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DISSERTAÇÃO DE MESTRADO

PREVENDO MUDANÇAS CLIMÁTICAS: RESPOSTA DA MEIOFAUNA A MUDANÇAS NA DISTRIBUIÇÃO ESPACIAL DE ESPÉCIES-CHAVE

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Botucatu-SP
-2016-

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Prevendo mudanças climáticas: resposta da meiofauna a mudanças na distribuição espacial de espécies-chave

Dissertação apresentada ao curso de pós-graduação em Ciências Biológicas: Zoologia, do Instituto de Biociências, Universidade Estadual Paulista (UNESP), Campus de Botucatu, como parte dos requisitos para a obtenção do título de Mestre em Ciências Biológicas – Área de Concentração: Zoologia.

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Palavras-chave: Deslocamento de espécies; Espécies chave; Meiofauna; Mudanças climáticas; Uca.

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SUMÁRIO

APRESENTAÇÃO

O aumento das emissões de gases do efeito estufa, principalmente o dióxido de carbono, metano e óxido nitroso desde a revolução industrial tem gerado aumentos significativos na temperatura atmosférica. Esse aumento da temperatura está relacionado a outas mudanças climáticas, tais como alterações nos padrões de precipitação e na frequência de extremos climáticos (secas, inundações e tempestades severas, por exemplo), além de aumento de temperatura na superfície dos oceanos, aumento do nível do mar, e derretimento de geleiras (IPCC, 2014).

As alterações climáticas e mudanças físicas e geomorfológicas nos oceanos podem afetar os ecossistemas costeiros, uma vez que mudanças nos padrões de pluviosidade e no nível dos oceanos podem provocar variações de salinidade (Lee *et al*., 2011) e aumento de sedimentação em zonas litorâneas (Ralston *et al*., 2013). Áreas costeiras de média e alta latitude, por exemplo, provavelmente enfrentarão mudanças na biodiversidade devido à entrada de novas espécies oriundas de regiões adjacentes (Chen et al., 2011; Molinos et al., 2015).

Modelos climáticos têm mostrado mudanças nos padrões de distribuição de espécies de caranguejos do gênero *Uca*. Devido às alterações no clima, espécies de *Uca* tenderão a ampliar sua distribuição espacial migrando em direção aos polos (Nabout *et al*., 2009, tese). Atualmente, existem 11 espécies de *Uca* com ocorrência na costa Atlântica da América do Sul (Bezerra, 2012). O limite máximo de distribuição destas espécies ao Sul é a cidade de Florianópolis, porção central de Santa Catarina, com exceção da espécie *Uca uruguayensis* que tem ocorrência até Mar Chiquita, província de Buenos Aires, Argentina e *Uca mordax* que ocorre até Torres no Rio Grande do Sul (Thurman *et al*., 2013).

Caranguejos do gênero *Uca*, conhecidos como caranguejos chama-maré, são organismos típicos de áreas estuarinas e ocupam diferentes porções da zona entremarés em regiões de clima tropical e subtropical (Crane, 1975). Caranguejos chama-maré são considerados espécies chave uma vez que atuam como engenheiros de ecossistema alterando as propriedades físicas e químicas do ambiente durante suas atividades de escavação e alimentação (Payton *et al*., 2002). Além disso, estudos recentes sugerem que caranguejos *Uca* desempenham um papel fundamental controlando as comunidades meiobênticas estuarinas (Ólafsson, 2003; Citadin *et al*., 2016, no prelo).

Assim, no trabalho apresentado a seguir, buscamos avaliar os efeitos causados na estrutura e funcionamento meiofauna devido à entrada de uma nova espécie chave na região sul da América do Sul, como resposta às variações climáticas. Para isto, foram usadas duas espécies de caranguejos chama-maré (*Uca uruguayensis* e *Uca leptodactyla*) em experimentos em laboratório usando mesocosmos nos quais a densidade e a diversidade de *Uca* foram manipuladas. Ao final dos experimentos, amostras de sedimento foram coletadas para análise da meiofauna. Nossos resultados mostraram efeitos significativos na meiofauna devido ao aumento de densidade e diversidade de caranguejos do gênero *Uca.*

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Projecting climate changes: response of meiofauna communities to shifts in spatial distribution of keystone species

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ABSTRACT

Aim Current trend of climate changes may affect biodiversity by shifting species distributions patterns. *Uca* crabs, keystone species of intertidal estuarine sediments, are supposed to shift their spatial distribution by poleward displacement as a response of global warming. We develop and test models assessing whether the entrance of a new fiddler crab species, which directly affects the diversity and abundance of keystone species, may affect the structure and functioning of meiobenthic communities in intertidal areas.

Location The southern Atlantic coastal zone of South America.

Methods We used replacement series and partial additive design in indoor mesocosms experiments, manipulating the diversity and density of keystone species. Experiments lasted for 20 days and then sediment samples were taken for meiofaunal analysis. The effect of keystone species was quantified by structural and functional univariate descriptors and structure of multivariate meiofaunal communities. We utilized permutational analysis of variance (PERMANOVA) to assess differences in nematodes assemblages among treatments.

Results Nematodes were the most abundant meiofaunal group at both experiments. At the replacement series experiment, the dominant genera of nematodes were *Microlaimus*, *Terschellingia*, and *Anoplostoma*. Increasing keystone species diversity did not affect the structure of nematodes assemblage, however it affected the functioning by reducing predators/omnivores nematodes. *Microlaimus* was the dominant genus at the partial additive design experiment. Increasing keystone species diversity and density affected the univariate descriptors of structure and functioning of nematodes assemblage as well as the multivariate structure. The total density of nematodes and density of *Microlaimus* were reduced with the increase of *Uca* density. Increasing fiddler crabs density also led to changes in the multivariate structure of nematodes assemblage. The increase of diversity of keystone species reduced non-selective deposit feeders nematodes in the high level of *Uca* density.

Main conclusions Our findings suggested that the entrance of new species of *Uca* crabs in the Southern of South America as a response of global warming may affect meiobenthic communities. Increasing density and diversity of *Uca* may affect the structure and the functioning of meiofauna due changes in top-down control.

