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RESPOSTA DA MACROFAUNA BÊNTICA ESTUARINA AO ESTRESSE INDUZIDO POR TAPETES DE ALGA

ANA PAULA MARIA CAVALCANTI VALENÇA

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, da Universidade Federal de Pernambuco (UFPE), como requisito parcial para obtenção do título de Doutor em Biologia Animal.

Orientador:
Prof. Dr. Paulo Jorge Parreira dos Santos

Co-Orientadora:
Prof^{a}. Dr^{a}. Mônica Lúcia Botter-Carvalho

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Aprovada em 23 de fevereiro de 2015.

Banca Examinadora:

Prof. Dr. Paulo Jorge Parreira dos Santos (orientador)
Departamento de Zoologia/UFPE

Prof^{a}. Dr^{a}. Tatiana Fabrício Maria (titular externo)
Departamento de Ecologia e Recursos Marinhos/ UNIRIO

Prof^{a}. Dr^{a}. Joseline Molozzi (titular externo)
Departamento de Biologia/ UEPB

Prof. Dr. Jesser Fidelis de Souza Filho (titular externo)
Departamento de Oceanografia/ UFPE

Prof^{a}. Dr^{a}. Paula Braga Gomes (titular interno)
Departamento de Zoologia/ UFPE

Prof. Dr. José Roberto Botelho de Souza (titular interno)
Departamento de Zoologia/ UFPE

À minha família.

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>
> Chico Xavier.

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Mahatma Gandhi

RESUMO

A presente tese avaliou os possíveis impactos gerados pelos tapetes de alga na macrofauna estuarina. Em uma planície estuarina lamosa, situada no Complexo Estuarino do Canal de Santa Cruz, litoral norte de Pernambuco, foi registrada a ocorrência de tapetes de algas filamentosas em diferentes períodos (Setembro-2012, Setembro a Dezembro-2013 e Maio a Junho-2014). Através de estudos observacionais e manipulativos *in situ* foram testados: (I) o efeito do tapete algal na biogeoquímica sedimentar e na fauna, considerando a presença da alga e o seu estágio fisiológico; (II) a recolonização e a recuperação da macrofauna em sedimentos com remoção algal considerando os efeitos da compactação sedimentar gerada pelo tapete e do tamanho da área algal removida; a existência de efeitos (III) de barreira e (IV) de sazonalidade gerados pelo tapete algal e analisados sob a ótica da recolonização inicial. O estágio fisiológico da alga foi importante para a biogeoquímica sedimentar, enquanto que a presença do tapete algal se caracterizou como uma importante fonte de distúrbio para a fauna bêntica. A recolonização pela macrofauna nos sedimentos com remoção algal ocorreu de forma discreta e dependente da compactação sedimentar e do tamanho da área removida apenas para os parâmetros univariados. Além disso, não foi observada a recuperação das áreas experimentais até ao final do estudo (95 dias). A recolonização inicial (30 dias) não foi afetada pela presença do tapete algal circunvizinho às áreas experimentais. Embora tenha sido observado que a sazonalidade influencia no sedimento e na fauna, a análise da comunidade indicou uma tendência à convergência entre os tratamentos de remoção (AR) e sedimento natural (NA) independente da mesma. Além disso, foi observada uma relação entre o regime pluviométrico e o desenvolvimento do tapete algal. As consequências ecológicas do tapete algal, analisadas sob a recolonização a curto (30 dias) e médio prazo (95 dias), mostraram ser pouco previsíveis e extremamente influenciadas pela heterogeneidade ambiental. Além disso, a recuperação da comunidade em uma área afetada pelo desenvolvimento de tapete algal pode não apresentar a sequência sucessional observada em outros experimentos de colonização. Embora os estudos tenham sido conduzidos em um único local (na mesma planície estuarina), os resultados permitem uma melhor compreensão dos efeitos dos tapetes de algas na macrofauna bêntica estuarina.

Palavras-chave: Macrofauna Bêntica. Tapete Algal. Recolonização. Efeito de Barreira. Sazonalidade. Estuário.

ABSTRACT

This thesis assessed the possible impacts of algal mats on estuarine macrofauna. In an estuarine mudflat placed on Santa Cruz Channel Estuarine Complex, in the north coast of Pernambuco, the occurrence of filamentous mat-forming algae was registered at different periods (September-2012, September to December-2013 and May to June-2014). Observational and manipulative studies were conducted *in situ* and tested: (I) the effect of algal mats on sediment biogeochemistry and fauna, considering the presence of the algae and the physiological stage; (II) the recolonization and recovery of macrofauna in algal removal sediments relating to sediment compaction and the extension of algal removed (i.e. spatial scale); the existence of (III) boundaries and (IV) seasonal effects generated by the algal mat and analyzed using macrofauna recolonization data. The physiological stage affected significantly the sediment biogeochemistry and the presence of the algae was an important source of disturbance to benthic fauna. The recolonization of algal removal sediments varied regarding sediment compaction and spatial scale, but this was discrete and restricted to univariate measures. Besides, no recovery of any removal treatment was observed at the end of the experiment (95 days). Initial recolonization (30 days) was not affected by the surrounding algal mat found in the algal removal treatments. Although seasonal effects influence on sediment and fauna data, the analysis of benthic community showed a tendency of convergence between NA and AR treatments regardless of season. Moreover, it was possible to observe a relationship between rainfall seasonality and algal mat development. Ecological consequences of algal mats, analyzed through faunal recolonization within short- (30 days) and medium- (95 days) terms, showed to be less predictable and strongly influenced by environmental heterogeneity. Besides, community recovery in an area affected by algal coverage does not always include the successional sequence observed in other colonization experiments. Although the series of experiments have been conducted under one location (the same estuarine mudflat), the results provided strong insights on the effects of algal mats on tropical benthic macrofauna.

Keywords: Benthic Macrofauna. Algal Mat. Recolonization. Boundary Effects. Seasonality. Estuary.

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SUMÁRIO

GENERAL INTRODUCTION

Eutrophication, defined here as an increased loading of nutrients to the system (Rosenberg, 1985), is one of the most severe and widespread forms of disturbance and known to be a threat to marine and coastal ecosystems (Gray et al. 2002). A primary consequence of nutrient enrichment is the excessive proliferation of algal biomass or ‘algal blooms’ (Bolam et al. 2000; Anderson et al. 2002; Salovius et al. 2005; Arroyo et al. 2006). These dense accumulations have become quite common in intertidal sediments (Ellis et al. 2000). In estuaries and shallow systems, the increase of algal blooms’ frequency, magnitude and persistence enables the development of algal mats due to limited tidal exchange (Cummins et al. 2004). The algal mats may be composed of filamentous macroalgae (Thiel and Watling, 1998; Österling and Pihl, 2001; Cardoso et al. 2004a), diatoms (Oviatt et al. 1986; Beyene et al. 2009) or cyanobacteria (Barth, 2003; Watkinson et al. 2005; García and Johnstone, 2006).

The effects of algal mats on intertidal and subtidal soft-bottom communities have been extensively reported (e.g. Norkko and Bonsdorff, 1996a, b; Thiel and Watling, 1998; Raffaelli, 2000; Österling and Pihl, 2001; Wetzel et al. 2002). However, the potential impact of algal mats on benthic communities is largely unpredictable (Lauringson and Kotta, 2006), and generalizations are not straightforward (Everett, 1994). Differences in hydrodynamic environment, type of algae and the position of algal in the substrate (attached or floating) made comparisons difficult (Bolam et al. 2000). Most studies showed that the physical presence of mats alters the structural characteristics of the habitat. Initially, discrete algal patches might increase habitat complexity, providing available resources for herbivores (Norkko et al. 2000) and detritivores (Norkko and Bonsdorff, 1996a), refuge from predators (Aarnio and Matilla, 2000) and enhancing dispersal efficiency (Brooks and Bell, 2001) for some benthic species. Due to eutrophication of coastal waters, the role of algal mats has changed and their importance in structure and function of benthic habitats and faunal communities has increased (Raffaelli et al. 1998).

Overall, field experiments show that long periods of algal mats coverage in the sediment may affect negatively the zoobenthos, causing oxygen deficiency at the algal-sediment interface (Ellis et al. 2000), leading to mass mortality of benthic infauna (Norkko and Bonsdorff, 1996b; Lauringson and Kotta, 2006). Algal cover induces hypoxia or anoxia in the system and elevates the hydrogen sulfide levels, because of reduced light intensity plus an increasing in respiration and decomposition levels within the mat (Ford et al. 1999; Salovius and Bonsdorff, 2004; Engelsen et al. 2010). In anoxic conditions, chemical properties of sediment are altered (Lopes et al. 2000), with high accumulation of organic matter and nutrients (Hansen and Kristensen, 1997) as well as the release of phosphate and ammonium contents into the water column, sustaining even more the algal growth (Sundbäck and McGlathery, 2004).

The problem of increasing occurrences of algal mats has been recognized for some decades (Sundbäck and McGlathery, 2004). There is a vast literature considering their effects on benthos, but most studies are based on observations or descriptions of benthic species and macroalgae genera *Enteromorpha*, *Cladophora*, *Chaetomorpha* and *Ulva* (Chlorophyta) in sediments from temperate regions (e.g. Norkko and Bonsdorff, 1996a,b; Thiel and Watling, 1998; Bolam et al. 2000; Lopes et al. 2000; Raffaelli, 2000; Österling and Pihl, 2001; Cardoso et al. 2004a,b; Lauringson and Kotta, 2006). Basic information about ecological consequences of algal mats for soft-bottom benthic communities in tropical estuaries is still not well understood.

In general, the effects on macrobenthic communities subject to algal stress might be rapid, with behavioral (infauna leaves its protected position in the sediment and emerges at surface, making them an easy preys to epibenthic predators) (Ellis et al. 2000) and trophic responses (replacement of surface detritivores and suspension-feeders by burrowing detritivores) (Norkko and Bonsdorff, 1996b). Algal disturbances also include secondary impacts on macrofaunal consumers, such as the decline of birds (Lewis and Kelly, 2001) and fish species (Ford et al. 1999).

When the cause of disturbance, i.e. the formation of algal mats, ceases, it creates an opportunity for new individuals to become established (Ford et al. 1999). The recolonization (and recovery) trend depends, in part, on the resulting environmental alterations and the recruitment success from unaffected populations in surrounding areas (Guerrini et al. 1998; Deplege, 1999). Besides, habitat-complexity and the mobile components from benthic fauna are factors that influence community recovery (Chapman, 2007), either towards the original structure or to a different one (Guerrini et al. 1998; Rossi and Underwood, 2002). The seasonality of algal mats in some coastal ecosystems will imply in alteration in the behavior, use of habitat and survival of benthic species, depending on their functional group, motility and hypoxia’s tolerance (Ellis et al. 2000; Norkko et al. 2000).

The recolonization and recovery of a community post-disturbance have been particularly assessed in coastal environments (e.g. Beukema et al. 1999; Cristoni et al. 2004). In estuaries, the macrobenthos experience natural and human-induced disturbance at different scales (Günther, 1992; Flemer et al. 1999). Benthic infauna has been used successfully in spatial scale studies regarding hypoxia and anoxia (Morris and Keough, 2001; Van Colen et al. 2008), at meso- (between 1-10^4m²) and microscales (<1m²), with a direct relationship observed between the spatial scale of disturbance and successional dynamics (Zajac et al. 1998). In fact, recovery time of estuarine macrofauna from defaunated sediments by hypoxia/anoxia may range from very short time (few days) to long-term periods (several months), depending on disturbance scale (see Botter-Carvalho et al. 2011).

Field studies on benthic communities after disturbance show that patterns of recovery can be scale-dependent and influenced by site-specific environmental factors (Norkko et al. 2010). Amongst these local factors, changes in sediment properties might be related to excretion of extracellular polymeric substances (EPS) by mat-building cyanobacteria and diatoms (Yallop et al. 1994; Fenchel, 1998; Fenchel and Kühl, 2000; Stal, 2010), causing sediment compaction below the mat. Benthic organisms' response to this 'compaction effect' has received no attention. Besides, ecological data about recolonization and recovery of tropical macrobenthos taxa on disturbed sediments are still restricted (e.g. Faraco and Lana, 2003; Botter-Carvalho et al. 2011).

Manipulative field experiments represent a more precise method to assess species-specific interactions and to test hypothesis about cause-effect relationships (Lopes et al. 2000). These experiments have been used to study the impact of algal mats on benthic fauna, by adding algal material to undisturbed areas (e.g. Olabarria et al. 2007) or by removing algae from within affected areas (e.g. Everett, 1994), although both approaches seem to lead to similar conclusions (Raffaelli et al. 1998; Bolam et al. 2000).

This thesis aimed to further understands the effects of algal mats on soft-bottom macrobenthic community. In a tropical estuarine mudflat in the north coast of Pernambuco (Northeast Brazil), the occurrence of filamentous mat-forming algae and the development of an extensive algal coverage in the sediment created the opportunity to assess the possible impacts on benthic infauna. Observational and manipulative field experiments focused on the following objectives:

- To examine, *in situ*, whether sediment biogeochemistry and benthic macrofauna are affected by the presence of the algal mat (compared to bare sediments) and whether the physiological stage of the algae (live and decomposing algae) is an important factor in understanding algal-mat effects (CHAPTER I)
- To investigate through an experiment (95 days) the recolonization process of benthic macrofauna after removal of algal mat, considering the changes in physical features of sediment caused by the mat development (i.e. sediment compaction) and the extension of mat removal (i.e. spatial scale) (CHAPTER II)
- To assess the initial recolonization of benthic macrofauna after removal of algal mat as a function of distance from nearby unvegetated sediments, showing the existence of boundary effects created by the mat (CHAPTER III)
- To assess whether the recolonization of algal removal sediments by benthic macrofauna is affected by the season (CHAPTER IV)

CHAPTER I. Effects of algal mats on a tropical estuarine benthic system: Sediment biogeochemistry and macrofauna*

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I.1. Introduction

Eutrophication, defined as an increase of nutrient load into the system, is a worldwide environmental problem (Pusceddu et al. 2011). In many marine coastal areas, the occurrence of algal mats has become a recurrent feature (Österling and Pihl, 2001; Arroyo et al. 2006) that is clearly associated with eutrophication (Lewis and Kelly, 2001; Lewis et al. 2003; Holmer and Nielsen, 2007).

Increases in the frequency and magnitude of algal mats can affect biogeochemical cycles (Viaroli et al. 2005) and benthic macrofauna (e.g. Norkko and Bonsdorff, 1996a,b; Österling and Pihl, 2001; Bolam et al. 2000; Raffaelli, 2000; Arroyo et al. 2006). Mats of both macro- and microalgae uncouple sediment-water biogeochemical processes, reducing the export of nutrients from the sediment to the overlying water (Krause-Jensen et al. 1999; McGlathery et al. 2007). Therefore, algal mats provide additional sources of inorganic nutrients and organic matter to bottom layers of sediment (Krause-Jensen et al. 1996; Sundbäck et al. 2003; Sundbäck and McGlathery, 2004). The decomposition and mineralization of the mat often produce hypoxic or anoxic conditions, leading to the accumulation of sulfides and to faunal mortality (García-Robledo and Corzo, 2011).

The ecological consequences of algal mats for sediment biogeochemistry and benthic fauna have been described in field and experimental studies, mainly with drifting macroalgae (e.g. Norkko and Bonsdorff, 1996a,b; Arroyo et al. 2006; Lauringson and Kotta, 2006; Berezina and Golubkov, 2008). Very few studies have quantified sediment-associated compounds and fauna in compact, laminated mats such as microbial ones (e.g. Fenchel, 1998; García de Lomas et al. 2005). These microbial mats are usually confined to upper layers (photic zone) of the sediment (Sundbäck and McGlathery, 2004), actively changing the rheology of substrate surface (Schieber, 2007). The impact of these mats should be carefully examined in terms of sediment properties. However, the sediment has been evaluated merely as a nutrient source for the maintenance of drift algae (Sundbäck et al. 1996), which form loose-lying mats close to the sediment surface or floating on the surface of shallow water (Sundbäck and McGlathery, 2004).

Generalizations concerning the effects of algal mats are not straightforward (Everett, 1994) due to differences in the hydrodynamic environment, type of algae and the position of algae on the substrate (attached to the sediment or floating in the water) (Bolam et al. 2000). These observations raised the question of whether the development of compact mats attached to the sediment affects the benthic biogeochemistry and fauna similarly to the effects caused by drift mats. Moreover, in situ measurements present challenges, since live and decomposing algae can spatially coexist (García-Robledo and Corzo, 2011), so the mats may contains algae in more than one physiological stage.

The effects of different physiological stages of mats (live and decomposing algae) on the benthic system have not been studied until now, which impedes the development of predictive models of algal-mat impacts. Herein, we present the results of an in situ study conducted on a tropical estuarine mudflat where compact mats develop. We addressed two main questions: how are sediment biogeochemistry and benthic macrofauna affected by the presence of the algal mat (compared to bare sediments); and is the physiological stage of the algae (live and decomposing algae) an important factor in understanding algal-mat effects?

I.2. Material and Methods

I.2.1. Study area

The study was carried out on an estuarine mudflat (7º46.184’S and 34º52.926’W) at Itamaracá Island, adjacent to the Santa Cruz Channel Estuarine Complex (northern coast of the state of Pernambuco, Brazil), in September 2012 (Figure 1). Similarly to most estuaries, this ecosystem is exposed to multiple pressures from agro-industry, mainly sugarcane plantations, shrimp farming, intensive fisheries and urban growth (CPRH, 2006). Despite its eutrophic condition, the ecological quality of the Santa Cruz Channel, assessed using the AMBI index, has been classified as “slightly disturbed” (Valença and Santos, 2012).

Historical records of the distribution and taxonomic composition of benthic algae in this estuarine area are lacking. However, an overall increase in seasonal blooms of benthic filamentous algae on this mudflat has been observed since the end of the 1990s, with the intertidal zone covered by a continuous algal mat, especially during the rainy season (March through August; authors' personal observations). The development of algal mats attached to sediments in the study area was initially described by Botter-Carvalho (2007). These mats were composed of filamentous cyanobacteria (*Microcoleus chthonoplastes* Gomont and *Lyngbya* sp.) and diatoms (Santos et al. 2009). Dominant diatoms in the study area were *Cymbella* sp.1 and sp.2, *Navicula longa* Grunow, *Amphora augusta* Gregory and *Gomphonema* sp. (Trindade, 2007).

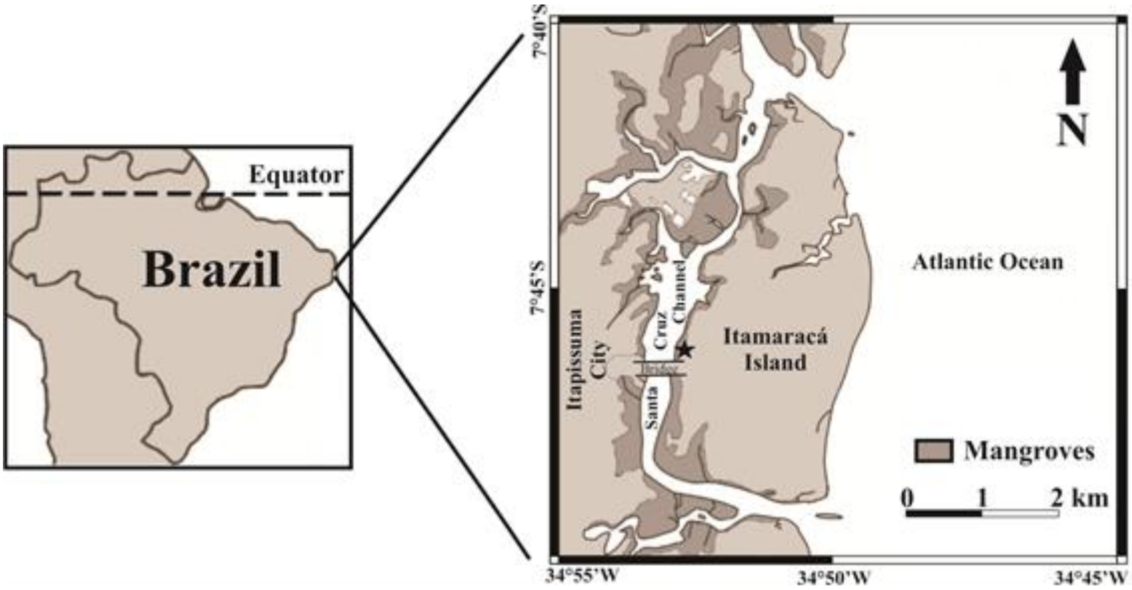


Figure 1. Itamaracá Island and the Santa Cruz estuarine system, showing the location of the estuarine mudflat (black star).

During the present study, due to a rainfall deficit (1.5 mm), the algal mat was reduced to discontinuous algal patches (Figure 2A) of varying sizes. These algal patches were composed of filamentous macroalgae from the family Ectocarpaceae (Phaeophyta) (TNV Reis; AJ Areces-Mallea and ALM Cocentino, personal observations), filamentous cyanobacteria, and diatoms. Despite the mixed composition with both macro- and microalgae components, features of the patches resembled those of classical microbial mats. The algae forming the patches were found in the uppermost layer (first millimeters). The patches were smooth, firm and fully attached to the sediment surface, with laminated sediment layers below (Figure 2B). The generic term ‘algal mat’ (as a layer of, usually, filamentous algae on marine and freshwater bottoms) is adopted in the text.

I.2.2. Sampling design

In the intertidal zone of the mudflat, two types of patches were visually recognized (Figure 2C-D): greenish-brown patches, without sediment coverage; and dark-brown patches, with signs of senescence, covered with sediment. In order to determine the relationship between visual types of patches and the physiological stage of the algae, measurements of chlorophyll-*a*, phaeopigments, proteins and carbohydrates from the first millimeters of the algal mat were obtained from three replicates of each type of patch. The algal patches were classified based on the physiological stage, as follows: Live algal patches (LAP) (greenish algal patches, without sediment coverage) and Decomposing algal patches (DAP) (dark-brown senescent algal patches, covered with sediment).

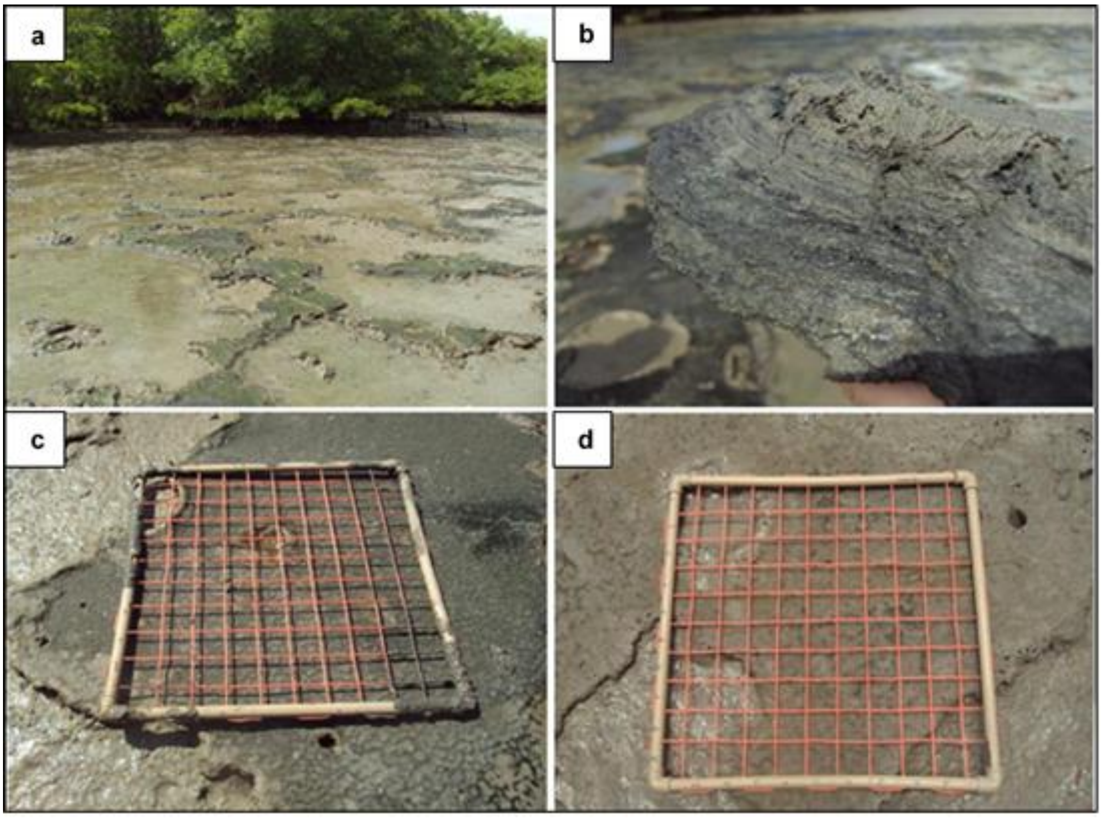


Figure 2. (a) Algal patches present in the study area. (b) Laminated sediment layers found below the algal patches (sediment layers inverted in photograph). (c) Live algal patch (LAP). (d) Decomposing algal patch (DAP). The patches were chosen based on size (scale used: 0.25 m² grid square).

