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Population structure of the tanaid *Sinelobus stanfordi* (Richardson, 1901) (Crustacea, Tanaidacea) associated with roots of the water hyacinth *Eichhornia azurea* (Sw.) Kunth. (Liliiflorae, Pontederiaceae), from a coastal lagoon in southern Brazil

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Abstract. **Population structure of the tanaid *Sinelobus stanfordi* (Richardson, 1901) (Crustacea, Tanaidacea) associated with roots of the water hyacinth *Eichhornia azurea* (Sw.) Kunth. (Liliiflorae, Pontederiaceae), from a coastal lagoon in southern Brazil**. This study aimed to investigate the population structure of *Sinelobus stanfordi* (Richardson, 1901) (Malacostraca, Tanaidae) in a coastal lagoon in the North coast in the Rio Grande do Sul state. The collections were performed weekly on banks of the aquatic hyacinth *Eichhornia azurea* (Sw.) Kunth during three months. Samples of water hyacinth were manually collected and washed on a sieve of 0.250µm mesh size. In the laboratory, the material was sorted and the tanaid lengths were measured and classified into following morphotype categories: Manca, Juveniles, Non-Reproductive Females (NRF), Preparatory Females (PREPF), Ovigerous Females (OVF) and Males. The distribution by frequency of body length classes presented a higher abundance of manca in the class of 0.75mm; juveniles in 2.19mm; non-reproductive females in 2.5mm; preparatory females in 2.7mm; ovigerous females in 2.85mm and males in 2.81mm. The average of sexual rate was 3.1:1 (females: males). It was observed the recruitment and predominance of juveniles in the eleven studied weeks, and the presence in lower proportion of PREPF, OVF and males. Based on the analyses of the population structure observed and data found in the literature, became evident the presence of juveniles, females and males during all the year, beyond a continuous reproduction activity of the species. The results have also suggested the species seems to have an opportunistic behavior, being able to colonize a high diversity of substrates.

Keywords: Coastal lagoon, macrobenthos, populational structure, Tanaidacea.

Resumo. Estrutura populacional do tanaídeo *Sinelobus stanfordi* (Richardson, 1901) (Crustacea, Tanaidacea) ocorrente em raízes do aguapé *Eichhornia azurea* (Sw.) Kunth. (Liliiflorae, Pontederiaceae), em uma laguna costeira do sul do Brasil. Este trabalho buscou obter informações sobre a estrutura populacional de *Sinelobus stanfordi* (Richardson, 1901) (Malacostraca, Tanaidae), em intervalos semanais por um período de três meses, em uma laguna costeira do Litoral Norte do Rio Grande do Sul. As coletas foram realizadas em estande da macrófita aquática *Eichhornia azurea* (Sw.) Kunth. Exemplares do aguapé foram coletados manualmente e lavados sobre uma peneira de 0.250µm de abertura de malha. Em laboratório, o material foi triado e os indivíduos medidos e classificados por morfotipos: Mancas, Juvenis, Fêmeas Não-Reprodutivas (FNR), Fêmeas Preparatórias

(FPREP), Fêmeas Ovadas (FOV) e Machos. A distribuição por frequências de classes de tamanho corporal demonstrou um maior número de indivíduos manca na classe de tamanho 0,75mm; juvenis, em 2,19mm; fêmeas não reprodutivas, em 2,5mm; fêmeas preparatórias, em 2,7mm; fêmeas ovadas, em 2,85mm e machos em 2,81mm. A média da proporção sexual foi de 3,1:1 (fêmeas: machos). Observou-se recrutamento e predomínio dos juvenis nas onze semanas de estudo e a presença, em menor proporção de FPREP, FOV e Machos. Com base na análise da estrutura populacional observada e dados encontrados na literatura, fica evidente a presença de juvenis, fêmeas e machos ao longo de todo ano, além de uma reprodução contínua da espécie. Estes resultados ainda sugerem que a espécie parece ter um comportamento oportunista, podendo, inclusive, colonizar uma diversidade de substratos.

