone of the majority of the stations in the present study. The faster flow observed (not measured), especially in the profundal zone of the lotic station, could affect the permanency of the specimens in this site. According to McMahon (1991), the young forms can be suspend in the current and disperse great distances downstream. Another factor to be considered is the tendency of the adults to dislocate to the banks to find better conditions, such as shallower waters and higher oxygenation. According to Gardner et al. (1976), adult bivalves prefer habitats that lie outside of the main channel, such as sandy or clay banks formed on the margins.

Studies that investigate the lengthweight relationship of organisms are indispensable in ecological approaches, in order to identify differences in growth patterns, age of specimens and, between different species (Gardner et al., 1976; Cataldo & Boltovskoy, 1999; Callil & Mansur, 2002). Significant differences in the lengthweight relationship of the C. fluminea and C. largillierti species were reported by Callil & Mansur (2002). These authors further emphasized the importance of biometric studies related to biotic factors, such as reproductive and growth patterns, as well as the size of specimens in a population. In the present study, the establishment of the length-weight relationship for C. fluminea revealed a pattern of exponential growth, and also enabled the elaboration of a model that estimates the C. fluminea biomass in this reservoir.

The majority of bivalves, among them C. fluminea, influence strongly ecological processes in freshwater systems, mainly because they are important filters of suspended material in the water column, constituted, for the most part, by phytoplankton and bacteria (Vaughan & Hakemkamp, 2001). Corbicula biomass, and its individual growth rate, has been positively correlated with the abundance of phytoplankton and/or the trophic status of the reservoir (Beaver et al., 1991). These mollusks can reduce the phytoplankton biomass of the environments they have colonized (Vaughan & Hakemkamp, 2001; Darrigran, 2002).

In the Rosana Reservoir, it has been observed through the values of chlorophyll

a, are low (Train et al., 2003). In fact, the data of phytoplankton biomass are typical of an oligotrophic environment, as the Rosana Reservoir (Tab. 1). This factor could interfere with the survival of C. fluminea. However, its biomass in the littoral zone remained high throughout the year. It is therefore probable that this species had used alternative food sources, due the lack of phytoplankton in the Rosana Reservoir. Vaughan & Hakemkamp (2001) stated that bacteria could be an important food source for C. fluminea in habitats where phytoplankton is scarce. In fact, this bivalve is considered to be a non-selective filter (Lauritsen, 1986), and could be consuming bacterioplankton, which was found to be present, with high density and biomass, in the water column from the Rosana Reservoir (Pagioro et al., 2003).

Thus, the longitudinal variation of the C. fluminea biomass could be explained by a combination of environmental factors, mainly depth and medium sand, as predicted by the linear regression. On the other hand, the temporal variability of the biomass probably was influenced to the availability of bacterioplankton in the Rosana Reservoir. Besides, this work also conduced to a length-weight model to calculate the C. fluminea biomass in this reservoir. So, studies about this exotic species become even more important, as the real impact on the aquatic environments it colonizes cannot be forecast.

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