The spatial distribution of the LAP and DAP did not follow any pattern on the mudflat, and both types occurred in similar frequencies. Fourteen individual patches were randomly selected, seven of each algal patch. The size of the patches was considered in the choice, and the sizes of the selected patches were between 0.25 m^2 and 1 m^2.

Coupled biotic and abiotic sediment samples were taken within the center of the patch (within LAP and within DAP) and outside each algal patch (outside LAP and outside DAP). The outside samples were taken at a standard distance of 0.5 m between the border of each type of algal patch and the matching outside point (bare sediment). Each sample (within LAP, outside LAP, within DAP and outside DAP) consisted of 7 replicates.

Samples for granulometry (corer area: 17 cm^2) and photosynthetic pigments (corer area: 1.1 cm^2) were obtained only for the 0-2 cm stratum. The sediment texture was analyzed based on the percentage of silt-clay content (Suguio, 1973). Photosynthetic pigments were extracted using 15 ml aqueous acetone, and the functional chlorophyll-*a* was measured in a spectrophotometer. A correction for phaeopigments was performed by acidification (HCl 0.1N). Both pigments were calculated in accordance with the equations of Lorenzen (1967), with some modifications (Colijn and Dijkema, 1981).

Sediment cores for organic matter, organic-compound composition and nutrient concentration analyses were collected with a cylindrical corer (area: 17 cm^2) and sectioned into strata of 0-2 cm and 2-5 cm. The amount of organic matter was estimated using the gravimetric method, with samples incinerated in a muffle furnace at 475 ºC for 4 h (Wetzel and Likens, 1990). Colorimetric methods were employed to determine organic compounds. Proteins were analyzed according to Smith et al. (1985), modified from Lowry et al. (1951), using Bicinchoninic Acid (BCA) reagent, with bovine serum albumin (BSA) as the standard. Carbohydrates were analyzed according to Gerchacov and Hatcher (1972), using glucose as the standard. Inorganic forms of nitrogen (total-N), phosphorus (total-P) and iron (total-Iron) were quantified using the Kjeldahl and Mehlich methods and atomic absorption spectrophotometry respectively (EMBRAPA, 1997).

For macrofauna, a larger corer was used (area: 41 cm^2) and samples were taken for the 0-2 cm, 2-5 cm and 5-10 cm sediment strata. All biological samples were washed through a 500-µm mesh, and the retained material was fixed in 4% formaldehyde and stained with rose bengal. The specimens were sorted, counted and identified to the lowest possible taxonomic level. The sampling procedure, corer size used and maximum sampling depth (10 cm) were chosen based on previous studies by

Botter-Carvalho et al. (2011) and Valença and Santos (2013) for the same estuarine area.

I.2.3. Statistical Analyses

Measurements of chlorophyll-*a*, phaeopigments, proteins and carbohydrates from the first millimeters of each type of algal patch were statistically compared by using the unilateral t-test considering the hypothesis that all these variables have higher values in the greenish-brown patches (LAP).

Analyses of Variance (ANOVA) were performed to determine whether the biogeochemical compounds in the sediment varied with the presence of algae (within vs. outside each algal patch) and the physiological stage (LAP vs. DAP). For organic matter, organic-compound composition and nutrient concentration, comparisons were also performed between the strata (0-2 cm vs. 2-5 cm). When a significant difference was found, Fisher's LSD test was used, and pairwise comparisons of main factor levels (presence of algae, physiological stage and strata) are shown in the figures. Homoscedasticity was verified with the Levene test (Sokal & Rohlf, 1997) and to correct heteroscedasticity the values of chlorophyll-a, phaeopigments, organic matter, proteins and carbohydrates were log(x+1) transformed due to asymmetric variable distributions whereas Total-N and Total-P data were arcsine square root transformed since values are shown in percentages. Variance analyses, LSD and Levene tests were conducted using the STATISTICA v12.0 program.

Macrofauna comparisons were assessed at the community level using multivariate techniques (Clarke and Gorley, 2006). Distance-based Permutational Multivariate Variance Analysis (PERMANOVA) was performed to test for differences considering the factors with the significance assessed with the Monte Carlo test (Anderson, 2005). BEST/BIOENV routine was applied to determine the possible association between environmental variables and macrofauna, based on the total sediment column. All multivariate analyses were conducted using the PRIMER v6.0+PERMANOVA statistical package. All statistical statements were based on a significance level of $\alpha = 5\%$.

I.3. Results

The abiotic variables obtained from the first millimeters of each type of algal patch (LAP and DAP) are reported in Table 1. The greenish-brown algal patches (LAP) showed significantly higher values of chlorophyll-*a* (t=3.52; *p*=0.012), phaeopigments (t=3.41; *p*=0.0135), proteins (t=13.30; *p*=0.0045) and carbohydrates (t=5.52; *p*=0.003) compared to dark-brown patches (DAP).

Table 1. Mean values (±SD) of chlorophyll-*a* (Chl-a), phaeopigment (Phae), proteins and carbohydrates in relation to the physiological stage of algae: Live algal patches (LAP) and Decomposing algal patches (DAP). Data were obtained from the algal fraction (uppermost layer of the sediment ≈ first millimeters).

	Chl-a ($\mu g/cm^2$)	**Phae ($\mu g/cm^2$)**	**proteins (mg/cm^2)**	**carbohydrates (mg/cm^2)**
Live algal patches (LAP)	33.71 (±11.4)	73.51 (±20.36)	9.87 (±2.27)	3.58 (±0.99)
Decomposing algal patches (DAP)	10.41 (±1.14)	32.15 (±5.04)	1.60 (±0.14)	1.37 (±0.04)

Silt-clay comprised 90% or more of the sediment (Figure 3). The presence of the algal patch increased the retention of fine sediments (Table 2), but significantly only for LAP (LSD test: *p*=0.004). Both sediment photosynthetic pigments were significantly higher within the algal patches than in the bare sediment (Figure 3 and Table 2). Sediment chlorophyll-*a* did not vary with the physiological stage of the patches. However, phaeopigments were higher within LAP than within DAP *p*=0.004), with differences for physiological stage x presence of algae (Table 2).

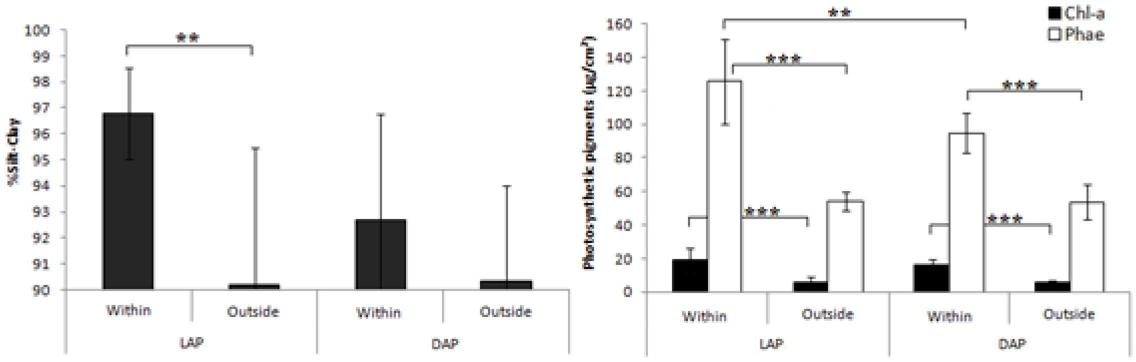


Figure 3. Mean values (±SD) of silt-clay content and photosynthetic pigments: chlorophyll-*a* (Chl-a) and phaeopigments (Phae) in relation to presence of algae (within and outside the patches) and physiological stage (LAP - Live algal patches and DAP - Decomposing algal patches). Significant differences (*p*) were determined using LSD tests (*p<0.05; **p<0.01; ***p<0.001).

Table 2. Two-way ANOVA results for silt-clay and photosynthetic pigments (chlorophyll-*a* and phaeopigments) in relation to presence of algae (PA) and physiological stage (PS). Note: Significant results are highlighted in bold.

Variable	Factor	df	MS	F (*p*)
Silt-clay	Presence of algae (PA)	1	141.0	**9.23 (0.005)**
	Physiological stage (PS)	1	27.1	1.78 (0.195)
	PA x PS	1	31.0	2.03 (0.167)
	Residual	24	15.3	
Chlorophyll-*a*	Presence of algae (PA)	1	1.64	**91.03 (0.000)**
	Physiological stage (PS)	1	0.007	0.39 (0.536)
	PA x PS	1	0.005	0.26 (0.618)
	Residual	24	0.018	
Phaeopigments	Presence of algae (PA)	1	0.65	**137.51 (0.000)**
	Physiological stage (PS)	1	0.027	**5.65 (0.026)**
	PA x PS	1	0.021	**4.60 (0.042)**
	Residual	24	0.005	

Additionally, the algal patches contributed to an increase in the organic-matter content in the 0-2 cm stratum compared to outside samples (Figure 4). Organic matter differed in terms of presence of algae and physiological stage x strata (Table 3). The first stratum (0-2 cm) of within and outside DAP had a higher organic content compared to within and outside LAP (*p*=0.03) (Figure 4).

Protein and carbohydrate contents of the sediment (Figure 4) were significantly higher within both algal patches than in the outside samples (Table 3). These organic compounds were more concentrated in the 0-2 cm than in the 2-5 cm layer. However, only carbohydrates varied, among all factors (Table 3). The difference in carbohydrate content regarding the physiological stage was restricted to the 2-5 cm layer (*p*=0.003) for both within and outside samples (Figure 4).

Nutrient concentrations tended to increase within the algal patches compared to the bare sediments (Figure 4). Total-N and iron became significantly higher only with the presence of the algae (Table 3). A reduction of total-N and iron contents was observed in both strata for LAP (within vs. outside). Nitrogen also differed between sediment strata (Table 3). The interaction of the presence of algae x physiological stage was important for total-N and total-P (Table 3). The total-N content was significantly higher in LAP than DAP, whereas the opposite distribution was found for total-P in both strata (Figure 4). Despite the differences in physiological stage, total-P content was the only parameter that did not vary between the sediments with or without algal cover (Figure 4).

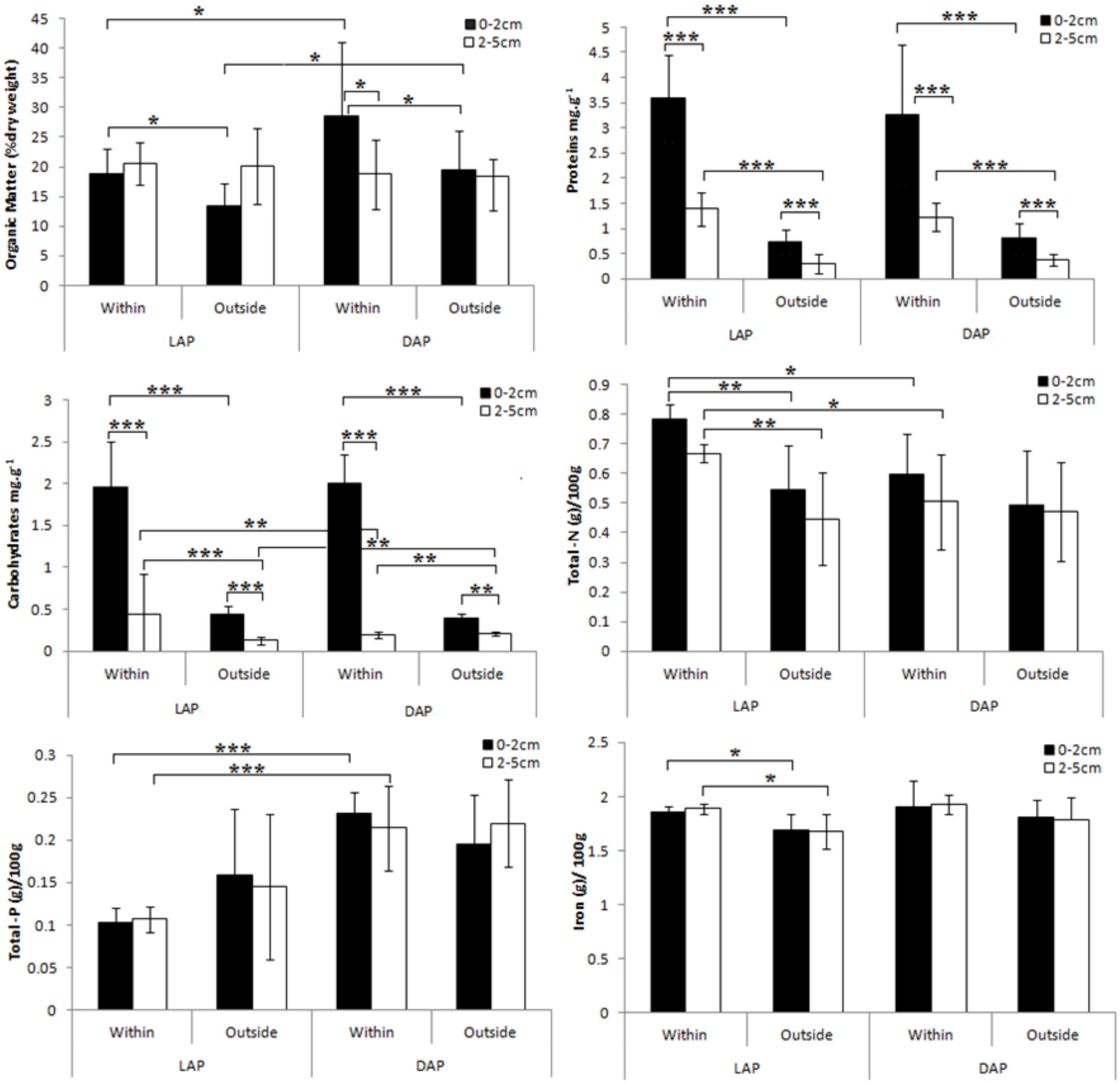


Figure 4. Mean contents (±SD) of organic matter, organic compounds (proteins and carbohydrates) and inorganic nutrients (total-N, total-P and iron) in relation to the presence of algae (within and outside the patches), physiological stage (LAP - Live algal patches and DAP - Decomposing algal patches) and strata (0-2 cm and 2-5 cm). Significant differences (*p*) determined using LSD tests (*p<0.05; **p<0.01; ***p<0.001).

Table 3. Three-way ANOVA results for organic matter and organic-compound composition (proteins and carbohydrates) and nutrient concentrations (total-N, total-P and iron) in relation to the presence of algae (PA), physiological stage (PS), and strata (S). Note: Significant results are highlighted in bold.

Variable	Factor	df	MS	F (*p*)
Organic matter	Presence of algae (PA)	1	0.105	**5.33 (0.025)**
	Physiological stage (PS)	1	0.046	2.35 (0.132)
	Strata (S)	1	0.0003	0.02 (0.899)
	PA x PS	1	0.0008	0.04 (0.845)
	PA x S	1	0.071	3.59 (0.064)
	PS x S	1	0.156	**7.94 (0.007)**
	PA x PS x S	1	0.0002	0.01 (0.917)
	Residual	48	0.019	
Proteins	Presence of algae (PA)	1	5.017	**243.01 (0.000)**
	Physiological stage (PS)	1	0.0000	0.00 (0.964)
	Strata (S)	1	1.904	**92.25 (0.000)**
	PA x PS	1	0.043	2.07 (0.157)
	PA x S	1	0.015	0.72 (0.401)
	PS x S	1	0.001	0.05 (0.824)
	PA x PS x S	1	0.0002	0.01 (0.932)
	Residual	48	0.024	
Carbohydrates	Presence of algae (PA)	1	2.740	**115.15 (0.000)**
	Physiological stage (PS)	1	0.0002	0.01 (0.919)
	Strata (S)	1	5.895	**247.78 (0.000)**
	PA x PS	1	0.176	**7.38 (0.009)**
	PA x S	1	0.776	**32.68 (0.000)**
	PS x S	1	0.0009	0.04 (0.846)
	PA x PS x S	1	0.287	**12.05 (0.001)**
	Residual	48	0.021	
Total-N	Presence of algae (PA)	1	0.348	**16.69 (0.000)**
	Physiological stage (PS)	1	0.136	**6.53 (0.013)**
	Strata (S)	1	0.113	**5.42 (0.024)**
	PA x PS	1	0.103	**4.93 (0.031)**
	PA x S	1	0.009	0.43 (0.516)
	PS x S	1	0.011	0.51 (0.478)
	PA x PS x S	1	0.002	0.72 (0.790)
	Residual	48	0.021	
Total-P	Presence of algae (PA)	1	0.005	0.97 (0.329)
	Physiological stage (PS)	1	0.203	**36.09 (0.000)**
	Strata (S)	1	0.00003	0.005 (0.943)
	PA x PS	1	0.023	**4.09 (0.048)**
	PA x S	1	0.0006	0.10 (0.751)
	PS x S	1	0.0005	0.09 (0.764)
	PA x PS x S	1	0.0063	1.12 (0.294)
	Residual	48	0.0056	
Iron	Presence of algae (PA)	1	0.332	**14.09 (0.000)**
	Physiological stage (PS)	1	0.086	3.64 (0.062)
	Strata (S)	1	0.0005	0.022 (0.883)
	PA x PS	1	0.021	0.90 (0.347)
	PA x S	1	0.004	0.18 (0.671)
	PS x S	1	0.0002	0.009 (0.924)
	PA x PS x S	1	0.0000	0.002 (0.965)
	Residual	48	0.024	

The benthic macrofauna was composed of 11 taxa (Table 4). The dominant taxa were, in descending order, the polychaete *Laeonereis culveri* Webster, 1879 (37% of total abundance), unidentified tubificid oligochaetes (23%), the polychaete *Capitella capitata* Fabricius, 1780 (18%) and unidentified juveniles of Nereid polychaetes (18%).

Table 4. List of taxa registered and their respective total mean densities(†) (individuals/m² ±SD) in relation to the presence of algae (within and outside the patches) and physiological stage (LAP - Live algal patches and DAP - Decomposing algal patches).

Taxon	**Within**	**Outside**	**Within**	**Outside**
	LAP		**DAP**	
Capitella capitata Fabricius, 1780	105 (±130)	5889 (±1838)		4599 (±1614)
Capitellides sp.		35 (±92)		
Heteromastus sp.		35 (±92)		
Laeonereis culveri Webster, 1879	209 (±296)	11742 (±4036)		9826 (±1508)
Nereis sp.		35 (±92)		
unidentified Nereididae polychaetes		3380 (±3996)		7108 (±5781)
Paraprionospio sp.				35 (±92)
Streblospio benedicti Webster, 1879		1045 (±942)		732 (±373)
Tellina sp.				70 (±119)
unidentified Tubificidae oligochaetes		8955 (±7829)		4564 (±3927)
unidentified Turbellaria		35 (±92)		

(†)Based on the total sediment column (0-10cm).

The presence of algae had a major impact on macrobenthos abundance. Both algal patches had a negative effect, with benthic depauperation (six individuals of *L. culveri* and three individuals of *C. capitata*) within LAP, and no fauna recorded in all strata within DAP.

Outside the algal patches (bare sediment), the macrofauna decreased in density with sampling depth, with most taxa concentrated in the upper sediment stratum (0-2 cm). This pattern was observed for the dominant taxa, i.e. *L. culveri* (7944 individuals/m² ±2776) and *C. capitata* (4652 individuals/m² ±1826) in 0-2 cm. Total densities of these polychaetes were, respectively, 2003 individuals/m² ±643 and 470 individuals/m² ±294 in the 2-5 cm stratum and 836 individuals/m² ±547 and 122 individuals/m² ±283 in the 5-10 cm stratum. Tubificids, however, were concentrated in the middle stratum (2-5 cm) with 4652 individuals/m² ±4523 against 854 individuals/m² ±1470 in the 0-2 cm stratum and 1254 individuals/m² ±1766 in the 5-10 cm stratum.

The fauna community within and outside algal patches was clearly separated with significant differences only for presence of algae (PERMANOVA: *pseudo*-F=114.62; *p*=0.0001).

The BIO-ENV procedure selected carbohydrates as the abiotic parameter that most closely matched the macrobenthic community structure (r_s= 0.734).

I.4. Discussion

The present study assessed the effects of compacted algal mats on a tropical estuarine benthic system. The presence of spatially coexisting live and decomposing algal patches allowed us to focus on comparisons within the mat and bare sediments, and also to take into account the physiological stage of the algae. Thus, the changes in sediment biogeochemistry and benthic macrofauna under both live and decomposing algal patches were evaluated.

I.4.1. Influence of benthic algal mats on sediment biogeochemistry

The sediment biogeochemistry was greatly affected under the algal patches. The algal patches enhanced retention of fine-grained and organic material, not only by the entangled framework of filamentous algae, but probably also by the production of mucilaginous secretions (extracellular polymer substances - EPS). The retention property observed in benthic microbial mats is mainly associated with mucilaginous secretions that trap and bind sediment particles, integrating them into a mat-bound surface (Gunatilaka, 1975; Malam Issa et al. 2001; Schieber, 2007; Stal, 2010). In some intertidal mudflat areas, the mucilage aids benthic microorganisms from the mat to attach firmly to their substrate and stabilizes the sediment surface, reducing its erodability (Decho, 2000; Stal, 2010). Microscopic investigations with benthic diatoms also revealed that extraction of the EPS could eliminate the retention properties that provide stability to the sediment (de Brouwer et al. 2005; Stal, 2010). Although the present study did not directly examine the production of mucilage by the algal patches, its presence is suggested by the characteristic laminations in the sediment layers found below them, as described in the literature (e.g. Jahnert and Collins, 2013).

Actively growing microbial communities also increase the chlorophyll-*a* content, as shown in the within-algal patch samples, and also by Pinckney et al. (1995) who in a one-year study, found that chlorophyll-*a* levels doubled in sediments with well-developed microbial mats in comparison to bare sand. In contrast, in drift algal mats, the chlorophyll-*a* concentration is generally higher in bare sediments than under the mats. Microphytobenthic photosynthesis at the sediment surface may decrease or even be completely prevented, due to light attenuation by the growth and deposition of drift algae (Krause-Jensen et al. 1996; Sundbäck et al. 2003; Sundbäck and McGlathery, 2004; Corzo et al. 2009; García-Robledo and Corzo, 2011). Although photosynthetic pigments in the algal mat allowed us to differentiate between live and decomposing algal patches (Table 1), the sediment results differed between the physiological stages only for phaeopigment concentrations per surface unit. The surplus concentration of phaeopigments in LAP is probably due to the greater retention of phytodetritus along with fine sediments (although this last parameter was not statistically distinct from the DAP). Contrastingly, the concentration of chlorophyll-*a* was not significantly different between LAP and DAP. Cartaxana et al. (2006) showed that the vertical distribution of the microphytobenthos in mud was highly stratified. These authors emphasized the importance of using thin sediment sections in order to correctly estimate algal biomass in intertidal sediments. This may explain the differences between results for sediment analysis (that used ~2 cm below the algae surface and thus mixes the superficial algal mat and buried older algal biomass) and samples collected to characterize the algal patch (based on only the superficial algal mat).