Palavras-chave: Tanaidacea, macrobentos, estrutura populacional, laguna costeira.

Introduction

Tanaids have a worldwide distribution and they are normally very abundant in marine and brackish-water habitats (Fonseca & D'incao, 2003). Furthermore, they can be associated with muddy and sandy substrates and stems and roots of aquatic macrophytes.

Sinelobus stanfordi (Richardson, 1901) (Tanaidae) is considered an opportunist species due to its wide geographic distribution and the high variety of niches that it can occupy (Bemvenuti *et al.,*1992**;** Rosa-Filho & Bemvenuti**,** 1998). Due to the abundance with which this species is found, its populations are very important in the benthic community structure of coastal lagoons, and they assume a significant role in the aquatic food chain (Larsen, 2002).

In Rio Grande do Sul, southernmost state of Brazil, this species has been recorded in lagoons from northern coast, living associated with freshwater macrophytes (Wiedenbrug, 1993), in mixohaline lakes of Tramandaí Lagoon System (Albertoni & Würdig, 1996; Rosa-Filho & Bemvenuti, 1998), in Rio Mampituba estuarine zone, the stream Chuí, in the channel Tramandaí estuary and in a lagoon called Lagoa do Peixe (Rosa-Filho *et al.*, 2004).

Studies about population structure, including distribution, patterns of occurrence, age-class structure and reproductive behavior of *S. stanfordi* were carried out by some authors (Santos, 2000; Toniollo & Masunari, 2007; Kassuga, 2009), however, the population dynamic of this species is poorly known and more studies are needed.

This study provides new information on the biology of *Sinelobus stanfordi*, which data were collected during a monitoring program carried out at weekly intervals.

Material and Methods

The present study was conducted at Gentil Lagoon (30°04'S, 50°13'W) (Fig. 1), which belongs to the group of lagoons of Tramandaí Estuarine System, located in the northern coast of Rio Grande do Sul State, southern Brazil. This lagoon has the bottom and margins colonized by aquatic macrophytes, which species can predominate differently according to morphometric characteristics of each lagoon (Albertoni & Würdig, 1996).

