Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Response of estuarine free-living nematodes assemblages to habitat conditions at Sado estuary (SW coast, Portugal)

Mário Nuno de Lima Pereira Pinto

Orientador(es) | Maria Adão

Soraia Vieira

Évora 2023

Esta dissertação não inclui as críticas e as sugestões feitas pelo júri.

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Dedicated to my godmother Sílvia Pereira Pinto and in Memory of my grandfather José Lima and my aunt Isabel Carvalho.

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Response of estuarine free-living nematodes assemblages to habitat conditions at Sado estuary (SW coast, Portugal)

Abstract

Estuarine ecosystems suffer significant pressures with the anthropogenic activities, the major challenge is monitoring these effects in order to preserve the ecosystem functioning. Sado estuary, at Southwest coast of Portugal provides habitat for many species, nevertheless is influenced by several anthropogenic activities and it is unknown how the ecological quality is being affected. How do communities shape themselves in response to different environmental conditions? To fill this gap, the diversity and density patterns of nematode assemblages will be analysed in spatial and temporal scale perspective. Three sites were selected with different ecological conditions and were sampled on two occasions. We hypothesised that significant differences were detected in nematode assemblages' diversity and density patterns between sites and across sampling occasions. PERMANOVA test reported significant differences only between sites ($p<0,05$). We concluded that different sediment biogeochemical composition in each site was determinant to shape in diversity and density the nematode assemblages.

Keywords: Nematodes; Meiofauna; Benthic assemblages; Sado Estuary; Sediment.

Response of estuarine free-living nematodes assemblages to habitat conditions at Sado estuary (SW coast, Portugal)

Resumo

Os ecossistemas estuarinos sofrem pressões significativas devido às atividades antropogénicas, o desafio é monitorizar esses efeitos para preservar o ecossistema. O estuário do Sado, costa sudoeste de Portugal, é influenciado por diversas atividades antropogénicas, não se sabendo os impactos na qualidade ecológica. Como é que as comunidades mudam em resposta a variações ambientais? Para preencher esta lacuna, padrões de diversidade e densidade das comunidades bentónicas serão analisados em escala espacial e temporal. Foram selecionados três locais com condições ecológicas diferentes e amostrados em duas ocasiões. A nossa hipótese é que diferenças há significativas na diversidade e nos padrões de densidade das comunidades de nematodes entre os locais e entre as ocasiões de amostragem. O teste PERMANOVA demonstrou diferenças significativas apenas entre diferentes locais ($p<0,05$). Concluímos que diferentes composições biogeoquímicas dos sedimentos foram determinantes para moldar, tanto a nível de diversidade como de densidade, as comunidades de nematodes.

1. Introduction

1.1 Estuarine ecosystems

Estuarine ecosystems are among the most productive ecosystems on the planet. An estuary consists of a partially coastal enclosed body of water connected, permanently or periodically, with the sea water and, at the same time, receiving freshwater inputs from rivers, streams, or seepage (Conley *et al.*, 2000; Potter *et al.*, 2010). Estuaries are included in the “transitional waters” category, being used as means of completing the continuum between freshwaters and coastal waters (Elliott & McLusky, 2002; European Union, 2000; McLusky & Elliott, 2007). Estuarine areas have long been considered as environmentally stressed areas because of the variation in their physico-chemical characteristics (Elliott & McLusky, 2002; Elliott & Quintino, 2007).

In estuaries the salinity is a structural factor that influences the composition and the distribution of all organisms. Usually decreases with proximity to the fresh water from rivers, while at the mouth of the estuary is similar to that in the seawater of the open ocean. Since in estuaries it follows a gradient, is categorized in three intervals within the estuary: oligohaline (0,5-5,0) near to freshwater inputs, mesohaline (5,0-18,0) in the middle of estuary and polyhaline (18,0-30,0) in mouth of estuary, near to the ocean. Near the connection with the open sea, estuarine waters may be euhaline (30,0-34,0) (Montagna, Palmer & Beseres Pollack, 2013; Alves *et al.*, 2013). The fresh water from rivers has salinity levels of 0.5 or less, so the further upstream, the lower the amount of salt dissolved in the water (Antonov *et al.*, 2006). The removal of freshwater from rivers and streams to supply the growing demand of water has caused a dramatic decrease in the amount of water reaching estuarine ecosystems. The salinity in estuarine waters depends on the daily tides, but also on the amount of freshwater inflows. The salinity of estuaries is, by definition, lower than in adjacent coastal areas. On the other hand, there is a high variability of this chemical characteristic within the same estuary, which also justifies that they are often considered as stressed ecosystems (e.g., Elliott & McLusky, 2002; McLusky & Elliott, 2007; Elliott & Quintino, 2007). If evaporation exceeds freshwater inputs, the estuary has a negative water balance and salinity gradually increases (Elliott & Quintino, 2007; Montagna *et al.*, 2013).

The salinity and temperature of the water directly affect the solubility, when they increase the dissolved oxygen decreases. Oxygen is a limiting factor for most organisms and influences the vertical distribution of the communities in sediments. The fine fraction is less permeable to oxygen than coarser sediments. Several studies have shown that oxygen and sediment granulometry highly influence the vertical distribution of organisms, due to the Redox Potential Discontinuity (RPD) (e.g., Adão, 2021; Kotwicki *et al*., 2005; Vieira *et al*., 2023). As cited by Gerwing *et al*. (2013), "the RPD marks the boundary between predominantly oxidizing and reducing conditions. RPD can vary extensively in time and space and is influenced by particle size, temperature, wave action, organic matter input, photosynthesis, light intensity, dissolved oxygen, bacterial activity and the presence of burrowing animals." Grain sizes were assigned to five classes: gravel (>200 mm), coarse sand (0,5–2,0 mm), mean sand (0,25–0,5 mm), fine sand (0,063–0,25 mm) and silt&clay (<0,063 mm) (McLachlan & Brown, 2006). Oxygen can be dissolved in water by diffusion from the atmosphere or through photosynthesis by aquatic plants. The movement of water by the wind or waves increases the rate at which oxygen from the air can be dissolved. On the other hand, the increase in turbidity negatively affects aquatic plants that are directly dependent on light. Freshwater inflows provide nutrients, sediment and organic matter which affects species movement, reproductive timing, and their survival rate (Montagna *et al*., 2013). Large nutrient inputs in estuaries, caused especially by organic discharges, sewage run-off and industrial effluent, gives rise to cascading effects that culminate in loss of biodiversity and increased development of opportunistic green algae, occasionally forming mats (Wilkinson, Telfer & Grundy, 1995; Elliott & de Jonge, 2002). Associated with the increase in green algae, there will be an increase in primary production, which will have an influence on biodiversity at all different levels. Among this biodiversity are benthic communities that play a very important role in marine benthic food chains, that is, acting as the trophic link between bacteria and larger fauna. Through predation and/or consumption of detritus by larger deposit-feeding invertebrates, meiofauna increase the rate of carbon mineralization, stimulating microbial activity (Heip, Vincx & Vranken, 1985; Moens, Bouillon & Gallucci, 2005; Alves *et al.*, 2013). Due to its ecological characteristics, namely, small size, high abundance, rapid generation times and absence of a planktonic phase,

meiofauna has recognized advantages compared to macrofauna as monitoring organisms (Schratzberger *et al.*, 2000; Austen & Widdicombe, 2006; Alves *et al.*, 2013).

1.2 Benthic communities

"Meiobenthos" is a term coined by Molly F. Mare in 1942 to define an assemblage of benthic metazoans with biomass and size within well-defined limits. Currently, meiobenthos are recognized as a valid intermediate between macrobenthos and microbenthos (bacteria, diatoms, and most protozoa). In addition to species considered "permanently" as meiofauna, there are some animals that have stages of their life cycle in which they meet the size requirements to be included in the meiofaunal category. That is, small organisms such as juvenile molluscs and annelids are "temporary" meiofauna until they grow beyond the upper limit of this category. Meiofauna is often found in and on soft sediments, but it is also possible to find it in hard substrates, such as animal tubes, surfaces of barren rocks or among epilithic plants (Giere, 2019; Adão, 2021). Methodologically, all the metazoans that are passing the coarse sieve (500 µm (micrometre) or 1000 µm) and are retained by the finer 38 µm sieve are defined as meiofauna e (Vincx, Meire & Heip, 1990). Nowadays, from a biological and ecological perspective, it is admitted that the meiofauna has an independent evolutionary history and represents a separate group of animals from larger macrofauna (Warwick & Gee, 1984).

Meiofauna organisms have a great ecological relevance and play an important role in the dynamics of aquatic food webs. Meiofauna have high estimated secondary production rates, supplying up to 80% of their predators' diet, being essential for juvenile fish and omnivorous meiofauna. Meiofauna also make a significant contribution to nutrient cycling, as their secretions and excretions provide matrix and inorganic nutrients that are easily metabolized by microorganisms (Adão, 2021).

1.2.1. Nematodes

Among all species belonging to meiobenthic communities, nematodes, particularly, have been recognized as good bioindicators (Schratzberger *et al.*, 2000; Steyaert *et al.*, 2007; Moreno *et al.*, 2008; Alves *et al.*, 2013). Nematodes are the most abundant and ubiquitous multicellular organisms on Earth and, in addition, they are easily sampled. Nemathelminthes occur in a wide range of habitats compared to other phylum and some species/genera have high tolerance to extreme environmental conditions (Austen & Widdicombe, 2006; Steyaert *et al.*, 2007; Adão *et al.*, 2009; Fonseca, Hutchings & Gallucci, 2011; Ramalho *et al.*, 2018; Adão, 2021). Some researchers consider that nematodes are a hyper diverse taxon with probabilities of reaching more than 1 million species, while others doubt this estimate (Lambshead & Boucher, 2003). So far, about 20,000 free-living species have been described, although the vast majority of them (90%) are living in marine habitats (Traunspurger, Michiels & Eyualem-Abebe, 2006). Some species/genera have a short life cycle and, in general, they are highly responsive to different types of pressures such as physical and chemical disturbances at spatial and temporal scales. Therefore, they have been used to determine the quality of the environment in several studies on marine and estuarine ecosystems (e.g. Austen & Widdicombe, 2006; Sroczyńska *et al*., 2021a).

Identification of meiobenthic nematodes is based mainly on characters that are visible without dissection, that is, taxonomically relevant external features such as marked buccal cavity diversity, general body shape and its external cuticle (Giere, 2019). Interpretation of nematode feeding strategy is generally based on the stoma and pharyngeal morphology. Several species have their mouths equipped with buccal armature (e.g., teeth, mandibles, and/or other sclerotized structures) that may serve to predatory feeding behaviour. Mainly based on the mouth size and presence or absence of prominent buccal armature, Wieser (1956) separated the species into two groups and four feeding types: “Group 1: without a buccal armature; 1A: selective deposit feeders; 1B: nonselective deposit feeders; Group 2: with buccal armature; 2A: epistrate, feeders; 2B: omnivores or predators”. Based on nematode life strategy, a colonizer-persister scale (cep scale) from 2 (colonizers) to 5 (persisters) could be

very useful. Taxa with characteristics such production of many progeny and rapid growth, usually also characterized by a high tolerance to disturbance, are considered colonizers. However, persisters have slow growing and often more sensitive taxa which thrive well in stable and pristine environments (Bongers, Alkemade & Yeates, 1991).

1.2.2. Influence of the abiotic variables

Mesoscale variability of nematode assemblages within estuaries, at the kilometre scale, may be more important than variability at the hundreds of kilometre scale or variability between systems. This is mainly due to estuarine gradients such as salinity changes and grain size differences (Adão *et al.*, 2009; Adão, 2021; Sroczyńska *et al.*, 2021b).

Estuarine meiofauna tend to decrease, in abundance as in number of species, from the sea to freshwater upstream. It is widely accepted that salinity is a factor influencing species distribution in estuaries. It is well documented that other natural gradients, such as grain size composition and dynamics, oxygen availability, temperature, and current speed, are factors that determine the temporal and spatial variations of meiofauna assemblages (e.g., Adão *et al*., 2009; Alves *et al*., 2013; Derycke *et al*., 2008; Steyaert *et al*., 2007).

Previous studies indicated that the majority of the meiofauna were restricted to the upper few centimetres in sandy habitats and to the upper few millimetres in muddy sediments (e.g., Li and Vincx, 1993; Kotwicki *et al.*, 2005; Steyaert *et al*., 2007). The fine sediments are less permeable to oxygen than coarser sediments, that is, there is an indirect effect of sediment granulometry on the vertical distribution and abundance of meiofauna in aquatic ecosystems (Adão, 2021). Vertical zonation is generally controlled by the position of the Redox Potential Discontinuity (RPD) layer. As one descends vertically in the sediment, oxygen availability decreases and the biological composition is altered, with meiofauna becoming absent and anaerobic metabolism increasing (Sampou & Oviate, 1991; Kotwicki *et al.*, 2005). Temperature and water content of the sediment, such as organic matter percentage and the presence of biogenic

structures (e.g. seagrass roots), can influence the meiofauna distribution patterns (Sampou & Oviate, 1991; Adão, 2021).