Keywords

Global warming, nematodes assemblage, poleward displacement, *Uca*.

INTRODUCTION

The increase of anthropogenic emissions of greenhouse gases since the beginning of industrial era has raised the atmospheric concentrations of carbon dioxide, methane and nitrous oxide resulting in higher air temperatures. Each of the last three decades has been successively warmer than any preceding decade since 1850. The period from 1983 to 2012 was likely the warmest 30-year period of the last 1400 years (IPCC, 2014).

Climate variability had altered marine environments. Among other effects general effects, warming temperatures in the atmosphere led to a decrease in permafrost and sea ice extent (Lawrence *et al*., 2008), increase in sea surface temperature (Levitus *et al*., 2008) and rise in sea level (Church & White, 2006). Regional effects also have been described, such as the current increase in rainfall along southwest Atlantic marine ecoregions (Bernardino *et al*., 2015), change in salinity along estuaries (Lee *et al*., 2011) and increase of sedimentation in coastal areas (Ralston *et al*., 2013). Against such stressful conditions, species are adapting to the new scenarios by shifting their phenology or changing their spatial distribution (Parmesan & Yohe, 2003).

Investigating changes in spatial distribution of species and interactions are key issues to predict the possible consequences of climate change (Van der Putten *et al*., 2010). Climate changes have increased the occurrence of invasive species, since changes in abiotic factors (e.g. temperature) increase the likelihood of species migrate to areas where the conditions are favorable, which could, in turn, affect local species and ecosystems (Walther *et al*., 2009; Burgiel & Muir, 2010). The entrance of a new species in an ecosystem may significantly affect native species. Non-native species may compete with native species (Mooney & Cleland 2001) and alter the availability of resources, causing suppression or enhancement of the relative abundance of native species (Didham *et al*., 2005) and thus affect species evenness.

Coastal areas of medium and high latitudes will probably face changes in biodiversity caused by the entrance of new species migrating from adjacent regions due to climate warming (Chen *et al*., 2011; Molinos *et al.,* 2015). This is the case of *Uca* crabs, an Ocypodidae keystone species, that is predicted to change their range of distributions next years, migrating poleward (J.C. Nabout *et al*., unpublished data). Keystone species are those whose effects are disproportionally large relative to their abundances (Power *et al*., 1996). *Uca* crabs, known as fiddler crabs, are keystone species as they modify the physical and chemical environment during their burrowing and feeding activities (Payton *et al*., 2002). These crabs also play a key role in controlling estuarine meiofauna (Ólafsson, 2003; Citadin *et al*., 2016 *in press*). Meiofauna is a group of small metazoans between 45 and 500 µm in size and constitutes the most abundant and diverse group of benthic marine invertebrates. Meiofaunal organisms contribute to the transfer of matter and energy by feeding on detritus, bacteria, microalgae and other microorganisms, and being a food source for higher trophic levels (Giere 2009; Schückel et al. 2013).

Here we focus our research on two abundant species of fiddler crabs, *Uca uruguayensis* and *Uca leptodactyla,* to investigate if changes in distributions of keystone species due climate changes may affect interactions in intertidal areas, by means of meiofauna. Fiddler crabs are conspicuous intertidal organisms from tropical and subtropical regions. Along the Atlantic coast of South America, *U. leptodactyla* ranges from Turiamo, Aragua State, Venezuela to Florianopolis, Santa Catarina State, Brazil meanwhile *U. uruguayensis* range of distribution is from Cabo Frio, Rio de Janeiro State, Brazil to Mar Chiquita, Buenos Aires Province (Fig. 1) (Thurman *et al*., 2013). The region of the Cape of Santa Marta at Laguna city, State of Santa Catarina, South Brazil, is a biogeographic transition zone. This region is the southern limit of mangroves along American continent (Schaeffer-Novelli *et al*., 2000). The State of Santa Catarina is also the southern limit of distribution of many species of fiddler crabs, with *U. rapaz*, *U. leptodactyla*, *U. burgersi* and *U. thayeri* occurring up to the central portion of the State. Southwards, only *U. uruguayensis* and *U. mordax* occurs (Thurman, 2013).

Figure 1 South America distribution of the fiddler crabs *Uca uruguayensis* and *Uca leptodactyla* according to Thurman *et al.* (2013).

Considering the putative trend of *Uca* species to expand southwards, and their strong top-down regulation on meiofauna (e.g. M.A. Fortuna *et al*., unpublished data; Citadin *et al*., 2016 *in press*), we develop and test models assessing whether the entrance of a new fiddler crab species, which directly affects the diversity and abundance of local keystone species, may affect the structure and functioning of meiobenthic communities in intertidal areas.

METHODS

We established two mesocosm setups to mimic the putative effects of the arrival of a new keystone species on meiofaunal communities. The experimental designs were based on the replacement series from De Wit (1960) and the partial additive design from Harper (1977) (Fig. 2). These designs differ in the way species are manipulated, in the replacement series the total density of species is held constant in single and multiple-species treatments while the proportion of each species is changed. In the partial additive design, density of one species is kept constant while other varies. The arrival of a new keystone species leads to increases in species density and diversity. Thus, firstly, to test the hypothesis of increase in keystone species diversity due shifts in distribution, as a result of climate change, may affect meiofaunal communities, we used the replacement series. Then, we tested the effects on meiofauna due changes in keystone species density together with diversity in a partial additive design.

