Marine Pollution Bulletin 90 (2015) 115–120

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Responses of estuarine nematodes to an increase in nutrient supply: An *in situ* continuous addition experiment

R.C. Ferreira [a], A.B. Nascimento-Junior [b], P.J.P. Santos [b,c], M.L. Botter-Carvalho [d], T.K. Pinto [a,e,*]

[a] Programa de Pós-graduação em Diversidade Biológica e Conservação nos Trópicos, UFAL, Maceió, AL, Brazil
[b] Programa de Pós-Graduação em Biologia Animal, UFPE, Recife, PE, Brazil
[c] Departamento de Zoologia, UFPE, Recife, PE, Brazil
[d] Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros s/n, Dois Irmãos, 52171-900 Recife, PE, Brazil
[e] Unidade de Ensino Penedo/Campus Arapiraca, UFAL, Penedo, AL, Brazil

ARTICLE INFO

Article history:
Available online 8 December 2014

Keywords:
Meiofauna
Mangrove
Inorganic enrichment
Fertilizers
Sugar cane

ABSTRACT

An experiment was carried out on an estuarine mudflat to assess impacts of inorganic nutrients used to fertilize sugar-cane fields on the surrounding aquatic ecosystem, through changes in the nematode community structure. During 118 days, nine quadrats each 4 m^2 were sampled six times after the beginning of fertilizer addition. The fertilizer was introduced weekly in six areas, at two different concentrations (low and high doses), and three areas were used as control. The introduction of nutrients modified key nematode community descriptors. In general, the nematodes were negatively affected over the study period. However, *Comesa*, *Metachromadora*, *Metalinhomoeus*, *Spirinia* and *Terschellingia* were considered tolerant, and other genera showed different degrees of sensitivity. Nutrient input also affect the availability and quality of food, changing the nematode trophic structure. The use of inorganic fertilizer should be evaluated with care because of the potential for damage to biological communities of coastal aquatic systems.

1. Introduction

Eutrophication is one of the most severe and widespread forms of anthropogenic impacts on aquatic environments (Ristou et al., 2012). The occurrence of hypoxia/anoxia as a result of eutrophication in coastal and estuarine areas is increasing worldwide, mainly as a result of human activities, such as the drainage of agricultural products into waterways and the release of industrial wastewater (Wolanski, 2007; Wu, 2002). These activities are the main sources of input of both organic and inorganic nitrogen and phosphorus (Gaudes et al., 2013), the main compounds related to eutrophication processes, into marine environments (GESAMP, 1990). Inorganic fertilizers used on sugar-cane fields are composed primarily of these two elements. It is assumed that slightly over 50% of fertilizers used in agriculture is consumed by plant biomass, with the remainder carried into rivers and estuaries (McLusky and Elliott, 2004). The limited availability of oxygen (hypoxia/anoxia) arising from eutrophication has been associated, in estuarine and marine areas, with changes in these systems that can lead to the decline of different biotic compartments, such as benthic communities (Modig and Olafsson, 1998; Rosenberg et al., 2002).

Among benthic organisms, the potential usefulness of nematodes for evaluating disturbances caused by humans is related to a number of characteristics of this taxon, such as close association with sediment, high abundances, low mobility, low dispersal ability and a short lifespan, resulting in a high capacity to reflect local events (Coull and Chandler, 1992; Souza et al., 2004). This indicator potential of nematodes is associated with the fact that sediments act as deposits for contaminants, and are strongly affected by the input of nutrients of allochthonous origin. Several studies have examined the response of nematode communities to conditions of hypoxia/anoxia induced in different ways, such as: controlling the oxygen level in the water (Steyaert et al., 2007; Modig and Olafsson, 1998); covering the sediment to exclude oxygen (Van Colen et al., 2009); adding contaminants such as heavy metals and organic carbon (Gyedu-Ababio and Baird, 2006); adding sucrose and organic matter (Gambi et al., 2009); and adding marine algae (*Ascophyllum nodosum*) (Schratzberger and Warwick, 1998). Most of these studies were conducted in temperate regions, with relatively few in tropical regions. In Cuba, Armenteros et al. (2010) induced a state of hypoxia/anoxia through the addition of *Spirulina* microalgae powder.

* Corresponding author at: Av. Beira Rio, s/n, Centro, Penedo, AL CEP 57200-000, Brazil. Tel.: +55 82 98284774.
E-mail address: tacianakp@gmail.com (T.K. Pinto).

http://dx.doi.org/10.1016/j.marpolbul.2014.11.012
0025-326X/

Understanding the problems caused by human intervention in aquatic environments is essential in order to develop measures to mitigate these impacts. The present study evaluated, through a 118-day *in situ* experiment, the response of nematode genera to induced hypoxia/anoxia, through continuous exposure to different concentrations of inorganic fertilizer used on sugar-cane fields.

2. Materials and methods

2.1. Study area

The study area is located in the coastal estuarine complex of the Ilha de Itamaracá region (7°46.184'S and 34°52.926'W), on the north coast of the state of Pernambuco, approximately 50 km from the city of Recife (Fig. 1). This part of the coast is distinguished by high primary productivity and faunal diversity (CPRH, 2001). The tidal flat area where the experiment was conducted is about 300 m from the waterline during average low water spring tides.