1.3. Research questions and Hypothesis

As mentioned, estuarine regions are naturally stressed areas with highly variable environmental conditions (Elliott & Quintino, 2007). However, these ecosystems are also known to be under significant pressure due to various anthropogenic activities (e.g., fisheries, harbour pollution, coastal development, mineral mining) (e.g., Hoegh-Guldberg and Bruno, 2010; Burrows *et al*., 2011). Therefore, it is particularly difficult to separate natural and anthropogenic stress using biological indicators (Elliott & Quintino, 2007; Borja *et al.*, 2016). Due to this problem, the "estuarine quality paradox" emerged, a concept created by (Dauvin, 2007). Depending on the parameter that varies and its degree of variation, there may be consequences for the fauna and flora, it may affect, for example, the survival rate of animal species (Elliott & Quintino, 2007). Biodiversity is fundamental to sustain estuarine and marine ecosystem services (Liquete *et al.*, 2016; Adão, 2021). So, to conserve the entire estuarine ecosystem, it is essential to be able to attribute each functional response to the natural disturbance or the type of anthropogenic stressor to which it is exposed. To make this understanding possible, it is crucial to expand our scientific knowledge about diversity and function within taxa and functional groups by analysing spatial and temporal distribution patterns of nematode assemblages. In Europe, particularly in the Iberian Peninsula, there is a lack of knowledge about meiobenthic communities and free-living nematodes in estuarine environments. Information on structural, functional, temporal and spatial distribution is fundamental to describe the biodiversity patterns of these species (Adão *et al.*, 2009; Sroczyńska *et al.*, 2021a b).

Sado estuary is one of the largest estuaries of Portugal thus providing habitat for many species. Nevertheless, this estuary has been suffering pressures due to various anthropogenic activities and it is not known how the ecological quality is being affected. Then arises the following scientific questions: How do communities shape themselves in response to different environmental

conditions? Gaining this understanding will provide a better knowledge of how we can protect the ecosystem and assess their ecological status. This study aims to investigate the effects caused by different environment conditions on nematode assemblages' composition and distribution patterns in Sado estuary. To achieve our objective, we will start by analysing the diversity and density patterns of the nematode assemblages in a spatial and temporal scale. The tree sampling sites were selected in accordance with different ecological conditions in Sado estuary with distinct anthropogenic influences across two sampling occasions. We propose two null hypotheses, 1) H0: There are no significant differences in nematode assemblages' diversity and composition between three sites with different sediment conditions. 2) H0: There are no significant differences in nematode assemblages' diversity patterns across two distinct sampling occasions (winter 2019 and summer 2020).; The composition and diversity of nematode assemblages will be analysed in order to assess the ecosystem integrity through the different environmental conditions of the estuary.

2. Material and Methods

2.1. Study area: Sado Estuary

Portugal is part of the Iberian Peninsula, which is in the extreme west of Europe. This country shares a land border only with Spain and has a considerable coastline. The Portuguese west coast extends for approximately 800 km along a straight North-South orientation and the south coast, with a West–East orientation, extends for approximately 200 km (Cunha, Assis & Serrão, 2013). The Portuguese coast is in a transitional zone between temperate and subtropical waters. Estuaries along this coastline vary significantly in geomorphologic and hydrologic features (Vinagre, Cabral & Costa, 2010). The Sado estuary (38° 31′ 14″ 'N, 53′ 32″ 8°W) is located on the west coast of Portugal and it is part of Sado hydrographic basin which has a total area of approximately 769 200 ha (Santos *et al.*, 2022). This is the second largest estuary in the country and has an area of approximately 24 000 ha (Caeiro *et al.*, 2005; Sroczyńska *et al.*, 2021b). The main bay is characterized by two navigation channels partially separated by intertidal sandbanks (Troia beach): the Northern channel, that allows access to

the Port of Setubal, and a highly dynamic Southern channel (Caeiro *et al.*, 2005; Biguino, Sousa & Brito, 2021; Sroczyńska *et al.*, 2021b). The Sado river has a low and seasonal flow rate (annual mean of 40 $m^3.s^{-1}$), with very low freshwater discharges occurring during summer. Consequently, there is a strong influence of the tidal currents on the estuary dynamics (Biguino *et al.*, 2021; Santos *et al.*, 2022). This estuary has a mean depth of 8 m and a semi-diurnal mesotidal system with tidal amplitude varying between 1.6 m, during spring, and 0.6 m, during neap tides (Gonçalves, Brogueira & Nogueira, 2015). This estuary can be considered a coastal plain estuary and according to water temperature variability and salinity range can be classified as well-mixed. The climate of Setúbal peninsula is dry sub-humid Mediterranean type, with a humid period covering from October to March and a dry period in the remaining months (Biguino *et al.*, 2021). In the bay there is the lowest hydrodynamics and the water residence time is higher compared to other parts of the estuary (Sroczyńska *et al.*, 2021b).

Due to its rich biodiversity, productivity and aesthetic value, most of the estuary has been considered a protected area since 1980, when the designation "Sado Estuary Nature Reserve" was created (Biguino *et al*., 2021). However, that does not mean that these ecosystems are not under pressure from anthropogenic activities. Setubal city is located precisely on the northern margin of the estuary and to the north of this estuarine system, about 40 km, is located the highly industrialized and populated metropolitan area of Lisbon. Thus, the Sado Estuary supports one of the most relevant ports in the country and the industrial zone of the Setubal city. Therefore, this estuary has a high socio-economic importance, but consequently also a high anthropogenic pressure (Lillebø *et al*., 2011; Brito *et al*., 2023). Harbor-associated activities, polluting industries (e.g. Navigator paper mill) that use the estuary for waste disposal purposes, and the wastewater from the city of Setubal itself are responsible for a high and poorly controlled input of organic matter into the ecosystem (Caeiro *et al*., 2005; Biguino *et al*., 2021). The surrounding areas of the estuary are intensively explored, mainly with rice fields, which also affect water quality. History of activities related to copper mines and pyrite outcrop erosion have contributed to the input of metals in the estuary water (Caeiro *et al*., 2005; Lillebø *et al*., 2011). The increase in aquaculture has also been reported in several previous studies (e.g., Brito *et al*., 2023). The Sado

estuary also supports other activities intrinsically associated with the major population in coastal areas, such as salt pans, fishing and recreational activities (Lillebø *et al.*, 2011). In the Northern channel, due to residual currents, there is a greater accumulation of sediments, which leads to the deposition of pollutants introduced locally instead of being transported away (Caeiro *et al*., 2005; Biguino *et al*., 2021).

2.2. Sampling Strategy

For this study, we selected 3 sampling sites: Navigator, which is a highly disturbed area with the influence of Navigator paper mild; Gambia, which is near to “Sado Estuary Nature Reserve” and has high residence time. However, Gambia is also located close to rice fields and aquaculture productions, therefore having a high input of organic matter; Troia, that is located at the mouth of the estuary, is a sandy area with high hydrodynamics (Fig.1).

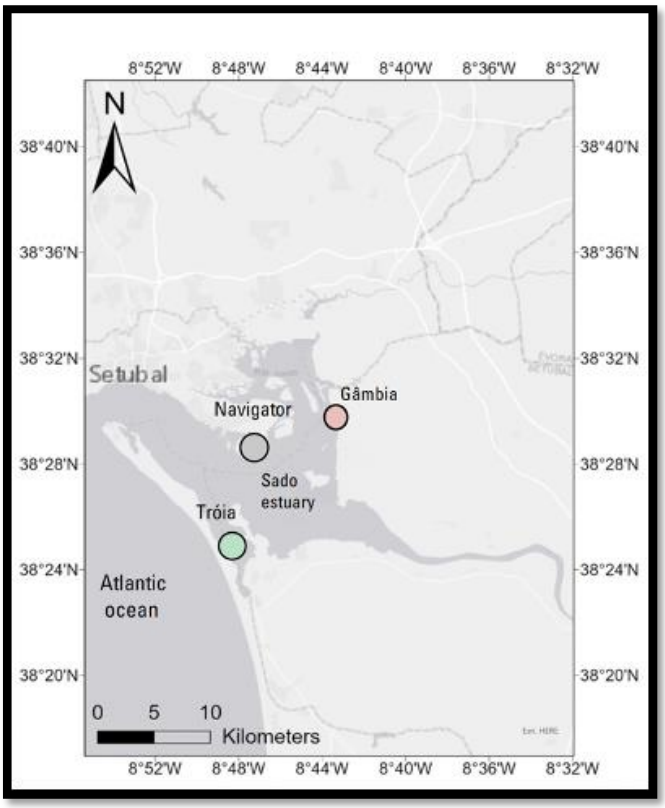

Figure 1: Sado estuary located at Southwest of Portugal (38◦ 31′ 14″ N, 8◦ 53′ 32″ W). The selected sampling sites: Navigator, (grey circle), highly industrialized area; Gambia (orange circle) with high organic inputs and Troia (green circle) with sandy sediment with high hydrodynamics.

In each sampling site were randomly selected 3 stations and collected 3 replicates of sediment in each station by forcing a hand core with 3,6 cm inner diameter and 3 cm deep. The samples were kept in thermal bags until they reached the laboratory and then were preserved in 4% 4% buffered formalin solution until analysis This procedure was repeated in two different sampling occasions: “winter 2019” and “summer 2020”.

To better analyse how nematodes assemblages respond to different environmental conditions was determined in each site the physical-chemical composition of the sediment, such as Total Organic Matter (TOM), sediment type and grain size. Salinity, Temperature (°C) and Dissolved Oxygen (DO) (mg L^{-1}) were measured in situ from the sediment interstitial water, using VWR phenomenal ® MU 600 H.

2.3. Sampling Treatment

In order to extract the nematodes, two sieves with different mesh were used: 1000 µm and 38 µm. Initially, the samples were rinsed in the sieve with a mesh of 1000 µm to separate macrobenthos, shells and detritus from the sample. The content, which was retained in a container, was poured through a 38 µm sieve. The fraction retained was vigorously washed, then the content from the sieve was collected into tubes for centrifugation. It was also added colloidal silica polymer LUDOX HS-40 60% with specific gravity (1.18g/cm^3) to separate marine nematodes from the sediment (Heip *et al.*, 1985). After mixing it thoroughly with a glass stick, the tubes were centrifuged for 10 min at 1700 rpm (revolutions per minute). The supernatant was removed after the centrifugation cycle and poured through the 38 µm sieve. After the material had been collected, LUDOX was again added to the tube and this process was repeated two more times. The final material was stored in a 4% formaldehyde solution.

Nematodes were counted using a counting dish and stereomicroscope LEICA M205 C.

Were randomly collected, 120 nematodes from each sample. Then they were transferred into a cavity box with Grisse I (Grisse I: 100ml = 99ml

formaldehyde 4% and 1ml glycerol (86% - 88%)), to prevent the damage of individuals. This cavity box was placed into another container that had alcohol inside and a Petri dish turned upside down. Then it was left for 12 hours at 35°C. Then the sample was taken from the container with alcohol and will start to evaporate. Afterwards, a few drops of Grisse II (Grisse II: 100 ml) = 95 ml of ethanol 95% + 5 ml of glycerol (86% - 88%)) were added to the cavity boxes three times in the interval of 2 hours. Afterwards, the samples were left again for 12 hours in the same conditions. We then made sure that the nematodes were really in glycerine before mounting the slides for further identification. In this step anhydrous glycerol was used (Vincx, 1996).

Taxonomic assignments were performed until genus level. For the identification was used a light microscope Olympus BX50 and Identification keys (Platt, Warwick & Furstenberg, 1985) as well as NeMys (Vanaverbeke, Soetaert & Vincx, 2004) and online databases.

2.4. Data Analysis

Multivariate analyses were performed to detect temporal and spatial significant changes in the nematode community structure in density and diversity. Two factors - PERMANOVA test was performed and the factors were “Sites” (fixed with 3 levels: Navigator, Gambia and Troia) and “Sampling occasions” (Random with 2 levels: “Winter 2019” and “Summer 2020”), considering $p<0,05$. For the factors with significant results, the paired-wise test (with 999 permutations) was performed to identify significant differences within levels, $p<0,05$. The statistical analyses of biological and environmental data were performed using the PRIMER v6 software package (Clarke & Warwick, 2001) with the PERMANOVA add-on package (Anderson, Gorley and Clarke, 2008).

2.4.1. Environmental variables

The following environmental variables were measured in situ: clay (%), sand (%), gravel (%), organic matter: OM (%), total carbon: TC (%), total nitrogen: TN (%), calcium carbonate: $CaCO_3$ (%), chlorophyll a: Chl a (mg/g),

phaeopigments: Phaeo (mg/g), temperature (ºC), salinity: Sal, dissolved oxygen: O_2 (mg/L) and pH. Sediment physicochemical processing was performed according to Vieira et al., 2023.