An increasing number of studies have devoted some attention to determining the biochemical composition of sediment organic matter (e.g. Dell'Anno et al. 2002; Pusceddu et al. 2009; Pusceddu et al. 2011), since this information allows an assessment of the origin, quality and availability of the deposited organic material (Manini et al. 2003). Here, the organic matter was composed of proteins and carbohydrates from the sediment; these are compounds of labile organic matter, i.e. the fraction of organic matter that is assumed to be assimilated by benthic consumers (Rodil et al. 2007; Pusceddu et al. 2009). However, very few studies (e.g. Joseph et al. 2008; Renjith et al. 2013) have assessed these compounds in estuarine sediments. Protein and carbohydrate levels in the algal samples fell within the ranges observed by Joseph et al. (2008) on the southwest coast of India (data converted to the same units; ranges for total proteins: 0.20 – 1.90 $mg.g^{-1}$ and 0.70 – 4.61 $mg.g^{-1}$, total carbohydrates: 0.25 – 1.23 $mg.g^{-1}$ and 0.51 – 2.46 $mg.g^{-1}$, in estuarine and mangrove sediments, respectively) and by Renjith et al. (2013) in Cochin Estuary on the west coast of India (data converted to the same units; ranges for total proteins: 0.024 – 2.76 $mg.g^{-1}$ and total carbohydrates: 0.17 – 6.34 $mg.g^{-1}$). Filamentous algae enriched the sediments with labile organic matter (Sundbäck et al. 2003; Holmer and Nielsen, 2007), as shown by significantly higher protein and carbohydrate contents within algal patches compared to outside samples. Although differences in biochemical composition are likely to be dependent on the input of algal material (Manini et al. 2003; Pusceddu et al. 2011), only carbohydrates allowed us to discriminate the physiological stage in the 2-5 cm stratum. As observed for photosynthetic pigments, the biochemical composition of organic matter differentiated the algal patches (Table 1), in contrast to the sediment results for the 0-2 cm stratum. The large amount of the sediment layer used in the analyses (~2 cm) in relation to the much smaller layer used to characterize the algal patches may also explain the lack of significance regarding the physiological stage, based on the biochemical data in the 0-2 cm stratum. This experimental procedure may have confounded the effect of the factor physiological stage on this layer for the sediment samples. All biological activity in the mat likely occurred in the uppermost millimeters of sediment (Fenchel, 1998). Thus, the sediment layer below the mat may possibly reflect the accumulation of decaying algae material (e.g. empty microbial cells, mucous matrix of cyanobacterial filaments, heterotrophic bacteria and fungi), contributing to the similar concentration of organic matter and organic compounds in this layer in the two physiological stages.

In the present study, the total-N and total-P contents varied as a function of the physiological stage of the algae, suggesting that the algal patch developed in response to nutrient availability. A general precept is that the relative abundance of macronutrients such as nitrogen and phosphorus controls algal growth, productivity and collapse (Kuffner and Paul, 2001; Sundbäck et al. 2003; Viaroli et al. 2005). Algal mats enhance nutrient cycling in the sediments in response to the input of organic matter, regardless of their position in relation to the substrate (Sundbäck and McGlathery, 2004). Both macro- and microalgae take up and store nitrogen for their immediate physiological needs (Stal, 1995; Sundbäck et al. 1996; Viaroli et al. 2005; Corzo et al. 2009). Thus, the amount of total-N in the LAP likely resulted from the accumulation of this nutrient. Thick mats lying on the sediment surface usually generate hypoxic conditions, resulting in the release of phosphorus (Sundbäck and McGlathery, 2004) in the form of algal debris (Corzo et al. 2009). The incorporation of this algal debris in the sediment surface could explain the higher levels of total-P in the DAP. Camacho and de Wit (2003) found in mesocosm experiments that the effect of the nutrient supply regimes (inorganic nitrogen and phosphorus) on a microbial mat was linked to changes in the taxonomic structure of the microphytobenthos, shifting from diatom dominance with the addition of nitrogen to cyanobacteria dominance with phosphorus inputs. Although in the present study the dominance of these microphytobenthic groups was not assessed in both types of algal patches, the observed differences in microphytobenthic pigments and biochemical composition of the algae (Table 1) reinforce the distinction of the patches based on the physiological stage rather than the dominant microphytobenthic group.

Despite the differences with respect to physiological stage, total-P levels did not differ between sediments with or without algal patches, which is attributable to the great differences between the algal samples (total-P differences were much higher between the LAP and DAP, than between within and outside each type of algal patch). In contrast, iron became more significant with the presence of algae, regardless of the physiological stage. Iron is an essential component of photosynthetic and respiratory electron transport chains, and is associated with the stimulation of metabolic processes (Watkinson et al. 2005; Ahern et al. 2008).

I.4.2. Benthic mats as structuring factor for macrobenthic community

The results clearly showed that the macrofauna declined markedly in both types of algal patches, with strong effects regardless of the physiological stage. Contrastingly, both meiofauna and harpacticoid copepod species exhibited significant differences between live and decomposing algal patches (Neves et al. unpublished data). Negative impacts have also been recorded on macrobenthic communities in estuaries in the presence of drift macroalgal cover (e.g. Norkko and Bonsdorff, 1996a,b; Bolam et al. 2000; Österling and Pihl, 2001; Berezina and Golubkov, 2008). In these areas, decomposition of small amounts of algal material may stimulate the production of many deposit-feeders, but larger amounts reduce oxygen concentration and cause faunal migration or mortality (Norkko and Bonsdorff, 1996a; Hansen and Kristensen, 1997).

The effects of algal mats on benthic fauna communities are associated with the development of the mat. When drifting algae are abundant, oxygen deficiency in the sediment below them leads to the impoverishment of benthic fauna, resembling the effects of organic enrichment (e.g. Norkko and Bonsdorff, 1996a,b; Bolam et al. 2000; Lauringson and Kotta, 2006). In contrast, few studies have quantified benthic fauna in microbial mats (Fenchel, 1998; Pinckney et al. 2003; García de Lomas et al. 2005; Al-Zaidan et al. 2006), with no direct relationship to oxygen. García de Lomas et al. (2005) compared three microbenthic communities (non-cohesive sediment, fluffy microbial mat and compacted-laminated microbial mat) using qualitative and quantitative analyses of microalgae, meio- and macrofauna composition. The most compact mat, dominated by the filamentous cyanobacterium *Microcoleus chthonoplastes,* had the highest photosynthetic biomass and highest fluxes of oxygen and sulfide, while meio- and macrofauna were nearly absent. The authors concluded that the benthic fauna abundance was controlled by the hydric stress conditions observed in the area, which in turn contributed to the maintenance of the compact mat structure. In our study all macrobenthic samples (within and outside algal patches) were collected in the same estuarine intertidal zone and it was the compact mat that developed in the study area that controlled benthic fauna abundance, by changing sediment properties and probably creating adverse conditions for the macrofauna to remain there. Experiments considering sediment compaction within and outside algal patches were performed a posteriori in the study area with a hand-held penetrometer. These data showed that sediment was clearly more compact within the patches (DAP: 0.43 $kg.cm^{-2}$ ± 0.03 and LAP: 0.32 $kg.cm^{-2}$ ± 0.03) than outside (bare sediment: 0.14 $kg.cm^{-2}$ ± 0.07), with significant differences observed among all conditions ($F_{2,18}$= 58.96; p=0.000; LAP x DAP: p=0.002; LAP x outside: p=0.0001; DAP x outside: p= 0.0001). Although not analyzed in the present study, it cannot be ignored possible toxic effects in benthic communities subjected to higher organic loads described in literature as a result of end products of organic matter degradation associated with the microbial breakdown processes (Tagliapietra et al. 2012).

Meio- and macrofauna can control the development of microbial mats in coastal environments, by reducing benthic microalgal producers through grazing (Fenchel, 1998; Pinckney et al. 2003; García de Lomas et al. 2005; Al-Zaidan et al. 2006). An experimental microscosm study also found that microbial mats developed only when grazers were excluded (Fenchel, 1998). No macrofaunal grazer was recorded on the algal patches; the few individuals found in LAP were the polychaetes *L. culveri* and *C. capitata,* both classified as deposit-feeders (Pearson and Rosenberg, 1978; Venturini and Tommasi, 2004). Besides, macrobenthos from the samples outside the algal patches was dominated by taxa tolerant to hypoxic-anoxic environments, which are typical of organically enriched sediments (Norkko and Bonsdorff, 1996a,b; Bolam et al. 2000; Lauringson and Kotta, 2006). The polychaetes *L. culveri* and *C. capitata* were abundant in the upper sediment layer, following a pattern similar to that reported by Botter-Carvalho (2007) in a nutrient-addition experiment performed in the same mudflat. These opportunist species (Zajac and Whitlatch, 1982) normally occupy surface sediments (Posey et al. 2002) and are commonly dominant in estuaries on the

Pernambuco coast (Valença and Santos, 2013). The tubificid oligochaetes were concentrated in the middle strata. These worms feed head down in the sediment, living as subsurface deposit feeders (Gaston et al. 1998). In Botter-Carvalho (2007) nutrient-addition experiment, benthic macrofauna was assessed in 0-2cm, 2-5cm, 5-10cm and 10-20cm strata, with tubificids concentrated mainly in the intermediate strata (2-5cm and 5-10cm).

Multivariate comparisons of macrofauna data confirmed only the distinction between algal patch areas and bare sediment, because most species and individuals were absent below the mats.

Although the quantity and quality of organic matter were different between the sediments with and without algal coverage, the BIOENV routine associated only carbohydrates with the macrobenthic fauna. There is a strong relationship between food availability and the biochemical composition of organic matter (Rodil et al. 2007). The response of consumers to an increased organic-matter supply is influenced more by the quality (e.g. bioavailability) of organic matter than by its bulk concentration (Pusceddu et al. 2011), particularly in naturally enriched organic sediments, such as occur in tropical estuaries. Even so, higher levels of carbohydrates were found in the algal patches and appeared not to be exploited by the macrofauna. Pusceddu et al. (2009) explained that in eutrophic systems, where large amounts of organic matter accumulate at the subsurface, potentially labile molecules (proteins, carbohydrates) may be encapsulated due to an increase in the complexation of buried organic molecules with the inorganic matrix, thus making them less ‘available’ for heterotrophic nutrition. The accumulation of mucilage produced by the algal mat may increase this encapsulation, although further studies of this possibility are required.

I.5. Conclusion

The interactions among algal mats, macrobenthic fauna and biogeochemistry appear to be complex, often depending on the characteristics of the mat, such as the composition of the algae and its position in relation to the sediment. In the Santa Cruz Estuarine Complex, the input of organic wastes and inorganic nutrients from domestic effluents and agro-industrial activities may enhance coastal eutrophication, increasing the primary productivity and organic matter exported to the benthic system. These conditions favor the development of compacted mats, which influence both the sediment biogeochemistry and the benthic macrofauna.

The present in situ study was spatially and temporally constrained to a single sampling occasion, and caution is needed in drawing conclusions for larger scales. Nevertheless, the results showed that the effects of compacted mats on environmental quality depends on the physiological stage of the algae, and this should be considered in future studies. There is a need to explore the impact of compacted mats on sediment biochemistry and their effects on the accumulation and bioavailability of organic and inorganic compounds in the benthic system. Furthermore, the presence of algal patches (irrespective of their physiological stage) caused extreme adverse effects on benthic organisms, with a strong reduction (within LAP) or exclusion (within DAP) of tolerant species that are commonly found in hypoxic-anoxic sediments. These results suggest that compacted mats are likely to significantly reduce macrofauna densities, as observed in the higher algal deposition from drift mats. Although algal cover played an important role in structuring benthic assemblages, the mechanisms by which these effects are brought about are poorly understood (Bolam et al. 2000), especially in compacted mats. These present findings underline the necessity of including monitoring and management of algal mats as a critical part of conservation efforts in tropical estuaries.

CHAPTER II. Macrofaunal recolonization on estuarine sediments after experimental removal of algal mats

II.1. Introduction

The seasonal occurrence of mat-forming algae has become a recurrent event in many marine coastal areas (Österling and Pihl, 2001; Franz and Friedman, 2002), increasing their frequency and dimension over the last decades (Wetzel et al. 2002).

In estuarine systems, dense algal-covered sediments experience periods of oxygen depletion and develop toxic hydrogen sulfide because of bacterial activity (Hull, 1987; Lewis and Kelly, 2001; Lewis et al. 2003). In addition, the presence of the mat also changes physical sediment features (e.g. erodability, cohesion, etc.) providing a stable geochemical interface underneath. In muddy substrates, the production of copious amounts of sticky extracellular substances binds sediment particles, forming laminated sedimentary structures below the mat (Schieber, 2007). All these physical and chemical changes impose significant effects on macrofaunal communities and local ecosystem processes as a whole. Thus, the algal mat represents an important source of disturbance on benthos (see Norkko and Bonsdorff, 1996a,b; Ellis et al. 2000; Österling and Pihl, 2001; Lauringson and Kotta, 2006, among others), often resulting in severe modification of community structure towards smaller, pollution tolerant, opportunistic taxa (Franz and Friedman, 2002).

Field benthic studies have shown that the rates of recolonization and recovery are dependent on scale or disturbance size (Smith and Brumsickle, 1989; Whitlatch et al. 1998; Norkko et al. 2006). Besides, site-specific environmental characteristics (Thrush et al. 1996; Zajac et al. 1998; Norkko et al. 2010) as well as biotic factors such as the availability of individuals and their substrate preferences (Guerra-García and García-Gomez, 2009) might influence on recolonization. Thus, the response to disturbed habitat is frequently associated to habitat conditions, to species’ life history and to mobility patterns (Whitlatch et al. 1998).

In situ experiments are considered useful tools for the solution of ecological questions (Guerra-García and García-Gómez, 2009). Manipulative field experiments have been used to examine the effects of algal mats on invertebrates and most have used the addition of algae material in unaffected areas (Hull, 1987; Lopes et al. 2000; Olabarria et al. 2007), especially where there was no previous history of algal cover

(Lewis et al. 2003). The present study adopted a different design, by removing algae from a mudflat area where cohesive mats occur, leading to biostabilization of the sediment surface. Despite that, both approaches seem to generate similar conclusions (Raffaelli et al. 1998; Bolam et al. 2000), in terms of assessing the impacts of algal mats on benthos.

Herein, the algal removal experiment was used to assess the recolonization (and possible recovery) of benthic macrofauna following mat displacement (simulated by removal of the algal mat). Specifically, this study focused on whether (1) changes in physical features of sediment caused by the mat development (i.e. sediment compaction) and (2) the extension of mat removal (i.e. spatial scale) influence benthic species' colonization pattern after disturbance ceases (represented by the algal removal). It was tested the null hypothesis (H_0) that macrofauna recolonization and recovery are not affected by either sediment compaction or spatial scale. Over the course of the experiment macrofauna and sediment data in the algal-removed areas are compared to nearby unvegetated sediment.

II.2. Material and Methods

II.2.1. Study area

The study area lies on an estuarine mudflat (7º46.184'S and 34º52.926'W), situated at Itamaracá Island, adjacent to the Santa Cruz Channel Estuarine Complex (northern coast of Pernambuco State, Brazil). In the last decade, it has been observed that benthic algae form gradually increasing cohesive mats (Botter-Carvalho, 2007), probably associated with eutrophication and rainfall regime (authors' personal observations).

In September 2013, when the experiment began, most of the upper and middle zones of the mudflat was covered by continuous healthy-looking algal mat, with the exception of a flat zone between the mat and the mangrove trees, placed a few meters away. In this flat zone, filamentous algae could be found, but with no algal mat development. During the present study the algal mat was composed by cyanobacteria *Microcoleus chthonoplastes* Gomont and *Oscillatoria* sp., diatoms and a sporadic record of macroalgae *Solieria filiformis* (Kützing) P.W. Gabrielson (ALM Cocentino, personal observations). The generic term 'algal mat' (as a layer of usually filamentous algae on marine or freshwater bottoms) was adopted in the text.

II.2.2. Experimental design and Sampling

The experimental design area was about 400m². It consisted of fifteen square areas (ten with 1m² and five with 0.25m² surface areas) established along the continuous algal mat, parallel to the tide edge. Besides them, an uncovered sediment area (Flat zone) where five units of unvegetated sediment, which naturally don't have algal mat (named as No algal mat treatment) were randomly sampled (Figure 1).

In all square areas, the uppermost layer of sediment (formed by the algal layer) was carefully removed with plastic spatulas (Figure 2A-C). In five 1m²-algal removed squares, the sediment below the algal layer was also mechanically reworked using a metal rake, in order to break the compact nature of the sediment created by the mat (Figure 2B). In the remaining squares (the other five 1m² and five 0.25m²) only the algal layer was removed (Figures 2A and 2C, respectively). No standardized distance was used among the square areas, but the minimum measure between two square areas was 1.5m. A randomized design was chosen in order to prevent any gradient in sedimentological parameters or algal cover from masking the effects of treatments in the analyses.

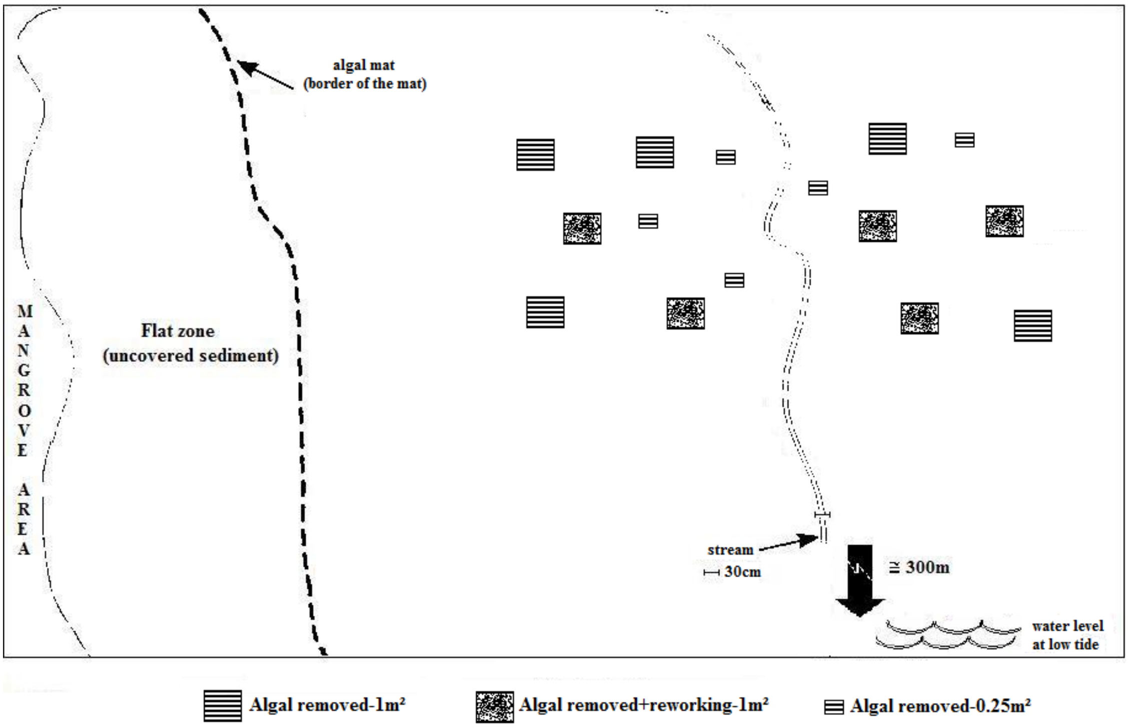


Figure 1. Schematic design of treatments (Algal removed-1m²; Algal removed+reworking-1m² and Algal removed-0.25m²) and the flat zone (where samples from No algal mat treatment were obtained) location in the estuarine mudflat.

Samples for sediment parameters and macrofauna were taken in four treatments: No algal mat (NA); Algal removed-1m² (AR-1m²); Algal removed+reworking-1m² (AR+R-1m²) and Algal removed-0.25m² (AR-0.25m²). Each square or unit represented a replicate from each treatment. The maintenance of the algal removed sediments was checked every two weeks, avoiding the re-establishment of the mat within the experimental treatments.

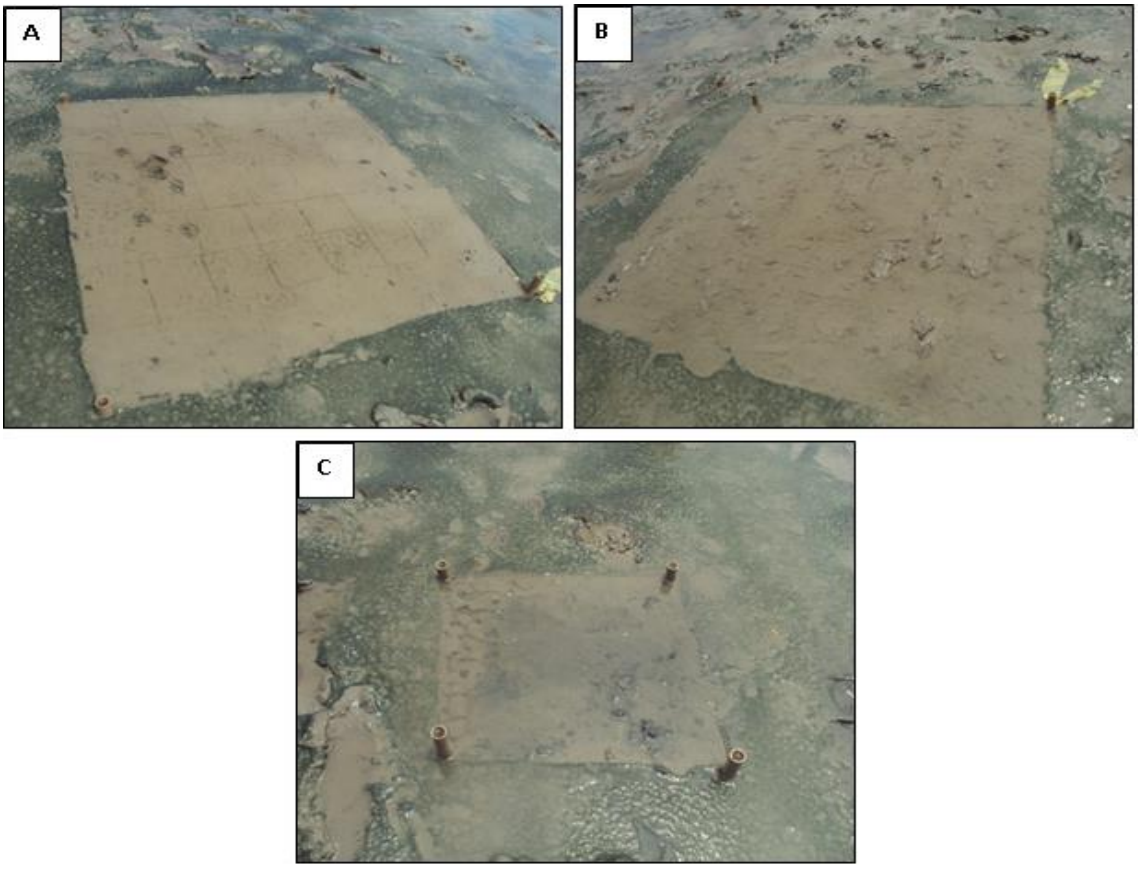


Figure 2. Algal removal treatments: (A) Algal removed-1m² (AR-1m²); (B) Algal removed+reworking-1m² (AR+R-1m²) and (C) Algal removed-0.25m² (AR-0.25m²). Note the algal mat surrounding the treatments.

The experiment was carried out from September to December 2013. Samplings were taken in seven days (0, 15, 30, 45, 60, 75 and 95 days). The first day of experiment (Day 0) was sampled before the algal layer has been removed. The last day of experiment (Day 95) was chosen following Botter-Carvalho et al. (2011) results for macrofauna abundance recovery (3 months).

Daily rainfalls from September to December 2013 were obtained with open data from APAC (Pernambuco Agency for Water and Climate) for Itamaracá station.