2.2. Experimental design and treatments

The experiment was conducted in nine quadrats of 4 m^2, 3 m apart. Six of these areas were enriched weekly with inorganic fertilizer (NPK TREVO: 20% nitrogen, as ammonium sulfate; 10% phosphorus, as water-soluble P_2O_5; and 20% potassium, as water-soluble K_2O). Three of these areas received 750 g/4 m^2 (low dose, LD) and three were treated with 1500 g/4 m^2 (high dose, HD). The remaining three areas were not enriched and were considered as control (C) areas.

Granulated fertilizer was applied directly on the sediment surface. The amounts of fertilizer were calculated to increase nutrient concentrations in the sediment, disregarding solubility in overlying water, by 12.5% and 25% weekly at the low dose and 25% and 50% weekly at the high dose for *N* and *P* respectively, compared to the concentrations analyzed before the experiment. The samples were collected at low tides on six occasions: day 0 (05/10/2005) when fertilizers were first added, day 26 (31/10/2005), day 43 (17/11/2005), day 71 (15/12/2005), day 91 (04/01/2006) and day 118 (31/01/2006).

A detailed description of the area and the environmental parameters measured during the experiment is available in the studies of Santos et al. (2009) and Botter-Carvalho et al. (2014). They reported that the sediment conditions before the experiment were similar in the three treatment areas, with fine-sediment percentage ranging from 16.9% to 31.3%, organic matter-content 2.04% to 6.65% and oxidation–reduction potentials below −84 mV. During the experiment, some differences were noted: the HD treatment showed the highest total-nitrogen concentration (1.8 mg/kg), organic-matter content (6.8%), and chlorophyll-a concentration (20 μg/cm^2), and the lowest oxidation–reduction potential (−359 mV). The total-phosphorus concentrations increased over time and between treatments, and Dunnett's test revealed differences between the control and HD treatments on days 71 ($p = 0.017$), 91 ($p = 0.010$) and 118 ($p = 0.010$).

2.3. Nematofauna

To assess the nematode fauna, a sample was taken in each treatment area (LD, HD and C) with the aid of a corer with inner area of 6.15 cm^2. The samples were fixed in the field with 4% saline formalin. The samples were processed according to Elmgren (1976), involving wet washing in a geological sieve with apertures of 0.5 mm and 0.063 mm. The first 60 nematodes (from the sample) were removed for mounting on permanent slides and identification at the genus level (Warwick et al., 1998; Deprez, 2005). Nematodes were classified in trophic groups according to Wieser (1953).

2.4. Data analysis

The STATISTICA v.10 statistical program was used to compare richness, diversity and number of genera between days and treatments, with analysis of variance (multifactorial ANOVA) and *post hoc* Tukey test for pairwise comparison. To assess the multivariate responses in the structure of the nematode community in relation to treatment and days, a multidimensional scaling (MDS) analysis was used to represent the similarity matrix, based on the Bray-Curtis index applied to genus densities. To test for significant

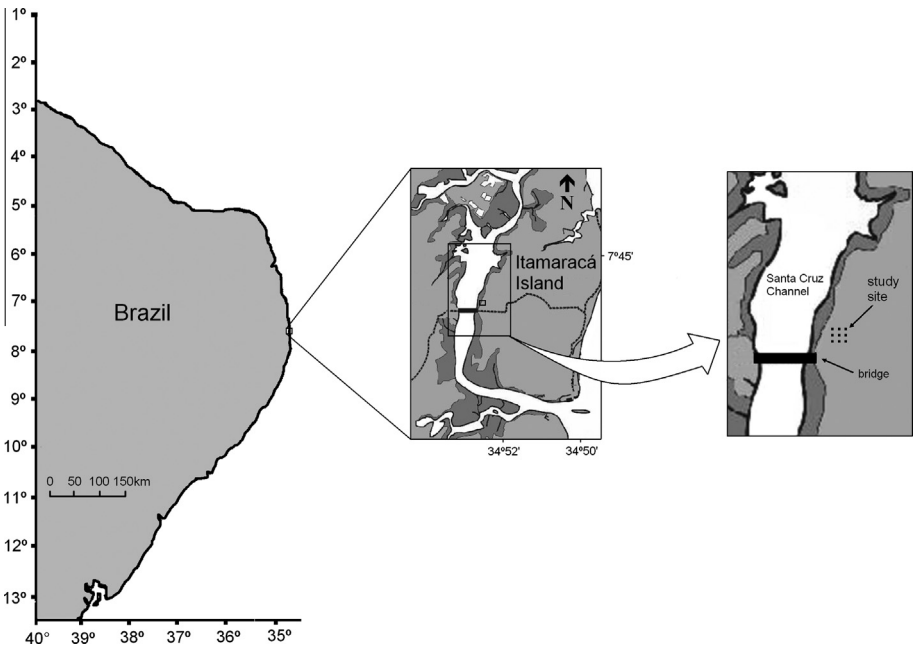

Fig. 1. Location of study area, Ilha de Itamaracá and Canal de Santa Cruz, Pernambuco, Brazil (adapted from Botter-Carvalho et al., 2014).