Principal Component Ordination (PCA) was performed for all environmental variables in order to characterize the multidimensional patterns of the studied sites. The high correlated variables were selected and removed from the analysis, then the others were Log(X+1) transformed and normalised (subtracting the mean and dividing by the standard deviation, for each variable) (Clarke & Warwick, 2001). The resemblance matrix was based on Euclidean distances and checked for uniform distribution.

2.4.2. Nematode assemblages

Principal Coordinate Analysis (PCO) was performed to determine the similarity patterns of the nematode assemblages, the density data was previously square rooted transformed, and it was resembled in Bray Curtis similarity matrix. Then the relative contribution of each taxon to the (dis)similarities between sites and within assemblages was calculated using the Bray Curtis method, SIMPER two-way crossover similarity percentage analysis (100% cut-off percentage).

Diversity is measured by two main components: species richness (the number of species), species evenness (how relatively abundant each species is). Margalef's Richness Index (d) (Margalef, 1958), Shannon-Wiener Diversity Index (H') (Shannon and Weaver, 1963), Simpson's Index (1-Lambda') (Simpson, 1949), and Pielou's Eveness (J') (Pielou, 1966) were calculated using the nematode dataset from each site and sampling occasion to measure the diversity of nematode assemblages. Values of the Margalef Index range from 0 (no diversity) to 8 (higher diversity of species); Shannon Wiener Diversity Index ranges from 0 (no diversity) to 1 (higher diversity of species); Simpson's Index ranges from 0 (higher diversity of species) to 1 (no diversity); Pielou's eveness ranges from 0 (no evenness, the relative abundances of the species diverge from evenness, that is, a few species dominate the community) to 1 (complete evenness).

Nematode genera were assigned in four feeding groups classified by Wieser (1956). They were mainly based on the mouth morphology: “selective (1A) and non-selective (1B), deposit feeders, epigrowth feeders (2A) and omnivores/predators (2B)”. Based on this feeding type classification, the Index of Trophic Diversity (ITD) was calculated as the sum of the squared proportional abundances of each feeding type (Heip *et al.*, 1985), and the reciprocal trophic diversity index (ITD^{-1}) was used, so that the higher values of the index correspond to higher trophic diversity. The Maturity Index (MI) (Bongers *et al.*, 1991) was used as a measure of nematode life strategy. The nematode genera were assigned a value on a colonizer-persister scale (*c-p* scale) from 2 (colonizers) to 5 (persisters). Taxa with characteristics of good colonizers, such production of many progeny and rapid growth, are usually also characterized by a high tolerance to disturbance. However, persisters are frequently more sensitive taxa (Bongers *et al.*, 1991)

A two-way permutational analysis of variance (PERMANOVA) was applied to test the hypothesis that significant differences existed between "Sites" among “Sampling occasions” in nematode assemblage descriptors: d, J', H', 1-Lambda', MI, ITD^{-1} indexes, $p<0,05$.

3. Results

3.1. Environmental variables

The environmental variables measured in sediments revealed distinct characteristics between sampling sites (Table 1). Troia's sediment was predominantly characterized by the highest mean values (%) of sand (97,3±0,2), while sediments from Gambia and Navigator presented the highest values of clay (17,4±5,1 and 30,1±3,1; respectively). Gambia has the highest values of gravel (15,1±6,4). Navigator has the highest values of OM (4,0±0,6). These results were in accordance with the clear separation between sites in the PCA (Fig.2). The first axis PC1 and PC2 (PC1:40,1% and PC2 22,2%) explain 62,3% of the total variation of environmental variables. The samples collected at Troia are completely separated from the others and are mainly due to the highest values of sand and $CaCO_3$. However, Gambia sediment was mainly associated with high values of Chl *a* and phaeopigments. In a temporal perspective the sampling occasion "summer 2020" is highlighted by the presence of high concentration of the pigments and salinity.

Table 1: Mean density ± standard error (SE) of environmental variables measured in situ (clay (%), sand (%), gravel (%), organic matter (%), total carbon (%), total nitrogen (%), calcium carbonate (%), chlorophyll *a* (mg/g), phaeopigments (mg/g), temperature (ºC), salinity, dissolved oxygen (mg/L) and pH).

Environmental Variables	Navigator		Gambia		Troia	
	Winter 2019	Summer 2020	Winter 2019	Summer 2020	Winter 2019	Summer 2020
Clay (%)	30,1±3,1	21,0±1,0	13,5±0,2	17,4±5,1	1,8±0,2	1,7±0,2
Sand (%)	66,2±2,6	73,1±1,7	71,5±5,5	67,5±4,1	93,8±1,9	97,3±0,2
Gravel (%)	3,8±0,8	5,9±1,5	15,0±5,6	15,1±6,4	4,4±1,8	1,0±0,2
Organic Matter (%)	4,0±0,6	2,3±0,3	0,6±0,04	1,7±0,4	1,9±0,3	0,4±0,02
Total Carbon (%)	1,0±0,3	1,1±0,6	0,7±0,4	0,4±0,1	0,6±0,1	0,7±0,1
Total Nitrogen (%)	0,1±0,02	0,1±0,01	0,1±0,02	0,03±0,01	0,02±0,01	0,01±0,003
Calcium Carbonate (%)	2,4±0,5	2,6±0,5	1,0±0,1	1,6±0,4	4,3±0,3	4,6±0,9
Chlorophyll *a* (mg/g)	15,6±3,9	9,8±0,1	10,3±1,0	55,5±2,4	26,7±2,1	11,1±1,4
Phaeopigments (mg/g)	14,7±3,7	10,7±1,2	7,8±0,5	115,1±32,8	14,5±1,0	5,2±0,9
Temperature (°C)	15,3±0,1	28,5±0,6	11,1±0,3	24,4±0,2	15,3±0,1	23,9±0,1
Salinity	31,9±0,9	36,5±0,3	30,6±0,7	17,7±0,1	31,9±4,4	16,4±0,04
Dissolved Oxygen (mg/L)	9,2±0,1	8,3±1,3	14,6±2,5	9,5±0,4	9,2±0,1	12,1±0,4
pH	7,9±0,01	8,2±0,1	8,0±0,1	7,8±0,02	7,9±0,01	7,9±0,04

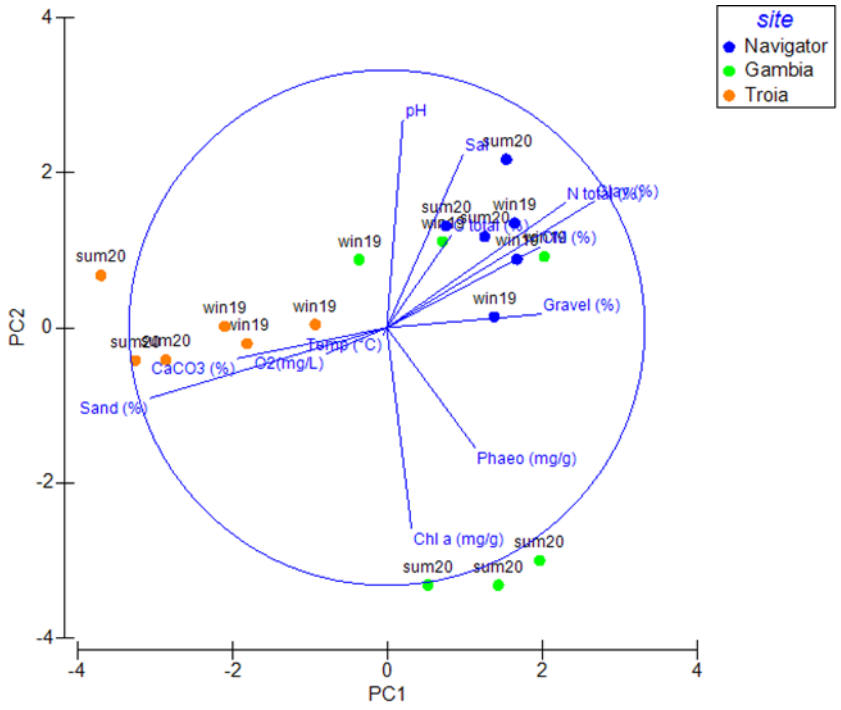

Figure 2: Principal Component Analysis (PCA) plot based on Euclidean distances, according to environmental variables measured (clay (%), sand (%), gravel (%), organic matter (%), total carbon (%), total nitrogen (%), calcium carbonate (%), chlorophyll a (mg/g), phaeopigments (mg/g), temperature (ºC), salinity, dissolved oxygen (mg/L) and pH) at each sampling site: Navigator, Gambia and Troia, at each sampling occasion: Winter 2019 and Summer 2020. PC1:40,1% and PC2 22,2%.

3.2. Density and structural diversity of nematode assemblages

3.2.1. Spatial scale perspective

Nematode assemblage's density in each site and across sampling occasions is summarized in the Table 2. Total density of nematode communities (ind.10 cm^2) were calculated in each site (Fig.3). The highest densities were obtained in Gambia and Navigator assemblages, (3849,9 ± 495,8; 3785,9 ± 958,2 ind. 10 cm^2 respectively), while the lowest density values were obtained in Troia assemblages (1277,8 ± 252,4 ind.10 cm^2).

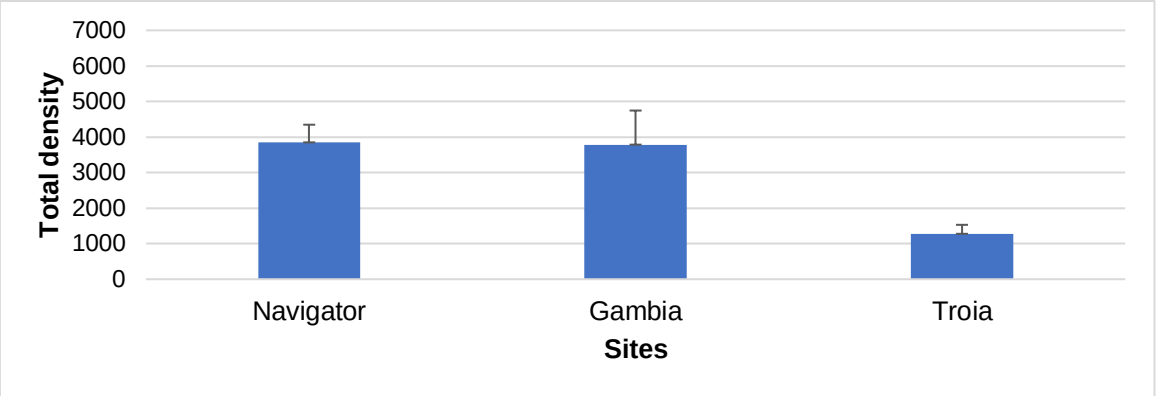

Figure 3: Density of nematode communities (ind.10cm^2) on each sampling occasion (Winter 2019, Summer 2020), at each site (Navigator, Gambia, Troia).

Table 2: Mean density ± standard error (SE), n=3 of nematode genera (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia) across 2 sampling occasions (Winter 2019 and Summer 2020).

Genera	Navigator		Gambia		Troia	
	Winter 2019	Summer 2020	Winter 2019	Summer 2020	Winter 2019	Summer 2020
Acantholaimus	0	0	0	0	0,7±0,6	2,0±1,6
Actinonema	0	0	0	0	5,4±3,1	41,7±13,3
Aegialoalaimus	10,5±4,1	15,7±6,6	18,7±4,0	9,3±4,6	0	0
Ammotheristus	0	0	0	0	3,2±1,7	1,5±1,2
Amphimonhystera	3,3±2,5	0	0	0	0	0
Anoplostoma	251,1± 127,7	120,0±19,7	108,1±11,2	61,5±25,5	0	1,9±1,5
Anticoma	0	0	6,7±5,2	0	3,7±0,4	6,2±0,6
Antomicron	1,6±1,3	7,3±3,7	0	6,0±2,4	0	0
Aponema	58,3±45,2	4,4±3,4	0	0	0	1,6±1,3
Axonolaimus	45,7±24,7	43,8±4,1	164,8±52,1	4,2±1,7	3,7±2,9	0
Bathyeurystomina	0	0	0	0	1,0±0,8	0
Bathylaimus	0	0	0	0	1,4±1,1	0
Bolbolaimus	21,5±9,5	0	0	0	0	2,0±1,6
Calyptronema	36,7±22,6	23,2±18,0	45,2±24,0	15,7±7,2	0	2,0±1,6
Camacolaimus	0	2,0±1,5	7,3±5,7	0	0	5,7±4,4
Campylaimus	0	6,9±5,3	0	0	0	0
Ceramonema	0	0	0	0	0	14,5±2,9
Cervonema	94,3±28,0	54,7±25,9	60,9±30,9	15,5±6,7	2,5±1,9	4,1±3,2
Chromadora	70,3±35,9	12,1±2,6	91,1±11,9	0	0,7±0,6	1,9±1,5
Chromadorina	0	0	49,2±27,3	0	0	1,5±1,2
Chromadorita	15,9±9,5	0	43,0±19,0	0	13,0±6,9	42,5±9,1
Chromaspirina	0	0	0	0	0,7±0,6	0
Cobbia	0	0	0	0	0	7,7±3,9