Cumulative bi-weekly rainfalls exceeded 60mm until Day 15 of experiment, decreasing to less than 10mm on Day 30. After that, values slightly increased towards 25mm until last day of experiment.

The sampling procedure (cylindrical corers area and sampling depth) and the processing protocols for sediment parameters and macrofauna are summarized in Table 1. Silt-clay content and nutrients concentration (total-nitrogen, total-phosphorus and iron) were assessed only for days 0 and 95. Microphytobenthic pigments, organic matter, organic-compound composition (proteins and carbohydrates contents) and *in situ* redox potential (Eh) data were assessed for all days of the experiment. Water content of sediment from AR-1m² and AR+R-1m² treatments were determined by weight loss on drying at 80°C.

Macrofauna samples were collected with a cylindrical corer and subsequently sifted through a 500µm mesh. Then, the retained material was fixed in 4% formaldehyde and stained with rose Bengal. Sorting and counting were performed under stereomicroscope and specimens identified to the lowest possible taxonomic level.

Table 1. Sampling procedure and processing protocols for sediment parameters and macrofauna.

Parameter (unit expressed)	**Sampling procedure (corer area, depth)**	**Method/Equipment**	**References**
Silt-clay content (%)	17cm², 0-2cm	Wet Sieving	Suguio (1973)
Total-nitrogen (%)	17cm², 0-5cm	Kjeldahl method	EMBRAPA (1997)
Total-phosphorus (%)	17cm², 0-5cm	Mehlich method	EMBRAPA (1997)
Iron (%)	17cm², 0-5cm	Atomic absorption Spectrophotometry	EMBRAPA (1997)
Microphytobenthic pigments (µg/cm²)	1.13cm², 0-2cm	Spectrophotometer	modified from Colijn and Dijkema (1981) and Lorenzen (1967) equations
Organic matter (%)	17cm², 0-5cm	Incineration	Wetzel and Likens (1990)
Proteins content (mg/g)	17cm², 0-5cm	Spectrophotometer	Smith et al. (1985)
Carbohydrates content (mg/g)	17cm², 0-5cm	Spectrophotometer	Gerchacov and Hatcher (1972)
Redox potential (Eh) (mV)	measured *in situ*(†)	Platinum electrodes	APHA (1989)
Macrofauna (ind./m², number of species and community structure)	41cm², 0-10cm	described in the text	Botter-Carvalho et al. (2011); Valença and Santos (2013)

(†) in surface (2cm) and bottom (10cm) layers

II.2.3. Statistical Analyses

Analyses of Variance (ANOVA) were performed to determine whether the sediment parameters varied in terms of sediment compaction (AR-1m² vs. AR+R-1m²) and spatial scale (AR-1m^2 vs. AR-0.25m^2). Besides, comparisons were also performed between the algal removal treatments and unvegetated sediment (NA).

The breaking of sediment compaction created by mechanical reworking was validate through the statistical analysis (ANOVA two-way) of sediment water content from AR-1m^2 and AR+R-1m^2 treatments and days (0, 15, 30, 45, 60, 75 and 95). ANOVA one-way was used to analyze sediment parameters considering treatment-related differences prior to experimental removal (Day 0). Due to absence of AR-0.25m^2 data from Day 0, silt-clay content and inorganic nutrients were tested for treatments (NA, AR-1m^2, AR+R-1m^2 and AR-0.25m^2) only for Day 95.

Repeated-measure ANOVA tests compared redox potential, organic matter, proteins, carbohydrates, chlorophyll-*a* and phaeopigments among the treatments (NA, AR-1m^2, AR+R-1m^2 and AR-0.25m^2) over days (15, 30, 45, 60, 75 and 95). The absence of AR-0.25m^2 data from Day 0 also leaded to exclusion of this day from these analyses. Redox potentials (Eh) were also compared between surface (2cm) and bottom (10cm) layers. Sediment data were transformed to meet ANOVA assumptions. When a significant difference was observed, Tukey HSD tests were applied (Zar, 1996). ANOVA and Tukey tests were performed using the STATISTICA v7.0 program.

Abundance (individuals), density (individuals/m^2) and richness (S) from all treatments (NA, AR-1m^2, AR+R-1m^2 and AR-0.25m^2) were used to estimate univariate biological descriptors. Density and richness results were compared among all treatments with ANOVA and Tukey tests. Besides ANOVA, a paired t-test was also performed to compare paired observations between AR-1m^2 vs. AR+R-1m^2 (sediment compaction) and between AR-1m^2 vs. AR-0.25m^2 (spatial scale) using means of sampling days (15, 30, 45, 60, 75 and 95). Macrofauna data were also analyzed using multivariate techniques. A MDS ordination was carried out using the Bray-Curtis similarity measure with the log (x+1) transformed data (Clarke and Gorley, 2006). A virtual dummy variable (+d) was included because of the large number of zero values (Clarke et al. 2006). PERMANOVA (n° of permutations: 9999) was applied to test for effects of sediment compaction, spatial scale and sampling days, with significance given by the Monte Carlo *p*-value (Anderson, 2005). All multivariate analyses were conducted using the PRIMER v6.0+PERMANOVA statistical package.

For all analyses, differences were accepted as significant at $p<0.05$.

II.3. Results

II.3.1. Sediment parameters

Water content was higher in the sediments mechanically reworked (AR+R-1m²) when compared to those only with removal of the algal layer (AR-1m²) for most days (treatments x days: $F_{1,6}$= 2.47; *p*<0.05). However, differences between AR+R-1m² and AR-1m² were statistically found at Day 15 (Tukey test: *p*<0.01).

At the start of the experiment (Day 0), sediment parameters in the algal treatments presented higher values when compared to unvegetated sediments (NA). Sediment analyses revealed significant differences among treatments, except for total phosphorus and chlorophyll-a (Table 2). Tukey tests confirmed the differences between the algal removal treatments (AR-1m² and AR+R-1m²) and NA, but for all sediment parameters, AR-1m² and AR+R-1m² were not statistically distinct.

Table 2. One-way ANOVA results from Day 0 for treatments on sediment parameters. *p<0.05; **p<0.01; ***p<0.001; n.s.: not significant.

Sediment Parameters	Factor: treatments$^{(\dagger)}$ (NA, AR-1m² and AR+R-1m²)	
Day 0	DF	F
Silt-clay	2	19.67***
Total nitrogen	2	27.22***
Total phosphorus	2	2.13 n.s.
Iron	2	20.19***
Organic matter	2	57.23***
Proteins	2	8.57**
Carbohydrates	2	8.11**
Chlorophyll-a	2	3.03n.s.
Phaeopigments	2	44.62***

$^{(\dagger)}$ absence of AR-0.25m² data from day 0

Three months later (Day 95), there was an increase on silt-clay content in AR-1m², AR+R-1m² and AR-0.25m² after the removal of the algal layer, enhancing the variation in relation to NA (Figure 3). Conversely, in the removal treatments, the levels of nutrients slightly dropped for total-nitrogen, whereas they remained close to their initial values (Day 0) for iron and total-phosphorus (Figure 3). Although differences were found for silt-clay ($F_{3;16}$= 58.50; *p*<0.001) and inorganic nutrients (total-nitrogen: $F_{3;16}$= 6.84; *p*<0.01 and iron: $F_{3;16}$= 72.66; *p*<0.001) among treatments for Day 95, no significant differences related to sediment compaction or spatial scale were observed.

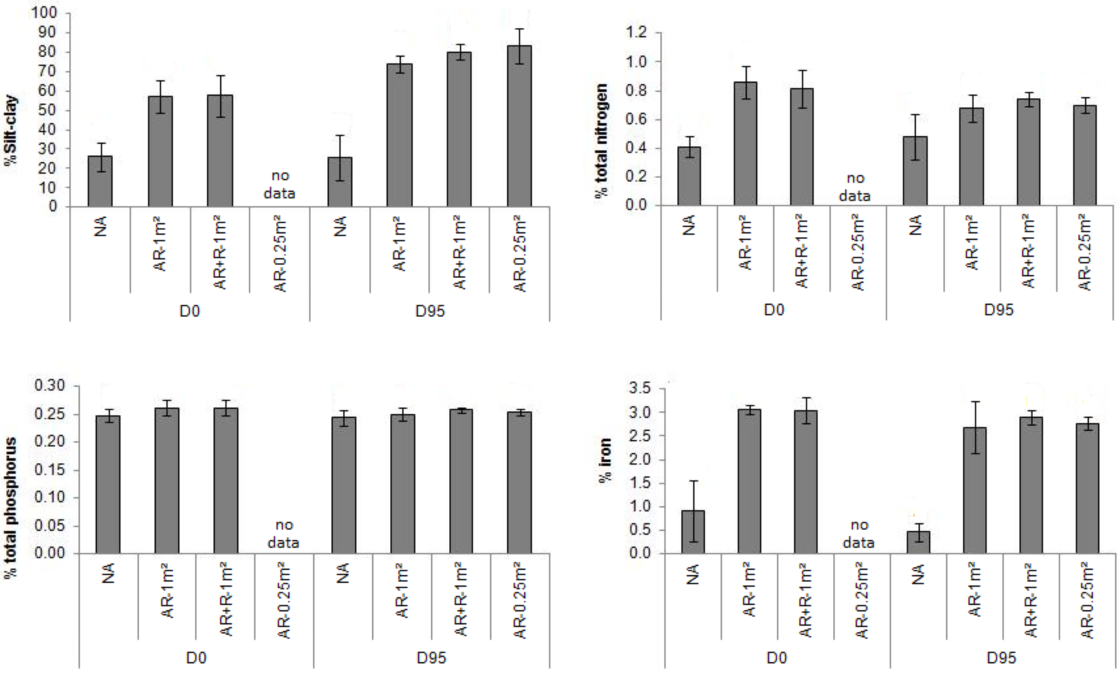


Figure 3. Mean values (±SD) of silt-clay content (%Silt-clay) and nutrients concentration (%total nitrogen, %total phosphorus and %iron) in relation to treatments: No algal mat (NA), Algal removed-1m² (AR-1m²), Algal removed+reworking-1m² (AR+R-1m²) and Algal removed-0.25m² (AR-0.25m²) and days: 0 (D0) and 95 (D95).

The redox potential (Eh) values indicated that the unvegetated sediments (NA) had more reduced conditions when compared to algal treatments (Figure 4). Sediments without (NA) and with algal mat (AR-1m²) were distinct before its removal (Day 0, Treatment: $F_{1,16}$= 43.87; $p<0.001$), in both surface (NA x AR-1m²: $p<0.01$) and bottom (NA x AR-1m²: $p<0.001$) layers, but not between the sampling depths (surface x bottom) within each treatment. Temporally, Eh values from surface layers of removal treatments tended to increase until Day 45, with values reducing in all treatments (except for AR+R-1m²). Conversely, Eh values from bottom layers of removal treatments gradually decreased towards NA values. Repeated measures ANOVA detected differences in treatments ($F_{18,77}$= 7.67; $p<0.001$) and sampling depths ($F_{6,27}$= 7.99; $p<0.001$). Again, these differences were restricted to removal treatments and unvegetated sediment (NA) in all days and depths. However, in Day 95, the removal and NA treatments were not distinct in the bottom layer.

Overall, the contribution of the algae to sedimentary organic matter, organic compounds (proteins and carbohydrates) and microphytobenthic pigments (chlorophyll-*a* and phaeopigments) is observed by reduced values in the experimental areas after fifteen days of algal removal, except for carbohydrates content from AR-1m² treatment (Figure 5A-E).

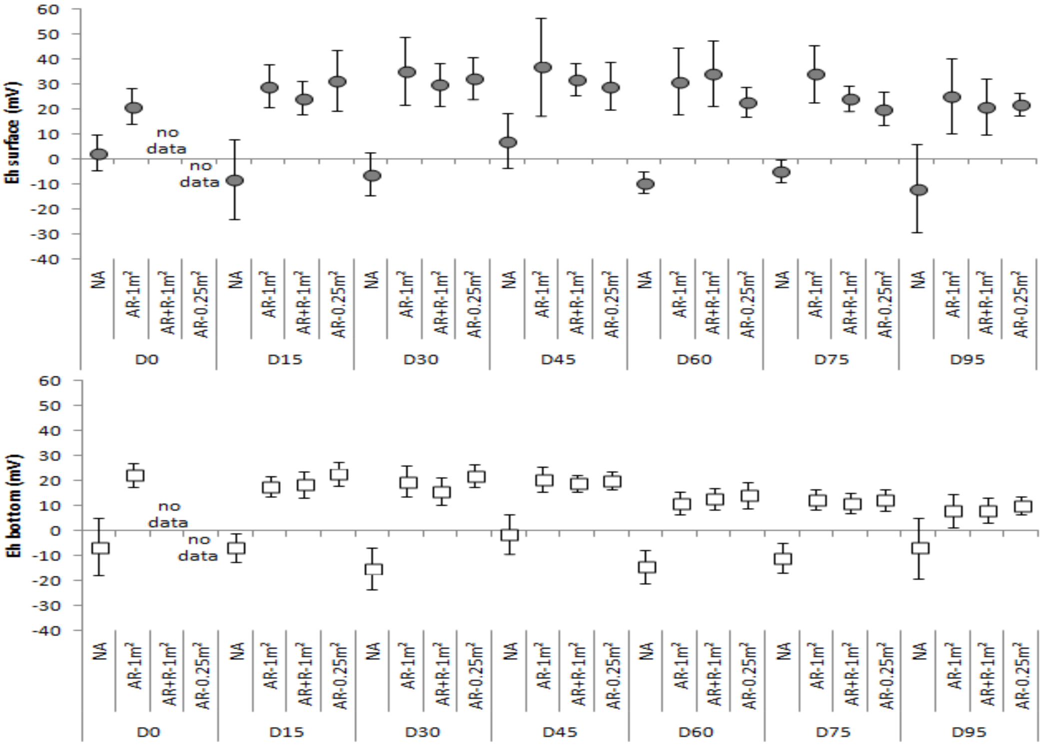
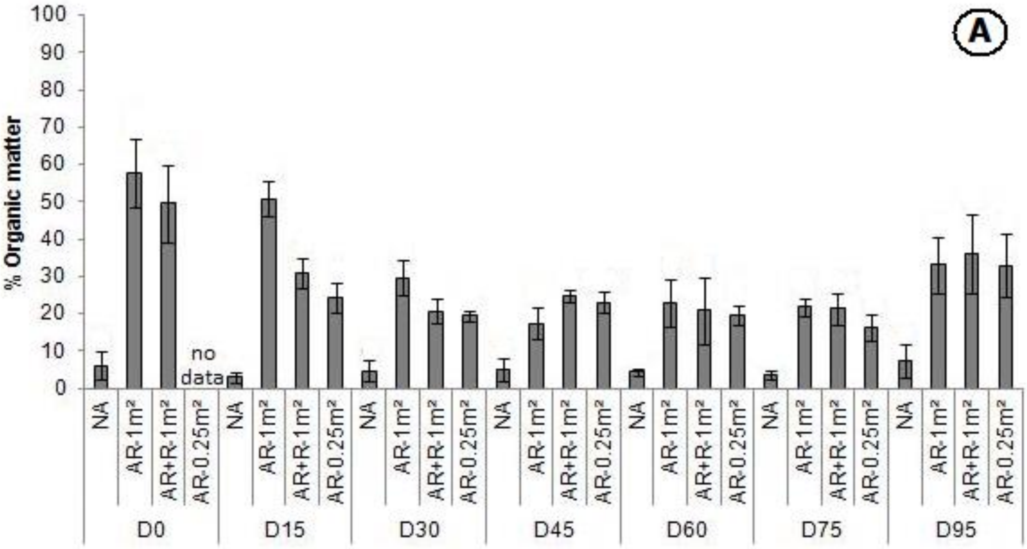
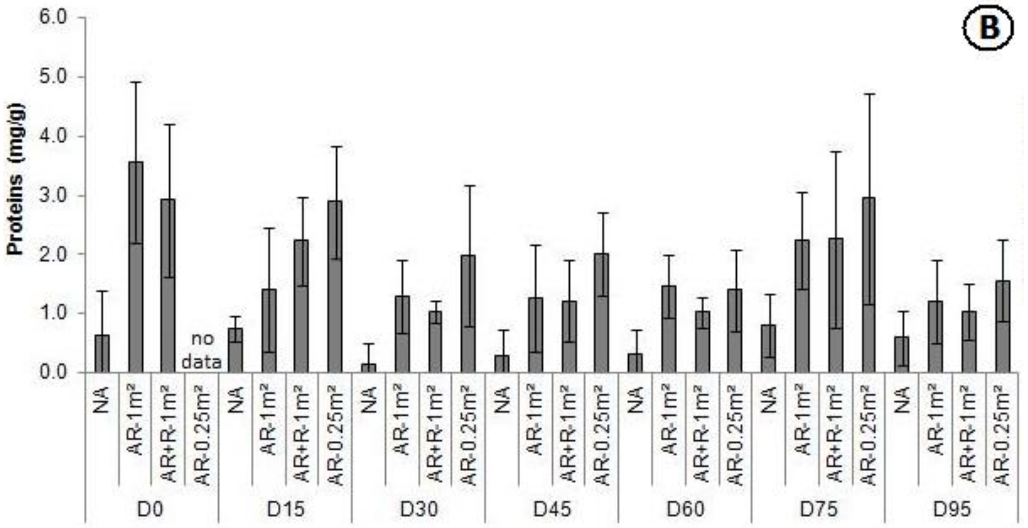
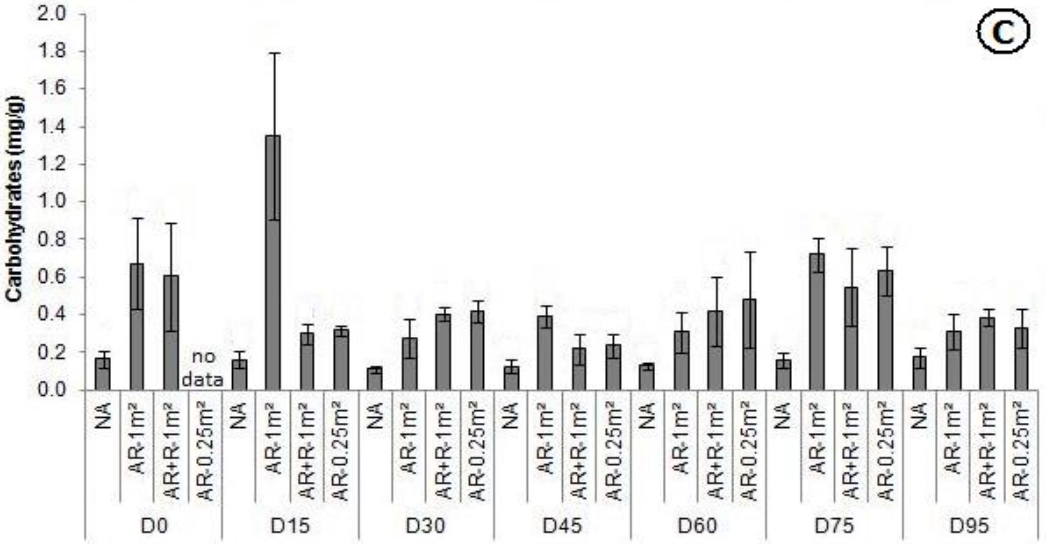
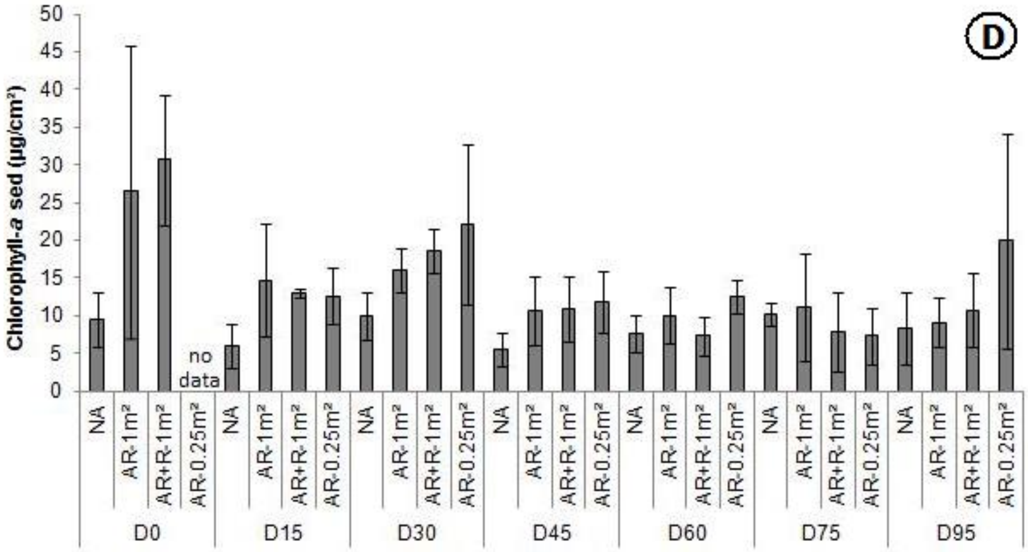


Figure 4. Mean values (±SD) of the redox potential (Eh mV) from surface and bottom layers in relation to treatments: No algal mat (NA), Algal removed-1m² (AR-1m²), Algal removed+reworking-1m² (AR+R-1m²) and Algal removed-0.25m² (AR-0.25m²) and sampling days: 0 (D0), 15 (D15), 30 (D30), 45 (D45), 75 (D75) and 95 (D95).




B
Proteins (mg/g)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
no data
NA
AR-1m²
AR+R-1m²
AR-0.25m²
D0
D15
D30
D45
D60
D75
D95


C
Carbohydrates (mg/g)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
no data
NA
AR-1m²
AR+R-1m²
AR-0.25m²
D0
D15
D30
D45
D60
D75
D95


D
Chlorophyll-a sed (µg/cm²)
50
45
40
35
30
25
20
15
10
5
0
no data
NA
AR-1m²
AR+R-1m²
AR-0.25m²
D0
D15
D30
D45
D60
D75
D95

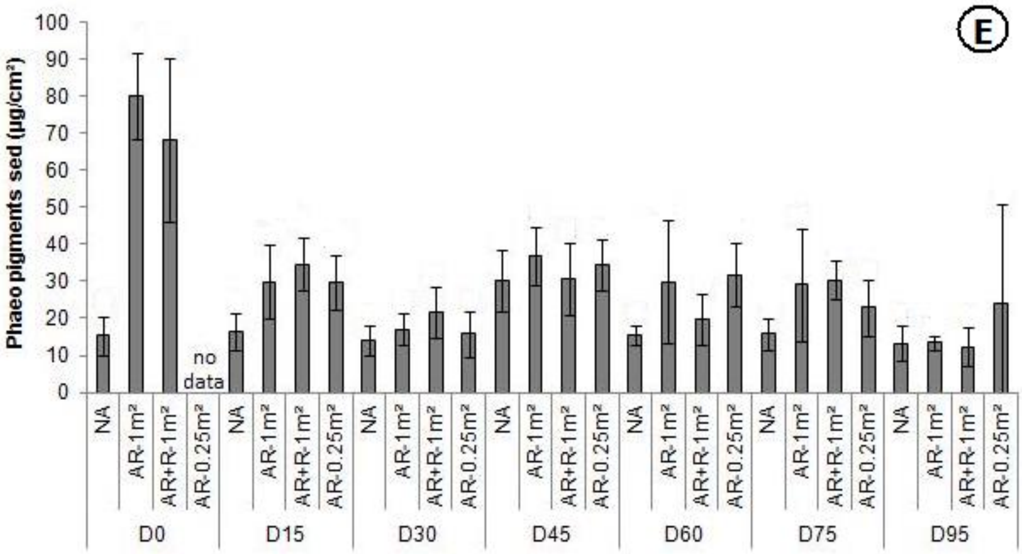


Figure 5. Mean values (±SD) of (A) organic matter content (%organic matter), (B) proteins (mg/g), (C) carbohydrates (mg/g), (D) chlorophyll-*a* (µg/cm²) and (E) phaeopigments (µg/cm²) in relation to treatments: No algal mat (NA), Algal removed-1m² (AR-1m²), Algal removed+reworking-1m² (AR+R-1m²) and Algal removed-0.25m² (AR-0.25m²) and sampling days: 0 (D0), 15 (D15), 30 (D30), 45 (D45), 75 (D75) and 95 (D95).

