Blooms of bryozoans and epibenthic diatoms in an urbanized sandy Beach (Balneário Camboriú - SC - Brazil): dynamics, possible causes and biomass characterization*

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ABSTRACT

Balneário Camboriu (SC - Brazil) is a touristic city where the disordered growth of the urban population and the implementation of coastal works without proper evaluation generated environmental impacts and affected the sanitary quality of water and sediment of Camboriu River and marine adjacent area. One of the most recent and alarming phenomena observed are the blooms of invasive bryozoans (*Arboscuspis bellula* and *Membraniporopsis tubigera*) associated with epibenthic diatoms (*Amphitetras antediluviana* and *Biddulphia biddulphiana*). Several clues associate these phenomena, started in 2003, with the excess of nutrients and organic matter in the Camboriú cove and large coastal works such as dredging, landfills and construction of jetties, leading to changes in benthic ecological structure. Being an aesthetic and environmental health problem, the concern of the scientific community and government agencies intensified as the occurrences become more frequent and persistent. This research addresses this

RESUMO

Balneário Camboriú (SC - Brasil) é uma cidade turística, onde o crescimento desordenado da população urbana e a implementação de obras costeiras sem uma avaliação adequada tem gerado impactos ambientais e afetado severamenteou a qualidade sanitária da água e do sedimento do rio Camboriú e da área marinha adjacente. Um dos fenômenos mais recentes e alarmantes observados são as florações de briozoários invasivos (*Arboscuspis bellula* e *Membraniporopsis tubigera*) associados com as diatomáceas epibênticas (*Amphitetras antediluviana* e *Biddulphia biddulphiana*). Vários indícios associam esses fenômenos, iniciados em 2003, com o excesso de nutrientes e matéria orgânica na enseada Camboriú e com grandes obras costeiras locais, como dragagem, aterro e construção de molhes, levando a mudanças na estrutura ecológica bêntica. Sendo um problema de estético e ambiental, a preocupação da comunidade e órgãos governamentais gerou uma intensificação nos estudos, uma vez que as ocorrên-

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issue through environmental and experimental studies. Samplings of the benthic material collected by boat and diving, and blooms monitoring were the environmental approach. The laboratory work included the algal isolation and culture, in addition to growth conditions assessment and chemical biomass analysis. Monitoring data showed a seasonal trend in the blooms, with more conspicuous events in warmer months. Diatoms increase in abundance in colder months and bryozoans in the warmer ones. The diatom *A. antediluviana*, predominant in the blooms, grew satisfactorily in laboratory cultivation, showing better growth in media with higher concentrations of silicate and phosphate. Bryozoans showed slow growth in laboratory conditions. The deposited material collected in the environment showed low concentrations of saturated fatty acids, but the high biomass suggest a possible use for biofuels production. Biomass samples dominated by bryozoans showed moderate antimicrobial activity against *Klebsiella pneumoniae*. The explanation for the occurrence of these blooms are still inconclusive, but there is considerable evidence that it is a synergistic effect between the high concentration of bacteria and organic debris in the water related to local pollution and the elimination of natural competitors by coastal works.

Descriptors: Bioinvasions, Coastal Pollution, Sandy Shores, Benthic Ecology, Phytoplankton.

cias se tornaram mais frequentes e persistentes. O presente trabalho aborda este problema através de estudos ambientais e experimentais. A coleta de material bêntico com embarcação e mergulhos e monitoramento florações junto a praia compuseram a abordagem ambiental. Os trabalhos de laboratório incluíram o isolamento e a cultura de algas e briozários, bem como a avalição do crescimento desses organismos, além da análise e avaliação química biomassa. Os dados de monitoramento mostraram uma tendência sazonal nas florações, com eventos mais evidentes nos meses mais quentes. Diatomáceas aumentaram em abundância nos meses mais frios e briozoários nos mais quentes. A diatomácea *A. antediluviana*, predominante nas florações, cresceu de forma satisfatória em cultivo laboratorial, mostrando um melhor crescimento em meios com concentrações mais elevadas de silicato e fosfato. Os briozoários mostraram crescimento lento em condições de laboratório. O material depositado no ambiente mostrou baixas concentrações de ácidos graxos, predominantemente saturados, mas a elevada biomassa sugere um possível uso para a produção de biocombustíveis. Amostras de biomassa dominadas por briozoários apresentaram atividade antimicrobiana moderada contra *Klebsiella pneumoniae*. A explicação para a ocorrência destes eventos ainda são inconclusivas, mas há evidências consideráveis de que há um efeito sinérgico entre a alta concentração de bactérias e detritos orgânicos na água, relacionada com a poluição local, e a eliminação de competidores naturais pelos impactos das obras costeiras.

Descritores: Bioinvasões, Poluição Costeira, Praias Arenosas, Ecologia Bêntica, Fitoplâncton.

INTRODUCTION

The appearance of blooms or biomass accumulations of different organisms (wracks) along the beaches are always events that draw attention. It appears as aesthetic problems to the beaches, with possible health risk, causing concern to communities and public authorities. Moreover, these phenomena represent hot spots for understanding ecological and oceanographic processes, since a very specific set of factors contribute to promote the proliferation and accumulation of a particular species.

In sandy beaches, several phenomena of accumulation and biomass deposition are widely known. Probably the most studied is the case of the surf zone diatoms, a group of microalgae that have special adaptations to stay at the surf zone of exposed, intermediate to dissipative, sandy beaches. These adaptations involve migration cycles physiologically controlled associated with advective processes. The surf diatoms populations remain in the epibenthic environment in calm conditions and are resuspended and transported onshore by storm events, which increase wave energy and surf zone width (TALBOT et al., 1990). The accumulated biomass are so huge that the surf zone water becomes flocculent brown, being among the largest known phytoplankton biomass in natural environments. These phenomena sustain the food webs from the sandy beaches where they occur, and also export biomass to adjacent systems (LEWIN; SHAEFER, 1983; MCLACHLAN; ROMER, 1990; LERCARI et al. 2010).

Another type of microalgae accumulation in sandy beaches are the neustonic blooms of *Trichodesmium* spp., a group of diazotrophic cyanobacteria that thrive in oceanic waters and are eventually carried to the coast by the wind. The blooms are relatively persistent in oligotrophic waters where they originate, but occur seasonally in the coastal zone, when tropical currents approach the coast, such as the case of the Brazilian coast, where the influence of the Brazil Current increases in spring and summer (GUIMARÃES; RÖRIG, 1997; CARVALHO et al., 2008).

Blooms of the bioluminescent dinoflagellate *Noctilucca scintillans* are recurrent on beaches producing spectacular effects of blue green light in the surf zone during the night and, sometimes, red patches during the day. Some blooms can be formed by harmful algae (cyanobacteria and dinoflagellates), and in this case represent dramatic health and ecological problems, causing damage to mariculture and shellfish extraction (ANDERSON, 2007). Despite the harmful blooms occur in different environments, there is a greater tendency to occur in coastal areas, where chemical and physical conditions are more variable and altered by interaction with the continental margin and human activities.