Comesa	0	0	0	0	0	2,6±2,0
Comesoma	9,5±0,7	0	27,4±21,2	0	0	0
Cyatholaimus	15,2±7,9	0	71,037,9	5,2±2,3	1,0±0,8	0
Dagda	0	16,8±13,0	0	0	0	6,5±5,0
Daptonema	41,7±22,0	62,8±32,2	31,1±19,4	44,7±9,3	67,2±9,1	26,3±7,9
Daptonema sp1	56,6±15,8	122,8±45,0	212,5± 125,2	18,7±5,5	0	38,4±27,6
Dasyhemoides	0	0	0	0	0	1,7±1,3
Desmodora	0	0	0	0	116,4±43,7	112,7±52,8
Desmoscolex	0	0	0	0	2,9±2,2	29,3±20,5
Dichromadora	3,6±2,8	0	23,9±18,5	8,7±3,5	17,0±5,0	82,7±0,9
Eleutherolaimus	0	3,9±3,0	0	0	0	0
Endeolophos	0	0	0	0	0	6,6±3,1
Enoploides	0	0	0	0	12,8±5,0	9,8±7,6
Eumorpholaimus	0	0	0	0	0	5,7±4,4
Gerlachius	0	0	0	0	0	0
Halalaimus	2,6±2,0	18,9±1,5	0	0	1,0±0,8	3,9±3,0
Halichoanolaimus	3,6±2,8	0	0	0	2,7±2,1	5,6±0,3
Hypodontolaimus	0	0	0	0	0	5,7±4,4
Innomonema	0	0	0	0	1,4±1,1	9,8±2,9
Kraspedonema	0	0	0	0	0	1,9±1,5
Leptolaimus	2,6±2,0	10,4±4,1	0	0	0	0
Linhomeus	2,6±2,0	46,3±30,3	0	57,5±15,8	0,9±0,7	15,4±9,7
Marylynia	0	0	0	0	1,2±1,0	1,5±1,2
Metachromadora	1776,2± 282,0	2044,9± 273,9	2082,4± 1083,3	0	3,7±2,8	12,7±5,6
Metacyatholaimus	0	0	0	0	0,9±0,7	0
Metadesmolaimus	0	0	0	0	1,9±0,8	0
Metalinhomoeus	54,3±18,0	36,5±14,6	10,2±6,4	43,6±9,7	2,3±1,0	64,1±34,6
Microlaimus	11,0±4,4	0	0	8,7±3,6	0	0
Molgolaimus	0	2,0±1,5	0	0	0	21,3±16,5
Monoposthia	4,7±3,7	0	0	0	206,1±32,2	229,8±78,2
Nannolaimoides	0	0	0	0	7,4±5,8	0
Nemanema	10,0±4,0	10,2±4,0	16,7±8,5	0	0	0
Neochromadora	0	0	0	0	1,8±1,4	0
Odontophora	134,3±42,7	105,5±44,7	15,5±6,2	8,1±3,7	5,5±1,1	7,6±1,7
Oncholaimellus	7,4±4,0	19,1±7,4	1,39±1,1	0	0,7±0,6	18,812,3
Onyx	0	0	0	0	16,0±6,4	32,1±10,8
Oxystomina	0	16,8±10,3	0	2,5±2,0	0	5,7±4,4
Paracanthonchus	1,6±1,3	0	4,9±3,8	0	0,9±0,7	2,0±1,6
Paracomesoma	47,9±14,3	63,1±31,9	12,3±5,7	13,0±2,9	0,9±0,7	1,6±1,3
Paracyatholaimus	0	0	8,5±3,4	2,5±2,0	2,5±1,2	0
Paradesmodora	0	0	0	0	2,7±1,0	5,7±4,4
Paralinhomeus	0	0	3,6±2,8	0	0	11,4±8,9

Paramicrolaimus	0	0	2,4±1,9	0	0,7±0,6	0
Paramonohystera	0	0	0	0	5,5±2,1	5,7±4,4
Paraticoma	0	0	0	0	0	3,5±1,4
Pareurystomina	0	0	0	0	4,0±0,1	8,6±4,9
Pomponema	0	5,8±4,5	0	0	8,2±2,7	22,4±6,9
Praeacanthonchus	8,4±3,9	22,0±12,2	47,4±17,0	14,0±10,9	0	0
Prochromadora	0	0	22,0±8,7	0	0	0
Prochromadorella	0	0	0	0	0,7±0,6	0
Promonhystera	0	0	0	0	0	1,6±1,3
Prooncholaimus	0	0	0	0	0	1,9±1,5
Pselionema	0	0	0	0	2,9±2,2	5,6±2,2
Pseudolella	34,6±13,2	143,1±8,0	28,3±6,9	54,8±13,4	0	2,0±1,6
Pterygonema	0	0	0	0	0	4,2±1,7
Ptycholaimellus	10,9±8,5	100,3± 45,3	229,5± 101,7	72,1±14,5	0	17,7±10,8
Rhabdocoma	0	0	0	0	1,4±1,1	15,1±7,5
Rhinema	0	0	0	0	0	7,2±4,0
Rhips	0	0	0	0	6,9±2,7	29,8±21,1
Rhynchonema	0	0	0	0	1,4±1,1	12,0±5,0
Sabatieria	268,3±99,7	345,6± 116,8	935,4± 423,1	529,3±74,4	80,5±35,8	343,7± 153,0
Scaptrella	0	0	0	0	3,7±2,9	1,6±1,3
Sphaerolaimus	42,5±18,8	61,1±18,6	179,4±72,5	76,3±12,5	0	0
Spilophorella	0	0	0	0	0	2,0±1,6
Spirinia	2,6±2,0	0	0	0	11,3±3,7	5,7±4,4
Symplocostoma	0	0	0	0	0	0
Synonchiella	0	0	0	0	0,9±0,7	0
Terschellingia	434,0± 136,1	353,9±73,9	273,1±89,1	141,8±22,7	3,2±2,5	3,5±1,4
Thalassoalaimus	0	5,9±4,5	0	0	0	6,0±4,7
Theristus	0	0	0	0	2,6±2,0	0
Trefusia	0	0	0	48,1±37,2	76,3±43,2	188,7±39,3
Trileptum	0	0	0	0	30,6±10,1	84,1±47,8
Triplyloides	10,4±8,1	7,2±5,6	0	0	1,0±0,8	9,8±5,4
Trochamus	0	0	0	0	0	1,9±1,5
Viscosia	65,3±28,4	113,3±17,0	117,5±5,3	117,7±26,1	31,4±6,5	66,5±14,7
Xyala	0	0	0	0	0	1,6±1,3
Total Density	3671,7± 746,8	4028,1± 193,8	4951,6± 1184,9	2620,3± 276,1	786,9± 61,4	1768,7± 104,6

The nematode assemblages of Sado estuary were mainly composed by the orders *Chomadorida*, *Monhysterida*, *Enoplida* and *Desmodorida* (Fig.4; Appendix 1). which were identified approximately 100 nematodes genera (Table 2) belonging to 29 families.

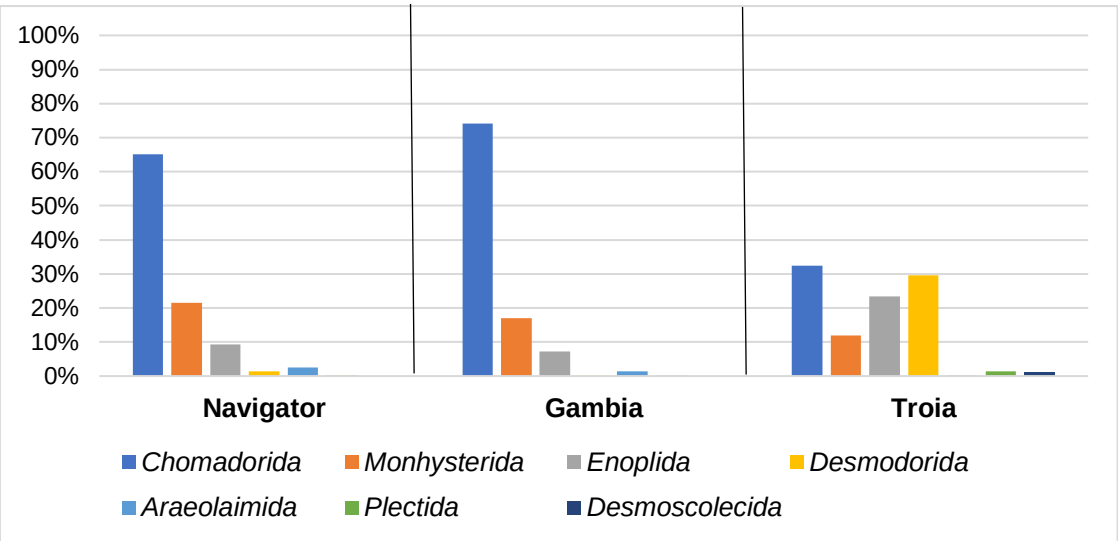

Figure 4: Mean of relative density (%) of nematodes on each sampling site (Navigator, Gambia and Troia) per order.

Navigator was composed by 20 families where the most abundant were *Desmodoridae*, *Linhomoeidae* and *Comesomatidae* (Fig.5; Appendix 2) that were represented by the generas *Metachromadora* (49,6%), *Terschellingia* (10,2%) and *Sabatieria* (8%) with the 67,8% of the total relative density (Fig.6; Appendix 3; Table 2). On the Gambia site, 18 families were registered and the most abundant generas were *Metachromadora* (43,9%), *Sabatieria* (19.3%) and *Terschellingia* (5,5%) (Fig.6). Troia's nematode assemblages was composed by 25 families and the most abundant were *Monoposthiidae*, *Comesomatidae*, *Trefusiidae* and *Desmodoridae* (Fig.5) represented by the generas *Monoposthia (*17,1%*)*, *Sabatieria* (16,6%), *Trefusia* (10,4%) and *Desmodora* (9%) with the 53,1% of the total relative density (Fig.6; Table 2).

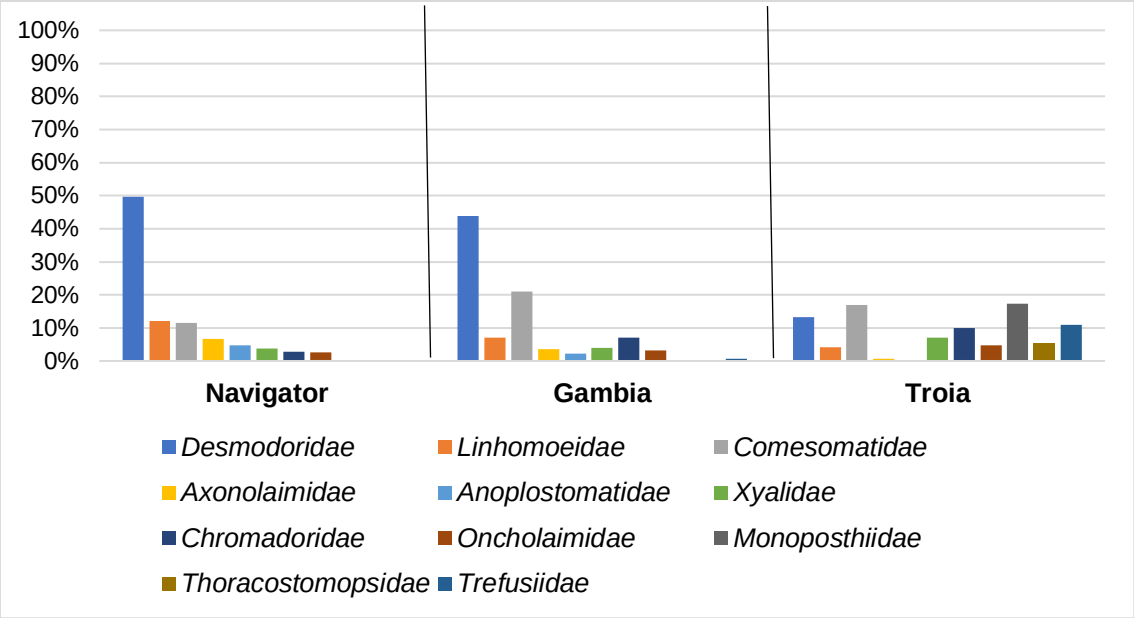

Figure 5: Mean of relative density (%) of nematodes on each sampling site (Navigator, Gambia and Troia) per family.

