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**Biological response to microhabitat formation by rocky shore
intertidal benthic macroalgal canopies**

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Presenta:

Scheherazada Umanzor Rodríguez

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Tesis defendida por
Scheherazada Umanzor Rodríguez

y aprobada por el siguiente Comité

Dra. Lydia Betty Ladah
Director de tesis

Dr. Saúl Álvarez Borrego

Dr. Luis Eduardo Calderón Aguilera

Dr. José Luis Ochoa de la Torre

Dr. José Antonio Zertuche González



Dra. Lucila del Carmen Lares Reyes
Coordinador del Posgrado en Ecología Marina

Dra. Rufina Hernández Martínez
Directora de Estudios de Posgrado

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Abstract of the thesis presented by **Scheherazada Umanzor Rodríguez** as a partial requirement to obtain the Doctor of Science degree in Marine Ecology.

Biological response to microhabitat formation by rocky shore intertidal benthic macroalgal canopies

Abstract approved by:

Dra. Lydia Betty Ladah
Director de tesis

Intertidal macroalgae modulate their biophysical environment by ameliorating physical conditions and creating habitats. Thereby, influencing the abundance and distribution of associated species, and altering ecosystem dynamics within their local environment. The modification ability of macroalgae and the relative importance of their bioengineering outcomes may change rapidly in response to shifting levels of environmental stress. Exploring how seaweed aggregations made up of distinct species at different densities modify their local environment may help explain the processes, patterns and mechanisms driving species distribution and community composition, and how the connections between these organisms are affected. Using *Silvetia compressa*, *Halidrys dioica*, *Chondracanthus canaliculatus* and *Pyropia perforata*, I constructed monocultures representing the leathery, corticated and foliose functional-forms, as well as a mixed tri-culture assemblage including the former three, at four different densities. Treatment quadrats were installed in the intertidal where I measured irradiance, temperature, particle retention, desiccation bulk water flow underneath the canopies. Additionally, I examined the response by the understory microphytobenthos and by a targeted group of macroinvertebrates. I found that the density per unit area and species composition influence the magnitude in which macroalgae modify extreme physical conditions in their local environment. Macroalgal aggregations of greater spatial complexity, showed a greater attenuation of understory bulk water flow, irradiance, temperature, particle transport and water loss. I also found that increasing the number of species within a patch do not always results in increased spatial complexity and therefore, did not necessarily cause greater ameliorated conditions. Moreover, I found that a further modulation on the microphytobenthos occurred, and that the response by macroinvertebrates differed based on the baseline conditions of the environment. Results indicate that the environmental modifications driven by these macroalgae as ecosystem engineers play a significant role in promoting distinct temporal and spatial patchiness of associated organisms. Consequently, affecting the biological dynamic pathways and influencing the local diversity at various scales and in turn, affecting the strength and nature of interspecific interactions.

Key words: biofilm, density, environmental stress, macroalgae, macroinvertebrates, microphytobenthos, morphology, seaweeds

Resumen de la tesis de **Scheherazada Umanzor Rodríguez**, como requisito parcial para la obtención del grado de Doctor en Ciencias en Ecología Marina.

Respuesta biológica a la formación de microhábitats por macroalgas del intermareal rocoso

Resumen aprobado por:

Dra. Lydia Betty Ladah
Thesis Director

Las macroalgas del intermareal rocoso modifican su entorno al atenuar las condiciones físicas extremas que afectan su entorno inmediato. En consecuencia, crean una serie de microhábitats bajo su dosel que a su vez influyen sobre la abundancia y distribución de especies asociadas. En la zona intermareal, la capacidad de las macroalgas para modificar físicamente su entorno, y la importancia relativa que estas modificaciones tienen sobre la biota, están directamente asociados a los niveles de estrés ambiental. Por ende, ambas están sujetas a cambiar rápidamente. Explorar cómo agregaciones de macroalgas con distintas morfologías y a distintas densidades modifican su entorno físico puede ayudar a explicar los procesos, patrones y mecanismos que dictan la distribución de especies y la composición de las comunidades en el intermareal. Para evaluar la influencia de las macroalgas en su papel de ingenieros del ecosistema en la zona intermareal, utilicé ejemplares de *Silvetia compressa*, *Halidrys dioica*, *Chondracanthus canaliculatus* y *Pyropia perforata* para construir cuadrantes experimentales. Estos cuadrantes consistieron en cuatro monocultivos y un cultivo mixto, cada uno a cuatro densidades. Una vez instalados en la zona intermareal, medí la irradiancia, temperatura, retención de partículas, flujo bruto del agua y pérdida de agua debajo de cada dosel. Además, examiné el microfitobentos asentado bajo el dosel y la presencia de un grupo determinado de macroinvertebrados móviles. Encontré que tanto la especie de macroalga como su densidad por unidad de área tienen un efecto en la atenuación de las variables físicas medidas. En general, los ensambles de macroalgas con mayor complejidad espacial mostraron mayor habilidad para atenuar físicamente su entorno inmediato. Adicionalmente, encontré que aumentar el número de especies dentro de una agregación macroalgal no necesariamente se traduce en un aumento de la complejidad espacial, y, por lo tanto, no necesariamente causa una atenuación significativa en las variables físicas que afectan su entorno. Por otro lado, los datos obtenidos muestran que la atenuación de las variables físicas producto de las macroalgas tiene un efecto significativo sobre la abundancia del microfitobentos, y que de manera similar las macroalgas modulan la abundancia y distribución de algunos grupos de macroinvertebrados en el intermareal. Sin embargo, este efecto de las macroalgas sobre los macroinvertebrados, está sujeto al nivel de estrés ambiental al que ambos estén expuestos. Los resultados evidencian que las modificaciones ambientales impulsadas por estas macroalgas en su rol de ingenieros del ecosistema juegan un papel importante en propiciar la heterogeneidad espacial y temporal, tanto de los organismos asociados, como de la estructura física de su entorno. En consecuencia, las macroalgas pueden influir la dinámica y diversidad local a varias escalas, que a su vez afectan la fuerza y naturaleza de las interacciones interespecíficas en el intermareal.

Palabras clave: biofilm, densidad, estrés ambiental, macroalgas, macroinvertebrados, microfitobentos, morfotipo.

Dedicatoria

*A todos aquellos idealistas que se atreven a soñar.
A quienes más allá de los problemas piensan en las soluciones.
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1. Introduction

1.1 Ecosystem engineers

Ecosystem engineers are organisms that alter their local environment, causing a variation in the flow of resources and/or creating or modifying habitats (Jones et al., 1994). These engineers include all varieties of life forms, ranging from microorganisms (Gerbersdorf et al., 2009) to large plants and animals (Dubi and Tørum 1994; Pringle, 2008), including humans. They physically modify environs in a range of ways. For instance, by stabilizing sediments, burrowing and creating channels, shifting hydrodynamic regimes and reef building. All of which ultimately have an impact on the abundance, distribution and composition of other associated communities, ecological processes, and overall ecosystem functioning.

Ecosystem engineering addresses four cause-effect relationships that together explain the system dynamics of bioengineered environments (Gutiérrez et al., 2011). First, bioengineers cause changes in the physical structure of their local environment. Second, the spatial changes cause by the bioengineers trigger abiotic changes. Third, both the introduced spatial changes and the subsequent abiotic changes could cause biotic changes. Lastly, the former three could affect the bioengineer back (Gutiérrez et al., 2011; Jones et al., 2010; Jones, Lawton and Shachak, 1997). For example, by increasing the complexity of the substrate, aggregations of mussels or macroalgae modify the physical structure of the intertidal. This could promote an increase of sediment deposition or a micro scale shift in local hydrodynamics, which in turn could enhance settlement or gathering of associated organisms. Consequently, an increase in the abundance of associated organisms could increase nutrient or food availability for the bioengineer triggering this chain of events (Figure 1).

Because of the nature of the ecosystem engineers, they can either have direct or indirect effects on the environment. Autogenic bioengineers, such as coral reefs or kelp forests, cause direct changes to their environment due to their own spatial complexity. For instance, with their mere presence both corals and kelp modify water flow and light penetration (Ferrario et al., 2014; Tait et al., 2014).