Organic matter, carbohydrates and phaeopigments statistically differed regarding treatments in contrast of proteins and chlorophyll-*a* (Table 3). Despite the organic matter and carbohydrates contents presented fluctuations in most days, their values were always higher in the removal treatments than in NA. In terms of sediment compaction, organic matter and carbohydrates differed AR-1m² from AR+R-1m² until Day 45, remaining similar between these treatments for the following days. Furthermore, they were also important considering the spatial scale effect, with differences between AR-1m² and AR-0.25m² registered for days 15, 30 and 75 (organic matter) and until Day 45 (carbohydrates). Organic matter and carbohydrates also distinguished the unvegetated sediment (NA) from removal treatments (AR-1m², AR+R-1m² and AR-0.25m²) daily varying considering the sediment parameter. As for phaeopigments, no effect of sediment compaction or spatial scale was observed, with differences found only for Day 15 between NA and AR+R-1m².

Table 3. Repeated-measures ANOVA results for treatments on sediment parameters. *p<0.05; **p<0.01; ***p<0.001; n.s.: not significant.

Sediment Parameters	Factor: treatments (NA, AR-1m², AR+R-1m², AR-0.25m²)	
Days 15 to 95	DF	F
Organic matter	18	11.56***
Proteins	18	1.64n.s.
Carbohydrates	18	8.89***
Chlorophyll-a	18	1.70n.s.
Phaeopigments	18	2.15*

II.3.2. Benthic macrofauna

The benthic macrofauna was composed of 8,511 individuals belonging to 22 taxa (Table 4). The dominant taxa were tubificid oligochaetes (40.82% of total abundance), *Capitella capitata* Fabricius, 1780 (26.58%), *Laeonereis culveri* Webster, 1879 (24.06%) and *Streblospio benedicti* Webster, 1879 (6.00%). These taxa were found in all treatments. Conversely, 17 taxa occurred exclusively in the treatment NA and did not colonize any removal treatment (Table 4).

The treatment NA showed mean densities greater than 25,000 individuals/m² and a taxonomic composition similar to total macrobenthos sampled, since this treatment accounted for 95% of the total abundance. During the entire experiment, the mean densities from NA differed from removal treatments by an order of magnitude, whereas the richness was almost five times higher in NA (Figure 6).

At the start of the experiment (Day 0), it was observed that the algal removal treatments were not completely defaunated, with two individuals of *L. culveri* in AR-1m² and one of *C. capitata* in AR+R-1m². Fifteen days after the removal of the algal layer, all experimental treatments (AR-1m², AR+R-1m² and AR-0.25m²) increased its densities. These treatments were colonized by polychaetes *L. culveri*, *C. capitata*, *S. benedicti*, tubificid oligochaetes and sporadically (i.e. one or two individual considering all samples) of *Sigambra grubii* Müller in Grube, 1858 and the bivalve *Macoma* sp.

The recolonization by most abundant polychaetes species varied across removal treatments and through time. The polychaete *L. culveri* comprised 73.6% of fauna followed by *C. capitata* (23.2%) from AR-1m² sediments. These two species also predominated in AR+R-1m² sediments with similar proportions (74.8% and 22.8%, respectively). As for the treatment AR-0.25m², *C. capitata* dominated (53.7%), followed by *L. culveri* (38.9%) with a slightly contribution of *S. benedicti* (3.7%).

Table 4. List of taxa registered and their respective total mean densities(†) (individuals/m^2 ±SD) in the treatments No algal mat (NA), Algal removed-1m^2 (AR-1m^2), Algal removed+reworking-1m^2 (AR+R-1m^2) and Algal removed-0.25m^2 (AR-0.25m^2).

Taxon/ Species	**NA**	**AR-1m^2**	**AR+R-1m^2**	**AR-0.25m^2**
unidentified Amphipoda	98 (±218)			
Anomalocardia brasiliana (Gmelin, 1791)	1659 (±1802)			
Boccardia sp.	341 (±679)			
Capitella capitata Fabricius, 1780	105561 (±47847)	1415 (±1752)	1951 (±2549)	1415 (±1752)
Capitellides sp.	1463 (±1992)			
Corbula sp.	146 (±327)			
Exogone sp.	439 (±774)			
Heteromastus sp.	98 (±218)			
Laeonereis culveri Webster, 1879	88000 (±23075)	4488 (±7722)	6390 (±4530)	1024 (±1868)
Macoma sp.	634 (±922)			49 (±109)
Mediomastus sp.	244 (±461)			
Nereis sp.	98 (±218)			
Paraprionospio sp.	98 (±218)			
Polydora sp.	195 (±352)			
Pygospio sp.	2244 (±3447)			
Sigambra grubii Müller in Grube, 1858	1171 (±1463)		98 (±218)	49 (±109)
Sphaerosyllis sp.	585 (±753)			
Streblospio benedicti Webster, 1879	24780 (±18380)	49 (±109)		98 (±218)
Tagelus plebeius (Lightfoot, 1786)	146 (±327)			
unidentified Tubificidae oligochaete sp1	156244 (±189468)	146 (±327)	98 (±134)	
unidentified Tubificidae oligochaete sp2	12976 (±12531)			
unidentified Turbellaria	683 (±953)			

(†)Based on the sum of the mean densities of each sampling day

Temporally, AR+R-1m^2 presented higher densities than AR-1m^2 whereas AR-0.25m^2 had the lowest values until Day 45. After that, no defined pattern was observed in the algal treatments. At the end (Day 95) the algal treatments had very few individuals and species. Mean densities varied from 98 (Day 0) to 1,965 (Day 30) individuals/m^2 in AR-1m^2; 49 (Day 0) to 4,176 (Day 15) individuals/m^2 in AR+R-1m^2 and 147 (Day 75) to 737 (Day 30) individuals/m^2 in AR-0.25m^2. Meanwhile, richness values showed great variability among treatments and days (Figure 6).

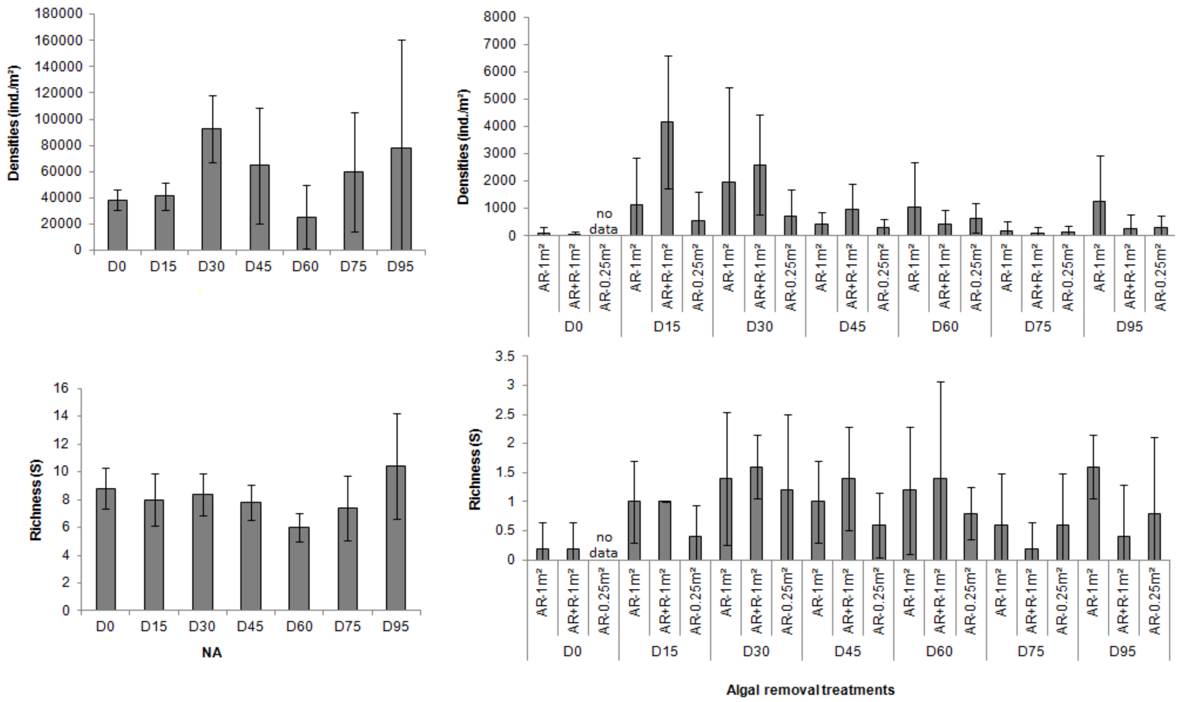


Figure 6. Mean values (±SD) of densities (individuals/m²) and richness (S) in relation to treatments: No algal mat (NA), Algal removed-1m² (AR-1m²), Algal removed+reworking-1m² (AR+R-1m²) and Algal removed-0.25m² (AR-0.25m²) and sampling days: 0 (D0), 15 (D15), 30 (D30), 45 (D45), 75 (D75) and 95 (D95). Note that density and richness values from NA are presented separated from algal removal treatments (AR-1m², AR+R-1m² and AR-0.25m²) due to large differences between these treatments.

At the start of the experiment (Day 0), total density and richness values were statistically distinct among treatments (total density: $F_{2,12}$= 31.84, $p<0.001$; richness: $F_{2,12}$= 106.15, $p<0.001$), with differences found between NA and algal removal treatments, but not between AR-1m² and AR+R-1m². In general, this pattern was maintained along all days (total density: $F_{18,32}$= 3.69, $p<0.001$; richness: $F_{18,32}$= 5.18, $p<0.001$), except for densities values from AR-1m² vs. AR+R-1m² of Day 95 ($p<0.05$). Despite that, results from a paired t-test performed with mean values of total density and richness from the days revealed the effect of spatial scale, with both univariate measures significantly higher in AR-1m² than in AR-0.25m² (total density: t_6=2.97, $p<0.05$ and richness: t_6=3.46, $p<0.01$; one tailed).

Sucessional patterns varied among the removal treatments, but trends were not consistent. During the experiment, the removal treatments remained separated from the unvegetated sediments (NA). Besides, temporal dissimilarities among algal removal treatments were greater from days 0 to 15 with a tendency of reducing them observed at Day 75 (Figure 7).

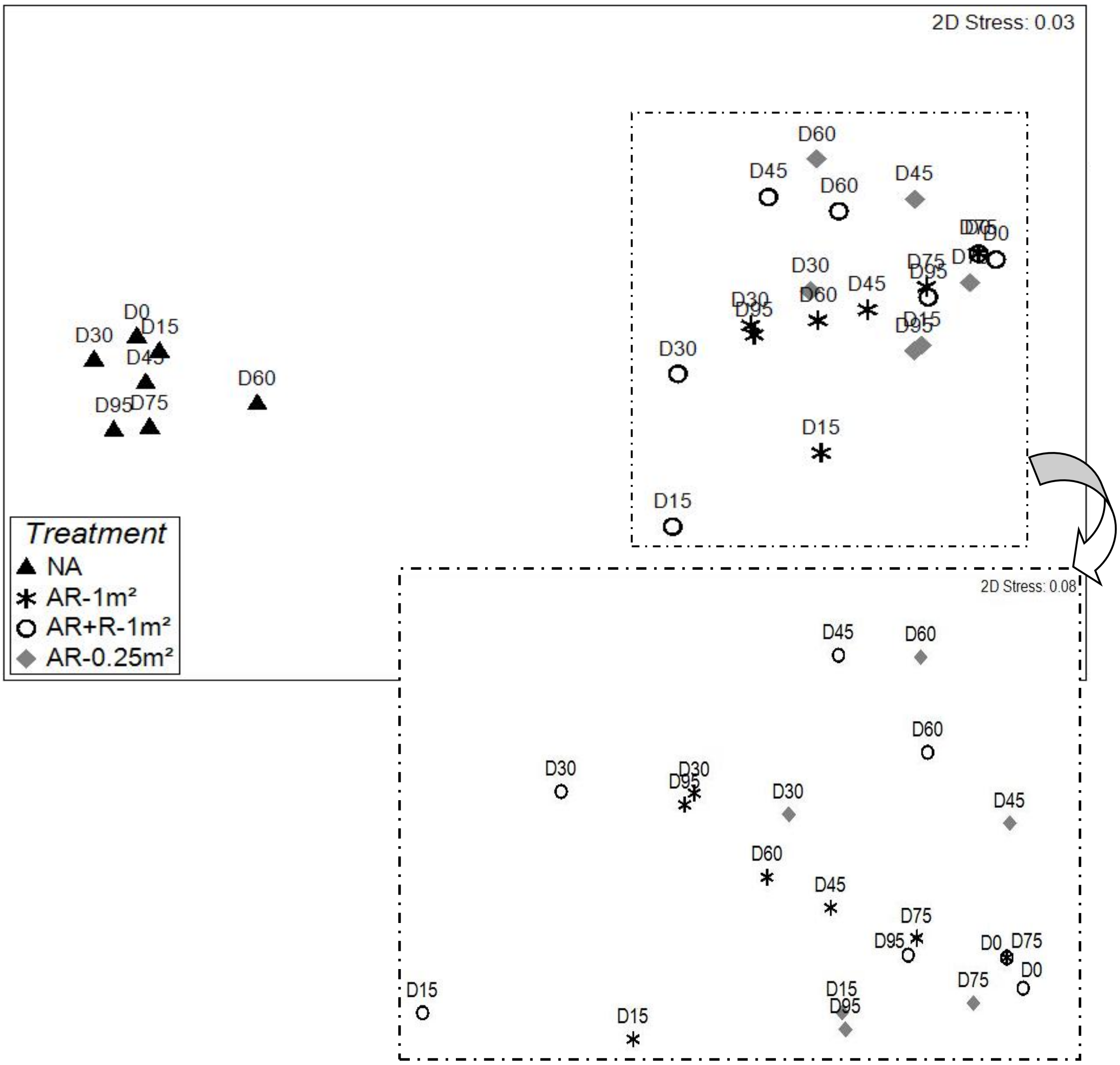


Figure 7. Two-dimensional MDS ordination plot of macrobenthos in relation to treatments: No algal mat (NA), Algal removed-$1m^2$ (AR-$1m^2$), Algal removed+reworking-$1m^2$ (AR+R-$1m^2$) and Algal removed-$0.25m^2$ (AR-$0.25m^2$) and sampling days: 0 (D0), 15 (D15), 30 (D30), 45 (D45), 75 (D75) and 95 (D95).

PERMANOVA tests formally confirmed the observations made on the MDS plots, with significant differences among treatments (*pseudo*-F = 88.47; $p = 0.001$), days (*pseudo*-F = 5.14; $p = 0.001$) and interaction of factors (*pseudo*-F = 2.15; $p < 0.01$). Again, the distinction was detected between NA and the removal treatments along the experiment (all pairwise tests, $p<0.01$), with no significant differences related to sediment compaction or spatial scale.

II.4. Discussion

In this study, it was investigated benthic recolonization and recovery after experimental removal of the algal mat and the possible effects of sediment compaction and spatial scale on the process. The results showed that, within fifteen days of algal removal, all the experimental areas increased its densities. However, more than three months after the beginning of the experiment, macrofauna univariate attributes remained lower and statistically distinct in all removal treatments (AR-1m², AR+R-1m² and AR-0.25m²) compared to nearby unvegetated sediments (NA). Furthermore, macrofauna structure from all three-removal treatments diverged in position at the MDS ordination plot in relation to NA, and their trajectories had not overlapped by the end of the experiment. Consequently, the removal treatments did not show a complete recovery, since it would have required high similarity with NA. Therefore, the recovery time for these experimental areas exceeds three months. Botter-Carvalho et al. (2011) have reported a faster recovery time in a mesoscale field experiment performed in the same studied mudflat, with species number and abundance data statistically similar between control and experimental sediments in 35 and 93 days, respectively.

There was some evidence from the present study to support the hypothesis that macrofaunal assemblages recolonization varied regarding to sediment compaction and spatial scale. Nevertheless, this was discrete and restricted to univariate measures. A lack of response from community structure to these effects was observed and possibly attributed to site-specific environmental factors (Norkko et al. 2010), early sucessional characteristics presented by the colonizers (Bolam et al. 2004; Norkko et al. 2006) or the physicochemical barrier imposed by algal mats (Hull, 1987; Everett, 1994; Bolam et al. 2000).

Field studies of benthic communities have shown that after disturbance event, the patterns of recovery might be influenced by site-specific environmental factors (Zajac et al. 1998; Norkko et al. 2010). In fact, benthic infauna presents such sediment fidelity that the differences in sediment characteristics could affect recolonization and succession (Zajac et al. 1998; Bolam et al. 2004). Significant differences in relation to sediment parameters were found among the removal treatments and unvegetated sediments. The algal removal treatments had higher contents of silt-clay, inorganic nutrients (total-nitrogen and iron), organic matter, carbohydrates and phaeopigments, but also higher values of redox potential in comparison to unvegetated sediments. This scenario found in the removal treatments (higher organic contents and oxygenate sediments) should have benefited the recolonization, which was not observed. It is possible that macrofauna from unvegetated sediments (and reduced redox potential values) could be adapted to low oxygen concentrations, adopting physiological requirements that made them tolerant to reduced oxygen availability. This implies that high oxygen concentrations in the removal treatments might cause some adverse effects on macrofauna. The exposure to hyperoxic conditions is a problem, especially to soft-bodied animals (such as found in the studied area), since elevated O_2 concentrations are related to oxidative stress responses (Abele et al. 2007). Such oxygenate conditions in the removal treatments is due to algal activity of the surrounding mat (see Figure 2). In microbial mats the oxygen levels can be increased to supersaturation values (200-300%) especially in the euphotic layer of the mat (Stal, 1995). Therefore, the ‘failure’ of assemblages to be established on the created areas (i.e. the removal treatments) could be partly explained by differences in sediment characteristics between created (removal treatments) and reference (i.e. unvegetated sediments) areas (Bolam and Whomersley, 2005).

The environmental heterogeneity observed in the mudflat was maintained during the 95 days of experiment. Unfortunately, the present study is limited in drawing conclusions about the origin of this heterogeneity, which could lead to a “chicken-and-egg” situation - if the environmental heterogeneity was produced by the algal mat or the algal mat was benefited by natural heterogeneous gradient found in the mudflat, allowing its growth and development in specific zones. In a previous study (Valença et al. *in press*), even when the mat was reduced to algal patches, samples taken within and outside these patches showed this heterogeneity on most sediment parameters measured suggesting it is the algal mat that contributes to environmental heterogeneity.

The stability (and compaction) of sediment created by the algal mat leads to spatial patterns and might increase resilience and resistance towards external forces (Stal, 2010). Until now, the consequences of such effect on the structure of benthic environment and fauna have not been explored. The compact nature of sediment below algal layer was important for organic matter and carbohydrates, since AR-1m² and AR+R-1m² treatments were significantly different for 45 days. The mat growth is frequently associated with accumulation of empty cyanobacteria and/or diatom cells mineral deposition (de los Ríos et al. 2004). Besides, the sediment contains particularly amounts of extracellular polymeric substances (EPS) composed by polysaccharides (polymeric carbohydrate molecules) which tend to better preservation of organic matter (Decho, 2000; Schieber, 2007; Stal, 2010). The removal of the upper algae layer (in AR-1m² and AR+R-1m²) could take out the younger filaments, but deeper sedimentary layers – an indication of previous growth phases of the mat – might have remained regardless of the mechanical reworking of sediment (in AR+R-1m²). Therefore, in this treatment, the mechanical reworking (represented by a single revolving of sediment at Day 0) was probably inefficient to break down completely the physicochemical nature of sediment compaction.

Some studies have indicated that sediments with higher organic contents negatively interfered macrofaunal recolonization on intertidal mudflats (e.g. Ford et al. 1999; Bolam et al. 2004). Moreover, the compact nature of sediment observed in the removal treatments may have affected recovery by preventing some taxa settling and/or burrowing into the sediment surface. Indeed, the single revolving in the AR+R-1m² treatment also promote an increase in macrofauna density, with values higher than in AR-1m² treatment until Day 45. Despite that, densities from AR-1m² and AR+R-1m² became statistically distinct only in the last day of experiment (Day 95), with higher values in AR-1m² than AR+R-1m², which reinforce that this single revolving of sediment might be not sufficient to overcome this compaction feature.

The distribution of algal mats is heterogeneous over a range of both spatial and temporal scales (Raffaelli, 2000). Here, the scale of observation considering the removal of algal layer (AR-1m² and AR-0.25m²) did not affect the interpretation of results for most environmental parameters (excluding organic matter and carbohydrates). This suggests that removal treatments were located within a homogeneous algal mat, which did not appear to have any visually discernible gradient, or patches. However, organic matter and carbohydrates seemed to be scale-dependent and extended for a longer period (30 and 45 days, respectively). In general, environmental factors exert control over multiple scales (Zajac et al. 1998) and in muddy sediments, geochemical gradients down the sediment column become increasingly important (Ellis et al. 2000).

The importance of scale has been identified as a central theme in understanding recovery dynamics in benthic ecology (e.g. Thrush et al. 1996; Whitlatch et al. 1998; Norkko et al. 2006, Ellis and Schneider, 2008; Norkko et al. 2010, among others). The present results demonstrated that the spatial scale had effect on macrofaunal univariate descriptors. Norkko et al. (2006) have shown through experimental and natural defaunation events that the intensity and magnitude of disturbance play an important role in determining the sucessional processes. Here, the intensity of disturbance could be represented more in terms of size area, with more extension of surrounding algal mat exerting a stronger effect on smaller removal areas ($0.25m^2$) rather than in larger ones ($1m^2$).

Low-diversity assemblages dominated by opportunistic taxa in mudflats seem to be in a continuous state of early succession. In intertidal mudflats, this early succession should be interpreted in terms of increases/decreases of dominant species (Van Colen et al. 2008). Therefore, it would be expect fast recovery rates in those habitats (Bolam et al. 2004; Bolam and Whomersley, 2005). Conversely, the maintenance of disturbance seems to shape the development of opportunistic responses by early colonizers. The colonizers found in the study were *L. culveri, C. capitata* and *S. benedicti*, classified as opportunists in the literature since they are able to reach high densities in areas with higher organic contents (see Pearson and Rosenberg, 1978). However, these taxa occurred at lower densities in the removal treatments compared to unvegetated sediments. Botter-Carvalho et al. (2011) have also shown that *C. capitata* and *S. benedicti* did not present classic opportunistic behavior in their experiments. The lack of clearly defined opportunistic responses in both surveys seemed to be corroborated by the observations of Norkko et al. (2006) that opportunistic responses are commonly associated with larger-scale disturbances.

Recovery from disturbance event is also dependent to a certain extent on the ability of surrounding undisturbed sediments to provide migrating adults and/ or recruiting larvae (Zajac and Whitlatch, 1982; Ford et al. 1999; Bolam et al. 2004). The organisms may colonize partly or totally defaunated areas as a strategy to search for available resources (space or food), so the intensity and scale (size) of disturbance should be taken into consideration (Günther, 1992; Norkko et al. 2006). The removal treatments were placed in the middle of the continuous algal mat (see Figure 1), and surrounding them there were a few meters of algal coverage (approx. 3-4m) separating the removal treatments from the border of the algal mat (and the flat zone with unvegetated sediment). It is likely that the surrounding mat (Figure 2) acted as a physical barrier, preventing dispersal of macrofauna from the unvegetated sediments (and the main source of colonizers). Besides, chemical cues released by the algae in the sediment, and not removed with the mat, could be considered, since many benthic species of algae and cyanobacteria (as found in the study area) produce a wide range of secondary metabolites that can act as inhibitors against competitors or predators (LeFlaive and Ten-Hage, 2007), affecting benthic fauna settlement and colonization.