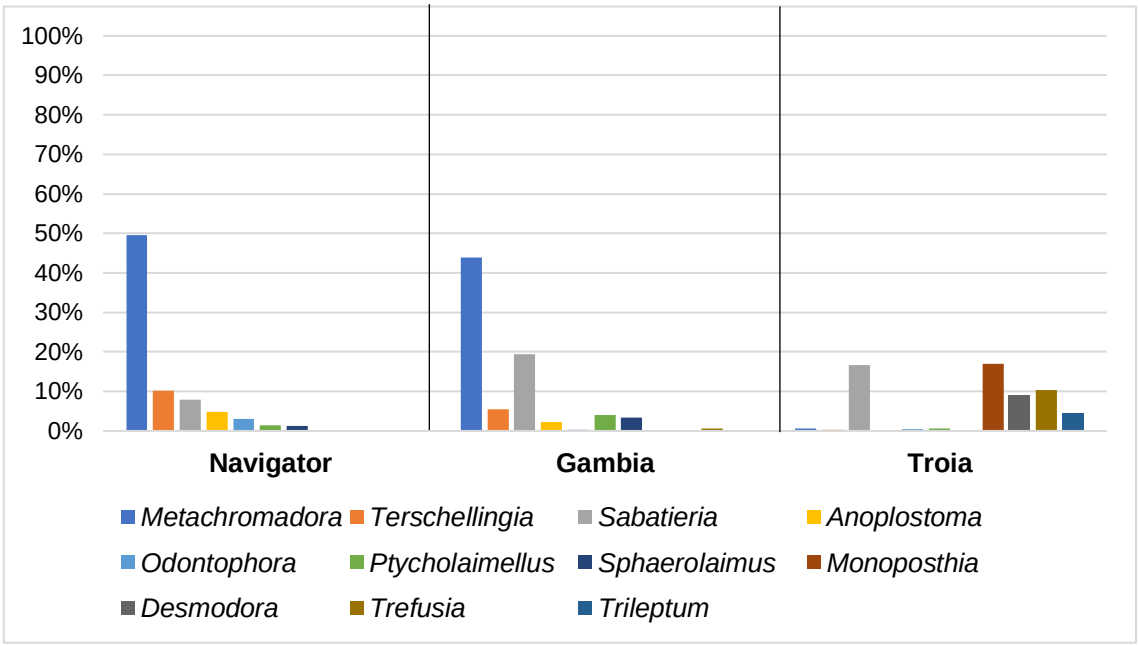

Figure 6: Mean of relative density (%) of nematodes on each sampling site (Navigator, Gambia and Troia) per genera.

PCO analysis highlights the high variability between-sites of nematode assemblages, especially Troia that shows an evident departure from the other sites (Fig.7).

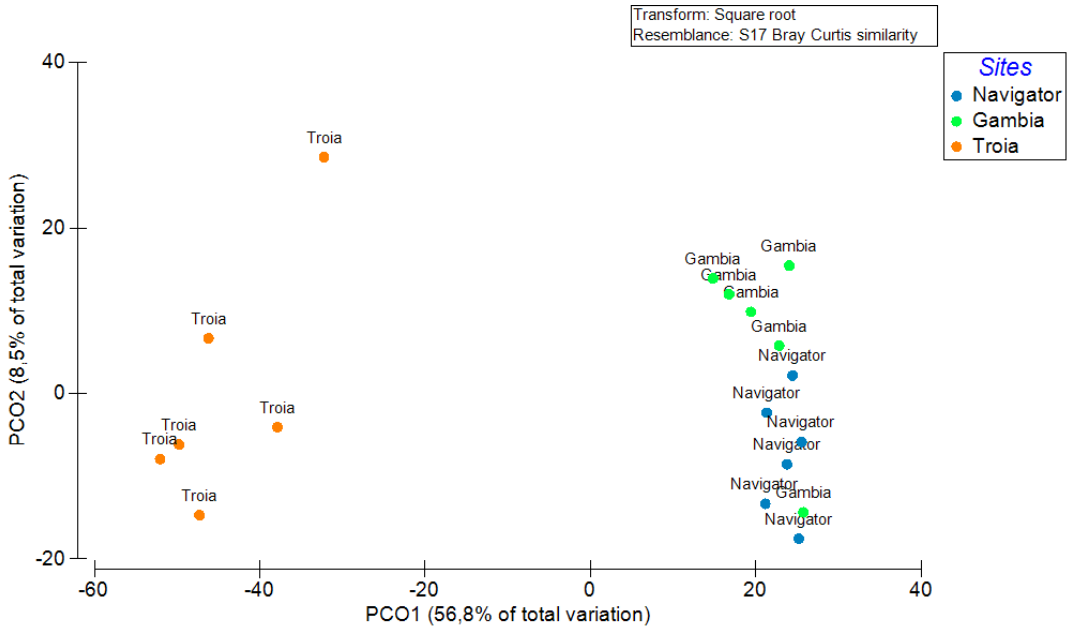

Figure 7: Principal Coordinates Analysis (PCO) based on the nematode dataset at each "Site" Navigator, Gambia and Troia (3 levels, fixed). PCO1 56,8%, PCO2 8,5% of total variation.

The SIMPER analysis revealed the nematode genera that most contributed to the similarity within sites and dissimilarity between sites. The genera *Metachromadora, Sabatieria and Terschellingia* were the main contributors for the similarity within sites, in Navigator and Gambia assemblages while Troia the main contributors for similarity within site were *Monoposthia, Sabatieria* and *Trefusia*. However, the highest contribution to the dissimilarity between Navigator and Gambia assemblages were from *Metachromadora*, *Sabatieria* and *Odontophora.*

The genera *Metachromadora*, *Terschellingia* and *Monoposthia* presented the highest contribution to the dissimilarity between Navigator and Troia. The average dissimilarity was 78,84. The genera *Metachromadora*, *Sabatieria* and *Monoposthia* presented the highest contribution to the dissimilarity between Gambia and Troia.

PERMANOVA analysis for nematode assemblages' density showed significant differences between "Sites" (p=0,0158) and a significant interaction (p < 0,05) between factors ("Site" x "Sampling occasions"). To better analyse this interaction was performed the pairwise test, and it was detected significant differences between the levels Troia vs Navigator, p= 0,0039 and Troia vs Gambia, p= 0,0313. These results suggested that there is a clear separation between sites and the spatial scale differences overcome the temporal variations in the nematodes assemblage's structure.

Concerning the diversity and composition of the assemblages, the highest values of richness and evenness (d, J and H) were obtained in Troia, more specifically at the sampling occasion "summer 2020", while Gambia assemblages presented the lowest values (Table 3). The same tendency was detected for the MI and ITD^{-1} indexes with the high values in Troia at sampling occasions "winter 2019" and "summer 2020".

Table 3: Mean ± standard error (SE), n = 3 of diversity descriptors of nematode assemblages (density, number of taxa, number of individuals, Margalef's Richness Index (d), Shannon-Wiener Diversity Index (H'), Simpson's Index (1-Lambda'), Pielou's Eveness (J'), Trophic Diversity Index (ITD^{-1}) and Maturity Index (MI) from each sampling site (Navigator, Gambia and Troia), at each sampling occasion (winter 2019 and summer 2020).

	Navigator		Gambia		Troia	
Diversity indices	**Winter 2019**	**Summer 2020**	**Winter 2019**	**Summer 2020**	**Winter 2019**	**Summer 2020**
Number of taxa	26,0±0,9	24,3±1,7	24,3±1,7	20±1,2	30,7±0,7	42±2,7
Number of individuals	216,3±21,6	229,3±18,1	245,7±24,1	167,7±9,4	118,7±3,8	213±10,6
Margalef's Richness Index	4,7±0,2	4,3±0,3	4,3±0,4	3,7±0,2	6,2±0,2	7,6±0,5
Shannon-Wiener Diversity Index	2,9±0,03	2,9±0,1	2,9±0,1	2,7±0,04	3,2±0,04	3,5±0,1
Simpson's Index	0,9±0,003	0,9±0,01	0,9±0,01	0,9±0,003	1±0,003	1,0±0,002
Pielou's Eveness	0,9±0,004	0,9±0,02	0,9±0,01	0,9±0,005	0,9±0,01	0,9±0,003
Index of Trophic Diversity	2,3±0,02	2,3±0,03	2,3±0,05	2,2±0,02	2,7±0,1	2,8±0,1
Maturity Index	2,5±0,1	2,4±0,2	2,6±0,3	2,5±0,1	2,6±0,4	3,0±0,2

Analysing the significant differences of the diversity indexes between sites and across sampling occasions, p<0,05, significant interactions were detected for S, N, d and H', (p < 0,05) (Table 4). Performing the pairwise test for the significant interactions, the S detected significant differences between the levels Navigator

and Troia p=0,0332, within level “Winter 2019”. The N shows significant variability between Navigator and Troia p=0,0299 and between Gambia and Troia p=0,0153, within level “Winter 2019”. For the d was detected significant differences between Navigator and Troia p=0,0082, as well as between Gambia and Troia p=0,0038, within level “summer 2020”. H' index revealed that there are significant differences between Navigator and Troia P(MC)=0,0254, within level “Winter 2019”. Significant differences were also revealed between Navigator and Troia p=0,0255 and between Gambia and Troia P(MC)=0,0003, within level “summer 2020” (Table 4). The MI only revealed significant differences between sites p=0,0115.

Table 4: Two-factor PERMANOVA test with “Sampling occasion” (2 levels, random) and “Site” (3 levels, fixed) for density, number of taxa, number of individuals, Margalef’s Richness Index (d), Shannon-Wiener Diversity Index (H’), Simpson’s Index (1-Lambda'), Pielou's Eveness (J'), Trophic Diversity Index (ITD^{-1}) and Maturity Index (MI). Bold values highlight significant effects and interactions ($p < 0,05$).

	Source of variation	Degrees of freedom	Sum of squares	Mean square	Pseudo-F	P(perm)	perms	P(MC)
Density	**Site**	2	18884	9441,9	8,2413	**0,0158**	60	0,0022
	Sampling Occasion	1	1541	1541	2,0558	0,1223	9929	0,1034
	Site x Sampling Occasion	2	2291,4	1145,7	1,5284	0,1764	9940	0,179
	Residual	12	8995,2	749,6				
	Total	17	31711					
Number of taxa	**Site**	2	10,83	5,4151	3,1729	0,1931	30	0,2385
	Sampling Occasion	1	0,23031	0,23031	1,094	0,3234	7694	0,3204
	Site x Sampling Occasion	2	3,4133	1,7067	8,1068	**0,0056**	9765	0,0051
	Residual	12	2,5262	0,21052				
	Total	17	17					
Number of individuals	**Site**	2	3,5905	1,7952	0,46442	0,7336	60	0,6887
	Sampling Occasion	1	0,14917	0,14917	0,32373	0,5724	9754	0,5765
	Site x Sampling Occasion	2	7,731	3,8655	8,3892	**0,0072**	9951	0,0053
	Residual	12	5,5293	0,46078				
	Total	17	17					
Margalef’s Richness Index		2	13,061	6,5304	8,1689	0,15	60	0,1123
	Sampling Occasion	1	5,08E-02	5,08E-02	0,26644	0,6046	9843	0,6076
	Site x Sampling Occasion	2	1,5988	0,79942	4,1899	**0,0447**	9951	0,0418

	Residual	12	2,2896	0,1908				
	Total	17	17					
Shannon-Wiener Diversity Index	**Site**	2	10,787	5,3933	3,9609	0,217	60	0,2099
	Sampling Occasion	1	2,86E-02	2,86E-02	9,93E-02	0,7557	9831	0,7617
	Site x Sampling Occasion	2	2,7232	1,3616	4,7202	**0,0337**	9959	0,0311
	Residual	12	3,4616	0,28847				
	Total	17	17					
Simpson's Index	**Site**	2	9,5466	4,7733	9,3779	0,151	60	0,0945
	Sampling Occasion	1	3,78E-02	3,78E-02	7,09E-02	0,7947	9842	0,7973
	Site x Sampling Occasion	2	1,018	0,509	0,95474	0,4189	9939	0,4138
	Residual	12	6,3975	0,53313				
	Total	17	17					
Pielou's Eveness	**Site**	2	5,8795	2,9398	5,51	0,1808	60	0,1552
	Sampling Occasion	1	2,93E-02	2,93E-02	3,50E-02	0,8578	9851	0,8529
	Site x Sampling Occasion	2	1,0671	0,53354	0,6387	0,5345	9950	0,5414
	Residual	12	10,024	0,83535				
	Total	17	17					
Index of Trophic Diversity	**Site**	2	1,4233	0,71166	1,4538	0,39	60	0,4057
	Sampling Occasion	1	6,20E-02	6,20E-02	5,12E-02	0,8208	9833	0,8261
	Site x Sampling Occasion	2	0,97904	0,48952	0,40412	0,6804	9962	0,6751
	Residual	12	14,536	1,2113				
	Total	17	17					
Maturity Index	**Site**	2	13,337	6,6685	88,893	0,1693	60	0,0115
	Sampling Occasion	1	1,63E-02	1,63E-02	5,60E-02	0,807	9806	0,8119
	Site x Sampling Occasion	2	0,15003	7,50E-02	0,25745	0,7717	9943	0,7678

3.2.2. Temporal scale perspective

Total density of nematode communities (ind.10cm^2) were calculated on each sampling occasion, at each site. The mean density was the highest at Gambia "winter 2019", while Troia "winter 2019" had the lowest value (Fig.8). The mean density ± SE was 3671,7±746,8 ind.10 cm^2 at Navigator "winter 2019", 4028,1 ± 193,8 ind.10 cm^2 at Navigator "summer 2020", 4951,6±1184,9 ind. 10 cm^2 at Gambia "winter 2019", 2620,3±276,1 ind. 10 cm^2 at Gambia "summer 2020", 786,9±61,4 ind. 10 cm^2 at Troia "winter 2019" and 1768,7 ± 104,6 ind.10 cm^2 at Troia "summer 2020".