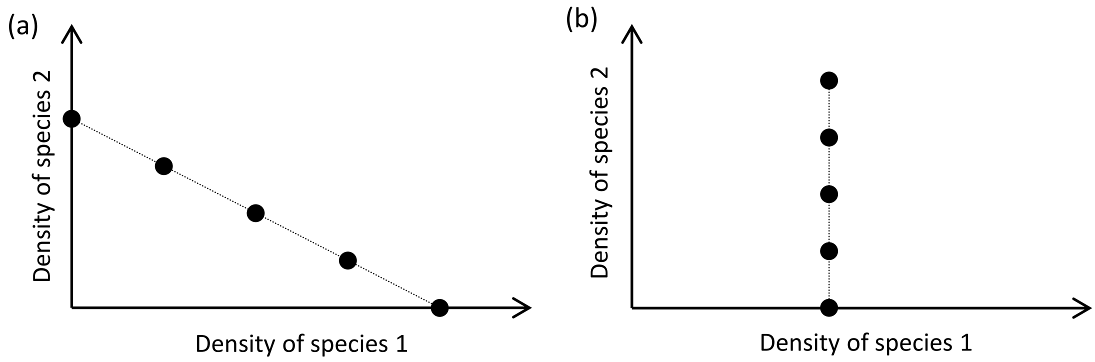

Figure 2 Experimental models used in this study: (a) in the replacement series, total density is constant and the proportion of each species changes; (b) in the partial additive design, the total density varies as density of one species is constant and other varies.

Set-up Experiment 1 – Replacement series

The replacement series experiment was performed between the 27th of October and the 16th of November 2014. Total density of keystone specimens was fixed in 8 inds/mesocosm (natural field densities may reach 240 crabs/m² (Masunari, 2006), *i.e.* 8,15 inds/mesocosm). The experiment included four treatments - one with a single keystone species and three with two keystone species. The single-species treatment was composed by 8 *U. uruguayensis* (8U), and two-species treatments comprised: 6 *U. uruguayensis* + 2 *U. leptodacyla* (6U+2L); *4 U. uruguayensis* + 4 *U. leptodatyla* (4U+4L) and 2 *U. uruguayensis* + 6 *U. leptodactyla* (2U+6L) (Fig. 3). Each treatment was replicated four times.

Set-up Experiment 2 – Partial additive design

The partial additive design was performed between the 26th of January and the 15th of February of 2015. In this experiment, both keystone species density and richness were manipulated. First, densities of *U. uruguayensis* were kept constant (two individuals) and *U. leptodactyla* were added two by two through the treatments to mimic the entrance of this fiddler crab in temperate zones due to climate change. So that, these treatments were composed by: 2 *U. uruguayensis* + 2 *U. leptodactyla* (2U+2L); 2 *U. uruguayensis* + 4 *U. leptodactyla* (2U+4L) and 2 *U. uruguayensis* + 6 *U. leptodactyla* (2U+6L). Besides, single-species treatments composed only by *U. uruguayensis* were also included with the same levels of total density than two-species treatment (4U; 6U and 8U) (Fig. 4) to test if the potential effects on meiofauna were due by the increase of richness or density of the keystone species.

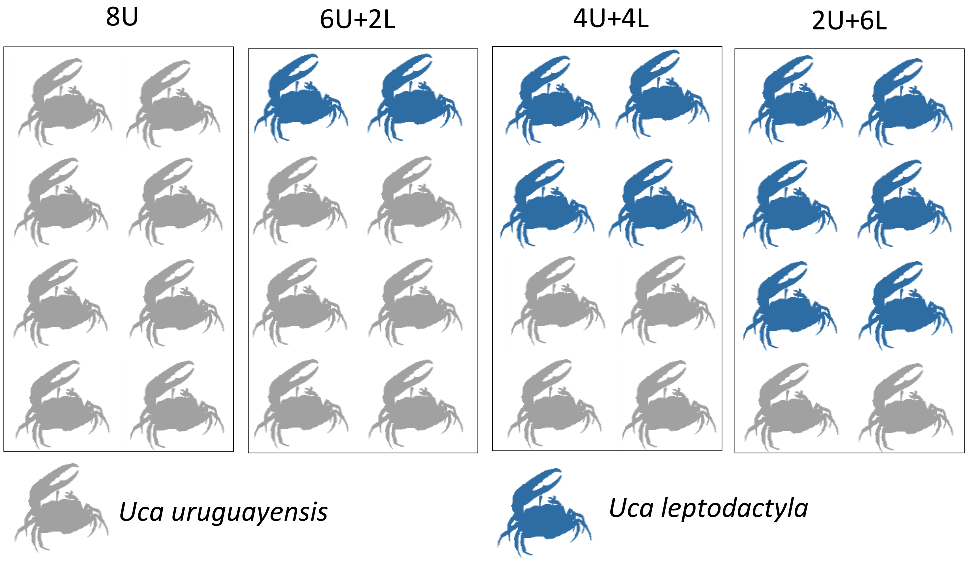

Figure 3 Set-up experiment 1 – replacement series. U: *Uca uruguayensis*; L: *Uca leptodactyla*.

Experimental conditions

In both experiments, *Uca* crabs were maintained in indoor mesocosms consisting in glass aquariums (29.5x11.5x20 cm, length x width x height). Sediments (upper than 5 cm) and fiddler crabs were collected on an unvegetated sandflat along the Una estuary, south-eastern Brazil (24°24’50”S, 47°04’14”W), at the Juréia-Itatins Reserve, part of a network protected areas of Atlantic rainforest (Marques & Duleba 2004), and transported to the laboratory inside plastic boxes. Only adult males of the two crabs species (from 8 to 10 mm of carapace width) were used in both experiments. Sediment were composed by very fine sand with an average total organic matter of 1.52% (L.F. Natálio *et al*., unpublished data), and chlorophyll *a* content ranging from 0 to 11.98 μg g^{-1} (Citadin *et al*., 2016 *in press*). In laboratory, sediments were gently homogenized, and subsamples for the mesocosms were taken up to complete a layer of 5 cm of sediment. Fiddler crabs were included in mesocosms only 48 h after sediments to permit meiofauna stratification and crabs acclimation at laboratory. The experiments ran with constant temperature (28° C). By the end of the experiments, crabs were counted and sediment samples were collected from each mesocosm to evaluate the changes in meiofauna.