Macroalgae deposits on beaches may be natural, but it has been recorded a large increase of occurrences associated with coastal eutrophication and / or abnormal events of water warming. Good examples are the accumulations of the green algae *Ulva* spp. (green tides) and brown algae like *Sargassum* spp. (brown tides) (SMETACEK; ZINGONE, 2013; QUILLIEN et al., 2015). Normally the macroalgae grow nearshore on hard substrates and after excessive growth they are pulled out by the hydrodynamics and dragged to the beach face.

Beach accumulations formed by invasive species are becoming progressively more frequent. Many species are transported by ballast water and ship hulls or also introduced by aquaculture. The proliferation of invasive species generally follows the same pattern: the species find suitable environmental for establishment and express aggressive growth, since they do not have natural predators or other factors that would control their populations. The blooms or accumulations of invasive organisms on sandy beaches refers not necessarily to cases where they established and proliferate in the beach environment, but also to cases where the source environment is offshore and grown biomass is released to the beach by physical processes. This condition makes these events less predictable and more difficult to control.

In all cases above, wind and waves are the factors that determine the accumulation of material on the inner surf zone, where they are trapped by circulation cells and become more susceptible to onshore transport by breaking waves. Once they reach the beach face the materials accumulate, although eventually return to the sea by tidal fluctuations.

Bryozoans are among the invasive organisms that can accumulate on sandy beaches. These benthic animals are ordinary members of fouling marine communities that grow on ship hulls, natural or artificial hard substrates, harbors and bays (GAPPA et al., 2010). Many nonindigenous bryozoans has been recorded in southwestern Atlantic (SCARABINO, 2006; VIEIRA; VIEIRA et al., 2008), where the species *Membraniporopsis tubigera* Osburn has appeared suddenly in Brazilian and Uruguayan coasts (21° S to 33° S) since 1997, forming large deposits in sandy beaches (GAPPA et al., 2010). These accumulations draw attention not only by their large biomass but also because the high speed in which they spread southward since it has been detected at first in southeastern coast of Brazil.

A few years later, in December 2003, extensive deposits of the bryozoans *Arbocuspis bellula* (Hincks) Nikulina (formerly *Electra bellula* Hincks) and again, *M. tubigera* started to appear in Camboriú sandy beach, Santa Catarina, Brazil (27° S) (GORDON; RAMALHO; TAYLOR, 2006; RAMALHO; DIEHL, 2007). Both species are probably originated from the Gulf of Mexico, despite several records have been done in Southeastern Asia (MORETZSOHN et al., 2015; GAPPA et al., 2010). These phenomena became recurring and eventually associated with the epibenthic diatoms *Amphitetras antediluviana* Ehrenberg and *Biddulphia biddulphiana* (Smith) Boyer. Since Camboriú is an important touristic beach, the deposits were considered aesthetic and economic problems, demanding daily cleaning of the whole 6 km beach face. No other beach in the region presents these phenomena, at least in the extreme intensity registered in Camboriú beach. Faced with this situation people and political authorities resorted to the scientific community making questions like: Why is this happening? Can we control it? Is it harmful? Is it useful for some purpose?

To address these questions and try to understand the phenomenon, a mutidisciplinary team performed environmental approaches and experimental procedures consisting of sampling benthic materials by boat and diving; monitoring the occurrence and composition of blooms; cultivating and determining the growth conditions of the main organisms involved, and also the chemical characterization of the biomass. This article presents the results of this effort.

MATERIAL AND METHODS

Study area

Camboriú beach is set in the cove of the same name, located in the state of Santa Catarina, Brazil, at latitude 27 ° S (Figure 1). It is 6 km long dissipative arc bay beach delimited by two igneous and metamorphic Pre-Cambrian headlands (KLEIN et al., 2002). The beach is NW-SE oriented and is relatively exposed to NE winds which prevalent in the area throughout the year (TRUCCOLO, 2011).

Both ends present inlets - the Marambaia channel to the north and the Camboriú river estuary to the south, the latter being the main inflow of materials for the cove, with about 120 m wide, 2 m deep and an average flow of 3 $m.s^{-1}$ (SIEGLE et al., 1999). Most of the surrounding mangrove areas of Camboriú river estuary has been claimed for a disordered urban growth during the last decades, and also receives the untreated or poorly treated sewage of around 200,000 people throughout the year and up to 1,000,000 people in the summer, during touristic season (PEREIRA-FILHO et al., 2001). The Camboriú river basin is about 200 km^2 and is not industrialized, but has large areas of irrigated rice cultivation, which affect the river flow and contribute to a high load of nutrients and pesticides (MACEDO et al., 2005).

In 2002, Camboriú river mouth was dredged and the sediment deposited on the South Beach portion (Barra Sul landfill). Since these sediments had different characteristics to the naturally found in the area, severe changes in the beach sedimentological patterns were observed, resulting in increased water turbidity (PEZZUTO et al., 2006). The sediment mobilization also removed a sandbar with around 20,000 m^2 at the river mouth, eliminating dense populations of the filter feeding bivalves *Tagelus plebeius* and *Anomalocardia brasiliana* (PEZZUTO et al., 2006). Estuarine banks of suspension feeders often act as natural biological filters, removing excess of organic matter from continental drainage (NYBAKKEN, 1993). Despite no specific studies were carried out, removing these “natural filters” certainly determined an increase in the organic discharge to the adjacent cove. The subsequent ecological impact was recorded during 2003, when mass mortalities of the suspension feeder bivalve *Tivela mactroides* were recorded along Camboriú beach. This phenomenon was the result of fine sediments migration from the landfill area to deeper parts of the bay, with consequent suffocation of organisms (PEZZUTO et al., 2006). Due to the large biomass revealed by the deposition of dead organisms, this bivalve probably was a dominant species in the shallow subtidal benthic assembly. Its removal significantly altered the local ecosystem trophic structure. Finally, in December 2003, accumulations of bryozoans and algae started to occur persisting to date.