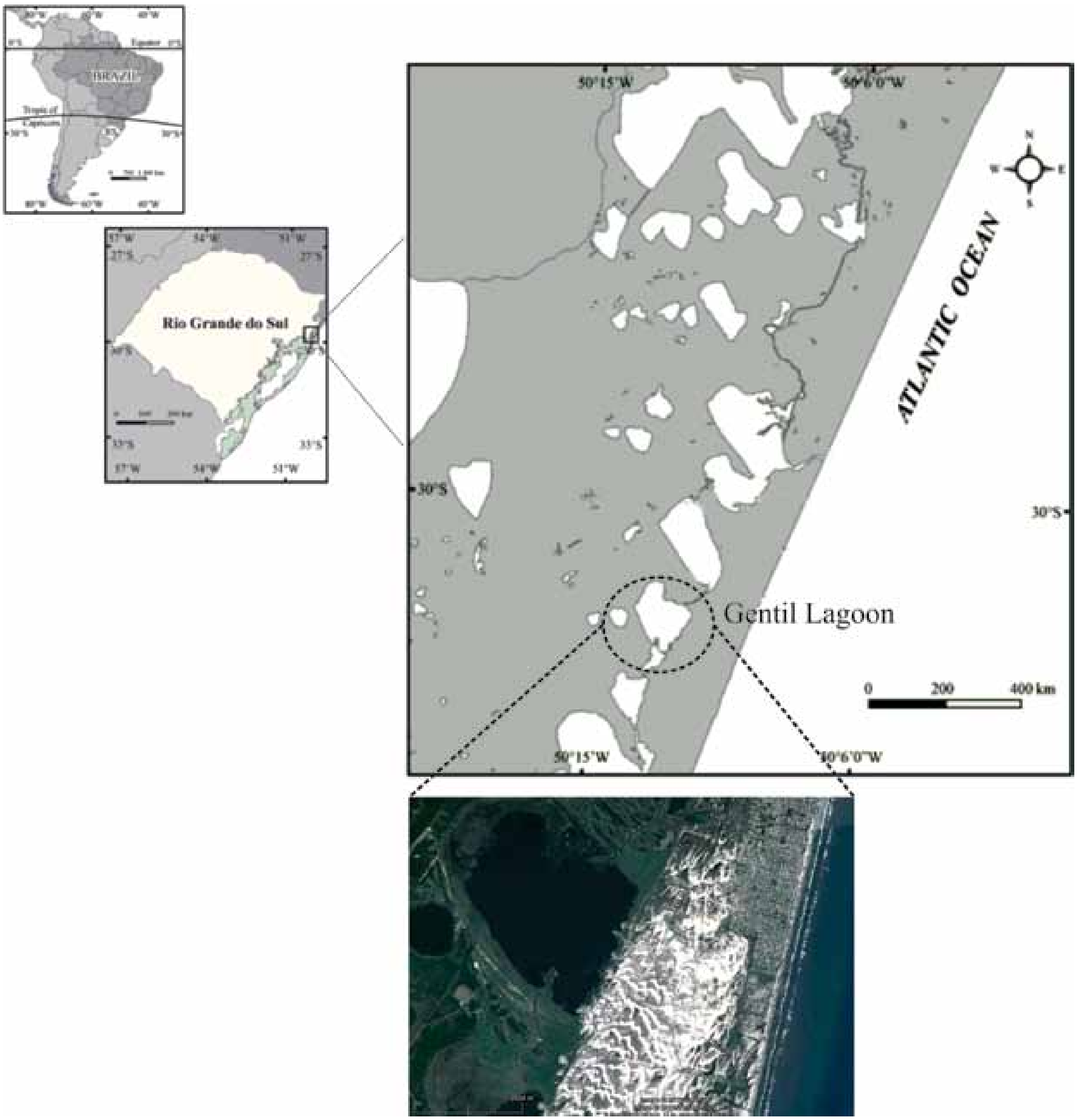

Figure 1. Map showing the coastal lagoon system of Rio Grande do Sul State (RS), Brazil, and, in detail, the northern region of the system indicating the Gentil Lagoon. Adapted from Werner *et al*. (2008).

The samples were collected weekly in the eastern margins of the Gentil Lagoon, from October 30th, 2009 to January 12th, 2010. The sample point was determined through an exploratory expedition carried out on October 30th, 2009, and it was chosen the site that harboured the greatest number of individuals of *Sinelobus stanfordi* (30º03'20"S, 50º11'10"W). About 800 grams of the water hyacinth *Eichhornia azurea* (Sw.) Kunth (Liliopsida, Pontederiaceae), were collected and stored in plastic bags of 50 liters with local water. In the laboratory, the samples were washed and sieved in a 0.250µm mesh. The hyacinth samples were preserved in alcohol 70% for later analysis under stereomicroscope. The salinity of the water was measured at Laboratory of Chemistry Analyzes of the Coastal, Limnological and Marine Studies Center, through Chloride Method by precipitation titrimetry according to Strickland & Parsons (1972). The environmental variables such as the water temperature, dissolved oxygen and pH of the water were measured *in loco* of each collection.

All individuals were measured with a millimetric ocular from the tip of the carapace to the distal medial margin of the pleotelson (total length, mm) (Leite *et al.*, 2003). They were separated according to their body length and preserved in alcohol 70%. The individuals were recognized into five morphotypes according to the morphology of antennae and chelipeds, following Toniollo & Masunari (2007): adult Males have well-developed chelipeds; Ovigerous Females (OVF), marsupium filled with eggs or embryos; Preparatory Females (PREPF), presence of oostegites; Non-Reproductive Females (NRF), without oostegites nor marsupium (in this body length the males have already presented large chelipeds); Juveniles, those measuring less than the smallest individual with evident sexual characteristics (immature males could not be differentiated from immature females until exhibiting large chelipeds and antennae) (Leite & Leite, 1997); Manca, the first stage after birth, no visible pleopods.