Regardless of the dispersal modes, many of the dominant species found in the removal treatments were adult polychaetes. Burrowing activity and water transport of adult macrofauna are assumed to occur at small-scale dispersal, by less than a meter (Günther, 1992; Pacheco et al. 2012). Large and deep-burrowing infauna (i.e. *A. brasiliana*, *T. plebeius* and *Corbula* specimens) and other species with swimming abilities (i.e. amphipods) were not detected in the experimental treatments, even though they occur in the nearby-unvegetated sediments.

II.5. Conclusion

Environmental disturbances are rapidly occurring over greater scales and represent significant threats to marine benthic biodiversity (Ellis and Schneider, 2008). The present study shows that algal mats contribute to substantial variation in estuarine macrofauna. Densities' and richness peaks were observed in the initial days of removal treatments, but they did not sustain as expected. Moreover, most sediment parameters, which are potentially important for habitat suitability to colonizers were remarkably different from unvegetated sediments, and showed little changes after three months of algal removal. Although some evidence of macrofaunal recolonization varied in terms of sediment compaction and spatial scale was found, it was discrete and restricted to univariate measures. A lack of response from community structure to these effects was observed and associated to (1) environmental heterogeneity observed between the unvegetated sediments and the removal treatments; (2) random patterns presented by early colonizers species following post-disturbance event (i.e. removal of the algal layer) and (3) the physicochemical barrier imposed by the surrounding mat. Clearly, the generality of the conclusions obtained from these algal removal experiments to different scale observations should be seen with caution, given that, for logistical reasons, manipulative experiments are conducted at limited spatial and temporal extents. Particularly, more research is required considering whether the recolonization potential could be affected when benthos faced a barrier, as observed here by surrounding mat.

CHAPTER III. Recolonization of estuarine macrofauna in algal removal experiments: are there any boundaries created by the algal mat?

III.1. Introduction

Disturbances are often considered to exert a central role in dynamics of assemblages by creating a mosaic of patches at different stages of recovery (Airoldi et al. 2000; Olabarria, 2002; Ledger et al. 2008), reflecting the heterogeneous nature of the environment (Dyson et al. 2007). In estuarine mudflats, an important source of environmental heterogeneity caused by disturbance is the development of algal mats and the associated physicochemical features on its underlying sediment (Bolam et al. 2000; Ellis et al. 2000).

The spatial distribution of algal mats is restricted to certain sections of an estuary and within mat-affected sediments, there are often mat-free ones (Raffaelli, 2000). Such heterogeneous arrangement may shape ecological patterns, because of the vegetation interspersed with bare substrate. The interface between two different areas (e.g. mat-affected and mat-free sediments) can interfere on both physical and biological processes (Bologna and Heck Jr., 2002) and the proximity to a boundary may determine the degree of changes in the response variable (Warry et al. 2009).

In marine systems, connectivity between populations (and areas) is assumed to occur by passive or active behavioral dispersal via hydrodynamic currents (Darcy and Eggleston, 2005), but the scale of dispersal vary with size and age of involved animals (Günther, 1992). For instance, benthic macrofauna is assumed to reach small distances (less than a meter) due to burrowing activity and water transport (Günther, 1992). In many dispersal models, the presence of habitat boundaries is taken into account to explain movements of individuals (Matthysen, 2002). Therefore, the colonization success will depend on faunal assemblages’ responses to, and preferences for, edge and interior conditions (Warry et al. 2009).

Recolonization of disturbed areas is a continuous process in coastal sedimentary habitats (Negrello Filho et al. 2006). Therefore, it is essential to understand which factors affect the arrival of new organisms into disturbed areas (Olabarria, 2002). Many estuarine benthic organisms lack long-distance dispersal mechanisms, and dispersal within and among habitats is more limited – in this case, colonization is expected to be strongly influenced by local neighborhoods (Lundquist et al. 2004). In previous field experiments, we examined the possible effects of sediment compaction (i.e. compact features of sediment attributed by algal mats) and spatial scale (i.e. extension of mat removal) on the recolonization of benthic macrofauna after disturbance ceased (represented by the removal of algal mat). A lack of response from macrobenthic community structure to these effects was observed and possibly attributed to the presence of the continuous algal mat surrounding the removal treatments. Therefore, it was hypothesized that the surrounding mat could act as a barrier, preventing dispersal of individuals from nearby unvegetated sediments to colonize the experimental treatments. Then, the overall goal of this study was to assess the initial recolonization by estuarine macrofauna as a function of distance from nearby unvegetated sediments, showing the existence of boundary effects created by the algal mat.

III.2. Material and Methods

III.2.1. Study area and Experimental design

The study area lies on an estuarine mudflat (7º46.184’S and 34º52.926’W), situated at Itamaracá Island, adjacent to the Santa Cruz Channel Estuarine Complex (northern coast of Pernambuco State, Brazil). Benthic algae form dense mats in this mudflat possibly due to eutrophication and rainfalls regime (authors' personal observations).

In May-2014 most of the upper and middle zones of the mudflat was covered by continuous algal mat, with the exception of a flat zone between the mat and the mangrove trees, placed a few meters away. In this flat zone, filamentous algae were observed, but with no algal mat development. During the present study, the algal mat was composed of unidentified filamentous cyanobacteria, diatoms and sporadic records from macroalgae *Chaetomorpha* sp. and *Gracilaria* sp. (ALM Cocentino, personal observations). Despite the mixed composition with both macro- and microalgae components, the generic term ‘algal mat’ (as a layer of usually filamentous algae on marine or freshwater bottoms) was maintained in the text.

The experimental design consisted of 15 square areas (1m²) established parallel to the tide edge (Figure 1). Five squares were placed in the flat zone (named here No algal mat treatment), five others located in the border of the algal mat (edge – Figure 2) and the remaining ones, within the continuous algal mat. In the removal treatments (border and within the mat) the uppermost layer of sediment, relating to the algal layer, was carefully removed using plastic spatulas. No standardized distance was used among the square areas, but the minimum measure between two square areas was 3 m and the maximum was 13m. A randomized design was chosen in order to prevent any gradients in sedimentological parameters or algal cover from masking the effects of treatments in the analyses.

Samplings were taken in three treatments: No algal mat (NA); Algal removed-edge (ARE) and Algal removed (AR), in three days (0, 15 and 30 days). Each square represented a replicate from each treatment (Figure 1). The first samples (day 0) were taken before the removal of the algal layer. The maintenance of the algal removed sediments was verified at two-week intervals, checking for algal growth within the experimental treatments. The experiment was carried out from May to June 2014.

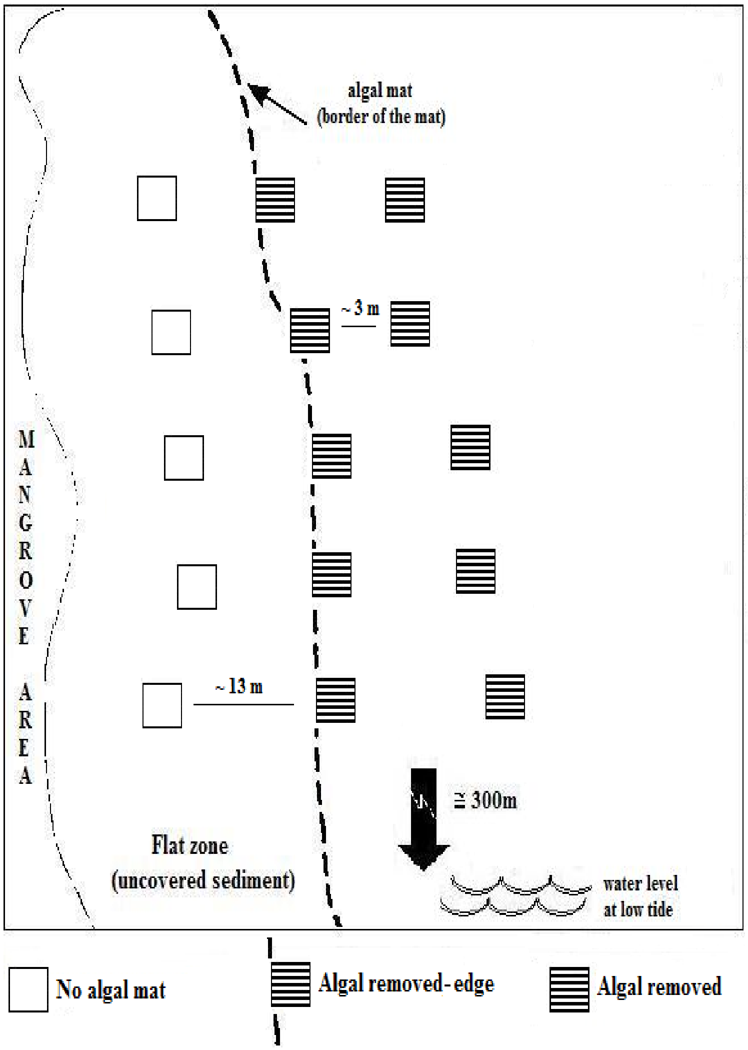


Figure 1. A schematic design of experimental treatments (No algal mat, Algal removed-edge and Algal removed) positions in the estuarine mudflat.

Figure 2. The algal mat border. Up arrow showed the location of a square from the Algal removed-edge (ARE) treatment.

Daily rainfalls from May to June 2014 were obtained with open data from APAC (Pernambuco Agency for Water and Climate) for Itamaracá station. Cumulative bi-weekly rainfalls showed similar values along the entire experiment: 76mm two weeks before the beginning of the experiment (Day 0), 75.5mm from Day 0 to Day 15 and 71.1mm from Day 15 to Day 30.

Sediment samples were taken with cylindrical corers in all days (0, 15 and 30) for microphytobenthic pigments (corer area: $1.13cm^2$ and sampling depth: 2cm), organic matter, proteins and carbohydrates (corer area: $17cm^2$ and sampling depth: 5cm) analyses. Microphytobenthic pigments were estimated using aqueous acetone for extraction (Colijn and Dijkema, 1981 and Lorenzen, 1967 equations). The amount of organic matter was estimated using gravimetric method, with incineration in a muffle furnace at 475 ºC for 4 h (Wetzel and Likens, 1990). Proteins were quantified following Smith et al. (1985) and data were expressed as bovine serum albumin equivalents. Carbohydrates were analyzed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents. Redox potential values were measured *in situ* for each square at 2- and 10-cm sediment depths in all days.

Macrofauna samples were collected with a cylindrical corer (area: $41cm^2$; 0-10cm) subsequently sifted through a 500µm mesh. Then, the retained material was fixed in 4% formaldehyde and stained with rose Bengal. Sorting and counting were performed under stereomicroscope and specimens were identified to the lowest possible taxonomic level. All sampling procedures adopted for macrofauna followed Botter-Carvalho et al. (2011) and Valença and Santos (2013) methods for the same studied area.

III.2.2. Statistical Analyses

Analyses of variance (ANOVA) were used to assess sediment and macrofauna univariate data (density and richness) in relation to treatment (NA, ARE and AR) and day (0, 15 and 30) factors. Redox potentials were also compared regarding sediment depths (2- and 10-cm). All data were tested for heteroscedasticity (Levene test) and log (x+1) transformed when necessary. A Tukey HSD test was used to detect post hoc differences among means (Zar, 1996) and comparisons among treatments within each sampling day are presented in the figures. All these tests were performed using the STATISTICA v7.0 program.

Macrofauna data were also analyzed using multivariate techniques. A MDS ordination was carried out using the Bray-Curtis similarity measure with the log (x+1) transformed data (Clarke and Gorley, 2006). A virtual dummy variable (+d) was included due to the large number of zero values (Clarke et al. 2006). PERMANOVA analysis (n° of permutations: 9999) was applied to test for effects of treatment and day factors, with significance given by the Monte Carlo test (Anderson, 2005). All multivariate analyses were conducted using the PRIMER v6.0+PERMANOVA statistical package. For all statistical analyses, the level of significance adopted was $p<0.05$.

III.3. Results

III.3.1. Sediment parameters

Initial sediment parameters showed higher values in the algal removal treatments (ARE and AR) in relation to unvegetated sediments (NA) (Figure 3). At Day 0, organic matter and microphytobenthic pigments did not differ the removal treatments. Conversely, more proteins and carbohydrates contents were observed in AR than ARE. Fifteen days after the removal of the algal layer, organic matter and phaeopigments were still higher in the algal removal treatments and distinct from NA treatment, whereas chlorophyll-*a*, proteins and carbohydrates decreased to become statistically similar to those found in the NA treatment. In the last day of experiment (Day 30), the levels of proteins, carbohydrates, chlorophyll-*a* and phaeopigments at both ARE and AR were comparable to NA treatment. Sediment analysis revealed significant differences among treatments, days and treatments x days (except for phaeopigments)

(Table 1). Tukey tests pointed out differences between the removal treatments and NA during the entire experiment period only for organic matter (Figure 3).

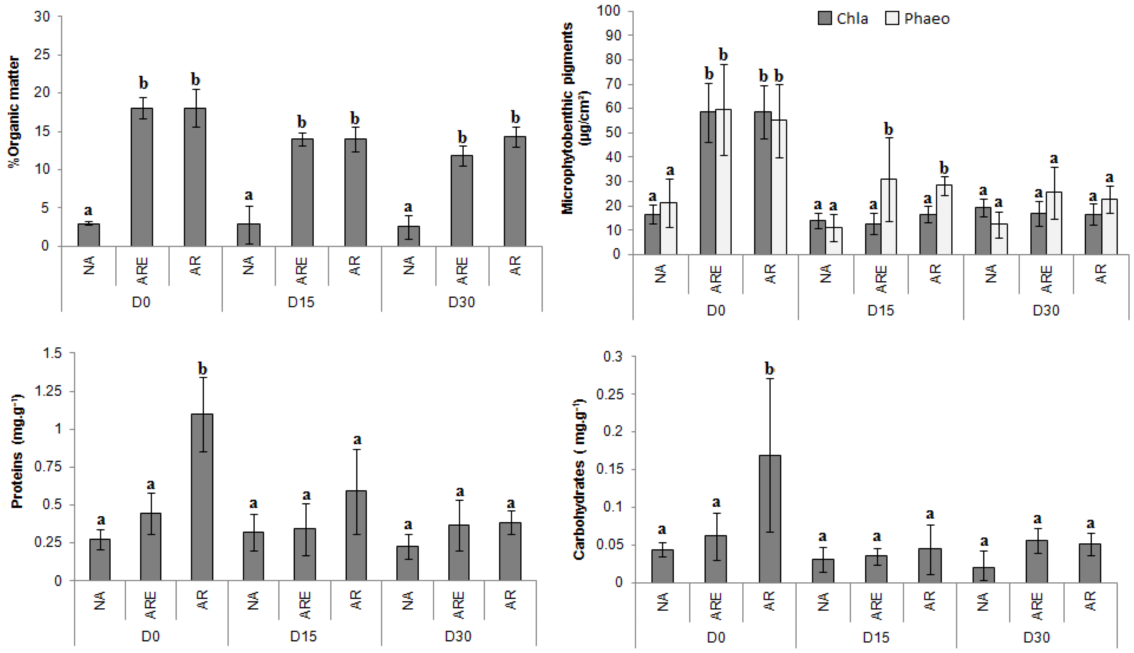


Figure 3. Mean values (±SD) of organic matter content (%), carbohydrates (mg/g), proteins (mg/g), microphytobenthic pigments (µg/cm²) chlorophyll-*a* (chla) and phaeopigments (phaeo) in relation to treatments No algal mat (NA), Algal removed-edge (ARE), Algal removed (AR) and days 0 (D0), 15 (D15) and 30 (D30). Treatments' means followed by the same letter (a,b) did not differ by the Tukey test within each day.

Table 1. ANOVA two-way results for sediment parameters in relation to treatments No algal mat (NA), Algal removed-edge (ARE), Algal removed (AR) and days 0, 15 and 30. *p<0.05; **p<0.01; ***p<0.001; n.s.: not significant.

Sediment parameters	**Edge effect** (treatments: NA, ARE and AR) (days: 0, 15 and 30)					
	Treatment		**Day**		**Factors Interaction**	
	DF	F	DF	F	DF	F
Organic matter	2	250.51***	2	16.52***	4	4.43**
Proteins	2	23.54***	2	9.85***	4	5.66***
Carbohydrates	2	8.06***	2	9.10***	4	4.01**
Chlorophyll-*a*	2	21.07***	2	97.33***	4	24.54***
Phaeopigments	2	22.24***	2	14.01***	4	0.49 n.s.

The redox potential (Eh) presented more reduced values in the NA treatment when compared to removal treatments (Figure 4). Sediment redox varied significantly with treatments ($F_{2,72}$= 74.65; p<0.001), days ($F_{2,72}$= 43.90; p<0.001), sampling depths

($F_{1,72}$= 6.32; p<0.01), treatments x days ($F_{4,72}$= 4.17; p<0.01), days x sampling depths ($F_{2,72}$= 28.11; p<0.001) and treatments x days x sampling depths ($F_{4,72}$= 5.92; p<0.001). At Day 0, differences between the removal treatments and NA were registered only in the bottom layer (10cm). At Day 15, Eh surface values from removal treatments (ARE and AR) become positive and statistically different from NA in both sampling layers. At Day 30, neither treatments nor sampling depths were distinct from each other. In any period of the experiment, no significant differences were observed between ARE and AR treatments.

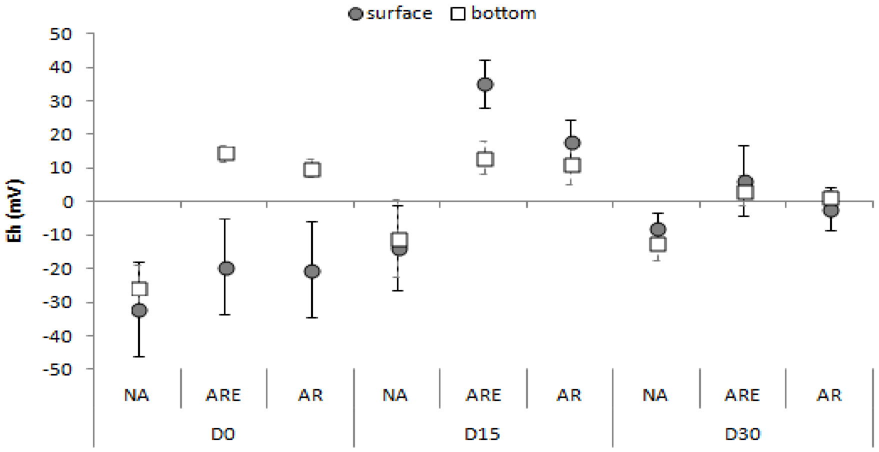


Figure 4. Mean values (±SD) of the redox potential (Eh mV) in relation to treatments No algal mat (NA), Algal removed-edge (ARE), Algal removed (AR); sampling depths (surface and bottom layers) in sampling days (Day 0, 15 and 30).

III.3.2. Recolonization by estuarine macrofauna

In the experiment, the benthic macrofauna was composed of 2,864 individuals belonging to 11 taxa (Table 2). The samples were dominated by polychaete *Laeonereis culveri* Webster, 1879 (49.03% of total abundance), followed by two species of tubificid oligochaetes (34.17%) and another polychaete species, *Capitella capitata* Fabricius, 1780 (7.86%).

At the start of the experiment (Day 0), both algal treatments had few individuals and species, with one individual of *L. culveri* and *C. capitata* in ARE treatment and one tubificid species in AR treatment. Fifteen days later, these treatments increased its densities and number of species (Figure 5). The polychaete *Sigambra grubii* Müller in Grube, 1858 and tubificid sp2 colonized exclusively ARE treatment.

Table 2. List of taxa registered and their respective total mean densities[(†)] (individuals/m² ±SD) in the treatments No algal mat (NA), Algal removed-edge (ARE) and Algal removed (AR).

Taxon/ Species	**NA**	**ARE**	**AR**
Capitella capitata Fabricius, 1780	9659 (±9967)	390 (±442)	244 (±313)
Heteromastus sp.	98 (±218)		
Laeonereis culveri Webster, 1879	26341 (±15219)	19610 (±12278)	18244 (±14325)
Lucina sp.	98 (±134)		
Macoma sp.	146 (±327)		
Mediomastus sp.	146 (±243)		
Pygospio sp.	390 (±506)		
Sigambra grubii Müller in Grube, 1858	6146 (±5018)	49 (±109)	
Streblospio benedicti Webster, 1879	4293 (±3893)	195 (±436)	146 (±218)
unidentified Tubificidae oligochaete sp1	39512 (±36734)	146 (±327)	49 (±109)
unidentified Tubificidae oligochaete sp2	4293 (±4304)	732 (±1636)	

[(†)]Based on the sum of the mean densities of each sampling day

Ninety-nine percent of the individuals colonizing the removal treatments fell into one of the aforementioned taxa. The recolonization by most abundant species was proportional to the fraction of extant populations in the nearby sediments, with maximum number of *L. culveri* (NA: 70.8 ± 39.8 individuals; ARE: 69 ± 39.8 individuals and AR: 65.6 ± 53.6 individuals) and *C. capitata* (NA: 17.8 ± 18.1 individuals; ARE: 1.2 ± 0.8 individuals and AR: 0.8 ± 0.8 individuals) registered at Day 30 in all treatments. However, tubificid oligochaetes did not follow a trend in recolonization: a decline on its abundance was observed from Day 0 (0.2 ± 0.4 individuals) to Day 30 (no individuals) in the AR treatment whereas in the ARE and NA treatments, the maximum number of oligochaetes was recorded, respectively, at Day 15 (3.2 ± 7.2 individuals) and Day 30 (79.2 ± 77.8 individuals).

The mean density of macrofauna was statistically different regarding treatments ($F_{2,36}$= 19.15; p<0.001), days ($F_{2,36}$= 29.14; p<0.001) and treatments x days ($F_{4,36}$= 5.57; p<0.01). Despite that, the removal treatments did not significantly vary according to the distance from nearby unvegetated sediments, since both ARE and AR treatments were colonized in a similar way (Figure 5). At the end of thirteen days, although the mean densities from these removal treatments remained lower than NA treatment, no significant differences were detected among the treatments.

The number of species (S) was also statistically different regarding treatments ($F_{2,36}$= 46.77; p<0.001) and days ($F_{2,36}$= 9.88; p<0.001), but with no distinction between

ARE and AR. At Day 30, no significant differences were found between NA and ARE treatments (Figure 5).

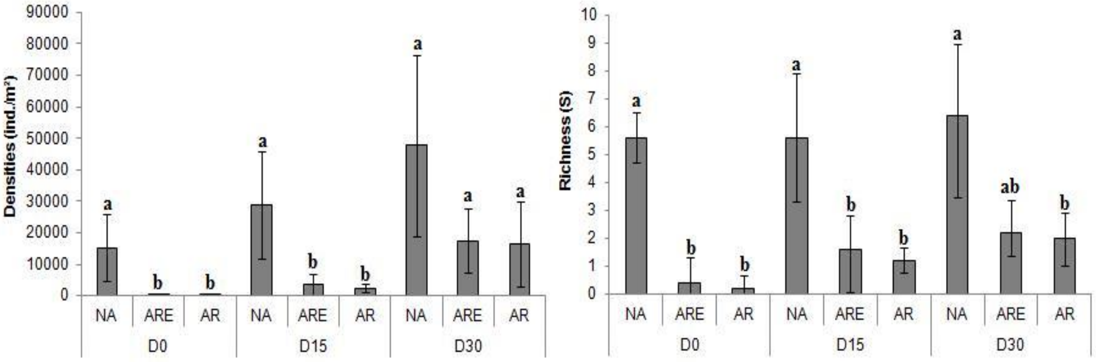


Figure 5. Mean values (±SD) of densities (individuals/m²) and number of species/ richness (S) in relation to treatments No algal mat (NA), Algal removed-edge (ARE), Algal removed (AR) and days 0 (D0), 15 (D15) and 30 (D30). Treatments' means followed by the same letter (a,b) did not differ by the Tukey test within each day.

The MDS ordination plot clearly distinguished the unvegetated sediments (NA) from the removal treatments in all sampling days. Although the macrofauna from the removal treatments started at different points (Day 0), a higher similarity was observed between AR and ARE treatments in the Day 30, and a tendency to go towards NA treatment. PERMANOVA test pointed out significant differences for treatments (*pseudo*-F = 23.88; $p = 0.0001$), days (*pseudo*-F = 13.33; $p = 0.0001$) and interaction of factors (*pseudo*-F = 3.25; $p < 0.01$). Pairwise comparisons among the treatments within each experimental day corroborated the results observed from MDS plot (Table 3).