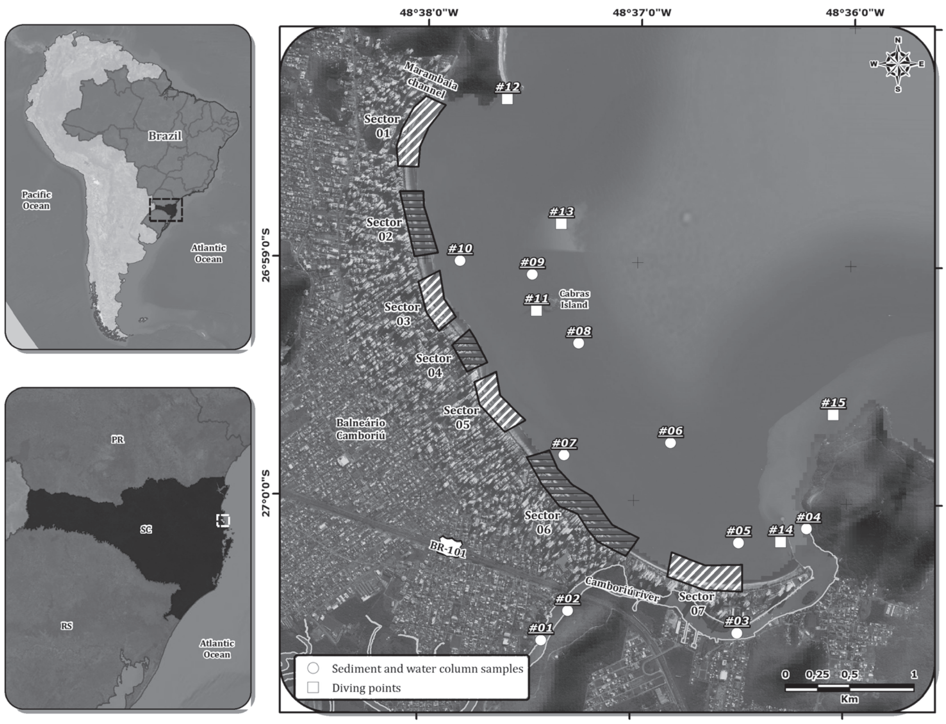

Figure 1. Camboriú beach location showing main physical and geographical features, sampling points and beach sectors defined according to the distribution of bryozoans and diatoms deposits (see text for details).

Sampling and Monitoring

Five types of sampling or monitoring were performed in the study area: (a) daily monitoring of deposit occurrence, (b) water column, (c) sediment, (d) scuba diving and (e) the relative composition of the deposited organisms. Table 1 shows the features and goals of these samplings and Figure 1 shows the location of sampling points during sampling cruises, when water and sediment were collected.

Table 1. Types of sampling or monitoring performed in Camboriú beach and bay between 2011 and 2013, their aims, procedures and periods.

Type	Aim	Procedures	Period
(a) Daily monitoring of deposit occurrence*	Determination of spatial and temporal occurrence of deposits on Camboriu beach	Daily record of deposits location and approximate weight across the beach, by weighing the collected material in bags	August/2011 to April/2013
(b) Water column	Qualitative analysis of phytoplankton at the bay and estuarine zone; Checking for the presence of epibenthic diatoms in the water column	Vertical hauls using phytoplankton net 20µm mesh on motorized boat; Analysis of presence / absence of diatoms under a microscope	11/11/2012 and 03/28/2012
(c) Sediments	Checking for the presence of target organisms (epibenthic diatoms and bryozoans) on bay and estuarine sediments	Sediment sampling with Ekman dredge and beam–trawl hauls	11/11/2012 and 03/28/2012
(d) Scuba diving	Checking for the presence of target organisms	Diving with movie camera and collection of hard substrates for analysis. Diving and collecting from motorized boat.	12/07/2013
(e) Relative composition of the deposited organisms	Determination of the relative amount of species; Biomass analysis	Manual collection (composed samples, with rates by at least 6 different points)	11/18/2011 01/05/2012 02/01/2012 03/28/2012 06/29/2012

** Procedure executed by workers from Coneville Company (Environmental Sanitation and Concessions Ltd.) that monthly sent a spreadsheet with the following data: date, place (street), time, mass of material per location, total mass of material collected and images (pictures) of collection sites.*

PHYTOPLANKTON ANALYSIS

Phytoplankton samples were collected with 20 µm mesh plankton net by vertical hauls throughout the water column, and fixed with 2% Lugol's solution. In each sampling point (Figure 1) 5 hauls were carried out. In the laboratory, samples were homogenized and aliquots of 1 mL analyzed under a microscope in Sedgewick-Rafter chambers, registering organisms with special attention to the occurrence of the epibenthic diatoms *A. antediluviana* and *B. biddulphiana*.

Sediment sampling

Sediments samplings were carried out by 10 m bottom trawl (Beam-trawl) at the bay area (predominantly sandy bottom) and 5-8 Ekman dredge throws per sampling point near and inside estuarine area (muddy bottom). The collected material was deposited on trays and homogenized for further separation of a subsample of about 5 L which was labeled and packaged for later analysis in the laboratory. In laboratory, samples were resuspended with filtered seawater and an aliquot of about 100 ml of the supernatant organic/biological material was fixed in 2% formaldehyde. The presence of target species was verified under stereoscopic microscope.

Processing and analysis of the deposited material

On five occasions in which large deposits of target organisms were recorded in Camboriú beach (Table 1), the material was collected in 6-8 points randomly distributed along the beach for determine the relative proportion of organisms and for further analysis of biomass. The material was manually collected and stored in plastic bags until processing in the laboratory, no later than three hours after collection. In the laboratory, the material was resuspended and washed with filtered seawater, separating organisms from sand and debris by using a small sieve with 60 µm mesh. From this material, about 1 kg of each sample was frozen for subsequent analysis of chemical and biological activities of biomass, and an aliquot of about 50 ml was completed to 100 ml with filtered seawater and fixed with 2% formaldehyde.

For analyzing the proportion of diatoms and bryozoans three 1 ml Sedgewick-Rafter chambers of each sample of the set were mounted in which 5 to 8 microphotography were taken randomly with 100x magnification. The abundance of each species was determined by counting cells/organisms on the digital photos using the software LEICA 3.0©, where results were automatically recorded into an Excel spreadsheet.

Frozen aliquots of biomass were freeze-dried in small portions (lyophilizer Terroni Ravel, LT model 1000/8), being kept at – 17°C until analysis. The chemical characterization of biomass involved analysis of lipids, fatty acid profile and Total Organic Carbon (TOC). The aim of this analysis was to evaluate the biomass as a possible raw material for obtaining oils for biofuel (biodiesel) production, or eventually for other purposes.

Lipid analysis and fatty acid profile

Total lipids were extracted and quantified by the method of BLIGH; DYER (1959). Aliquots of 0.2 g of freeze-dried biomass from were transferred to test tubes covered with aluminum foil, in which 1.5 mL of chloroform:methanol (1:2 v/v) was added. Each tube was shaken for 5 min in vortex. Then, 0.4 mL of distilled water was added and the tube was vortexed for more 2 min. Further, 0.5 mL of chloroform was added and again vortexed for 2 min. Finally, another 0.5 mL of distilled water was added followed by vortexing for more 2 min. After this process, the tubes were centrifuged for 5 min at 3,000 rpm to phase separation. For full extraction of lipids samples remained for 24 h at 4°C in dark. The lipid extract was recovered with Pasteur pipette and separated into another tube with known mass. To the residual biomass in the tubes, 2 mL chloroform were added, then vortexed and centrifuged recovering the whole lipid phase to the first tube. The chloroform was then evaporated and the tubes with total lipids sample were weighed discounting the weight of the tube to find the amount of lipids in each sample.

With the obtained extracts it was also performed a qualitative and quantitative analysis of fatty acids by gas chromatography. The lipid fraction was esterified to obtain the methyl esters of fatty acids, according to the methodology proposed by PALAU et al., (2007). The fatty acid analysis was performed on a Varian gas chromatograph model - 3400CX equipped with a flame ionization detector and a fused silica column of 30 m length and 0.32 mm in diameter, containing polyethylene glycol as stationary phase. The carrier gas was nitrogen at 0.5 mL.min-1. Temperatures of the injector and detector were 250°C and 280°C, respectively. The initial column temperature was 100°C then increased at 8°C.min-1 until 230°C, staying at this temperature for 20 min. The fatty acids were identified by comparison of retention times with standard (Sigma Supelco; Bellefonte, USA) and quantified by area normalization.