The degree of correlation between the abundance of each morphotype and the environmental variables (water temperature, salinity and pH) was tested using Spearman's correlation coefficient (r) at a significance level of 5% (Zar, 1999).

The Kruskal-Wallis test was used to compare the abundance of *S. stanfordi* morphotypes among the size classes. Differences among values of abundance of each morphotype during the sampled weeks were analyzed through ANOVA test. Paired tests, T test and Wilcoxon test were also developed to compare the total abundance between or within some categories. To identify the existence of significant differences between the proportions of each sex, we employed a X^2-test. All the tests were performed in the software Past 1.34 (Hammer *et al.*, 2001).

Results and Discussion

The record of *Sinelobus stanfordi* associated with hyacinths in the northern coast of Rio Grande do Sul widened the range of habitats that the species can colonized. It builds its tubes attached to stems, roots or thallus of macrophytes and algae; they can also live in sandy and muddy substrates. However, the occurrence of these tanaids attached to hairy roots of hyacinths is the first record. Santos (2000) recorded a high density of *S. stanfordi* over the banks of *Chara zeylanica* Willd, a benthic algae with filamentous branches provided with internodes that retain suspended material that, in turn, is used by the tanaids to build their tubes. Albertoni & Würdig (1996) also reported the presence of *S. stanfordi* in high densities in the southeast margin at Gentil Lagoon, which were associated with the macrophytes *Bacopa monieri* (L.) Pennell and *Websteria confervoides* (Poir.) S.S.Hooper. In this substrate, *S. stanfordi* constructed its tube over filamentous algae that, in turn, are harboured on macrophytes. In the present study, no banks of *C. zeylanica* were recorded, over which this species usually predominates. As the hyacinth *Eichhornia azurea* is a floating macrophytes very common in coastal lagoons in southern Brazil (Würdig & Dornelles da Silva, 1990), it is possible to deduce that large populations of tanaid are living in this habitat. In the study area, during the entire sampled period, the water temperature varied from 23.0 to 29.9°C. The salinity oscillated between 0.198 to 0.394 and the pH from 5.00 to 7.53 (Tab. 1). The climatic conditions were practically constant and, hardly they had some influence on the population structure of *S. stanfordi*. Through a Spearman's Correlation, no relation was verified between the environmental variables and the total abundance of *Sinelobus stanfordi* collected in each week of the sampling period (p>0.05). However, Santos (2000) compared the *S. stanfordi* abundance in the mixoalins and freshwater lagoons from Tramandaí Estuarine Complex and suggested a tendency to find more abundant populations in environments with salinity influence.

Table 1. Environmental variables: T water (water temperature), salinity and pH in the weekly samplings at Gentil Lagoon, RS.

Samples	T water (° C)	Salinity (‰)	pH
1st week	25	0.394	5
2nd week	23	0.316	6.7
3rd week	23.9	0.412	6.98
4th week	28.8	0.384	6.95
5th week	26.4	0.215	6.03
6th week	28.2	0.235	7.53
7th week	27.6	0.255	7.4
8th week	29.9	0.201	7.17
9th week	29,7	0.257	7.19
10th week	28.1	0.282	7.21
11th week	27.6	0.198	7.3

A total of 2,721 individuals of *Sinelobus stanfordi* were collected, of these 1,446 were Juveniles, 296 Males, 279 Manca, 159 Ovigerous Females (OVF), 129 Preparatory Females (PREPF) and 412 Non-Reproductive Females (NRF). The difference of specimens abundance among the different categories was significant (ANOVA, F=27.1, p<0.001), and all the morphotype categories were present in the sampled period, except during the sixth week when ovigerous females were not recorded. Through a Spearman's Correlation, no correlation was found between the environmental variables and the ovigerous females. Probably, other environmental variables or biological aspects of *S. stanfordi* not totally analyzed here, could explain the absence of OVF. Figure 2 shows that juveniles were the most abundant category during all the sampled period, as observed by Santos (2000) and Kassuga (2009). Manca was highly abundant in the fourth week, although it had presented at low abundance when considered all the sampled period. This fact could be explained by the highest fragility of manca stage (Toniollo & Masunari, 2007).