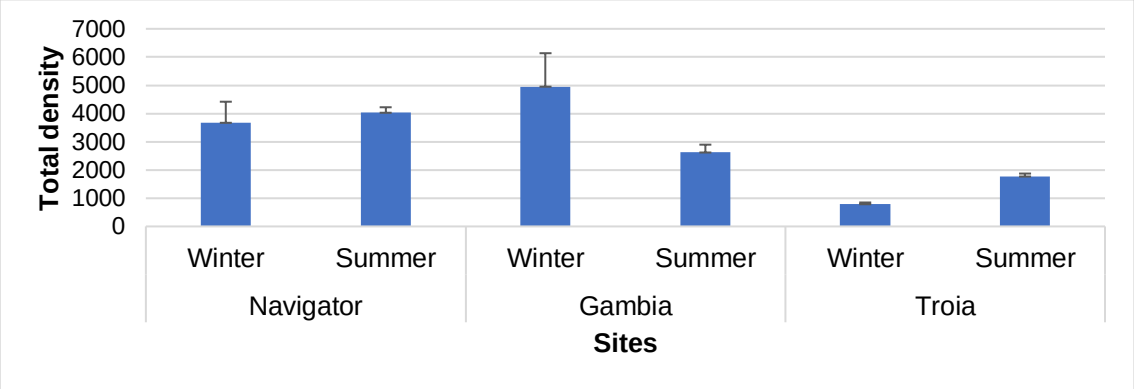

Figure 8: Density of nematode communities (ind.10cm^2) on each sampling occasion (Winter 2019, Summer 2020), at each site (Navigator, Gambia, Troia).

The order *Chomadorida* was the most predominant order at Navigator, Gambia sampling sites. At Troia sampling site, specifically at the sampling occasion "summer 2020", the order that represented the highest density values was also *Chomadorida*, while at the sampling occasion "winter 2020" was *Desmodorida* (43,4%) (Fig.9; Appendix 4). *Desmodoridae* is the family that, globally, stands out the most, although the density is lower in the Troia sampling occasions compared to the others. *Comesomatidae* remain relatively uniform density values across all sampling sites, on all sampling occasions. *Monoposthiidae* stands out in Troia on both sampling occasions (winter 2019 and summer 2020), being non-existent or practically non-existent on the remaining sampling occasions (Navigator "winter 2019" and "summer 2020", Gambia "winter 2019" and "summer 2020") (Fig.10; Appendix 5).

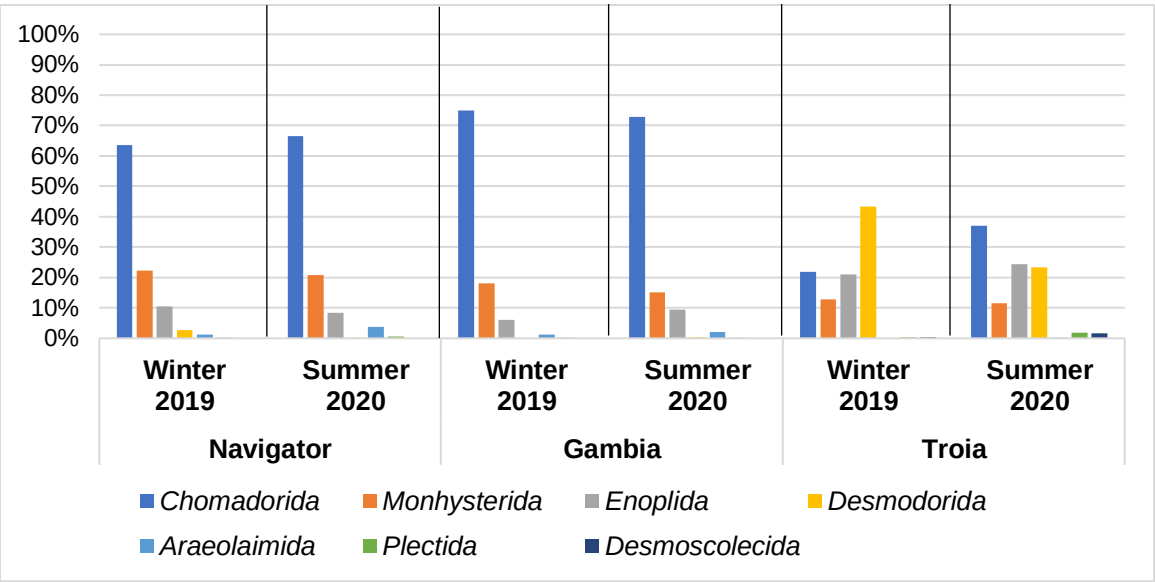

Figure 9: Mean of relative density (%) of nematodes on each sampling occasion (Winter 2019 and Summer 2020), at each site (Navigator, Gambia and Troia) per order.

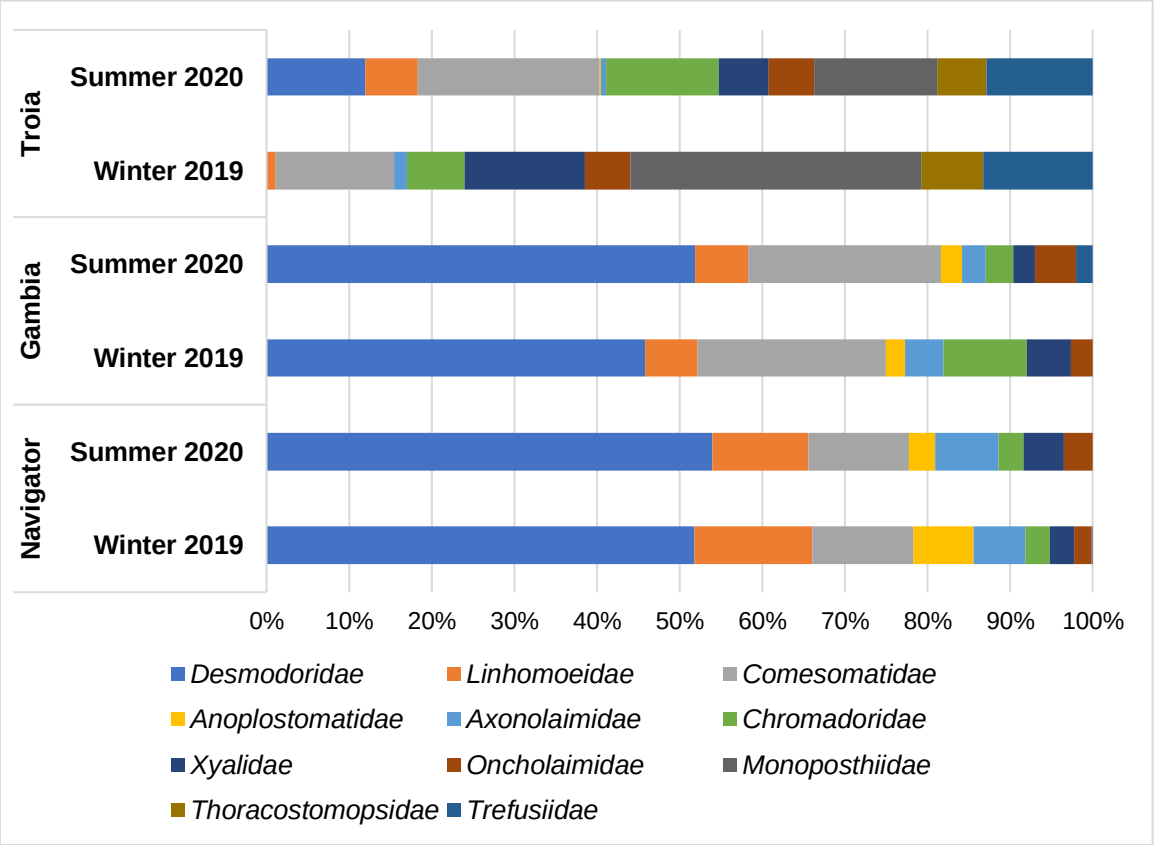

Figure 10: Mean of relative density (%) of nematodes on each sampling occasion (Winter 2019 and Summer 2020), at each site (Navigator, Gambia and Troia) per family.

The 10 genera with the highest density on each sampling occasion (winter 2019 and summer 2020), at each site (Navigator, Gambia and Troia) were chosen. The only genera that stand out in the 3 sampling sites, on each sampling occasion, is *Sabatieria*. The genera *Sphaerolaimus*, *Monoposthia*, *Desmodora*, *Trefusia* and *Trileptum* are not present on all sampling sites. *Metachromadora* and *Terschellingia* have high densities on Navigator and Gambia, both sampling occasions "winter 2019" and "summer 2020", but have a considerably lower percentage in Troia, on both sampling occasions. *Monoposthia*, on the other hand, has a high density in Troia, both sampling occasions, but at Navigator the sampling occasion "winter 2019" it has a reduced density, being non-existent at the remaining sampling occasions (Navigator "summer 2020", Gambia "winter 2019" and Gambia "summer 2020") (Fig.11; Appendix 6).

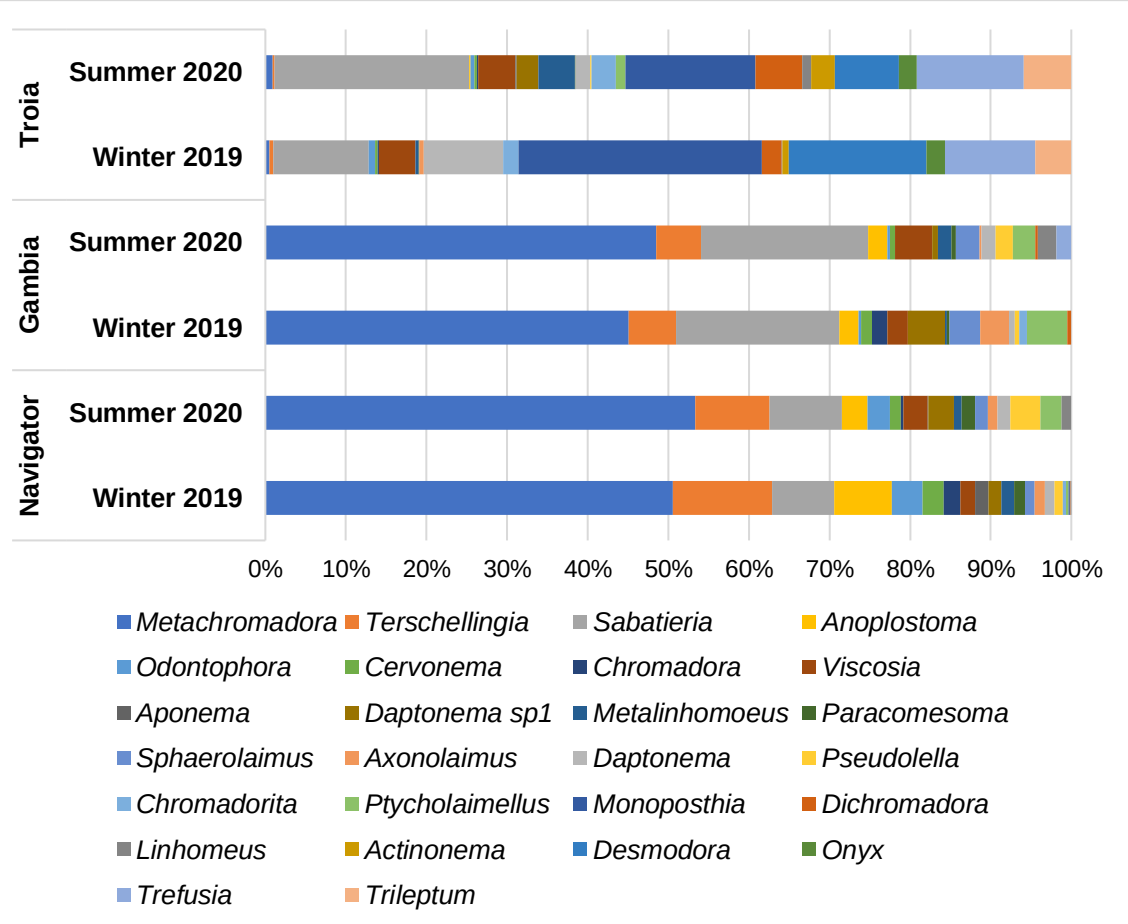

Figure 11: Mean of relative density (%) of nematodes on each sampling occasion (Winter 2019 and Summer 2020), at each site (Navigator, Gambia and Troia) per genera.

PCO analysis indicates that there is a low variability of nematode densities between sampling occasions. (Fig.12).