Total Organic Carbon (TOC)

For determining total organic carbon (TOC) 2 mg of freeze-dried biomass were used. Samples, in triplicate, were submitted to catalytic combustion (TOC Analyzer Vario-Cube, Elementar®) by sample digestion in reaction column at 950°C and detection by infrared (NDIR detector) with a standard deviation detection of ± 1%. The analytic gas used was 99.99% purity oxygen under 1200 mbar pressure and 200 $mL.m^{-1}$ drag flow. The average value of triplicates was only accepted if coefficient of variation among replicates was smaller than 10%, otherwise a new set of replicates was used.

Antimicrobial activity of the biomass

The antimicrobial activity characterization refers to samples collected in 01/02/2012, when bryozoans were widely dominant. These samples were chosen because this group of organisms is known to produce molecules with different biological activities (FIGUEROLA et al., 2014). The methodology followed the recommendations of ALMEIDA et al. (2012), where 10g of freeze-dried biomass were initially washed with ammonium formate (0.5 M) at a ratio of two parts of reagent to one part of biomass (2:1), in order to remove the salt. Formate was allowed to act for 5 min, then the sample was centrifuged for other 5 min at 4,000 rpm. The supernatant was removed and the sedimented biomass was again resuspended with formate (2:1), repeating this process three more times. The desalted biomass was exhaustively extracted with 92% ethanol. The resulting extract was concentrated under pressure resulting in an aqueous fraction, a fraction of hexane and a butane fraction. All fractions were tested for antifungal and antibacterial activities. The antimicrobial activity was evaluated by the disk diffusion test (Oliveira et al., 2005) with some modifications. The filter paper discs (6 mm) were impregnated with 20µL of extract solutions and then placed on Muller-Hinton agar plates (HIMEDIA), which were inoculated with the microorganism to be tested according to the standard protocol described by CLSI / NCCLS (2002). The plates were incubated at 35 ± 1 °C, and after 18h the diameter of the inhibition zone were measured. Paper discs containing only the solvents were used as negative control. The tested organisms were: *Sporogenes perfringens* (ATCC 11437), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Streptococcus pneumonie* (ATCC 49619), *Streptococcus pyogenes* (ATCC 19615), *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 25922), *Enteroccocus faecalis* (ATCC 29212), *Klebsiella pneumonie* (ATCC 13883), *Salmonella typhimurium* (ATCC 14028), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231) e *C. tropicalis* (ATCC 13803).

Isolation and culturing of the diatoms

Live samples from deposits containing diatoms were collected on several occasions, in order to isolate the two species in laboratory and evaluate if their growth rates could explain the high biomass in the beach deposits. Three culture media were tested: F/2 according to ANDERSEN (2006), F (F/2 medium with double concentrations of the components) and IMR medium according to EPPLEY et al. (1967), which presents differences in the concentrations of components in relation to the F/2 medium, especially because of the higher concentration of phosphate and silicate. Table 2 shows a comparison among these culture media regarding the macronutrients (N, P and Si).

Table 2. Molar concentrations of macronutrients (N, P and Si) in the diferent culture media tested and as their atomic proportions.

Nutrient	Culture medium		
	IMR	F	F/2
Nitrate (N-NO3-)	0.00412 M	0.024 M	0.012 M
Phosphate (P-PO4-3)	0.000775 M	0.00224 M	0.00112 M
Silicate (Si(OH)4)	0.012 M	0.001 M	0.005 M
Si:N:P Ratio	15:5:1	4.5:11:1	4.5:11:1

Diatom cells or chains were isolated through the capillary isolation method (ANDERSEN, 2006). Cultivation conditions were: irradiance of 50µMol.m^{-2}.s^{-1} (58W daylight fluorescent lamps), photoperiod of 12 hours and temperature of 22° ± 2°C. Cultures were maintained in 250mL Erlenmeyer flasks with 100 mL of culture media. Once the isolates showed physiological acclimation to the laboratory conditions (visible growth in the flasks), weekly subcultures were performed for maintenance.

Viable cultures were grown in five different salinities (15, 20, 25, 30, 35) and the three culture media above referred. The tests were carried out in 24-well microplates with 2 mL of culture medium and 0.5 mL of inoculum into each well with four replicates for each treatment. Cell counting was performed under inverted microscope at 250x increase. The counts were made immediately after the inoculation (T_0) and following 96 hours (T_n). Data of initial and final cell density of each sample, were used to generate growth rates using the the exponential growth equation (equation 1). Growth rates were compared by two-way ANOVA (Bonferroni post test) to verify significant differences among treatments using GraphPad Prism 6.0 software. The purpose of these tests was to find out the best growth conditions for the diatoms, trying to understand if they are fast-growing algae, which could explain their abundance in the beach deposits.

Equation 1.

where: µ is the specific growth rate; N_0 is the cell density at t_0 (cells.mL^{-1}); N_n is the cell density at t_n (cells.mL^{-1}); t_n is the time of the final measure (96h).

Isolation and cultivation of bryozoans

On March 8, 2013 live samples of bryozoans from deposits were collected in order to try to cultivate the dominant species *Arbocuspis bellula*. The material was kept in water from the sampling site, inside Styrofoam boxes until processing in the laboratory (maximum 3 h after collection). In the laboratory, 4 bryozoan fragments of about 3g were inoculated into each of 3 tissue culture flasks of 410 mL (TPP tissue culture flaks, filter cap), filled to 2/3 of the volume with sterile filtered seawater at the same salinity and temperature of the sampling site (salinity 30, temperature 23° C). These bottles were kept in a room with the same light conditions, temperature and photoperiod described above for diatoms. After acclimation for 72 hours the flasks received weekly 5 ml of an exponentially growing culture of the microalgae *Isochrysis galbana*. Survival and growth of the fragments were observed over 60 days by weight measurements every 15 days in semi-analytical balance (precision 0.001 g). Average growth rate was determined as daily percentage increase of wet weight. The rates were determined using only the initial and final data (day 1 and day 60, respectively). For weighing, colonies were carefully removed from the bottles and dry on paper towels. The purpose of these experiments was the same described above for the diatoms.

RESULTS

Occurrence of the beach deposits

During the 18 months of daily monitoring along Camboriú beach conspicuous deposits of the target species (bryozoans and epibenthic diatoms) were recorded in 152 days, corresponding to around 30% of the period. The total amount (kg) as well as the relative proportion of species in the deposits varied throughout the year. Figure 2 shows the temporal and spatial distribution of deposits in different sectors of the beach (see Figure 1 for location of the sectors). Although no systematic analyzes have been made, it was visible to the naked eye a higher relative importance of bryozoans in warmer months, whereas diatoms were more common in winter and spring months. However, bryozoan abundance was always higher than diatoms.