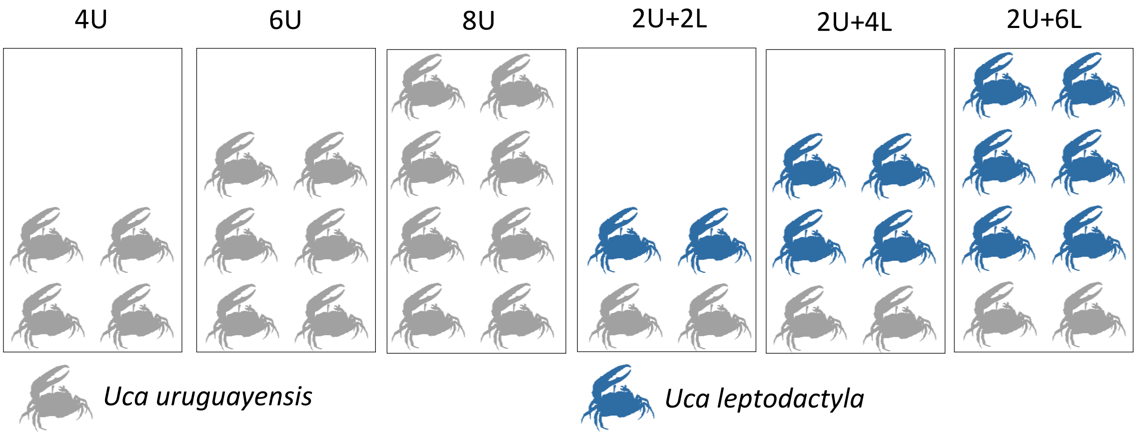

Figure 4 Set-up experiment 2 – Partial additive design. U: *Uca uruguayensis*; L: *Uca leptodactyla.*

Sampling and sample processing

A total area of 19.23 cm^2 of sediment was taken from each mesocosm from the experiment 1, (two PVC corers of 3.5 cm in diameter to a depth 1 cm), and 28.84 cm^2 for the experiment 2 (three PVC cores of 3.5 cm x 1 cm). Differences in volume of sampled sediment were due to the difference in density of meiofauna inside the mesocosms, lower in experiment 2. All sediment samples were fixed in 4% formalin and processed following Somerfield *et al.* (2005). Sediment was washed in fresh water, sieved through 500 and 63 μm mesh openings, and fauna on the smaller mesh was extracted by flotation in Ludox TM-50 (specific gravity of 1.15). The extracted fauna was placed in embryo dishes with glycerol (65% water, 30% alcohol and 5% glycerin) and left to evaporate for about 10 h, then permanent slides were made.

Data analysis

Two replicates, one from 2U+4L treatment and other from 2U+6L treatment of experiment 2 had crabs died by the end of the experiment, and were excluded from statistical analysis. For data analysis, only nematodes were used as they accounted for 97% of the total meiofauna. Both univariate and multivariate statistical methods were used to test the effects of fiddler crabs density and/or diversity on nematodes community. Permutational analysis of variance (PERMANOVA; Anderson *et al.*, 2008) were used to assess differences in nematodes univariate descriptors and multivariate structure among treatments. PERMANOVA was chosen because it allows for mixed designs, an interaction term, and does not assume a normal distribution of errors.

In univariate analysis both structural and functional nematodes descriptors were used. Structural descriptors included total density, number of genera, Shannon-Wiener diversity ($\log_2$) and the density of numerically dominant genera. Yet as functional attributes of the nematodes, we used the relative abundance of each feeding type (Wieser, 1953; 1960), the index of trophic diversity (Heip, 1985) and the maturity index (Bongers *et al.*, 1991).

For the experiment 1, univariate one-way PERMANOVA tests were run on Euclidean distance matrices with 999 permutations and with unrestricted permutation of raw data. Multivariate one-way PERMANOVA tests were run on Bray-Curtis similarity matrices. For both analysis, when significant differences were detected ($p<0.05$), pairwise tests P-value based on Monte Carlo (MC) were applied. Ordinations of the multivariate faunal data were represented by non-metric multidimensional scaling (nMDS).

For the second experiment two-way PERMANOVA were used to assess differences in univariate and multivariate faunal structure among keystone species' density (fixed factor with three levels: 4, 6 and 8 crabs) and richness (fixed factor with two levels: *U. uruguayensis* and *U. uruguayensis* + *U. leptodactyla*, nested in keystone species density). Univariate PERMANOVA tests were run on Euclidean distance matrices with 999 permutations, and the residuals were permuted under a reduced model. PERMANOVA tests for differences in the multivariate community structure were carried out on Bray-Curtis similarities matrices. When significant differences were detected ($p<0.05$), pairwise tests P-value based on Monte Carlo (MC) were applied. Ordinations of the multivariate faunal data were represented by non-metric multidimensional scaling (nMDS).

RESULTS

Experiment 1 - Replacement series

Nematodes accounted 97% of the total individuals, so that other meiofaunal groups were excluded from statistical analyses. Nematodes densities ranged from 36 to 126 inds/10 cm², distributed in 25 genera belonging to 14 families (Appendix 1). The genera *Anoplostoma* (Anoplostomatidae), *Microlaimus* (Microlaimidae) and *Terschellingia* (Linhomoeidae) were the numerically dominant genera contributing for 25,1%, 19,9% and 18% (respectively) of the total fauna.

The structural and functional descriptors of nematodes did not vary significantly with the increase in keystone species diversity (Table 1). The exception was the relative abundance of predators/omnivores nematodes (p(MC)=0,037). The pairwise PERMANOVA test showed difference between the groups of treatments 6U+2L and 4U+4L; and 8U and 4U+4L (results of pairwise tests are on Appendix 2), predators/omnivores nematodes were lower in the 4U+4L treatment (Fig. 5).

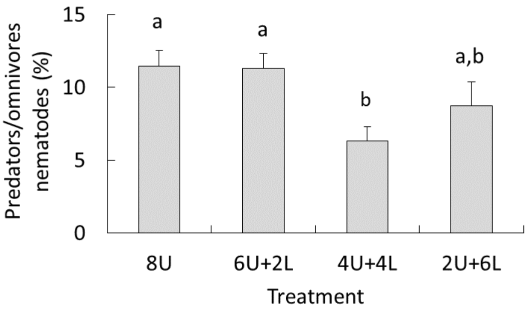

Figure 5 Mean values (±SE) of predators/omnivores nematodes on treatments. U*: U. uruguayensis*; and *L: U. leptodacyla.* Different letters mean differences found on pairwise tests.