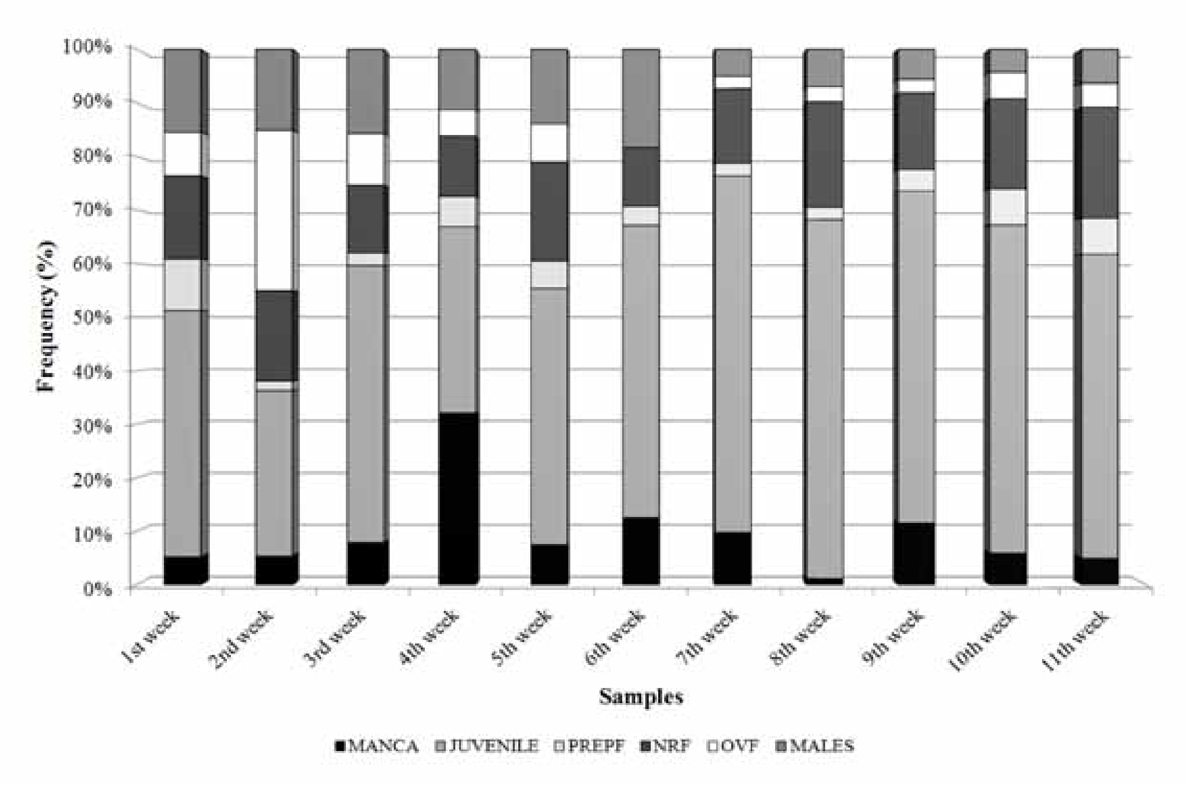

Figure 2. Frequency distribution of morphotype categories of *Sinelobus stanfordi* (Richardson, 1901) at Gentil Lagoon, RS, in weekly periods from October 2009 to January 2010.

The frequency of PREPF was highest in the first week (9.70%) than in other weeks. In the second week, OVF were the most frequent (30.1%) which suggests that PREPF developed to OVF. This highest frequency of OVF in the second week could explain the high number of manca in the fourth week (32%). According to Toniollo & Masunari (2007), who observed the post-marsupial development of this species in the laboratory, about eight days are spent from birth up to the second stage of manca. According to the authors the time of development from PREPF to OVF was from four to thirty days, in clear dependence of the seasons.