The weight of deposited material was higher during summer and autumn, with a peak of about 480 tons in March 2012, and lower in the winter and spring months (Figure 2A). The total weight deposited in the period was about 2,700 tons. Regarding the spatial distribution of deposits along the beach, a clear sectorization coul be seen, where the sectors 1, 3, 5 and to a lesser extent, sector 7 had the highest total mass in the period and the sectors 2, 4 and 6 showed much lower amounts (Figure 2B).

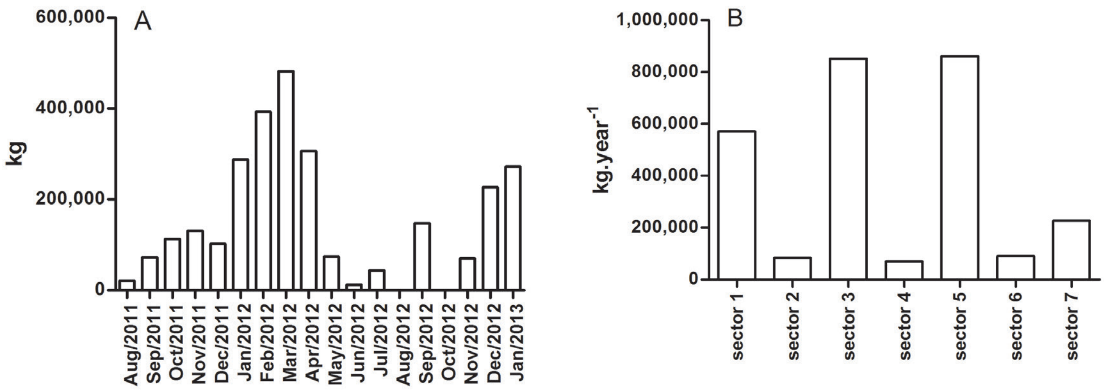

Figure 2. Biomass of the bryozoans and epibenthic diatoms deposited at Camboriú beach between August/2011 and January/2013. A: total biomass along the beach by month; B: total biomass along the entire period in different beach sectors. Sectors refer to figure 1.

Regarding the proportion of diatoms and bryozoans species in samples from the five defined sampling days, it was noted in the case of diatoms, a significant predominance of *Amphitetras antediluviana* (Table 3). Regarding bryozoans, *Arbocuspis bellula* was widely prevalent or exclusive (Table 2). Under the microscope was possible to see that diatoms grow intertwined with bryozoans, apparently are using these animals as a substrate.

Table 3. Proportion of epibenthic diatoms and bryozoans in samples collected from of Camboriú beach deposits.

	Diatoms		**Bryozoans**	
Sampling date	***Amphitetras antediluviana* (%)**	***Biddulphia biddulphiana* (%)**	***Membraniporopsis tubigera* (%)**	***Arbocuspis bellula* (%)**
01/05/2012	81.3	18.7	0	100
03/28/2012	58.9	41.1	0	100
06/25/2012	60.4	39.6	8.4	91.6
01/27/2013	64.0	36.0	1.1	98.9
02/07/2013	54.6	45.4	0	100
Mean	63.8	36.2	1.9	98.1

Presence of the target species in the water column and sediment samples

Checking the presence of the target species in water and sediment samples in Camboriú bay and Camboriú estuary is important for understanding the origin and dynamics of the deposits. No diatom cell or bryozoan zooid were found in the 10 water samples collected in subsurface and 1m above bottom during the cruises, despite being recorded on the beach as deposits in the same days. The same negative result was obtained from the phytoplankton net hauls. Phytoplankton was dominated by planktonic diatoms like *Skeletonema* spp., *Thalassiosira* sp., *Pseudo-nitzschia* spp., *Dactyliosolen fragilissimus* and *Chaetoceros* spp. There was also considerable importance of nanoflagellates. These results confirm the benthic characteristic of the diatoms *A. antediluviana* and *B. biddulphiana.*

The results for sediment sampling are shown in Table 4. During the scuba diving, it was verified the discreet presence of bryozoans in several rocky substrates in points # 11, # 12, # 13, # 14 and # 15, but belonging to species other than the found in the deposits. The bryozoan *A. bellula*, dominant in the deposits, was found predominantly on sandy bottom samples (points # 4, # 5, #6 and # 8). It is an indication that this species has epibenthic habitat in the region, growing not attached to hard substrates but free on the sandy bottom.

Table 4. Characteristics of the substrate/sediments from Camboriú bay collected or observed to verify the presence of the target organisms. The points refer to Figure 3.

Point	Depth (m)	Substrate type	Macroscopic characteristics	Presence of target species	Sampling date/ Observations
#1	1.5	Muddy	Biodetritic gravel	No	11/18/2011
#2	1.7	Sandy-muddy	Biodetritic gravel	No	11/18/2011
#3	3.0	Sandy-muddy	Absence of biological material	No	11/18/2011
#4	1.8	Sandy-muddy	Low quantity of target material and presence of other organisms	Yes	11/18/2011
#5	2.2	Sandy	High quantity of target material and presence of other organisms	Yes	11/18/2011
#6	3.8	Sandy	Low quantity of target material	Yes	03/28/2012
#7	1.8	Muddy	Vegetal debris and biodetritic gravel	No	03/28/2012
#8	3.6	Sandy	Vegetal debris e biodetritic gravel	Yes	03/28/2012
#9	3.2	Sandy	Many *Tagelus* shells	No	03/28/2012
#10	1.7	Sandy-muddy	*Tagelus* and other molluscan shells, anaerobic aspect	No	03/28/2012
#11	4.0	Rocky	Considerable presence of macroalgae on rocks; considerable diversity of animals like sponges, hydroids, some urchins; considerable deposition of fine sediments; some bryozoans present particularly on the lower face of rocks	Yes	12/07/2013
#12	4.5	Rocky	Clean rocks, few algae, low sediment deposition, moderate hydrodynamics, biogenic pebbles, absence of bryozoans	No	12/07/2013
#13	6.5	Rocky	Clean rocks, few algae, low sediment deposition, moderate hydrodynamics, biogenic pebbles, absence of bryozoans	No	12/07/2013
#14	1.0	Rocky	Shallow area, maximum 1.5m depth; presence of algae, especially *Ulva* spp. and no bryozoans.	No	12/07/2013
#15	4.0	Rocky	Few algae; Presence of bryozoans.	Yes	12/07/2013

BIOMASS CHARACTERIZATION

Total lipids and total organic carbon (TOC)

Table 5 presents the results for lipid content and TOC in samples collected on different dates. There was a trend of increased lipid content in the samples with the highest proportion of diatoms, indicating that they are the main contributors to lipids in the material analyzed.

Regarding the TOC, the highest value was obtained in the sample with the highest proportion of bryozoans, indicating the influence of these organisms in the organic content of the material. In general, the percentage of organic carbon was relatively low, indicating the influence of calcium carbonate from bryozoans and silica from diatoms. However, it must be considered the possible presence of sand in the samples. All samples contained sand, but its concentration could not be determined. So, it is expected a certain error in the determinations.

