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The influence of trampling disturbance on the fluorescence and pigment concentration of *Sargassum* beds (Fucales)

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ABSTRACT

Direct and indirect effects on ecological processes caused by natural and anthropogenic disturbances may alter the composition and relative abundance of marine benthic communities. Trampling is a source of impact that can lead to the removal of individuals, breakage or damage of structural components and alterations of physiological performance of sessile organisms, possibly affecting the population dynamics and indirectly promoting structural changes in the entire community. To better support management strategies for coastal environments and to evaluate the anthropogenic impacts on a seaweed bed dominated by species of the genus *Sargassum* inhabiting the subtidal rocky shores in southeastern Brazil, we used an experimental approach to assess the physiological responses of this seaweed to trampling. We applied different intensities of trampling expected during summer vacation and throughout different periods of the year and quantified the fluorescence and pigment concentration of *Sargassum* beds. Changes caused by trampling are reflected in the health status of *Sargassum*, showing a decrease in chlorophyll *a* fluorescence and concentration of photosynthetic pigments depending on the site where this kind of impact occurs.

1. Introduction

Both natural and anthropogenic disturbances may alter the composition and relative abundance of benthic communities inhabiting rocky shores (Benedetti-Cecchi et al., 2001; Keough and Quinn, 1998; Mangialajo et al., 2008). The removal of individuals or the damage of structural components of their bodies (Denis and Murray, 2001; Keough and Quinn, 1998; Povey and Keough, 1991; Schiel and Taylor, 1999) can affect their population dynamics. When the affected species serve as a secondary habitat for other organisms, as macroalgae do, disturbance of habitat-forming species will indirectly trigger cascade changes in the structure of the entire community, modifying the spatial complexity and physical conditions and altering light, temperature, hydrodynamics, sedimentation and biotic interactions (McCook and Chapman, 1991; Platt and Connel, 2003).

Herbivory and wave impacts are among the most important natural sources of disturbance on rocky shores, shaping the morphology and diversity of seaweed. Benthic grazers can change algal morphology by promoting the production of adventitious branching, thereby increasing the strength and toughness of thalli; can mediate competition for space between algae and corals; or can have a significant influence on the abundance of algal species, potentially modifying the spatial distribution of dominant species (Aguilera et al., 2015; Jompa and McCook, 2002; Lowell et al., 1991; Sala and Graham, 2002; Van Alstyne, 1989). Wave impacts can modulate the specific composition of macroalgae (*e.g.*, Jorge et al., 2012), since the increase in hydrodynamic activity can cause a shift from a community dominated by individuals vulnerable to mechanical disturbances to on dominated by resistant individuals (Guillemot et al., 2010). For seaweeds, the outcome of the hydrodynamic changes will depend on (1) the density of the canopy surrounding the thallus, (2) the position of the thallus within the canopy, and (3) the length of the stipe of the thallus relative to the height of the canopy (Johnson, 2001).

Trampling is another common mechanical disturbance along rocky shores that can affect benthic communities (Araújo et al., 2009; Ferreira and Rosso, 2009; Milazzo et al., 2004, 2002; Silva and Ghilardi-Lopes, 2012); damage mussel shells, increasing their susceptibility to predation (Cintra-Buenrostro, 2007); cause declines in the size of algal and barnacle populations (Brosnan and Crumrine, 1994; Schiel and Taylor, 1999); change the spatial and temporal distribution patterns of affected species and lead to replacement by opportunistic organisms (Bertocci et al., 2011); and reduce the abundance of canopy-forming fucoids (Araújo et al., 2012) and the habitat stability and biodiversity of rocky-shores (Davenport and Davenport, 2006). The recovery rate of

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communities after disturbance is generally related to the trampling intensity (Milazzo et al., 2002; Plicanti et al., 2016; Schiel and Taylor, 1999), which is worrisome considering that conservation areas attract visitors, which consequently facilitates this kind of impact (Pimm et al., 1995).

In macroalgae, mechanical damage, commonly observed in trampled communities, usually induces physiological responses, such as an increase in the concentration of phenolic compounds or alterations in heat stress metabolism (Amsler, 2001; Keough and Quinn, 1998; Pavia and Toth, 2000; Schiel and Taylor, 1999; Van Alstyne, 1989). However, the consequences of physical damage on the concentration of photosynthetic pigments and performance in macroalgae, which are also affected by other stressors such as light, UV radiation, organic pollution, high temperatures and heavy metals (Clark et al., 2013; Costa et al., 2017a; Scherner et al., 2013; Scherner et al., 2012; Schmidt et al., 2015), are still unknown.

Due to their sessile life history and measurable responses to environmental changes, benthic macroalgae are considered useful indicators of the action of environmental factors (Aevalo et al., 2007; Hellawell, 1986; Soltan et al., 2001). One example are macroalgae of the genus *Sargassum* C. Agardh (Ochrophyta, Sargassaceae), which are used as indicator species since they are nutrient opportunists and are sensitive to nutrient uptake (Alquezar et al., 2013; Matsuo et al., 2009; Roberts et al., 2008; Rossi et al., 2009).

Sargassum species are distributed throughout tropical and subtropical regions globally and are considered important components of the marine flora, playing a role as ecosystem engineers in subtidal rocky-shore habitats and other marine environments and providing shelter and nurseries for numerous marine species (Aburto-Oropeza et al., 2007; Tanaka and Leite, 2003). They are also economically important as sources of raw material for different products in the chemical and food industries (Borines et al., 2013; Marín et al., 2009).