The relative frequencies of manca and juvenile were higher than that of OVF in the samples (X^2=559.15, df=10, p<0.001). It is also noticeable a high abundance of OVF in the second week, juveniles in the eighth week and manca in the fourth week. These last individuals could coming from the hatching of mentioned OVF, considering a time of 6-7 days for egg incubation and 7-8 days for development of manca stages. The highest frequency of juveniles in the eighth week is a probable consequence of the development of individuals from manca stage over the sampled weeks. According Lewis (1998) a continuous recruitment is an advantageous strategy to small animals that carry few eggs.

Unfortunately it was not possible to distinguish clearly the change of modes during the sampled period. However, it is clear that there is a continuous reproductive activity.

The average of population body size was 2.53±1.10mm (Tab. 2). Abundances of morphotype categories were significantly different among the size classes (Kruskal-Wallis, H=156.9, p<0.001) (Fig. 3). The body length of manca and juveniles ranged from 0.63 to 1.00 mm and from 1.06 to 2.38mm, respectively. Toniollo & Masunari (2007) reported a body length range of 0.53 to 0.94mm for manca stage, a range relatively close to the present study. The value of 0.63mm found to the smallest manca can be related with the sieve mesh aperture (=250μm) used to wash the material, which did not retained the smallest specimens.

Table 2. Descriptive statistic of body length of *Sinelobus stanfordi*. (N) absolute number; (x) average; (Min.) minimum, (Max.) maximum; (S.D.) standard deviation.

Categories	N	x	Min.	Max.	S.D.
Manca	279	0.82	0.63	1	0.1
Juvenile	1446	1.72	1.06	2.38	0.4
PREPF	129	3.09	2.19	4.44	0.6
NRF	412	3.22	2.44	4.06	0.5
OVF	159	3.07	2.19	4.44	0.6
Males	296	3.16	2.44	4	0.5
TOTAL	**2.721**	**2.53**	**0.63**	**4.44**	**1.1**

Males (3.16±0.45mm) were only possible to distinguish from females in individuals longer than 2.44mm, when the sexual dimorphism of antennae and chelipeds came out. Between the size classes of 2.40 and 4.05mm, 30% of the specimens were males and 70% were females (22% OVF, 16% PREPF and 61% NRF). The categories OVF (3.07±0.59mm) and PREPF (3.09±0.6mm) did not differ significantly in body length. The NRF were more abundant in the classes of 2.55 and 2.7mm. In the classes from 4.05 to 4.5mm, females were the only category present, showing the largest body length in the population. This same pattern can be observed in the study of Toniollo & Masunari (2007), who found body length from 1.16 to 3.24 mm for females and from 1.16 to 3.00 mm for males, suggesting larger lengths for females than for males; actually, shorter lengths than these values were found by us. Although studying different species, Leite *et al*. (2003) also observed males of *Kalliapseudes schubartii* with shorter body length than females, which indicates to be a common feature for tanaidaceans.

The shorter body length in different development stages verified in the population of *Sinelobus stanfordi* in the present study when compared to population of Custódias Lagoon observed by Santos (2000) could be explained by different environmental conditions of these lagoons and by different sampling periods. The salinity recorded at Custódias Lagoon ranged between 6.0 and 8.5 (Santos, 2000), while at Gentil Lagoon, it was approximately zero. According to Passano (1960), the temperature can influence the moult and can also influence the processes that control it. In laboratory cultures, Gama *et al*. (2006) found out that the temperature has affected the intermoult period and the growth rate of mysids raised at 13º, 20º and 25° C. The highest estimated growth rate was observed in individuals created at 25° C, regardless the salinity concentration. Temperature and salinity are also able to cause changes in the metabolism of aquatic organisms, accelerating the growth and consequently altering the composition of length classes. In the present study, the substrate in which the specimens are found probably influenced the species development. The roots of *Eichhornia azurea* floating in the water column provide food supplies and most protection for preys.