When evaluating illustratively the proportion of lipids in the TOC, it is clear that the samples with higher proportion of diatoms (# 2 and # 4), over 60% of the organic content was represented by lipids, while in samples dominated by bryozoans this proportion was lower.

Table 5. Percentage of total lipids, TOC and proportion of diatoms and bryozoans in the five samples of deposited material analysed.

Sample ID	Sampling date	Total lipids (%) Average±SD	TOC (%) Average±SD	%lipids in TOC	% diatoms	% bryozoans
1	11/18/2011	3.00±0.31	-	-	80	20
2	01/05/2012	4.62±0.05	6.887±1.028	67.1	80	20
3	02/01/2012	3.28±0,21	9.481±0.806	34.6	10	90
4	03/28/2012	3.07±0.4	4.890±1.049	62.8	90	10
5	06/29/2012	0,98±0,49	5.966±0.980	16.4	5	95

Table 6 shows the fatty acids profile from 4 of 5 samples of deposits collected. The fatty acid profile showed minor differences among samples. In total, 14 different fatty acids (and/or isomers) were identified, both saturated as unsaturated. Saturated fatty acids were more representative, especially hexadecanoic acid (16:0, palmitic acid) with 50% on average in the samples, tetradecanoic acid (14:0, myristic acid) with about 31% on average and octadecanoic acid (18:0, stearic acid) with about 6% on average. These three compounds correspond to approximately 90% of the total fatty acids identified in the biomass. Among the unsaturated fatty acids the main was the (9Z)-Hexadecenoic acid (16:1 palmitoleic acid) with an average of 8% in the samples.

Table 6. Fatty acid profile (%) in the 4 deposit samples analyzed. Mean and Standard Deviation (DP) among three chromatographic injections. Obs.: cases where the sum was lower than 100% are related to the presence of unindentified fatty acids, not presented in the table.

		Samples			
Fatty acid name	C:D*	2 (01/05/2012)	3 (02/01/2012)	4 (03/28/2012)	5 (06/28/2012)
Tetradecanoic acid	C14:0	26.10±0.2	20.8±0.2	35.9±0.31	42.5±0.7
Pentadecanoic acid	C15:0	1.50±0.2	1.4±0.07	1.5±0.04	0.00
(9Z)-Hexadecenoic acid	C16:1	12.50±0.2	6.52±0.14	4.6±0.06	8.65±1.1
Hexadecanoic acid	C16:0	53.60±1	48.52±0.61	52.4±0.32	45.5±0.38
Heptadecanoic acid	C17:0	0.40±0.1	3.1±0.09	0.8±0.02	0.00
(9Z)-Octadecenoic acid	C18:1c	0.00	2.77±0.09	0.8±0.02	0.00
(E)-Octadecenoic acid	C18:1t	0.60±0.5	1.36±0.01	0.00	0.00
Octadecanoic acid	C18:0	3.10±0.1	13.68±0.08	3.9±0.05	3.07±0.14
(5Z,8Z,11Z,14Z)-Eicosatetraenoic acid	C20:4	0.10±0.1	0.00	0.00	0.00
Eicosanoic acid	C20:1	0.10±0.1	0.49±0.01	0.00	0.00
Docosanoic acid	C22:0	0.40±0	0.64±0.02	0.00	0.00
Tetracosanoic acid	C24:0	0.60±0	0.73±0.04	0.00	0.00
% Saturated		85.70	87.47	91.07	94.50
% Unsaturated		13.30	4.62	8.65	5.40

*C:D - notation where C is the number of carbon atoms in the fatty acid and D is the number of double bonds in the fatty acid.

Antimicrobial activity of the extracts

The biomass samples No. 2 and 3 (Table 5), which were subjected to extraction of possible active principles and tested showed no antimicrobial activity against most of the microorganisms. However, there was moderate activity against *Klebsiella pneumoniae*, a pathogenic bacteria that causes respiratory diseases. This result, although preliminary and little expressive, indicates a potential to be better explored in subsequent research.

Physiological tests with the diatoms

Both species of diatoms have been isolated, but only *A. antediluviana* grew satisfactorily in laboratory cultures. Thus, the physiological experiments were conducted only with this species, which also proved to be the predominant in the deposits. Even after isolated the species often presented smaller diatoms and other microorganisms as epiphytic contaminants, so that further treatment was required for final purification. The pre-isolated algae were repeatedly washed with sterile culture medium through a sieve with a 20 µM mesh for the epibionts to be removed.

Figure 3 shows the results of growth tests performed with three different culture media and 5 salinities. In general, *A. antediluviana* has low growth rates, which could be explained by its large cell size. Statistical analysis showed that there was no interaction between the factors and salinity culture medium (P>0.1), but the salinity and the culture medium alone highly significantly affected the growth of the species (P <0.0001). Comparing the growth in F and F/2 media, the latter showed significantly higher growth than in salinities 20 and 35. When comparing the IMR medium with the others, the growth was significantly higher in IMR medium than in F in salinities 20 and 25 and also higher than the medium F/2 in salinity 25. From this it was possible to conclude IMR medium was generally more suitable than the others. Apparently, taking into consideration the macronutrient composition of the media, the diatom in question requires higher concentrations of phosphate and silicon, features found in the IMR medium. The cultures grown in F and F/2 media, commonly used for marine diatoms, also showed morphological changes in frustules as well as frequent formation of auxospores. Cells grown in IMR medium appeared healthier and no auxospore formation was detected.

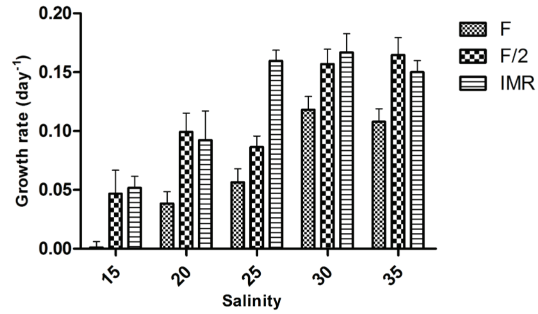

Figure 3. Growth rates of the diatom *Amphitetras antediluviana* in different culture media and salinities. Bars on columns indicate the standard deviation of the mean (n=4). See text for statistics details.

In vitro growth of *Arbocuspis bellula*

The fragments of *A. bellula* incubated had, in general, good survival and adaptation to laboratory conditions. Of the 12 inoculated fragments only 2 died, and it took place between the days 30 and 45. These colonies were removed from their flasks and not considered in the calculation of the growth rate. As can be seen in Figure 4, the growth pattern was very similar among fragments and among flasks, except for flask # 1 which had a considerable drop in biomass between days 15 and 30 because of a fragment which showed negative growth (lost part of the colony). However, this fragment recovered growth and the biomass data were similar to the other at the day 60. Average daily growth rate (daily percentage increase in fresh weight), calculated using only the initial and final data (T_0 and T_{60}) was 0.237%, with 16% coefficient of variation among the means of culture flasks.