Non-metric multidimensional scaling showed that the multivariate structure of nematodes did not differ between the treatments since no clear pattern of aggregation of the groups can be observed (Fig. 6). Multivariate PERMANOVA also showed no difference between treatments (p(MC)=0,263; Table 1).

Table 1 Results of PERMANOVA evaluating the increase in keystone species diversity on multivariate structure and on structural and functional univariate descriptors of nematodes. Bold values indicate p≤0.05. Pairwise tests are available on Appendix 2. df: degrees of freedom; SS: sum of squares; MS: mean squares; p(MC): p-value obtained with Monte Carlo permutation test.

Source of variation	df	SS	MS	F	p(MC)
Multivariate structure					
Treatment	3	1152,8	384,26	1,2007	0,263
Residual	12	3840,4	320,04		
Structural descriptor					
Density					
Treatment	3	3303,7	1101,2	1,5694	0,253
Residual	12	8420,1	701,68		
Number of genera					
Treatment	3	10,5	3,5	0,7433	0,568
Residual	12	56,5	4,7083		
Diversity (H’)					
Treatment	3	0,0151	0,0050	0,8040	0,526
Residual	12	0,0753	0,0062		
Anoplostoma density					
Treatment	3	155,83	51,943	0,7309	0,575
Residual	12	852,77	71,065		
Microlaimus density					
Treatment	3	278,87	92,957	1,5802	0,243
Residual	12	705,94	58,828		
Terschellingia density					
Treatment	3	301,93	100,64	2,3724	0,128
Residual	12	509,07	42,422		
Functional descriptor					
Selective deposit feeders					
Treatment	3	279,56	93,187	1,544	0,236
Residual	12	724,23	60,353		

Table 1 Results of PERMANOVA evaluating the increase in keystone species diversity on multivariate structure and on structural and functional univariate descriptors of nematodes. Bold values indicate p≤0.05. Pairwise tests are available on Appendix 2. df: degrees of freedom; SS: sum of squares; MS: mean squares; p(MC): p-value obtained with Monte Carlo permutation test.

Source of variation	df	SS	MS	F	p(MC)
Non-selective deposit feeders					
Treatment	3	116,44	38,814	1,1044	0,379
Residual	12	421,72	35,144		
Epistratum feeders					
Treatment	3	134,49	44,829	0,8939	0,496
Residual	12	601,78	50,148		
Predators/Omnivores					
Treatment	3	71,569	23,856	4,0687	**0,037**
Residual	12	70,36	5,8633		
Index of Trophic Diversity					
Treatment	3	0,0030	0,0010	2,0308	0,158
Residual	12	0,0059	0,0004		
Maturity Index					
Treatment	3	0,0069	0,0023	0,2092	0,872
Residual	12	0,1321	0,0110		

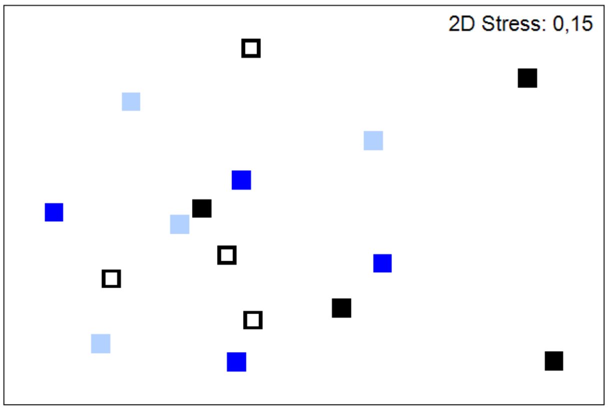

Figure 6 MDS ordination for log(x+1) transformed nematodes multivariate structure. Black squares 8U; dark blue: 6U+2L; light blue: 4U+4L and open squares 2U+6L. U: *U. uruguayensis*; and L: *U. leptodactyla*

Experiment 2 – Partial additive design

As in the replacement series experiment, nematodes were the dominant meiofaunal group and account more than 95% of the total fauna, so that, only nematodes were used on statistical analysis. Nematodes densities ranged from 19 to 64 inds/10^2, distributed in

18 genera belonging to 14 families. (Appendix 3). The genus *Microlaimus* (Microlaimidae) was the numerically dominant genera contributing for 82,1%, of the total fauna.

The total density of nematodes and the density of *Microlaimus* sp. were affected by the increase of keystone species density (both p(MC)= 0,017) but not by the increase of keystone species richness (p(MC)=0,331 and 0,0333, respectively) (Table2). For total density of nematodes, the pairwise test showed this descriptor varied between the levels of crabs' density 4 and 8, and 6 and 8 but not between 4 and 6 (Figure 7a). Although fiddler crabs diversity did not affect nematodes density, it can be seen in the Figure 7b that two species treatments with higher levels of density, the densities of nematodes were higher than in one-species treatment.

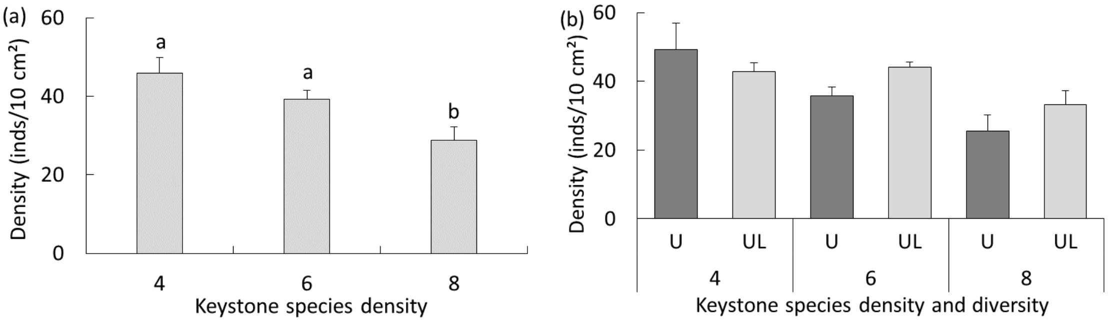

Figure 7 Mean values (±SE) of density of nematodes at (a) the densities of 4, 6 and 8 keystone species and at (b) the densities of 4, 6 and 8 keystone species divided by single and two-species treatments. U: *Uca uruguayensis*; UL: *Uca uruguayensis* and *Uca leptodactyla.* Different letters mean differences found on pairwise tests.