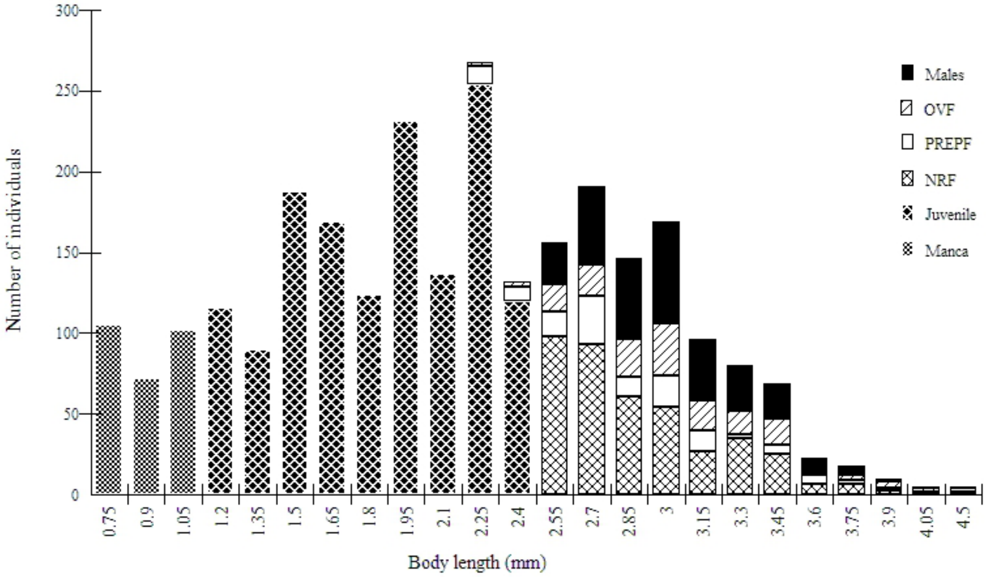

Figure 3. Distribution of absolute frequency in the body length classes of *Sinelobus stanfordi* collected from October 2009 to January 2010 at Gentil Lagoon, RS.

Sinelobus stanfordi showed a population sex ratio skewed toward females (Tab. 3). Although the deviation from the expected proportion of 1:1 (X^2=997.77, df=10, p<0.0001) may be explained by factors related to life cycles of each population (Wenner, 1972), it may also be attributed to the male reproductive behavior, which have to leave their tubes in the reproductive period to find a female tube, becoming vulnerable to biotic and abiotic factors, such as predation and range of temperature, among others (Johnson & Attramadal, 1982; Borowsky, 1983; Highsmith, 1983).

The predominance of females in some tanaidaceans could be related with the protogyny, e.g. *Leptochelia dubia* (Kroyer, 1842) which after reproductive period, the female changes her sex to male, trying to avoid the low frequency of males (Highsmith, 1983). However, as observed by Toniollo & Masunari (2007) and in the present study, the absence of external evidences of protogyny, protandry, or hermaphroditism among native populations confirms that *S. stanfordi* is a gonochoristic species. Moreover, through morphological evidences, such as the presence of copulatory cones in juveniles which always become males and the sexual differentiation that happen in a gradual way in *S. stanfordi*, would be improbable the existence of protogyny or hermaphrodism in this species.

Table 3. Sexual proportion in the population of *Sinelobus stanfordi* from Gentil Lagoon.

Samples	Females	Males	F:M
1st week	110	51	2.15:1
2nd week	55	17	3.23:1
3rd week	63	40	1.15:1
4th week	62	32	1.93:1
5th week	95	43	2.2:1
6th week	37	47	0.8:1
7th week	63	17	3.7:1
8th week	43	12	3.58:1
9th week	67	18	3.72:1
10th week	34	5	6.8:1
11th week	71	14	5.07:1
TOTAL	**700**	**296**	**3.11:1**

To a better understanding toward the biology and behavior of populations of *Sinelobus stanfordi*, more comprehensive studies about the reproductive strategies, fecundity, and life span of this species are necessary. These studies certainly will confirm if this species is suitable as bioindicator of anthropic action, as it has already been suggested by Ocon *et al*. (2008).

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