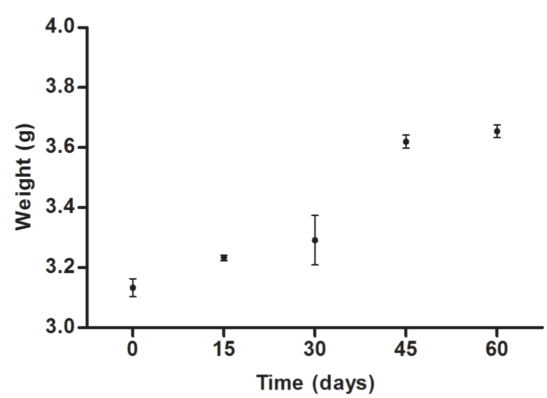

Figure 4. Growth data of the bryozoan *Arbocuspis bellula* in laboratory cultures. Each point is the mean weight (g) of three fragments/colonies. Bars represent the standard deviation of the mean.

DISCUSSION

Temporal and spatial dynamics of the events

The monitoring of beach deposits showed that the phenomenon is intermittent throughout the year. There was a tendency for greater biomasses in the months of December, January, February and March (austral summer), especially with regard to the dominant bryozoan, *Arbocuspis bellula*. Certainly the largest temperature increases metabolism and growth of these organisms, considering its tropical origin (HAYWARD; RYLAND, 1995). However, as they are suspension feeders, a possible additive effect related to the higher input of organic matter (bacteria and debris) in the summer period (local touristic season), must be considered. Then, it can be expected a probable synergism between temperature and pollution favoring the growth and deposition of organisms in summer.

An aspect that became evident is the epibenthic behavior of *A. bellula* in the area, although it is described as incrusting, especially on macroalgae (LISBJERG; PETERSEN, 2000). During the studied period it was not possible to detect large biomasses of the species attached to hard substrates, although the surveyed area has been restricted, not encompassing rocks and macroalgae located farther offshore. The species was only recorded at two points of rocky bottom, but in small amounts along with other species (Table 4). During the dives and observations at the surf zone it was possible to observe the material being gradually transported onshore along the sandy bottom. GAPPA et al. (2010) found the same behavior for *Membraniporopsis tubigera* in sandy beaches of the extreme southern Brazil and Uruguay. Apparently these species may switch between fouling and epibenthic (not attached to substrates), which gives adaptive advantage to different conditions. Thus it is very likely that the origin of these organisms in Camboriu beach are large areas of sandy bottom located inside and outside the bay. To confirm this a more extensive search is needed in the region, which can only be achieved by obtaining images of sandy and rocky bottoms with the help of remotely operated vehicles (ROV) or similar system.

Regarding the diatom *A. antediluviana* the events described in this study are the first records of the species forming blooms and deposits on beaches. This species has been eventually recorded in the plankton of the Brazilian coast (VILLAC et al. 2008), but never in high densities. *B. biddulphiana* has been already recorded forming turf like blooms on corals in the Midriff Islands, Gulf of California (GALLAND; PENNEBAKER, 2012). The authors considered the species as invasive, but report that the bloom quickly retreated and was not seen in the following years. In the material obtained from the deposits of Camboriú beach these diatoms always presented long chains interlaced with the bryozoans. This behavior suggests that the microalgae use the bryozoans as support for growth, probably benefiting from the CO_2 released from the respiration of these animals. The large size of these diatoms (80 to 150µm in major axis) and their habit of forming chains indicates that they are not used as food by the bryozoans, meaning that there is no trophic relationship between these species. The decrease of diatoms in the months of higher temperatures should be related to the intense growth of bryozoans, which can prevent the diatoms to fix on it. In periods of lower temperatures the smaller growth rate of bryozoans favors epibiosis.

The spatial distribution of deposits along the beach showed clear patterns. Camboriú beach seems to have two circulation cells induced by wind and waves, delimited by the central beach cusp associated with the Cabras Island, where there is a decrease in depth and hydrodynamics (Figure 1, sector 4). Thus, there are two areas relatively most exposed and with more energy (sectors 2 and 6) and four areas of lower energy (sectors 1, 3, 5 and 7), which favor the deposition of the debris transported by waves. It was in these sectors of lower energy which the largest biomasses were recorded throughout the study period. This trend of wrack deposition at the ends of arc beaches is well described in the literature, especially for low-energy beaches (JACKSON et al. 2002). An apparently random deposition of materials showed to follow a clear pattern related to beach morphodynamics.

Finally, it is noteworthy the fact that these events only occur in such large proportions in Camboriu beach. Regionally, the material was only detected in two other beaches, one distant 130 km to the north and another 100 km to the south, but with very small densities, nothing comparable to Camboriú events. This indicates that the forming organisms are not trapped in Camboriu bay and may be dispersed by a number of processes. It seems clear, then, that local factors are critical for such a large proliferation. The high levels of pollution and the environmental and ecological changes that have taken place at this beach are certainly among the explanations, as discussed below.

Possible causes of the events

The two species of bryozoans recorded in this study are alien species or bioinvaders. In this sense, they certainly take the advantages of this condition such as the absence or low incidence of natural predators, parasites and diseases, and possible adaptations that allow them to overcome and possibly displace resident species (ELTON, 2000). This partly explains the persistent and progressive increase of the events on Camboriu beach. However, the adaptive advantages of an invasive species do not explain why this intensive process is recorded about 12 years only on this beach and not in the neighboring beaches. This fact, as already mentioned, requires the identification of local factors that favor the involved species, more than in neighboring regions.

There are several features in Camboriú Beach that differ from nearby beaches, such as: a high population density; high pollution load of Camboriu river due to an under dimensioned and/or low efficiency sewage treatment system, or even the absence of sewage treatment in the upstream cities; the relatively heavy nautical traffic, which may contribute to the proliferation of invasive species, and the execution over the years of many coastal works such as landfills, dredging and without adequate technical criteria, resulting in the removal of native ecological components, facilitating the bioinvasion and niche substitution.

Intense pollution load represented by organic debris and bacteria favors suspension feeders like bryozoans. In addition, PEREIRA-FILHO et al. (2001) detected a high chlorophyll-a belt along the Camboriu river estuary, related to nutrient intakes. In recent studies it was recorded values up to 100 $\mu g.L^{-1}$ chlorophyll-a in the estuarine area (author's unpublished data). On the other hand, it can be expected high toxicity levels in these waters due to the complexity of pollution sources (sewage, pesticides, waste oil fuels, etc.). PEZZUTO et al. (2006) found highly toxic sediments in the area, attributing such toxicity to heavy metals. This reveals a history of severe contamination in the area. In this context, it is possible that the bryozoans have higher tolerance to the effects of these pollutants and benefit from the high primary and bacterial production.

In addition to the aspects related to pollution, ecological disturbances caused by technically inadequate coastal works (see study area) were certainly critical to determine the emergence and expansion of the events, as hypothesized by PEZZUTO et al. (2006). The physical removal or suffocation of the native suspension feeders near estuarine banks, opened the niche for opportunistic species which can be the case of the bryozoans studied here. Apparently, it is a case of persistent ecological disturbance, indicating loss of ecosystem resilience and the establishment of invasive species whose control will be very difficult.