As total density, densities of *Microlaimus* sp. also decreased with higher density of fiddler crabs (Fig. 8). *Microlaimus* sp. density varied among the levels 4 and 8 crabs, but not between 4 and 6, and 6 and 8 (results of pairwise tests are available on Appendix 4). All the other descriptors of nematodes structure was not affected by the increase of *Uca* density nor richness (p(MC)>0,05; Table2).

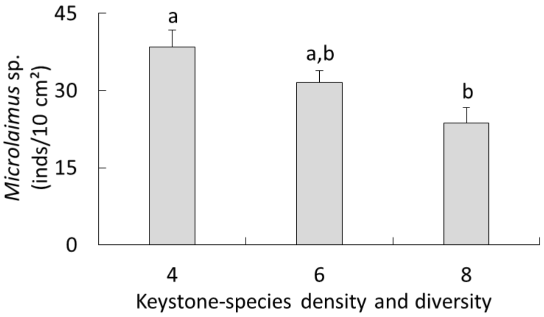

Figure 8 Mean values (±SE) of *Microlaimus* sp. density at the densities of 4, 6 and 8 keystone species. Different letters mean differences found on pairwise tests.

The relative abundance of non-selective deposit feeders nematodes did not vary significantly between keystone species density (p(MC)=0,924), but between keystone species richness it did (p(MC)=0,031; Table 2). Non-selective deposit feeders nematodes abundance was low in the presence of the two keystone species (Fig. 9a). This effect was observed at the high level of fiddler crab density (Appendix 4; Fig. 9b).

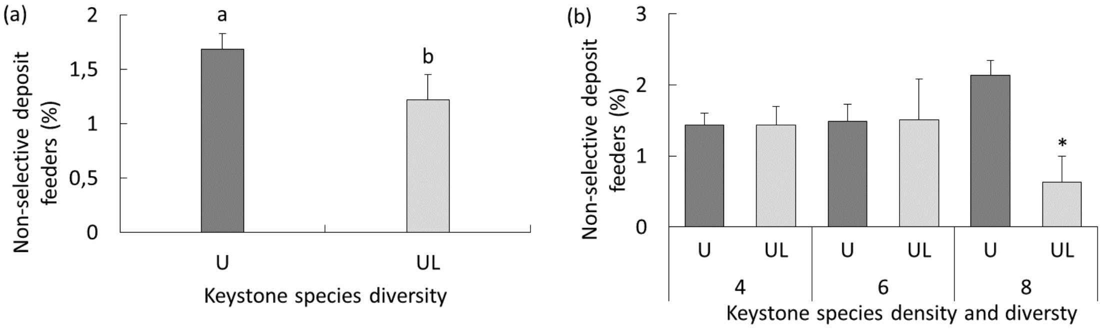

Figure 9 Log(x+1) transformed mean values (±SE) of non-selective deposit feeders nematodes on (a) single and two-species treatments and on (b) single and two-species treatments at the densities of 4, 6 and 8 keystone species. U: *Uca uruguayensis*; UL: *Uca uruguayensis* and *Uca leptodactyla.* Different letters and asterisk (*) mean differences found on pairwise tests.

The non-metric multidimensional scaling showed a clear separation between the groups. The treatments with 4 crabs are placed mostly on the left of the graph, while treatments with 8 crabs are mostly on the right, 6 crabs treatments are at the center, being intermediate between 4 and 8. Multivariate PERMANOVA showed that fauna varied with keystone species density (p(MC)=0,008; Table 2), but with keystone species richness (p(MC)=0,239). Pairwise tests showed difference between the groups of 4 and 8 crabs, but not between 4 and 6, and 6 and 8 (Appendix 4).

Figure 10 MDS ordination for nematodes multivariate structure at densities of 4 (dark blue); 6 (light blue) and 8 (open squares) crabs.

Table 2 Results of two-way PERMANOVA evaluating the increase in keystone species diversity and density on multivariate structure and structural and functional univariate descriptors of nematodes. Data were log(x+1) transformed when it was necessary. Bold values indicate p≤0.05. Pairwise tests are available on Appendix 4. df: dregrees of freedom; SS: sum of squares; MS: mean squares; p(MC): p-value obtained with Monte Carlo permutation test.

Source of variation	df	SS	MS	F	p(MC)
Multivariate structure					
Uca density	2	1940,2	970,1	4,0555	**0,008**
Uca richness (*Uca* density)	3	990,52	330,17	1,3803	0,239
Residual	16	3827,3	239,2		
Nematodes structure					
Density					
Uca density	2	1025,7	512,83	6,4585	**0,017**
Uca richness (*Uca* density)	3	300,02	100,01	1,2595	0,331
Residual	16	1270,5	79,403		
Number of genera					
Uca density	2	13,452	6,7259	1,9245	0,194
Uca richness (*Uca* density)	3	2,1548	0,7182	0,2055	0,907
Residual	16	55,917	3,4948		
Diversity (H’)					
Uca density	2	0,0292	0,0146	0,5067	0,628
Uca richness (*Uca* density)	3	0,0271	0,0090	0,3137	0,824
Residual	16	0,4610	0,0288		

Table 2 Results of two-way PERMANOVA evaluating the increase in keystone species diversity and density on multivariate structure and structural and functional univariate descriptors of nematodes. Data were log(x+1) transformed when it was necessary. Bold values indicate p≤0.05. Pairwise tests are available on Appendix 4. df: dregrees of freedom; SS: sum of squares; MS: mean squares; p(MC): p-value obtained with Monte Carlo permutation test.