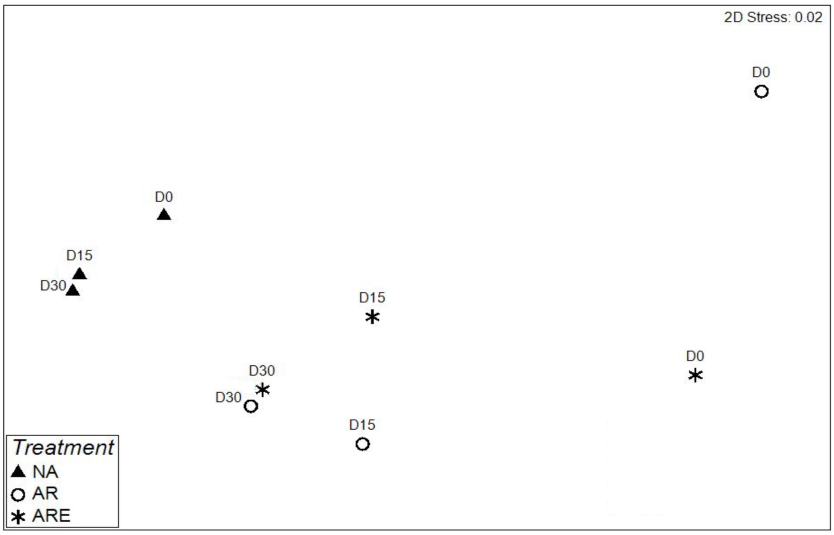


Figure 6. MDS ordination plot of macrofauna (mean abundances; n=5) in relation to treatments No algal mat (NA), Algal removed-edge (ARE), Algal removed (AR) and days 0 (D0), 15 (D15) and 30 (D30).

Table 3. Results from PERMANOVA analyses pairwise tests for Interaction factor (Treatment vs. Day) within each Day (0, 15 and 30) level. Legend: No algal mat (NA), Algal removed-edge (ARE), Algal removed (AR). *p(MC) <0.05; **p(MC)<0.01; ***p(MC)<0.001; n.s.: not significant.

	Day 0 **t-value; *p*(MC)**	**Day 15** **t-value; *p*(MC)**	**Day 30** **t-value; *p*(MC)**
NA vs. ARE treatments	6.12***	2.50**	2.05*
NA vs. AR treatments	7.31***	3.96***	2.49**
ARE vs. AR treatments	1.05 n.s.	0.64 n.s.	0.18 n.s.

III.4. Discussion

The results showed that benthic macrofauna recolonize the removal treatments regardless of their distance from the unvegetated sediments (NA treatment), indicating that individuals moved towards the experimental treatments rather than changing direction. Besides, univariate (total density and richness) and multivariate (community data) attributes from macrofauna border (ARE treatment) and within the mat (AR treatment) were not significantly distinct from each other. However, the number of species was firstly enhanced in the removal treatment closer to the unvegetated sediments. The knowledge of faunal responses to boundaries is specially explored near habitat edges (e.g. Hovel et al. 2002; Boström et al. 2006; Warry et al. 2009), with taxa showing variable responses (Warry et al. 2009). This variability depends on specific edge type encountered (Ries and Sisk, 2004) and may be due to species-specific interactions (Boström et al. 2006).

The dispersal of macrobenthic organisms has been shown to occur over smaller scales in many intertidal and shallow subtidal areas (Zajac et al. 1998, Norkko et al. 2000; Negrello Filho et al. 2006). The dispersal includes passive (resuspension) and active (swimming, crawling or burrowing) mechanisms through water column and sediment (Günther, 1992; Shull, 1997). Although, the present study did not test the colonization mechanisms used by macrofauna, it is suggested that organisms reached the experimental treatments within the sediment or at the surface by small-scale active movements (crawling and/ or burrowing). This evidence is partially supported by a simultaneous experiment using sediment traps placed into the removal treatments, with higher dominance of juvenile forms of polychaetes (212 and 188 juveniles against 10 and 5 adult polychaetes, respectively, in ARE and AR treatments) in contrast to sediment data, where adult macrofauna was abundant (author's personal observations).

Besides, colonization by adults through active movements is often responsible for initial establishment of small patches (Lundquist et al. 2006).

Early colonizers of soft-bottom disturbances often exhibit limited larval dispersal, with a short or non-planktonic stage (Shull, 1997; Lundquist et al. 2006). Among the colonists found in the removal treatments, *L. culveri* is a deposit feeder with free spawning and entirely benthic development (Mazurkiewicz, 1975; Wilson, 1991) whereas *C. capitata* reproductive mode and larval development pattern are variable in terms of geographic regions but breeding season of the whole populations extended throughout almost the whole year (Tsutsumi and Kikuchi, 1984). Besides embryos and larvae brooded in a parental tube, with a very short planktonic stage or passing through direct development (Wilson, 1991). These common life-history characteristics observed for these polychaetes allow a direct replenishment of specimens from surrounding sediments to disturbed ones.

Some studies have proposed that early colonizers in natural disturbances actively seek and exploit food resources made available by the disturbance (see Netto and Lana, 1994). Even with thirty days of algal removal, sediment organic content remained higher in both removal treatments compared to unvegetated sediments, which could benefit colonization by pioneer species here, in contrast to previous experiment (Valença et al. unpublished data) where the high amount of organic matter seemed to not favor fauna’s densities. In fact, densities of *L. culveri* were essentially the same between the removal treatments and unvegetated sediment. Botter-Carvalho et al. (2011) observed that *Laeonereis acuta* (synonym of *L. culveri*) rapidly colonized defaunated sediments in the same studied mudflat, reaching similar abundances from control areas with 18 days of experiment. For *C. capitata*, the recolonization rate was slower in both surveys: here, with 30 days, densities of this species were still lower in the removal treatments; in Botter-Carvalho et al. (2011), *C. capitata* was found in defaunated sediments after 18 days of experiment.

Moreover, the lack of boundary effects from estuarine macrofauna could be explained by resource availability. A neutral response is predicted when two adjacent areas contain resources of similar quality (Ries and Sisk, 2004) – so, being near the border of the mat would have no benefit with respect to access resources. Sediment organic compounds were almost similarly distributed in the removal treatments (ARE and AR), so the recolonization was not differentiated in these experimental areas in terms of resources.

Clearly behavioral processes are important in the colonization pattern in soft-sediment systems but the suitability of substrate should be considered (Lundquist et al. 2006), since the dispersal of organisms also depends on the physical and chemical characteristics of disturbed areas (Netto and Lana, 1994; Dyson et al. 2007). Here, the geochemical characteristics of sediment more than physical barrier should be considered. Apart from organic matter, sediment parameters measured in the removal treatments did not differ significantly from the unvegetated sediments (NA) in the last day of experiment. Unlikely, in a previous algal removal experiment (Valença et al. unpublished data), differences between the removal treatments and NA were maintained during 95 days for most sediment parameters (and named as environmental heterogeneity). This suggested that the environmental heterogeneity rather than distance from unvegetated sediments played a major role on recolonization processes in the removal treatments.

III.5. Conclusion

This small-scale experiment provided some insights into major processes of benthic recolonization in estuarine sediments impacted by development of algal mats. The proposed hypothesis was rejected - the initial recolonization observed in the removal treatments was not affected by the presence of the surrounding algal mat, although some chemical effects released by the mat (and not tested) could not be excluded. At the same time, a decrease of environmental heterogeneity between these experimental areas and the unvegetated sediments (not observed previously) associated to high resilience of mudflat communities, particularly those which are composed by dominant species with life-history characteristics and dispersal mechanisms used by typical early-colonizers species proved to be more important for assessing initial recolonization (and possibly recovery) based on macrofauna attributes. More studies are required in order to understanding these processes operating at various scales and possible cascading effects due to fauna-environment relationships in sediment landscape following mat deployment.

CHAPTER IV. Recolonization of estuarine macrofauna in algal removal experiments: Is there any seasonal influence on early successional pattern of macrofauna?

IV.1. Introduction

Understanding the process of community recolonization and recovery following a disturbance event is still complex and largely concerning for ecologists (Norkko et al. 2006; Walker et al. 2013). Although information on recolonization and sucessional trajectories in soft-bottom communities has been explored in literature, it is still not possible to develop a general model to most of benthic environments (Pacheco et al. 2010). Some challenges are recognized such as the dependency upon the magnitude, frequency and extent of disturbance (Norkko et al. 2006), the dispersal modes of animals (Günther, 1992; Whitlatch et al. 1998) and the poorly understood influence of spatial and temporal variability (Platt and Connell, 2003; Underwood and Chapman, 2006).

In muddy sediments, succession is more likely to occur following a deterministic pathway, passing through a few stages (Pearson and Rosenberg, 1978; Zajac et al. 1998; Rosenberg, 2001; Van Colen et al. 2008). The initial sucessional phase, dominated by early colonizers (i.e. opportunists), is usually followed by severe mortality and partial or total replacement by late-colonizing species (Zajac and Whitlatch, 1982; Underwood and Chapman, 2006; Pacheco et al. 2010). However, the variable supply of potential colonizers and the population dynamics of infaunal species in a particular area may result in more unpredictable recolonization and succession patterns than expected (Zajac and Whitlatch, 2003), which often require system-specific evaluations (Pacheco et al. 2010).

In the description and analyses of benthic community succession trajectories, seasonality represents an important source of variability. Seasonality has a role in changing metabolic rate, faunal activity and breeding behavior (Hall and Frid, 1998). Experimental studies on soft-bottom benthic communities are mainly from temperate regions and have shown that early succession is strongly affected by season (e.g. Zajac and Whitlatch, 1982; Norkko et al. 2006). Besides, seasonality may interfere on later sucessional stages and community recovery (Pacheco et al. 2010) and most studies of succession have limited temporal replication (Underwood and Chapman, 2006).

Here, the results from short-term recolonization experiments (30 days) in algal removal sediments were described and compared at two different seasons (Post-Rainy and Post-Dry seasons). Some studies showed that initial colonization by macrofauna is related to species availability at the moment when the substrate was made available (Zajac and Whitlatch, 1982; Lu and Wu, 2000). Within this context, the present study assessed whether the recolonization of these algal removal sediments by benthic macrofauna is affected by the season. The hypothesis tested was that availability and abundance of colonists may be dependent on seasonality and should play a key role in the recolonization of the algal removal sediments.

IV.2. Material and Methods

IV.2.1. Study area and Experimental design

The study area lies on an estuarine mudflat (7º46.184’S and 34º52.926’W), situated at Itamaracá Island, adjacent to the Santa Cruz Channel Estuarine Complex (northern coast of Pernambuco State, Brazil). In the last decade, mat-building filamentous algae have been developing in the area (see Botter-Carvalho, 2007), probably associated with eutrophication and rainfall regime (authors' personal observations).

The annual precipitation at Itamaracá Island is marked by two seasons: a rainy season from March through August, with mean monthly rainfall in excess of 200mm, and a dry season from September through February, with mean monthly rainfall less than 100mm (data provided by the Meteorological Laboratory of Pernambuco). Throughout the year, water salinity fluctuates between euryhaline and polyhaline levels in response to local meteorological conditions (Flores-Montes et al. 2002).

In two distinct periods, September-2013 (Post-Rainy season - PR) and May-2014 (Post-Dry season - PD), most of the upper and middle zones of the estuarine mudflat was covered by continuous algal mat with the exception of a flat zone between the mat and the mangrove trees, placed a few meters away. In this flat zone, filamentous algae were observed, but with no algal mat development.

The composition of the algal mat remained quite similar during experiment periods, with dominance of filamentous cyanobacteria *Microcoleus chthonoplastes* Gomont and *Oscillatoria* sp. in Post-Rainy season and unidentified species in Post-Dry season, diatoms and sporadic records of macroalgae *Solieria filiformis* (Kützing) P.W.

Gabrielson in Post-Rainy season and *Chaetomorpha* sp. and *Gracilaria* sp. in Post-Dry season.

Five 1m²-square areas were placed along the continuous algal mat, parallel to the tide edge. In these squares, the uppermost layer of sediment (the algal layer) was carefully removed using plastic spatulas (named here as Algal removal treatment) (Figure 1). A randomized design was chosen in order to prevent any gradients in sedimentological parameters or algal cover from masking the effects in the analyses. Besides, the maintenance of the algal removed sediments was verified at two-week intervals, checking for algal growth.

Five units of unvegetated sediment were randomly sampled at both seasons in the flat zone (named here as No algal mat treatment).

Samplings were taken in treatments No algal mat (NA) and Algal removal (AR); in days 0, 15 and 30; in PR and PD seasons. Each square from AR treatment represented a replicate. The first samples (day 0) were taken before the algal layer was removed. These data were obtained from Chapters 2 (Post-Rainy season) and 3 (Post-Dry season) of the present thesis.

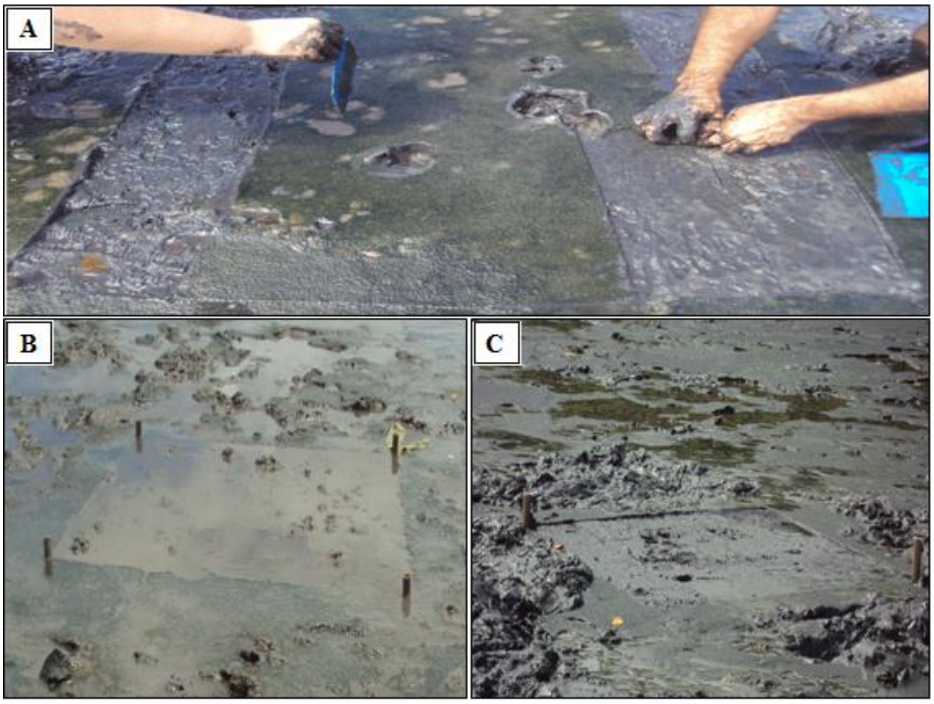


Figure 1. Photos showing (A) the procedure of removal of the algal layer; (B) and (C) the Algal removed treatments (AR) on the first day of experiment (Day 0) in Post-Rainy (PR) and Post-Dry (PD) seasons, respectively.

Daily rainfalls from September to October-2013 (PR season) and May to June-2014 (PD season) experimental periods were obtained with open data from APAC (Pernambuco Agency for Water and Climate) for Itamaracá station. Cumulative rainfalls during the thirty-day experiments in each season were 151mm (PR season) and 222.6mm (PD season).

Sediment samples were taken with cylindrical corers for microphytobenthic pigments (corer area: 1.13cm² and sampling depth: 2cm), organic matter, proteins and carbohydrates (corer area: 17cm² and sampling depth: 5cm) in all treatments, days and seasons. Each sediment parameter was determined according to specific literature: microphytobenthic pigments (Colijn and Dijkema, 1981 and Lorenzen, 1967 equations), organic matter (Wetzel and Likens, 1990), proteins (Smith et al. 1985) and carbohydrates (Gerchacov and Hatcher, 1972). Redox potential values were measured at 2- and 10-cm sediment depths in all treatments, days and seasons.

Macrofauna samples were collected with a cylindrical corer (area: 41cm²; 0-10cm) and processed through a sieve with 500µm mesh size. The retained material fraction was fixed in 4% formaldehyde and stained with rose Bengal. Organisms were sorted, counted and identified to the lowest possible taxonomic level. All sampling procedures used for macrofauna followed Botter-Carvalho et al. (2011) and Valença and Santos (2013) methods for the same studied area.

IV.2.2. Statistical Analyses

Analyses of variance (ANOVA) were used to assess sediment parameters and macrofauna attributes (density and number of species) in terms of treatment (NA and AR), day (0, 15 and 30) and season (PR and PD) factors. Redox potentials data from 2- and 10-cm sediment depths were grouped (mean depth values) and analyzed considering the abovementioned factors. All data were tested for heteroscedasticity (Levene test) and log (x+1) transformed when necessary. A Tukey HSD test was used to detect post hoc differences among means (Zar, 1996). All these tests were performed using the STATISTICA v7.0 program.

Macrofauna was also analyzed using multivariate techniques. A MDS ordination was obtained using Bray-Curtis index measure and log (x+1) transformed data (Clarke and Gorley, 2006). A virtual dummy variable (+d) was included due to the large number of zero values (Clarke et al. 2006). PERMANOVA analysis (n° of permutations: 9999) was applied to test for treatments, days and period effects with significance given by the Monte Carlo test (Anderson, 2005). SIMPER analysis (Clarke, 1993) was calculated to indicate the contribution of main taxa (>10%) to distinguish treatments (NA and AR) and seasons (PR and PD). All these multivariate analyses were conducted using the PRIMER v6.0+PERMANOVA statistical package. For all statistical analyses, the level of significance used was $p<0.05$.

IV.3. Results

IV.3.1. Sediment parameters

The distribution of sediment parameter in each treatment (NA and AR), day (0, 15 and 30) and season (PR and PD) is given in Figure 2. In general, AR showed higher values for most sediment parameters than NA, especially in PR.

NA treatment had more proteins and carbohydrates contents in PR. Besides, an increase in the reduction Eh potential was also observed in this season. Conversely, chlorophyll-*a* was slightly superior in PD. No obvious pattern was found for organic matter and phaeopigments.

In AR treatment, organic matter, proteins and carbohydrates presented the highest values in PR. Organic matter and carbohydrates contents along the sampling days remained higher in PR than PD. Proteins contents found before the removal of algal layer (Day 0) in PD were comparable to values after the removal (Day 15) in PR. Besides, more positive Eh values were registered in all days in this season. Microphytobenthic pigments observed in the beginning of the experiment (Day 0) showed different concentrations between seasons: more phaeopigments in PR and more chlorophyll-*a* in PD.

Sediment analysis revealed significant differences among treatments, days, seasons and the interaction of the three factors for all parameters (Table 1). Seasonal differences within each treatment and day (e.g. NA Day 0 PR x PD; AR Day 0 PR x PD) were evident especially for carbohydrates (in both treatments all days) and organic matter (in AR all days). Other significant seasonal comparisons were observed for Eh potential (in NA Day 0 and in AR Day 0, Day 30), proteins (in NA Day 30) and chlorophyll-*a* (in AR Day 0). Proteins' and phaeopigments' concentration were the parameters statistically similar between seasons (Table 1).

Differences between treatments within each season and day (e.g. NA x AR Day 0 PR; NA x AR Day 0 PD) were registered in both seasons for organic matter (all days in PR and PD), Eh (all days in PR; Day 0 and Day 15 in PD), carbohydrates (Day 0 and Day 15 in PR; Day 0 and Day 30 in PD) and phaeopigments (Day 0 in PR; Day 0 and Day 15 in PD). The treatments differed exclusively in PR for proteins (Day 0 and Day 30) and in PD for chlorophyll-*a* (Day 0).

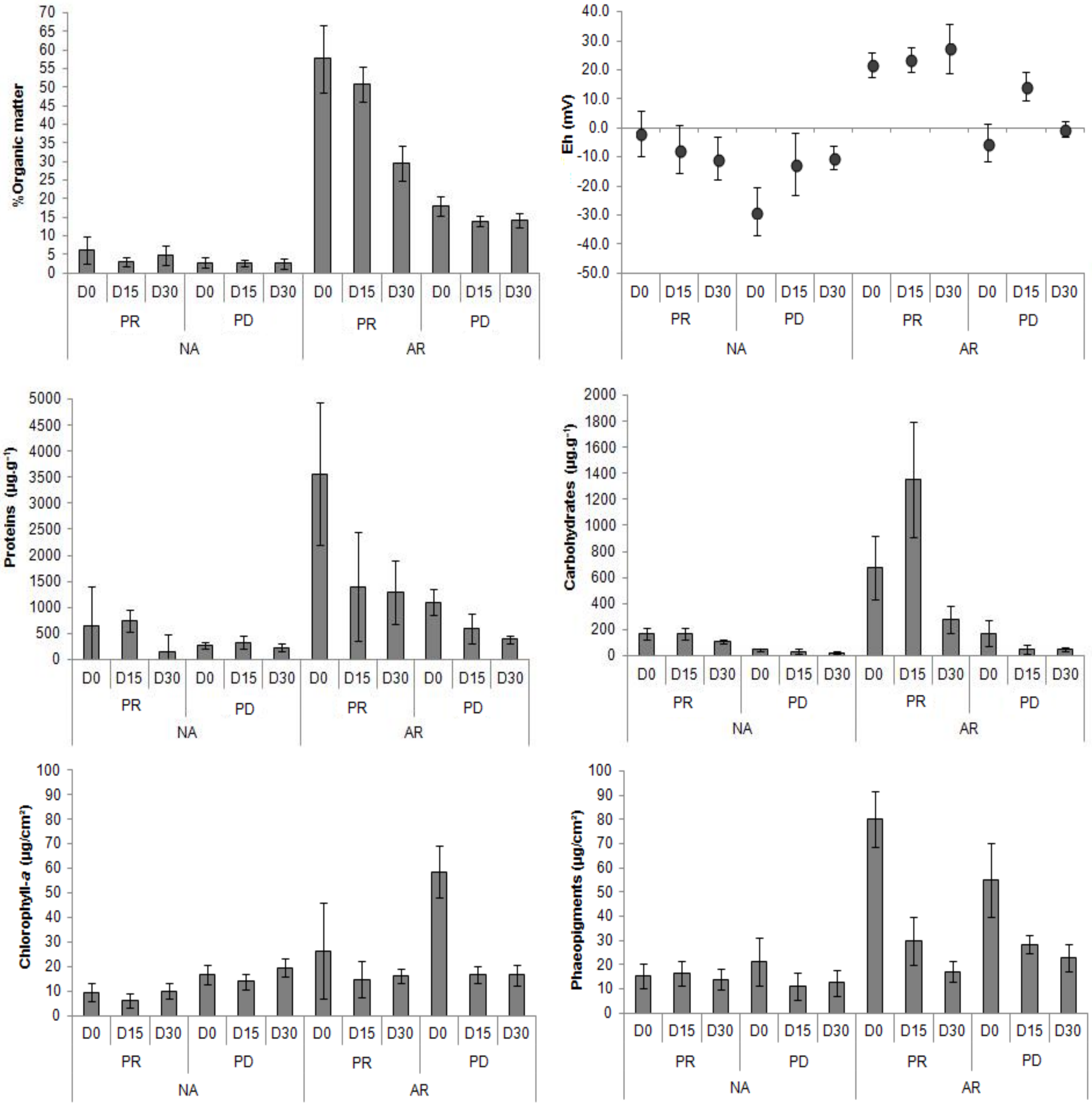


Figure 2. Mean values (±SD) of organic matter content (%organic matter), redox potential (Eh mV), proteins (µg/g), carbohydrates (µg/g), chlorophyll-*a* (µg/cm²) and phaeopigments (µg/cm²) in relation to treatments No algal mat (NA), Algal removed (AR); days 0 (D0), 15 (D15), 30 (D30) and seasons Post-Rainy (PR), Post-Dry (PD).