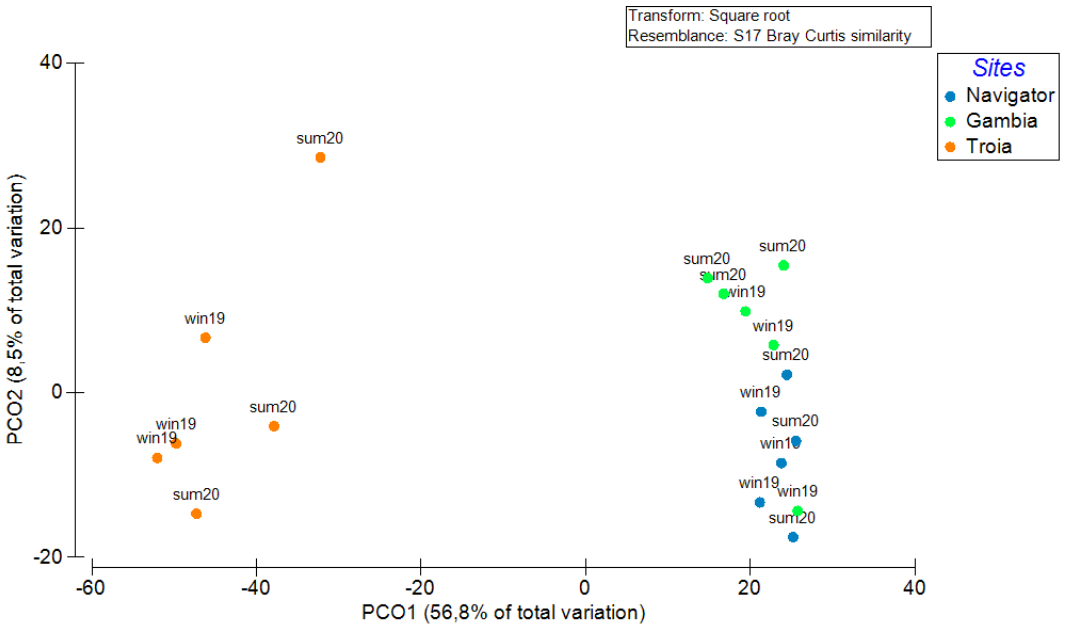

Figure 12: Principal Coordinates Analysis (PCO) based on the nematode dataset at each "Site", Navigator, Gambia and Troia (3 levels, fixed), and on each "Sampling Occasion", Winter 2019 and Summer 2020 (2 levels, random). PCO1 56,8%, PCO2 8.5% of total variation.

The SIMPER analysis showed how nematode genera contributed to the similarity values of a priori defined groups. Average similarity in Navigator was 65,50 and the genera that presented the highest contribution to the similarity between "winter 2019" and "summer 2020" were *Metachromadora*, *Terschellingia* and *Sabatieria*. At the Gambia site, the genera that presented the highest contribution to the similarity was *Metachromadora*, *Sabatieria* and *Terschellingia*. At Troia site, *Monoposthia*, *Sabatieria* and *Trefusia* was the genera that presented the highest contribution to the similarity within this sampling site.

PERMANOVA analysis showed no significant differences between "Sampling occasions". However, revealed significant interaction ($p < 0,05$) between factors ("Site" x "Sampling occasions"), as previously mentioned (Table 4).

4. Discussion

Benthic communities are known to have adaptive responses to variation in estuarine sediment (Sroczyńska *et al.*, 2021b; Vieira *et al.*, 2023). In this study, the spatial and temporal patterns of nematode assemblages were assessed in three distinct sites of Sado estuary (Navigator, Gambia and Troia), with different environment conditions. Through these conditions, communities' responses were tested, in diversity and density, between sites and across sampling occasions.

Our results revealed that assemblages were more responsive to the site-specific habitat conditions than to the temporal variations. Assemblages exposed through similar environmental conditions showed to be more similar (Navigator and Gambia) than the others, that are exposed to different conditions (Troia), especially sediment compositions. Troia sediments were more sandy presenting high contents of sand and $CaCO_3$ while Navigator and Gambia sediments were muddy with more silt in the case of Navigator and more gravel in the case of Gambia sediment. These differences were directly reflected on the composition and diversity of the assemblages. Organic matter was more present in the muddy sediments (Navigator and Gambia) which are exposed to low hydrodynamic waters, with high residence time. These conditions are also combined with the influence of surrounding anthropogenic activities. The same patterns in nematodes assemblages related with the sediment properties were already reported (e.g., Adão, 2021). Assemblages' densities are mainly due to the organic matter contents (Steyaert *et al.*, 2003; Soetaert & Middelburg, 2009). Comparing our results with other Portuguese estuaries, the density of nematode assemblages recorded in this study was high compared to Mondego (Alves *et al.*, 2013), however, in the Mira estuary some higher values were recorded (Materatski *et al.*, 2015). Comparing with (Vieira *et al.*, 2023), a study carried out in the same estuary, we have slightly higher density results on comparable sampling site, such as Navigator. Despite the lowest values of density, Troia assemblages presented the highest values of diversity. This is possibly due to the high amount of microhabitats available for nematodes in these types of sediments when compared to muddy ones (Steyaert *et al.*, 2003; Soetaert & Middelburg, 2009).

As concluded by Alves *et al*., (2013), the increase in taxonomic resolution, that is, from major meiofauna taxa to the nematode genera level, showed that at the genera level we were able to obtain more refined information to evaluate changes in the benthic community. PCO analysis confirms a high between-site variability of nematode densities, especially Troia that shows an evident departure from the other sampling sites. In Navigator and Gambia sampling sites, the dominant genera were, by far, *Metachromadora* being followed by the genera *Sabatieria* and *Terschellingia*. *Monoposthia* was the dominant genera in Troia sampling site, followed by the genera *Sabatieria*. *Metachromadora* and *Terschellingia* present low relative density values on the Troia sampling site compared to the other sampling sites, just as the genera *Monoposthia* presents a very low relative density value on the Navigator sampling site and is non-existent in Gambia. SIMPER shows that the genera with the highest variation in relative density between Navigator and Gambia are *Metachromadora*, *Sabatieria* and *Odontophora*. Comparing with (Vieira *et al.*, 2023), we observed that *Metachromadora*, *Sabatieria* and *Terschellingia* are also among the dominant genera. Comparing with the Mondego estuary (Alves *et al*., 2013), we observed that these 3 same genera are also dominant, depending on the salinity of each sampling site. The major importance of salinity in nematodes species distribution in transitional water systems, where salinity levels can vary from 0,5 to 30, is widely recognized (e.g., Vincx, Meire and Heip, 1990; Alves *et al*., 2013). At the Mondego estuary *Metachromadora* was dominant in euhaline sampling sites, *Sabatieria* in euhaline and polyhaline sampling sites and *Terschellingia* in mesohaline sampling sites and some polyhaline sampling sites. In our study the salinity was not a determinant variable, primarily because were instantaneously measured and the selected sampling sites did not follow the estuarine gradient as the (Alves *et al.*, 2013) study. Comparing with the Mira estuary (Materatski *et al.*, 2015) we can observe that the genera *Terschellingia* was one of the dominant genera in the before-collapse period and during early recovery. The *Sabatieria* and *Metachromadora* genera become more dominant during early recovery which is partially explained by the presence/absence of vegetation. We also verified through PERMANOVA analysis that there are no significant differences in nematode assemblages' diversity patterns across the sampling occasions (winter 2019 and summer 2020).

According with Wieser classification, *Terschellingia* is 1A (selective deposit feeders), *Sabatieria* is 1B (non-selective deposit feeders), *Monoposthia* is 2A (epistrate, feeders) and *Metachromadora* is 2B (omnivores or predators). The ITD^{-1} is usually used to correlate the trophic diversity of nematodes with pollution levels (e.g., Heip, Vincx and Vranken, 1985). Relatively to Maturity Index (MI), *Sabatieria* and *Metachromadora* are categorized as 2 (colonizers), *Terschellingia* and *Monoposthia* are categorized as 3. *Sabatieria* and *Metachromadora* are also considered as opportunistic genera with low MI values are dominant in disturbed and polluted environments (Gyedu-Ababio & Baird, 2006). Statistically significant changes can only be obtained when there are strong variations in the nematode assemblage structure, that's why previous studies highlight the lack of sensitivity of these indices to habitat changes (e.g., Moreno *et al*., 2008). In this study, none of these indices presented statistically significant values.

Gambia highlighted from the other samples mainly due to the high concentrations of Chl *a* and phaeo at "summer 2020". Gambia is located close to rice fields and aquaculture productions, therefore having a high input of organic matter, which gives rise to cascading effects that culminate in increased development of opportunistic green algae, occasionally forming mats (Wilkinson *et al.*, 1995; Elliott & de Jonge, 2002). Diatoms and other microalgae are important food sources for many epigrowth feeders (2A) (Moens and Vincx, 1997). However, phaeo, a non-photosynthetic pigment which is the degradation product of algal chlorophyll pigments, are also a food source for opportunistic species, which can lead to an increase in the number of individuals of less selective species (1B). Genera such as *Terschellingia* and *Sabatieria* have been described as indicators of a poor ecological quality status due to their well-known tolerance to pollution (e.g. Steyaert *et al*., 2007).It would be necessary to know, at least, for how long Chl *a* and phaeo values were actually high in order to be able to affirm that this parameter had a real impact on the abundance and/or diversity of the nematode community.

As previously mentioned, estuarine regions are naturally stressed areas with highly variable environmental conditions, however, these ecosystems are also known to be under significant pressure due to various anthropogenic activities. Therefore, it is particularly difficult to separate natural and

anthropogenic stress using biological indicators. Due to this problem, Dauvin, (2007) proposes to use the term "paradox of estuarine quality". All the indices which aim to determine anthropogenic stress, report to abundances of stress tolerant species, which may also be tolerant of natural stressors. Therefore, benthic indices and the diverse indicator species were used in coastal environments must be monitored carefully in transitional waters. Variation in the diversity and abundance of nematode species at different sampling sites or sampling occasions may be related to environmental variations, making it difficult to analyse the response of benthic communities to anthropogenic stressors.

5. Conclusion

Estuaries are included in the "transitional waters" category, being used as means of completing the continuum between freshwaters and coastal waters and therefore have very variable environmental conditions. Therefore, it is difficult in an estuary to attribute the variation in the composition and/or abundance of benthic species to a specific stressor, mainly because, in many cases, several environmental and anthropogenic factors may be contributing at different scales to this change.

The results showed an evident divergence in the composition of the benthic community of Troia comparing to Navigator and Gambia. Troia is located closest to the mouth of the estuary and, therefore, has the highest hydrodynamics and the lowest residence time. Troia differs from other sampling sites mainly due to its sandy sediment. Therefore, it was possible to conclude that there are significant differences in nematode assemblages' diversity and composition between sites with different sediment conditions, possibly influenced by the availability of organic matter, rejecting our first null hypothesis (H0: There are no significant differences in nematode assemblages' diversity and composition between three sites with different sediment conditions). Our second null hypothesis (H0: There are no significant differences in nematode assemblages' diversity patterns across two distinct sampling occasions (winter 2019 and summer 2020)) was confirmed, as no statistically significant variations were identified between sampling occasions.

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7. Supplementary data

Appendix 1: Mean density ± standard error (SE) and relative density (%) n=3 of nematode orders (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia).

	Navigator			Gambia			Troia		
Order	**Mean**	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Chomadorida	2504,1	291,7	65,0%	2809,1	763,0	74,2%	413,8	167,2	32,4%
Monhysterida	825,8	151,1	21,4%	642,5	195,9	17,0%	152,7	37,2	12,0%
Enoplida	359,1	92,5	9,3%	270,5	37,6	7,1%	298,9	96,3	23,4%
Desmodorida	51,0	38,0	1,3%	4,3	3,1	0,1%	377,2	83,1	29,5%
Araeolaimida	97,0	28,4	2,5%	55,3	12,5	1,5%	1,0	1,1	0,1%
Plectida	12,9	9,9	0,3%	4,2	2,1	0,1%	18,0	8,1	1,4%
Desmoscolecida	0	0	0%	0	0	0%	16,1	14,6	1,3%

Appendix 2: Mean density ± standard error (SE) and relative density (%) n=3 of nematode families (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia).

	Navigator			Gambia			Troia		
Family	**Mean**	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Desmodoridae	1912,8	256,9	49,7%	1661,6	725,7	43,9%	170,5	42,3	13,3%
Linhomoeidae	465,7	98,0	12,1%	221,1	65,3	7,0%	53,3	42,2	4,2%
Comesomatidae	441,7	138,9	11,5%	797,0	294,4	21,1%	216,7	119,8	17,0%
Axonolaimidae	253,5	60,2	6,6%	137,9	49,8	3,6%	9,4	2,8	0,7%
Anoplostomatidae	185,6	87,8	4,8%	84,8	21,0	2,2%	1,0	1,0	0,1%
Xyalidae	143,6	55,9	3,7%	153,5	85,1	4,1%	91,0	14,1	7,1%
Chromadoridae	106,6	41,7	2,8%	269,8	124,7	7,1%	128,4	48,4	10,0%
Oncholaimidae	102,6	27,2	2,7%	118,3	16,8	3,1%	59,6	16,8	4,7%
Monoposthiidae	2,4	2,6	0,1%	0	0	0%	221,6	52,7	17,3%
Thoracostomopsi--dae	0	0	0%	0	0	0%	68,7	31,9	5,4%
Trefusiidae	0	0	0%	24,0	26,3	0,6%	140,7	46,6	11,0%

Appendix 3: Mean density ± standard error (SE) and relative density (%) n=3 of nematode genera (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia).