Source of variation	df	SS	MS	F	p(MC)
Microlaimus density					
Uca density	2	750,82	375,41	5,8627	**0,017**
Uca richness (*Uca* density)	3	236,74	78,914	1,2324	0,333
Residual	16	1024,5	64,033		
Nematodes functioning					
Selective deposit feeders					
Uca density	2	60,901	30,451	1,0745	0,358
Uca richness (*Uca* density)	3	5,402	1,8007	0,0635	0,978
Residual	16	453,41	28,338		
Non-selective deposit feeders					
Uca density	2	0,0474	0,0237	0,0739	0,924
Uca richness (*Uca* density)	3	3,8954	1,2985	4,0454	**0,031**
Residual	16	5,1356	0,3209		
Epistratum feeders					
Uca density	2	89,319	44,659	1,327	0,291
Uca richness (*Uca* density)	3	47,47	15,823	0,4701	0,709
Residual	16	538,47	33,654		
Predators/omnivores					
Uca density	2	0,9453	0, 0472	2,1736	0,146
Uca richness (*Uca* density)	3	1,4923	0,4974	2,2875	0,109
Residual	16	3,4794	0,2174		
Index of Trophic diversity					
Uca density	2	0,0158	0,0079	1,2154	0,313
Uca richness (Uca density)	3	0,0118	0,0039	0,6036	0,63
Residual	16	0,1044	0,0065		
Maturity index					
Uca density	2	0,0017	0,0008	0,1780	0,856
Uca richness (*Uca* density)	3	0,0130	0,0043	0,8694	0,476
Residual	16	0,0799	0,0049		

DISCUSSION

As we hypothesized, the poleward displacement of keystone species as a response of climate change may affect the structure and functioning of the meiofauna communities of intertidal flats. In our experiments, changes in fiddler crabs diversity affected nematodes functioning, by altering the relative abundance of predators/omnivores nematodes and non-selective deposit feeders. Moreover, the increase in density of fiddler crabs affected negatively the nematodes assemblage decreasing their densites and the density of the most abundant genera. The entrance of a new invertebrate species in marine environments may affect the native community by altering food web dynamics and modifying physical environment (Crooks, 1998; Mistri, 2003; Greene *et al*., 2011; Chan & Bendell, 2013).

In our replacement series experiment, high fiddler crab diversity did not lead to changes in structure of meiofauna. This result may be due to *U*. *uruguayensis* and *U*. *leptodactyla* are similar (Crane, 1975) and may explore similar niches by reworking similar volumes of sediments at almost identical depths (Machado *et al.,* 2013). O'Connor & Bruno (2009) also did not found changes in the structure of diverse prey communities such as our study with meiofauna, with shifting predator richness. According to the authors, studies reporting significant effects on prey composition with the increase of predator richness, (e.g. Siddon & Witman, 2004; Finke & Denno, 2005; Byrnes *et al*., 2006; Douglass *et al*., 2008; Naddafi & Rudstam, 2013), used a small number of prey species (one to five species). The effects of predator richness therefore, seem to be more important under conditions of low prey diversity. If this is true, future experiments should be done including different nematodes communities with high and low levels of nematodes diversity.

While the increase of keystone species diversity did not affect the structure nematode assemblages, it did affect their trophic functioning. The relative abundance of predators/omnivores nematodes declined when more *U. leptodactyla* were present. The exact nature of this change in the trophic structure of the nematode assemblages is not easily to explain. The most abundant predators/omnivores nematodes found in this work belong to the genus *Oncholaimus* (Oncholaimidae), nematodes that usually have large body and occupy the surface sediment layers (Moens *et al*., 2013). Considering *Oncholaimus* size, or the superficial layer of sediment that these nematodes occupy, the genera could be more easily ingested by crabs or disturbed in their presence. However, although fiddler crabs may feed actively on meiofauna, they probably do not select species as they feed by flotation feeding (Quinn, 1980; Dittmann, 1993), so the size of the organism may be important during this process. Unfortunately, we did not measure the nematodes biomass to evaluate if there is a relationship between the size of nematodes and *Uca* species. Future studies should include analysis of nematodes biomass to explore this question.

When we evaluated the effects of keystone species diversity together with density in the partial additive design experiment, no effects of *Uca* diversity on nematodes assemblage structure were found, confirming our results from the replacement series. Increasing fiddler crabs density, however, affected significantly the structure of nematodes. The total density of nematodes and the density of the most abundant genus (*Microlaimus*) declined with increasing fiddler crabs density independently of the species used. Similarly, in a field enclosure experiment Guidetti (2007) observed a decrease in the density of sea urchin, caused by an enhancement of predation, when he increased predator density. Our results confirm the important top-down regulation of the meiofauna by fiddler crabs by means of sediment disturbance and predation observed by Ólafsson (2003) and Citadin *et al*. (2016 *in press*). The increase of keystone species therefore, may reduce nematodes density directly via predation, or indirectly via modification of the sediment environment.

Although *Uca* diversity caused no significant effects in nematodes structure, the mean density of nematodes was higher in the two-species treatments at high and mid levels of keystone species density. These results may suggest a reduction of the top-down control when both species of crabs are together in high densities. The reduction of predation is not unusual when two species forage together, Siddon & Witman (2004), for instance, reported a significant risk reduction for urchins when two predators were combined.

The abundances of non-selective deposit feeders nematodes decreased in the higher levels of keystone species diversity and density. This low abundance in this functional feeding group of nematodes may also be due the activities of bioturbation exerted by fiddler crabs. During burrowing activities, fiddler crabs manipulate large quantities of sediment increasing habitat heterogeneity both horizontally and vertically (Hoffman *et al*., 1984; Botto *et al*., 2008; Sayão-Aguiar *et al*., 2012). The maximum depth burrowed by *U. leptodactyla* is generally higher than *U. uruguayensis* (Machado *et al*., 2013), with high abundance of *U. leptodactyla* in mesocosms it is possible that more non-selective deposit feeders nematodes are being affected by *Uca*'s disturbance.