Table 1. ANOVA three-way results for sediment parameters in relation to (A) Treatment: No algal mat and Algal removed; (B) Day: 0, 15, 30 days and (C) Season: Post-Rainy and Post-Dry. *p<0.05; **p<0.01; ***p<0.001; n.s.: not significant.

Factors	**Sediment parameters (F-values)**					
	Organic matter	Eh	Proteins	Carbohydrates	Chlorophyll-*a*	Phaeopigments
(A) Treatment	797.98***	210.06***	31.28***	88.61***	19.46***	84.85***
(B) Day	25.85***	7.24**	6.04**	15.26***	6.17**	23.24***
(C) Season	287.43***	81.63***	0.52 n.s.	259.20***	23.99***	0.74 n.s.
(A) x (B)	22.37***	0.93 n.s.	4.50*	0.64 n.s.	3.33*	8.32***
(A) x (C)	225.05***	9.19**	11.59***	6.86**	0.95 n.s.	0.20 n.s.
(B) x (C)	16.02***	11.05***	3.08 n.s.	9.45***	1.71 n.s.	1.04 n.s.
(A) x (B) x (C)	16.48***	6.30**	3.92*	6.34***	3.26*	3.96*

IV.3.2. Recolonization by estuarine macrofauna at different seasons

The benthic macrofauna was composed of 5,827 individuals belonging to 22 taxa (Table 2). Among them, ten taxa were found in both seasons (seven polychaetes species, two tubificid oligochaetes and one bivalve), eleven taxa were exclusive for PR (seven polychaetes species, three bivalves, one amphipod) and a single taxa for PD (a bivalve). The most abundant species included *Laeonereis culveri* Webster, 1879 (34.94%), tubificid oligochaete sp1 (31.87%) and *Capitella capitata* Fabricius, 1780 (22.02%), all occurred in both treatments (NA and AR) and seasons (PR and PD).

In both seasons, the algal removal treatment (AR) was almost defaunated in the beginning of the experiment (Day 0), showing two individuals of *L. culveri* in PR and one tubificid oligochaete sp1 in PD. Fifteen days later, AR was colonized, increasing its densities and number of species (richness). However, with thirty days of experiment, a greater increment on densities values was observed in PD, while richness values increased similarly regardless of the season (Figure 3).

Density and richness values of total macrofauna in the NA samples were always higher than in AR (Figure 3). In both seasons and treatments, mean densities progressively increased from the beginning (D0) to the end (D30) of the experiment. This pattern was also observed for AR richness, while NA showed minor fluctuations over days. For both univariate measures, significant differences were found for treatment (density: $F_{1,48}$= 120.43; p<0.001 and richness: $F_{1,48}$= 298.21; p<0.001), day (density: $F_{2,48}$= 18.70; p<0.001 and richness: $F_{2,48}$= 7.11; p<0.01), treatment x day (density: $F_{2,48}$= 12.24; $p<0.001$ and richness: $F_{2,48}$= 7.91; $p<0.001$) and treatment x season (density: $F_{1,48}$= 9.07; $p<0.01$ and richness: $F_{1,48}$= 8.36; $p<0.01$). Mean densities varied between seasons (PR x PD) for AR treatment but richness accounted for season differences only in NA treatment (Tukey test $p<0.05$). Differences between treatments (NA x AR) were found in PR and in PD for both univariate measures.

Table 2. List of taxa registered and their respective total mean densities$^{(\dagger)}$ (individuals/m² ±SD) in the treatments No algal mat (NA) and Algal removed (AR) in Post-Rainy Season (PR) and Post-Dry Season (PD).

	NA		**AR**	
Taxon/ Species	**PR**	**PD**	**PR**	**PD**
unidentified Amphipoda	98 (±218)			
Anomalocardia brasiliana (Gmelin, 1791)	1268 (±1132)			
Boccardia sp.	49 (±109)			
Capitella capitata Fabricius, 1780	52098 (±18026)	9659 (±9967)	585 (±908)	244 (±313)
Capitellides sp.	1024 (±1342)			
Corbula sp.	49 (±109)			
Exogone sp.	98 (±134)			
Heteromastus sp.	49 (±109)	98 (±218)		
Laeonereis culveri Webster, 1879	52244 (±8954)	26341 (±15219)	2488 (±4257)	18244 (±14325)
Lucina sp.		98 (±134)		
Macoma sp.	146 (±218)	146 (±327)		
Mediomastus sp.	98 (±218)	146 (±243)		
Nereis sp.	49 (±109)			
Paraprionospio sp.	49 (±109)			
Polydora sp.	98 (±134)			
Pygospio sp.	976 (±1234)	390 (±506)		
Sigambra grubii Müller in Grube, 1858	780 (±932)	6146 (±5018)		
Sphaerosyllis sp.	98 (±134)			
Streblospio benedicti Webster, 1879	5854 (±2377)	3893 (±146)	49 (±109)	146 (±218)
Tagelus plebeius (Lightfoot, 1786)	49 (±109)			
unidentified Tubificidae oligochaete sp1	50976 (±38141)	39512 (±36734)	49 (±109)	49 (±109)
unidentified Tubificidae oligochaete sp2	5122 (±3891)	4293 (±4304)		

$^{(\dagger)}$Based on the sum of the mean densities of each sampling day

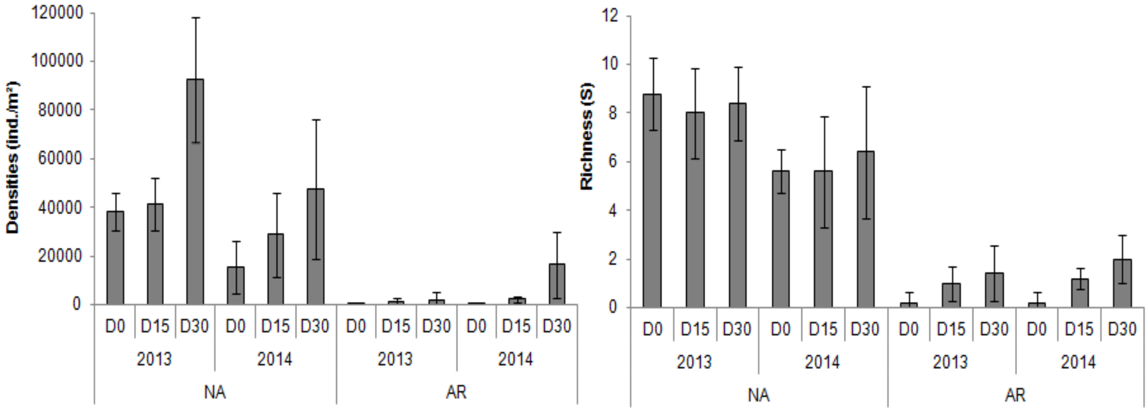


Figure 3. Mean values (±SD) of densities (individuals/m²) and number of species/ richness (S) in relation to treatments No algal mat (NA), Algal removed (AR); days 0 (D0), 15 (D15), 30 (D30) and seasons Post-Rainy (PR), Post-Dry (PD).

The distribution patterns of the most abundant species showed effect of treatments and days for all aforementioned taxa (Table 3). Season became important for the polychaetes *C. capitata*, with significant results observed for this factor (and for treatment x season, day x season and treatment x day x season) and *L. culveri* (treatment x season and day x season). The taxon *C. capitata* exhibited seasonal differences (PR x PD) restricted to NA treatment. Moreover, differences between treatments (NA x AR) were found only in PR for all days. For *L. culveri*, seasonal differences were observed in both treatments, but differences between treatments were found only in PR.

Table 3. ANOVA three-way results for most abundant taxa in relation to (A) Treatment: No algal mat, and Algal removed; (B) Day: 0, 15, 30 days and (C) Season: Post-Rainy and Post-Dry. *p<0.05; **p<0.01; ***p<0.001; n.s.: not significant.

Factors	**Most abundant taxa (F-values)**		
	L. culveri	tubificid oligochaete sp1	*C. capitata*
(A) Treatment	51.76***	29.16***	126.44***
(B) Day	13.86***	6.06**	13.12***
(C) Season	1.59 n.s.	0.47 n.s.	62.34***
(A) x (B)	0.08 n.s.	6.09**	11.57***
(A) x (C)	26.84***	0.47 n.s.	60.37***
(B) x (C)	10.58***	2.33 n.s.	8.69**
(A) x (B) x (C)	0.24 n.s.	2.34 n.s.	8.17**

Community structure from removal treatments showed different starting points, but a tendency to converge towards NA treatment regardless of the season (Figure 4). Clearly, a strong dissimilarity was observed between treatments (PERMANOVA: *pseudo*-F= 128.24; p<0.001) and among days (*pseudo*-F= 8.77; p<0.001). Besides, this dissimilarity was maintained from PR to PD (*pseudo*-F= 7.24; p<0.01). Seasonal comparisons pointed out significant variation in NA (t= 3.33; p<0.001) and AR (t= 2.51; p<0.01) treatments. Significant interactions were found for treatment x day (*pseudo*-F= 8.23; p<0.001) and for treatment x season (*pseudo*-F= 10.43; p<0.001). Pairwise comparisons for treatment x season confirmed MDS results, with differences between treatments in PR (t= 10.19; p<0.001) and in PD (t=6.50; p<0.001).

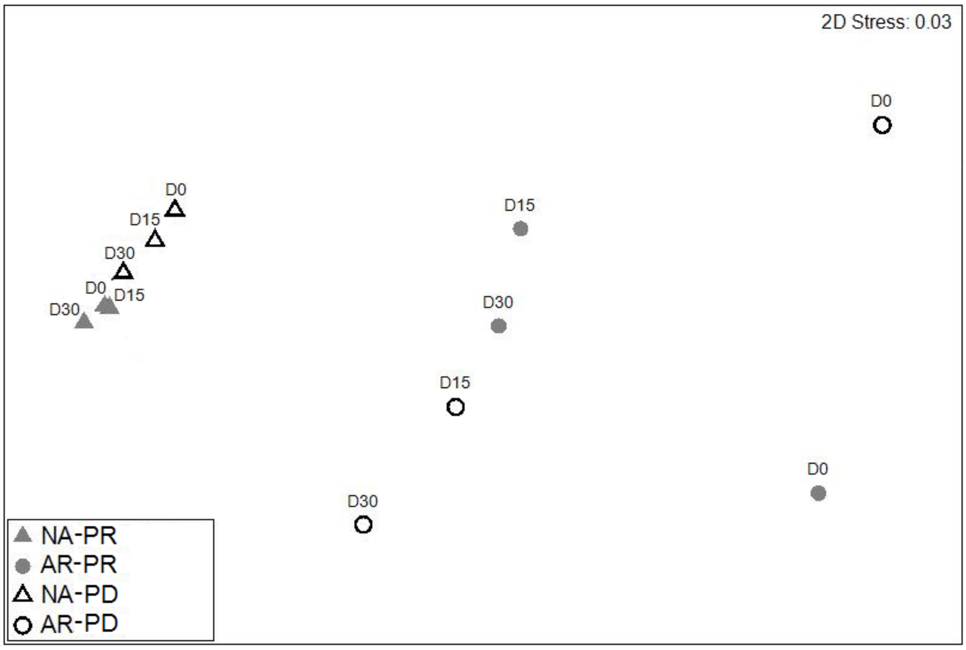


Figure 4. MDS ordination plot of macrofauna (mean abundances; n=5) in relation to treatments No algal mat (NA - triangles), Algal removed (AR - circles); days 0 (D0), 15 (D15), 30 (D30) and seasons PR (grey points), PD (white points).

SIMPER analyses showed that average dissimilarities between NA and AR slightly reduced from PR (89.45%) to PD (76.86%). The main taxa that contributed to distinguish seasonally the macrofauna in NA treatments (average dissimilarity= 40.03%) were *C. capitata* (17.02% of contribution), tubificid oligochaete sp1 (14.75%), *S. benedicti* (12.51%), *L. culveri* (11.67%) and *S. grubii* (11.17%). In AR treatment, differences between PR x PD (average dissimilarity= 80.47%) were due to *L. culveri* (71.04% of contribution) and *C. capitata* (19.39%).

IV.4. Discussion

Seasonal effects on initial macrofaunal recolonization in algal removal sediments were investigated in a tropical estuarine mudflat. Tropical ecosystems are known to be submitted to major hydroclimatic fluctuations, under rainfalls, winds and/or temperature regimes (Hernández-Guevara et al. 2008; Gaonkar et al. 2013), which may affect the process of recolonization and succession in these areas. In fact, Botter-Carvalho et al. (2011) described that macrofauna recolonization rates in control and defaunated sediments was directed influenced by pluviometric precipitation, with the start of rainy season benefiting the growth of some benthic species (*S. benedicti*, *L. acuta* and tubificid oligochaetes) in the same estuarine mudflat. For this reason, it was expected to find consistent seasonal differences in macrofauna attributes in NA and AR treatments.

Here, season exerted some influence on sediment parameters and macrofauna, but spatial variation (differences between NA x AR) was maintained regardless of the season.

In general, reductions in values of most sediment variables were observed from PR to PD specially in the removal treatment (AR), with significant differences regarding factor season found for organic matter, redox potential, carbohydrates and chlorophyll-*a*. These differences could be related to development of algal mats under different weather conditions. The most vigorous growth phase of the mats often corresponds to wet seasons (Gunatilaka, 1975). According to Zedler (1980) when rainfall is low, substrate becomes very dry and unsuitable for algal growth. Since 2013 samplings occurred right after the rainy season (mean monthly rainfall 224.7 ±139.3mm), it was likely that the algal mats developed during the rainy season have contributed more on sediment parameters than observed in the 2014 samplings, which occurred after the dry season (mean monthly rainfall 67.5 ±43.2mm).

When the rainy season comes, it is more probable for an overflow of nutrients from natural sources (fluvial discharges and terrestrial drainage) as well as from anthropogenic activities. This nutrient overload should contribute to algal mat development. Nutrients are suggested as one of the main factors influencing algal growth. As most estuaries, the Santa Cruz Channel Estuarine System is exposed to multiple pressures from industrial pollution, domestic sewage discharge, agriculture and fish farms (Flores-Montes et al. 2002; Botter-Carvalho et al. 2011). Besides, seasonal differences in the area are recognized for rainfalls (4.5x) and freshwater runoff (50x), and total average river discharge ranges for more than 55m^3s^{-1} at the rainy season and less than 1m^3s^{-1} at the dry season peaks (Medeiros et al. 2001).

The suggested changes in algal mat development influenced by rainfall regime might have consequences on the environmental heterogeneity (since major differences between NA and AR were found in PR), and also interfering in the competence of colonizers (larvae, juveniles and adult organisms) to explore resources available. Experimental studies examining the recolonization of small-scale disturbed sediment have described that recovery patterns depend on local environmental characteristics (Pacheco et al. 2010). Environmental heterogeneity is a driving force when considering recolonization and succession patterns, as observed in literature (e.g. Zajac et al. 1998) and previous experiments (Valença et al. unpublished data).

Due to the strong relationship between environment and recolonization dynamics (Zajac and Whitlatch, 1982), seasonal effects could be noted for total densities in AR, but not in terms of richness. Therefore, recolonization in these treatments was related to the availability of resident *C. capitata* and *L. culveri* species from the surrounding sediments. Besides, macrofauna from NA treatment was composed by fewer taxa after the dry season (PD). Gaonkar et al. (2013) described temporal changes in the macrobenthic assemblages of a tropical estuarine system associated with annual monsoon rainfalls, with low number of species during the non-monsoon/dry period.

Despite the different seasonal onset, a tendency of AR treatment to converge in direction to NA treatment was observed for both seasons. Evidences from other studies pointed out effects of season on early succession, but a convergence to similar community structure at later stages (e.g. Rosenberg, 2001; Pacheco et al. 2010). Several pathways, patterns and processes are known for succession of different types of assemblages (Whitlatch et al. 1998; Platt and Connell, 2003; Underwood and Chapman, 2006) but not always predictable: different starting sequences of arrivals and interactions can be followed by one climatic assemblage, but different starts can lead to different ends. Successional trajectories depended on the influences of biological interactions (e.g. competition), disturbances characteristics (e.g. magnitude) and prevailing environmental conditions, or variation in the pool of colonists (see Underwood and Chapman, 2006). Here, it is suggested that different early succession in the removal treatments was linked to the degree of environmental heterogeneity created by algal mats, which is affected by rainfall seasonality.

IV.5. Conclusion

The present results demonstrated that initial dynamics of recolonization was mainly dependent on spatio-temporal environmental variability. It is suggested that the increase in rainfalls favors the algal mat development, which drives major changes in sediment parameters, enhancing the environmental heterogeneity between disturbed (i.e. AR treatments) and reference (i.e. NA treatments) sediments. Despite that, macrofauna recolonization starting in different seasons presented a tendency to converge to a similar pattern. This provides the basis for further understanding of the recovery processes on sediments impacted by algal mats in tropical estuaries.

FINAL CONSIDERATIONS

The main aim of this thesis was to assess the effects of algal mats on benthic macrofauna. This was achieved through the analysis of fauna and sediment data from observational (Chapter 1) and manipulative (Chapters 2, 3 and 4) field experiments in a tropical estuarine mudflat where it has been observed an overall increase and persistence of benthic mat-forming algae.

Extensive development of algal mats is growing globally in significance of its impact on marine and coastal systems (Schlacher et al. 2010) and current understanding on the ecological consequences of algal mats on tropical estuaries are still scarce. Within this context, it was necessary to describe the possible effects of algal coverage on benthic system and fauna in a tropical estuaries. In Chapter 1, the effects of compacted algal mats grown attached to sediments on the benthic biogeochemistry and macrofauna were investigated in an estuarine mudflat in northeastern Brazil. During the study, the algal mat was restricted to algal patches and two types of patches were recognized based on the physiological stage (live and decomposing algae). Sediment biogeochemistry and fauna below the algal patches were significantly affected in comparison with sediment not covered by algae. Physiological stage influenced the sediment concentrations of organic matter, carbohydrates, phaeopigments, which were explained by retention properties of the algal mat, trapping sediment particles from the water column and binding them (Gunatilaka, 1975). Sediment concentrations of total-N and total-P also varied as a function of the physiological stage, suggesting that these nutrients control algal productivity (Sundbäck et al. 2003; Viaroli et al. 2005). Macrofauna declined markedly in both algal patches, with strong effects regardless of the physiological stage. These adverse effects observed on macrofauna highlight the importance of monitoring and managing the development of algal mats in tropical estuaries.

In 2013, rainfall regime exceeded the historical average precipitation in the northeastern Brazil, according to the INFOCLIMA Data Base of Climate System Data Sets. Probably, as a response to increased level of rainfalls, the algal patches have given place to a continuous algal mat, which covered most of the upper and middle zones of the estuarine mudflat. This enabled to perform a field experiment over a three-month period to investigate the recolonization and recovery of benthic macrofauna following mat displacement (simulated by removal of the algal mat). The results focused on two main factors which could potentially interfere in recolonization and recovery processes (Chapter 2): sediment compaction (laminated sedimentary structures found below the mat) and the extension of mat removal (named as spatial scale). The removal treatments (AR-1m², AR+R-1m² and AR-0.25m²) showed both uni- and multivariate significant differences compared to nearby unvegetated sediments (NA treatment) during the entire experiment. No recovery of any removal treatment was observed during the 95 days experiment. The general lack of community recovery response to algal removal was thought to be related to site-specific factors such as (1) specific environmental parameters (Norkko et al. 2010) due to environmental heterogeneity observed between the unvegetated sediments and the removal treatments, (2) early sucessional characteristics presented by the colonizers (Bolam et al. 2004; Norkko et al. 2006) and (3) the physicochemical barrier imposed by algal mats (Everett, 1994; Bolam et al. 2000).

In order to address some of these aforesaid speculations, another manipulative study (Chapter 3) was carried out during May-June 2014 to verify, through initial recolonization by macrofauna (thirty days), whether the surrounding mat may act as a barrier preventing dispersal of individuals from nearby unvegetated sediments to colonize removal treatments. Samplings were taken in nearby unvegetated sediments (NA treatment), in the border (ARE treatment) and within (AR treatment) the mat (before and after the removal of the algal layer in ARE and AR). It was observed that the initial recolonization in the removal treatments was not affected by the surrounding mat since benthic macrofauna colonized these treatments regardless of their distance (border or within the mat). At same time, the environmental heterogeneity decreased between unvegetated sediments and the removal treatments, unlike seen in the 2013 experiment. Based on results from both experiments (Chapters 2 and 3) it was suggested that the environmental heterogeneity rather than physical barrier played a major role on recolonization processes in the removal treatments. The suitability of substrate should be considered (Lundquist et al. 2006), because the dispersal of organisms also depends on the physicochemical characteristics of disturbed areas (Netto and Lana, 1994; Dyson et al. 2007). Although not tested, chemical cues released by the mat could not be neglected.

Seasonality was also considered to be an important factor when assessing and comparing the effects of algal mats on benthic fauna (Thiel and Watling, 1998; Ford et al. 1999; Norkko et al. 2000). The effects of season on initial recolonization (30 days) were explored in Chapter 4, using nearby unvegetated sediments (NA treatment) and algal removal (AR treatment) data from September-2013 (Post-Rainy season: PR) and May-2014 (Post-Dry season: PD). Season exerted some influence on sediment parameters and macrofauna, but spatial variation (differences between NA x AR) was maintained regardless of the season. The results suggested a relationship between rainfalls and algal mat productivity/development, as emphasized in other studies (e.g. Gunatilaka, 1975; Zedler, 1980). The increase in rainfalls favors an overflow of nutrients from natural sources as well as from anthropogenic activities – since the Santa Cruz Channel Estuarine System is constantly exposed to several sources of pollution. The excessive input of nutrients in the system favors the algal mat development, enhancing the environmental heterogeneity between disturbed (i.e. AR treatment) and unvegetated sediments (i.e. NA treatment). Therefore, seasonal effects could be noted for total densities in AR treatment, but not for richness. Despite the different seasonal onset, community structure from AR treatment presented a tendency of converge towards NA treatment. Rosenberg (2001) and Pacheco et al. (2010) also showed seasonal effects on early succession with convergence to control/reference samples at later stages.

Macroinfauna taxa might show variable tolerance responses to algal exposure, often linked to their resistance under longer exposure (Arroyo et al. 2006) due to changes on physicochemical properties of sediment (e.g. Hansen and Kristensen, 1997). In the present thesis, results showed that algal mats have had negative impacts on macrofauna. However, ecological consequences of algal growth, analyzed through faunal recolonization within short (few days) to medium (three months) periods in algal removal treatments were less predictable and strongly influenced by environmental heterogeneity.

The present findings also provided evidences that macrofauna succession in the estuarine mudflat affected by algal mat does not always include the successive sequence of community changes as observed in other colonization experiments (e.g. Botter-Carvalho et al. 2011). Among the early-colonizers taxa, none exhibited a true peak of opportunistic behavior. Although models based on successional continuum, as described by Pearson and Rosenberg (1978), are frequently applied in muddy organic enriched habitats (Rosenberg, 2001; Pacheco et al. 2010), they do not account for site-specific variations that may determine changes in the usual succession after a disturbance. Besides, in a low-diversity ecosystem, such as the studied mudflat, recovery should be faster and easier to predict. A slow rate of recovery may be a sign of declining resilience in the system (Norkko et al. 2010) or even the release of chemical cues by the algae in the sediment, which could perform as inhibitors of benthic fauna settlement and colonization.

Finally, linking local field experiments to broad-scale information in order to identify general mechanisms is essential in ecology, though spatial or temporal variation in the strength of site-specific relationships frequently makes it difficult (Thrush et al. 2000). Furthermore, disturbance may have transient or longer lasting effects on benthic communities (Norkko et al. 2010). Thus, to improve our understanding and to predict the impact of algal mats on structuring benthic communities, information gathered from numerous empirical studies, conducted in a wide range of environments and scales should be included. The series of experiments that were performed, although conducted under one location (a single estuarine mudflat), which limited the generality of conclusions, provided strong insights on the effects of algal mats on tropical benthic macrofauna.

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