Genera	Navigator			Gambia			Troia		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Metachromadora	1910,5	257,2	49,6%	1661,6	725,7	43,9%	8,2	4,5	0,6%
Terschellingia	393,9	99,9	10,2%	207,5	66,5	5,5%	3,4	1,8	0,3%
Sabatieria	307,0	99,0	8,0%	732,4	289,3	19,3%	212,1	118,5	16,6%
Anoplostoma	185,6	87,8	4,8%	84,8	21,0	2,2%	1,0	1,0	0,1%
Odontophora	119,9	39,7	3,1%	11,8	4,9	0,3%	6,6	1,4	0,5%
Ptycholaimellus	55,6	36,4	1,4%	150,8	75,6	4,0%	8,8	8,1	0,7%
Sphaerolaimus	51,8	17,3	1,3%	127,9	52,9	3,4%	0	0	0%
Monoposthia	2,4	2,6	0,1%	0	0	0%	218,0	53,8	17,1%
Desmodora	0	0	0%	0	0	0%	114,6	43,4	9,0%
Trefusia	0	0	0%	24,0	26,3	0,6%	132,5	46,1	10,4%
Trileptum	0	0	0%	0	0	0%	57,3	33,6	4,5%

Appendix 4: Mean density ± standard error (SE) and relative density (%) n=3 of nematode orders (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia), at each sampling occasion (Winter 2019 and Summer 2020).

Order	Navigator					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Chomadorida	2331,9	400,3	63,5%	2676,4	186,2	66,4%
Monhysterida	814,9	227,8	22,2%	836,6	71,4	20,8%
Enoplida	383,6	140,3	10,5%	334,6	36,9	8,3%
Desmodorida	95,5	49,1	2,6%	6,4	3,0	0,2%
Araeolaimida	44,1	13,9	1,2%	150,0	11,9	3,7%
Plectida	1,6	1,3	0,04%	24,2	13,0	0,6%
Desmoscolecida	0	0	0%	0	0	0%
Order	**Gambia**					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Chomadorida	3707,8	952,0	74,9%	1910,3	254,1	72,9%
Monhysterida	890,1	242,7	18,0%	395,0	16,4	15,1%
Enoplida	295,5	32,4	6,0%	245,5	46,0	9,4%
Desmodorida	0	0	0%	8,7	3,6	0,3%
Araeolaimida	55,7	14,5	1,1%	54,8	13,4	2,1%
Plectida	2,4	1,9	0,05%	6,0	2,4	0,2%
Desmoscolecida	0	0	0%	0	0	0%

Order	Troia					
	Winter			Summer		
	Mean	SE	Relative Density	Mean	SE	Relative Density
Chomadorida	172,2	40,8	21,9%	655,5	182,3	37,1%
Monhysterida	101,1	10,2	12,9%	204,4	41,9	11,6%
Enoplida	165,2	45,6	21,0%	432,5	101,8	24,5%
Desmodorida	341,9	73,7	43,5%	412,5	105,2	23,3%
Araeolaimida	0	0	0%	2,0	1,6	0,1%
Plectida	3,6	2,8	0,5%	32,5	5,7	1,8%
Desmoscolecida	2,9	2,2	0,4%	29,3	20,5	1,66%

Appendix 5: Mean density ± standard error (SE) and relative density (%) n=3 of nematode families (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia), at each sampling occasion (Winter 2019 and Summer 2020).

Family	Navigator					
	Winter 2019			Summer 2020		
	Mean	SE	Relative Density	Mean	SE	Relative Density
Desmodoridae	1778,8	280,0	48,5%	2046,8	275,4	50,8%
Linhomoeidae	490,9	150,1	13,4%	440,5	33,3	10,9%
Comesomatidae	420,0	134,4	11,5%	463,4	172,8	11,5%
Anoplostomatidae	251,1	127,7	6,8%	120,0	19,6	3,0%
Axonolaimidae	214,5	71,8	5,8%	292,4	54,6	7,3%
Chromadoridae	100,8	45,4	2,7%	112,4	47,6	2,8%
Xyalidae	101,6	31,8	2,8%	185,7	75,8	4,6%
Oncholaimidae	72,7	31,5	2,0%	132,4	17,8	3,3%
Monoposthiidae	4,7	3,7	0,1%	0	0	0%
Thoracostomopsidae	0	0	0%	0	0	0%
Trefusiidae	0	0	0%	0	0	0%
Family	**Gambia**					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Desmodoridae	2082,4	1083,3	42,1%	1240,7	191,8	49,0%
Linhomoeidae	286,9	96,8	5,8%	155,3	67,6	6,1%
Comesomatidae	1036,1	420,7	20,9%	557,9	73,8	22,0%
Anoplostomatidae	108,1	11,2	2,2%	61,5	25,5	2,4%
Axonolaimidae	208,6	53,4	4,2%	67,1	18,3	2,7%
Chromadoridae	458,8	130,9	9,3%	80,7	17,9	3,2%
Xyalidae	243,6	114,9	4,9%	63,3	4,5	2,5%
Oncholaimidae	118,9	4,8	2,4%	117,7	26,1	4,7%
Monoposthiidae	0	0	0%	0	0	0%
Thoracostomopsidae	0	0	0%	0	0	0%

Trefusiidae	0	0	0%	48,1	37,2	1,9%

Family	**Troia**					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Desmodoridae	150,8	46,0	19,2%	190,3	46,1	10,8%
Linhomoeidae	6,4	3,1	0,8%	100,2	56,0	5,7%
Comesomatidae	83,9	34,2	10,7%	349,4	155,4	19,8%
Anoplostomatidae	0	0	0%	1,9	1,5	0,1%
Axonolaimidae	9,2	3,0	1,2%	9,7	3,3	0,6%
Chromadoridae	40,7	3,6	5,2%	216,1	35,0	12,2%
Xyalidae	85,5	11,5	10,9%	96,5	18,7	5,5%
Oncholaimidae	32,1	6,9	4,1%	87,1	14,4	4,9%
Monoposthiidae	206,1	32,2	26,2%	237,0	75,9	13,4%
Thoracostomopsidae	43,4	5,5	5,5%	93,9	46,2	5,3%
Trefusiidae	77,7	42,7	9,9%	203,8	34,9	11,5%

Appendix 6: Mean density ± standard error (SE) and relative density (%) n=3 of nematode genera (number of individuals per 10 cm^2) in 3 sites (Navigator, Gambia and Troia), at each sampling occasion (Winter 2019 and Summer 2020).

Genera	**Navigator**					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Metachromadora	1776,2	282,0	48,4%	2044,9	273,9	50,8%
Terschellingia	434,0	136,1	11,8%	353,9	73,9	8,8%
Sabatieria	268,3	99,7	7,3%	345,6	116,8	8,6%
Anoplostoma	251,1	127,7	6,8%	120,0	19,6	3,0%
Odontophora	134,3	42,7	3,7%	105,5	44,7	2,6%
Cervonema	94,3	28,0	2,6%	54,7	25,9	1,4%
Chromadora	70,3	35,8	1,9%	12,1	2,6	0,3%
Viscosia	65,3	28,4	1,8%	113,3	17,0	2,8%
Aponema	58,3	45,2	1,6%	4,4	3,4	0,1%
Daptonema sp1	56,6	15,8	1,5%	122,8	45,0	3,0%
Metalinhomoeus	54,3	18,0	1,5%	36,5	14,6	0,9%
Paracomesoma	47,8	14,3	1,3%	63,1	31,9	1,6%
Sphaerolaimus	42,5	18,8	1,2%	61,1	18,6	1,5%
Axonolaimus	45,7	24,7	1,2%	43,8	4,1	1,1%
Daptonema	41,7	22,0	1,1%	62,8	32,2	1,6%
Pseudolella	34,6	13,2	0,9%	143,1	8,0	3,6%
Chromadorita	15,9	9,5	0,4%	0	0	0%
Ptycholaimellus	10,9	8,5	0,3%	100,3	45,3	2,5%
Monoposthia	4,7	3,7	0,1%	0	0	0%

Dichromadora	3,6	2,8	0,1%	0	0	0%
Linhomeus	2,6	2,0	0,1%	46,3	30,3	1,1%
Actinonema	0	0	0%	0	0	0%
Desmodora	0	0	0%	0	0	0%
Onyx	0	0	0%	0	0	0%
Trefusia	0	0	0%	0	0	0%
Trileptum	0	0	0%	0	0	0%
Genera	**Gambia**					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Metachromadora	2082,4	1083,3	42,1%	1240,7	191,8	47,3%
Terschellingia	273,1	89,1	5,5%	141,8	22,7	5,4%
Sabatieria	935,4	423,1	18,9%	529,3	74,4	20,2%
Anoplostoma	108,1	11,2	2,2%	61,5	25,5	2,3%
Odontophora	15,5	6,2	0,3%	8,1	3,7	0,3%
Cervonema	60,9	30,9	1,2%	15,5	6,7	0,6%
Chromadora	91,1	11,9	1,8%	0	0	0%
Viscosia	117,5	5,3	2,4%	117,7	26,1	4,5%
Aponema	0	0	0%	0	0	0%
Daptonema sp1	212,5	125,2	4,3%	18,7	5,5	0,7%
Metalinhomoeus	10,2	6,4	0,2%	43,6	9,7	1,7%
Paracomesoma	12,3	5,7	0,2%	13,0	2,9	0,5%
Sphaerolaimus	179,4	72,5	3,6%	76,3	12,5	2,9%
Axonolaimus	164,8	52,1	3,3%	4,2	1,7	0,2%
Daptonema	31,1	19,4	0,6%	44,7	9,3	1,7%
Pseudolella	28,3	6,9	0,6%	54,8	13,4	2,1%
Chromadorita	43,0	19,0	0,9%	0	0	0%
Ptycholaimellus	229,5	101,7	4,6%	72,1	14,5	2,7%
Monoposthia	0	0	0%	0	0	0%
Dichromadora	23,9	18,5	0,5%	8,7	3,5	0,3%
Linhomeus	0	0	0%	57,5	15,8	2,2%
Actinonema	0	0	0%	0	0	0%
Desmodora	0	0	0%	0	0	0%
Onyx	0	0	0%	0	0	0%
Trefusia	0	0	0%	48,1	37,2	1,8%
Trileptum	0	0	0%	0	0	0%
Genera	**Troia**					
	Winter 2019			**Summer 2020**		
	Mean	**SE**	**Relative Density**	**Mean**	**SE**	**Relative Density**
Metachromadora	3,7	2,8	0,5%	12,7	5,6	0,7%
Terschellingia	3,2	2,5	0,4%	3,5	1,4	0,2%
Sabatieria	80,5	35,8	10,2%	343,7	153,0	19,4%

Anoplostoma	0	0	0%	1,9	1,5	0,1%
Odontophora	5,5	1,1	0,7%	7,6	1,7	0,4%
Cervonema	2,5	1,9	0,3%	4,1	3,2	0,2%
Chromadora	0,7	0,6	0,1%	1,9	1,5	0,1%
Viscosia	31,4	6,5	4,0%	66,5	14,7	3,8%
Aponema	0	0	0%	1,6	1,3	0,1%
Daptonema sp1	0	0	0%	38,4	27,6	2,2%
Metalinhomoeus	2,3	1,0	0,3%	64,1	34,6	3,6%
Paracomesoma	0,9	0,7	0,1%	1,6	1,3	0,1%
Sphaerolaimus	0	0	0%	0	0	0%
Axonolaimus	3,7	2,9	0,5%	0	0	0%
Daptonema	67,2	9,1	8,5%	26,3	7,9	1,5%
Pseudolella	0	0	0%	2,0	1,6	0,1%
Chromadorita	13,0	6,9	1,7%	42,5	9,1	2,4%
Ptycholaimellus	0	0	0%	17,7	10,8	1,0%
Monoposthia	206,1	32,2	26,2%	229,8	78,2	13,0%
Dichromadora	17,0	4,3	2,2%	82,7	0,9	4,7%
Linhomeus	0,9	0,7	0,1%	15,4	9,7	0,9%
Actinonema	5,4	3,1	0,7%	41,7	13,3	2,4%
Desmodora	116,4	43,7	14,8%	112,7	52,8	6,4%
Onyx	16,0	6,4	2,0%	32,1	10,8	1,8%
Trefusia	76,3	43,2	9,7%	188,7	39,3	10,7%
Trileptum	30,6	10,1	3,9%	84,1	47,8	4,8%