The above explanations can also be applied to epibenthic diatoms. Diatoms are typically opportunistic primary producers, who have advantages over other groups of algae in conditions of high nutrient concentration. Blooms of diatoms in coastal areas are common and natural in the surf zone of some types of sandy beaches (surf-zone diatoms), and have been extensively studied both in Brazil and globally (TALBOT et al. 1990; RÖRIG; GARCIA, 2003). In this case, the processes are well known. But, for the species studied here, there is not enough knowledge on their physiology and ecology. On the other hand, there are studies that show affinity for certain bryozoan larvae to settle in biofilms of benthic diatoms (DAHMS et al. 2004). That insert a further complexity component for the understanding of Camboriu events.

The growth rates of the bryozoan *A. bellula* and the diatom *A. antediluviana*, determined in laboratory to evaluate the physiology of these organisms, showed low values. These low rates do not explain the sudden appearance of the beach deposits by overgrowth (bloom). Thus, it is more likely that the deposited biomass are the result of slow and gradual growth in extensive nearshore areas, accumulated beachward by increased wave energy due to the passage of cold fronts, which have a frequency 3-4 events per month in the region (TRUCCOLO, 2011). However, it should be considered that the laboratory conditions in which growth tests were performed may not be optimal for expression of maximum growth rates of these organisms. In the environment, the increased turbulence and possible existence of specific nutritional factors, can optimize the species growth. *A. antediluviana* was grown in liquid medium without the presence of a substrate, a possible requirement for good growth, which in the environment is represented by the bryozoans. Moreover, the greatest growth rates were achieved when using culture medium with higher concentrations of phosphate and silicon, indicating an opportunistic nature for this species. In fact, many other experiments varying growing conditions must be done to set up the real maximum potential growth rates for these algae. A reinforce to this hypothesis is the fact that, in the beach deposits, the species *B. biddulphiana*, was always present and looking healthy. In the laboratory this species simply was not cultivable under the tested conditions. For the bryozoan *A. bellula*, the experiments were static (without turbulence) and using a unique food source (*Isochrysis galbana*), conditions that may have limited its physiological performance. Moreover, the fact of *A. bellula* and *A. antediluviana* have remained alive and relatively healthy in laboratory cultures, despite the low growth, indicates that they are physiologically robust species.

At last, there are accumulated evidences indicating that the definitive explanation for the emergence and maintenance of these events in Camboriú seems to be a combination of factors, where invasive pollution tolerant species have found available niche and large amount of food for establishment. No conclusive answer was obtained to the question about the origin of the material. That is, if it comes from rocky bottoms of adjacent marine areas or if actually grows in the epibenthos of large areas of sandy bottom.

Possible use and importance of the biomass

The biomass deposited on Camboriu beach is removed every day and sometimes twice a day. This procedure is necessary because the appearance of the material is unpleasant, causing inconvenience to beach goers, who reach tens of thousands in summer days. However, public spending generated for such cleaning is very large. While the process is not sufficiently known to evaluate a possible way of control, it seems reasonable to give some useful purpose for the biomass, other than the simple deposition in landfills. However, the results generated until now were not very encouraging.

The biochemical composition of bryozoans is little known. SCHOPF; MANHEIM (1967), in a pioneer work, conducted chemical elementary analysis in several species of bryozoans, but they were more focused on organic matter, total protein and minerals. The organic matter content amounting to over 50% in species without calcite structures, but below 25% in species having these structures, and the proteins ranged from 0.6 to 11%. In the biomass from Camboriu beach, mostly made up of bryozoans, the organic carbon content was very low, indicating the importance of carbonate material from the colonial structures of bryozoans and to a lesser extent, the silica derived from diatoms. However, these organic carbon values may be underestimated due to possible contamination of the samples with sand, since the calculation is based on gravimetric percentage of dry mass. This low organic content is a limitation to the use of biomass.

With respect to lipids and fatty acids quantitative information for bryozoans is also limited. DEMIDKOVA (2010), which focused on the fatty acids profile of Gymnolaemata bryozoans, has found a great diversity of these compounds, with saturated fatty acids being most important. The lipid content analyzed in Camboriu deposits was low (around 3% on average) and higher in samples with higher amounts of diatoms. Most of the fatty acids was saturated. Oils mainly composed of saturated fatty acids are suitable for the production of biodiesel, having little importance as nutraceuticals, for example. Despite the low lipid content in the dry biomass, the large amount of material deposited represent a considerable mass of lipid that can be extracted. As the average moisture of the material was 63% (value obtained after biomass freeze drying) and the average wet mass was around 150 ton.month^{-1}, the average dry weight would be around 55.5 ton.month^{-1}. Considering the 3% on average lipids, this would result in about 1.6 ton of lipid per month that could be extracted from the material. However, in realistic scenario, economic calculations should be made, since the investment for these extractions would be high.

Another possibility is using this biomass as organic fertilizer. Although the organic content is low, calcium carbonate and other salts make the biomass interesting for soil enrichment. The use of biological materials of marine origin as fertilizer is not new, especially when composed by seaweeds, which besides being rich in phosphorus and nitrogen salts, act as soil conditioners (CRAIGIE, 2011). Using the biomass deposits here studied for this purpose would be appropriate in a broader planning context, including the handling of similar materials over long stretches of coastline. Exploitations like that occur in cold climate coasts, where deposits of seaweeds and other kinds of wracks are abundant, but the erratic supply conditions found in tropical and subtropical coastlines such as Brazil, limit the economic viability of this process.

Regarding the antimicrobial activity of the extracts, the results showed low potential for use. Among the 15 microorganisms tested, only one (*Klebsiella pneumoniae*) was moderately sensitive to the extracts. In other preliminary tests with extracts obtained from samples of other periods the biological activity was also found to be little, but positive against the pathogenic fungi *Candida albicans* (Gilsane Lino von Poser, Faculty of Pharmacy, Federal University of Rio Grande do Sul, personal communication). In one of the few studies on the species *A. bellula*, NAIR (1993) detected strong activity against the bacteria *Proteus vulgaris*, *Klebsiella pneumoniae* and *Shigella flexneri*. These data indicate that there is a variation in the chemical composition and biological activities of the biomass. Probably the water conditions are determinants of this chemical variation. Thus, despite the results of antimicrobial activity have not been promising, there is a possibility of finding molecules with interesting properties in this material, which deserves a more focused study and analysis with higher amount of samples.

None of the possible uses of biomass has shown promising in this first approach. However, it must be considered the ecological role that the whole process is performing as an organic and inorganic carbon sink, as well as an inorganic nutrient consumer. Since Camboriu bay is heavily polluted, and algae and bryozoans deposits grow in these waters assimilating these compounds, and then are removed from the beach and transferred to landfills, a considerable mass of carbon and nutrients is being removed from these waters. This is a kind of bioremediation. However, as the biomass is always removed, it would be more appropriate at least use it as raw material for fertilizer, rather than just waste it in landfills. Thus, we strongly suggest a directed evaluation of the material to this end.

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