Our predictions about the effects on nematodes assemblages due climate changes refer exclusively to those expected as a response to the increase of keystone species. Besides changes in patters of keystone species distributions, other points may be considered to evaluate the effects of climate variability on nematodes assemblage. For instance, rising temperatures may affect keystone-species behavior by increasing foraging activities, as observed by Sanford (1999) affecting even further the meiofauna. Increasing temperature may also affect nematodes structure, as seen by Gingold *et al*. (2013), resulting in loss of density, richness, diversity, abundance and community biomass.

The increase of keystone species diversity could cause fewer effects in lower latitudes where there is a bigger number of species (Fischer, 1961; Hillebrand, 2004) than in high latitudes. The high biodiversity found in low latitudes increases the possibility of several species perform the same function resulting in ecological redundancy, which is not observed at high latitudes. However, these patterns of species distributions are been modified by climate changes. Tropicalization of temperate zones due to species poleward displacements could lead to increase species richness on temperate zones and poles (Molinos *et al*., 2015).

In summary, our results suggest that global climate changes may indirectly affect meiofauna community via displacement of keystone species. Global warming and climate changes will continue unless anthropogenic emissions of greenhouse gases stop. More studies evaluating the direct and indirect impacts of climate changes on ecosystems should be done to predict future changes and explore ways to mitigate them.

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Appendix 1. Total abundance of nematodes genera in replacement series experiment on treatments: 8U; 6U+2L; 4U+4L and 2U+6L. U: *Uca uruguayensis* and L: *Uca leptodactyla.*

Genera	8U	6U+2L	4U+4L	2U+6L
Anoplostoma	104	147	171	140
Chromadorella	4	13	12	15
Chromadoridae indet	0	0	1	1
Dichromadora	60	83	102	90
Hypodontolaimus	0	1	0	0
Neochromadora	3	0	0	1
Ptychollaimelus	0	0	0	1
Paracanthonchus	7	9	14	11
Paracyatholaimus	0	0	1	7
Pomponema	1	0	0	0
Dorylaiminae	2	5	7	5
Ethmolaimus	1	3	4	5
Haliplectus	2	5	2	1
Leptolaimus	3	2	2	0
Linhomoeidae	0	3	2	1
Paralinhomoeus	0	0	2	0
Terschellingia	73	65	146	120
Microlaimus	57	120	131	138
Diplolaimella	0	1	0	0
Diplolaimelloides	2	2	0	1
Thalassomonhystera	5	7	1	5
Monhysteridae	0	1	0	1
Oncholaimus	39	59	40	49
Viscosia	1	1	2	1
Oncholaimidae	4	46	4	1
Halalaimus	4	8	10	9
Oxystomina	2	2	3	1
Subsphaerolaimus	1	1	1	1
Rhabdocoma	5	1	8	0
Trefusia	1	1	1	0

Appendix 2. Pairwise PERMANOVA tests from replacement series experiment for effects of keystone species treatments (8U; 6U+2L; 4U+4L and 2U+6L, U: Uca uruguayensis and L: Uca leptodactyla.) on predators/omnivores nematodes. t: pseudo-*t*; p(MC): p-value obtained with Monte Carlo permutation test.

Groups	t	p(MC)
8U, 6U+2L	0,083303	0,933
8U, 4U+4L	3,4642	**0,012**
8U, 2U+6L	1,3783	0,205
6U+2L, 4U+4L	3,5994	**0,011**
6U+2L, 2U+6L	1,3616	0,236
4U+4L, 2U+6L	1,2574	0,246

Appendix 3. Total abundance of nematodes genera in partial additive design experiment on two and single keystone species treatments and among levels of density.

Genera	*U. leptodactyla* + *U. uruguayensis*			*U. uruguayensis*		
	4	6	8	4	6	8
Anoplostoma	0	0	1	2	1	2
Axonolaimus	0	0	0	0	1	2
Chromadorella	0	1	0	0	1	1
Desmodora	1	0	1	0	1	0
Dichromadora	0	1	0	0	0	0
Diplolaimelloides	17	19	2	18	12	19
Dorylaiminae	0	1	0	0	0	0
Ethmolaimus	3	3	3	8	1	3
Halalaimus	0	0	1	1	0	1
Haliplectus	12	13	8	15	15	3
Linhomoeus	0	1	0	0	0	0
Microlaimus	410	303	243	477	333	234
Oncholaimidae	0	0	0	1	0	0
Oncholaimus	1	0	0	3	1	0
Paracanthonchus	1	2	0	0	0	1
Paracyatholaimus	1	0	0	0	0	0
Siphonolaimus	0	0	0	1	1	0
Terschellingia	31	34	26	32	35	27
Thalassomonhystera	1	0	0	0	1	0
Viscosia	0	0	0	0	1	0

Appendix 4. Pairwise PERMANOVA tests from partial additive design experiment for effects of keystone species density (4: four crabs, 6: six crabs and 8: eight crabs) and keystone species richness (U: *U. uruguayensis* and UL: *U. uruguayensis* + *U. leptodactyla*; nested in keystone species density) on nematodes multivariate structure and univariate descriptors. t: pseudo-*t*; p(MC): p-value obtained with Monte Carlo permutation test.

	Multivariate		Total Density		*Microlaimus* density	
Groups (keystone species density)	t	p(MC)	t	p(MC)	t	p(MC)
4,6	1,1293	0,279	1,2827	0,234	1,5337	0,163
4,8	2,6127	**0,013**	3,0847	**0,01**	3,109	**0,011**
6,8	1,8347	0,056	2,8677	**0,02**	2,1072	0,056

	Non-selective deposit feeders	
Groups (Keystone species richness nested in keystone species density)	t	p(MC)
U,UL (4)	0,013826	0,994
U,UL (6)	0,043613	0,963
U,UL (8)	3,8136	0,011