To support better management strategies for coastal environments and evaluate the anthropogenic impacts on a seaweed bed dominated by species of the genus *Sargassum* inhabiting the rocky shores of southeastern Brazil, we used a manipulative experimental approach to assess whether the effects of trampling were consistent among three study sites. To do so, we simulated different intensities of trampling expected during the summer holidays and quantified the chlorophyll *a* fluorescence and pigment concentration of the macroalgae throughout different periods of the year. We expected that chlorophyll *a* fluorescence and the concentration of antenna complex pigments (chlorophyll *c* and carotenoids) would be negatively affected by the stress caused by trampling given that mechanical damage influences the photosynthetic machinery (Mills et al., 1996). Additionally, we expected that recovery after disturbance would depend on the intensity of the disturbance and that determining this could help us propose management strategies related to coastal tourism.

2. Methodology

2.1. Study site

We conducted this study in 2015 at three rocky shore sites from the southwestern Atlantic, Brazil: Pernambuco Beach (23°58′S, 46°11′W), Cigarras Beach (23°43′S, 45°23′W) and Fortaleza Beach (23°32′S, 45°10′W) (Fig. 1). All three sites experience a high intensity of visitation during the weekends and summer holidays, and the rocky shores in the subtidal zone are covered by an extensive *Sargassum* bed.

Pernambuco Beach extends 1650 m and is shaped like a half moon. In the southern part of the beach, an island deflects the main swells (David et al., 2003), so the rocky shore in which we conducted this study is protected from strong wave exposure. However, among the three study sites, this is the most exposed to wave action and is predominated by *Sargassum vulgare* var. *nanum* E. de Paula (Paula, 1988). Cigarras Beach is bordered by rocky shores at both its northern and southern boundaries. It is a very urbanized beach with summer houses nearby. In addition, a submarine emissary discharges between 1.5 and 7.6 l of untreated sewage per second a few metres from the coast (Ferreira, 2008). Algae beds in Cigarras Beach are dominated by *Sargassum stenophyllum* C. Martius (De Széchy et al., 2001; Ferreira, 2008). Fortaleza Beach extends along a southwest-northeast direction (Jacobucci and Leite, 2006) and is surrounded by hotels. In its sheltered portion where we conducted our study, natural pools formed at low tide are frequented by tourists and small boats. In Fortaleza Beach, *Sargassum cymosum* C. Agardh and *Sargassum filipendula* C. Agardh are the dominant species (De Paula and Oliveira, 1982; Ferreira, 2008).

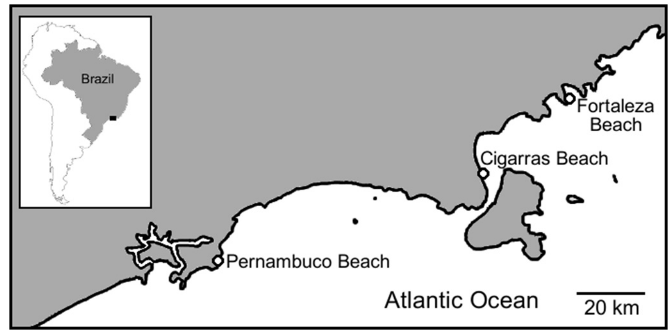

Fig. 1. Study sites from the Southwestern Atlantic (Brazil): Pernambuco Beach; Cigarras Beach and Fortaleza Beach.

2.2. Sampling method

To assess how trampling caused by visitation could affect the chlorophyll *a* fluorescence and pigment concentration of *Sargassum* spp., we selected a continuous and homogeneous *Sargassum* bed in the midlittoral zone of each site, where the human foot traffic is a common occurrence, and by using transect methodology, we randomly delimited nine 40 × 40 cm quadrat points, which were divided into three treatments that simulated different trampling intensities: 1) control (no trampling; C); 2) low trampling intensity (LT), in which we simulated 150 steps over the sampling units; and 3) high trampling intensity (HT), in which we simulated 300 steps over the *Sargassum* bed (Supplementary material Fig. S1). Additionally, to assess the potential consequences of trampling over the short and long term and the recovery from this impact, we evaluated the responses over time: 1) immediately after the trampling procedures (on the 5th day) (time zero; T0), 2) 90 days after trampling (T90), and 3) 180 days after trampling (T180), totalling nine treatments (three trampling intensities x three repetitions). The trampling intensities were applied by a person averaging 50 kg body weight using rubber soled boots over five consecutive mornings always during low spring tide. The sampling periods were during low tourism season (from March to October 2015), to avoid the intense visitation period. The chosen trampling intensities and periods of time were based on Denis and Murray (2001), Ferreira and Rosso (2009) and Schiel and Taylor (1999).

2.3. Physiological analysis

We assessed the response of the *Sargassum* bed to the stress caused by trampling through the *in vivo* chlorophyll *a* fluorescence of photosystem II (*PSII*) and through the concentration of photosynthetic pigments (chlorophyll *a*, chlorophyll *c* and carotenoids - denoted Chl*a*, Chl*c* and Carot respectively) in the laboratory. Photosynthesis, measured as *in vivo* chlorophyll *a* fluorescence, provides us with information about the state of excitation and electron transport of PSII, indicating the extent to which it is using the energy absorbed by chlorophyll *a*nd the extent to which it is being damaged by excess light (Maxwell and Johnson, 2000). Thus, it provides insights regarding the