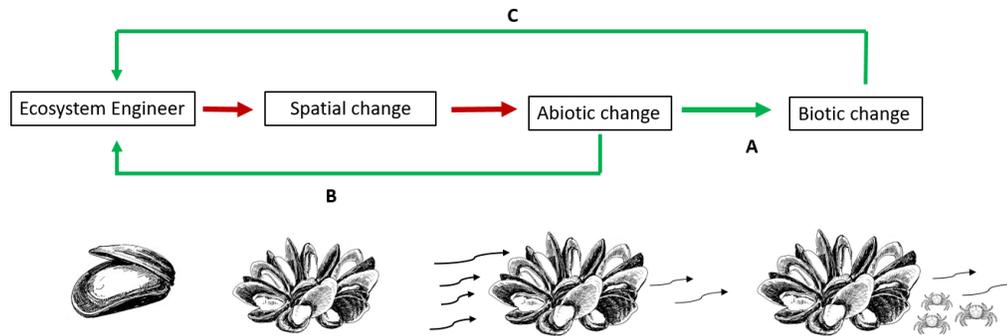


Figure 1. Pathways of ecosystem engineering process (red arrows) and biotic outcomes (green arrows). Biotic outcomes include effects of: a) abiotic changes on other organisms, b) feedback effects to the engineer driven by either by the abiotic change or c) by its biotic impact on other species. Ecosystem engineers such as mussels increase the spatial complexity of their environment causing changes in water flow and providing shelter to other invertebrates.

Other organisms, such as burrowing crabs are considered allogenic bioengineers, which cause indirect changes by modifying the materials that are available in their environment. For instance, by excavating crabs can modify sediments creating channels and thereby, causing changes in the flow of nutrients (Kristensen 2008). Interestingly, a third group of bioengineers can simultaneously cause direct and indirect effects on their local environment. For example, mangrove trees aid stabilizing sediments (direct effect) with their pneumatophores, but can also bioturbate the substrate (indirect effect), hence, promoting differences in the flow of resources through the sediments (Carlton, 1974).

Although theoretically, all organisms could be ecosystem engineers, as all modify the environment in one way or another. This is perhaps the greatest criticism made to the ecosystem engineering concept (Reichman y Seabloom, 2002; Wilby, 2002). However, this concept is far from trivial. The key lies in determining: 1) what modifications are being caused by the engineer, 2) how, where, and when these modifications have the greatest impact on the attenuation of the physical environment, 3) which organisms are favored or disfavored by such modifications, and 4) how the connection between ecosystem engineers and their associated organisms are affected.

The relative importance that an ecosystem engineer may have in the local environment will largely depend on physical baseline conditions where the engineer develops. Crain and Bertness (2006) theoretically predict that in relatively mild environments, where predation is the main community-structuring mechanism, bioengineers with the ability to provide “predation-free” spaces will have the greatest relative importance. Their model also predicts that as environmental conditions shift from mild to intermediately

stressful, competition becomes the main community-structuring mechanism and therefore, bioengineers with the ability to provide “competition- free” spaces gain a greater relative importance. Lastly, they describe that in extreme environments where the main community-structuring mechanism is physical stress; bioengineers with the ability to ameliorate the habitat will have the most relatively important of them all (Figure 2). This merits further testing as relatively few studies (Bertness et al., 1999; Wright et al., 2014) have simultaneously tested bioengineering outcomes across difference baseline conditions.

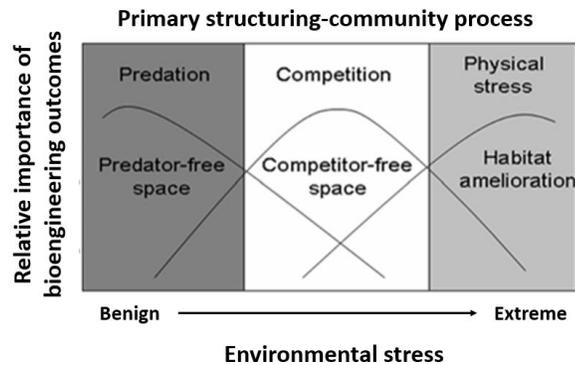


Figure 2. Relative importance of bioengineering outcomes as a function of environmental stress and primary community-structuring processes. Modified from Crain and Bertness (2006).

1.2 Rocky shores

Sea shores, the fairly narrow strips where land meets the sea, provide one of the most diverse and extreme habitats for organisms on Earth (Denny, 1987). From a biological perspective, there are two major types of shores. First, hard shores also known as rocky shores and second, soft shores, related to sandy and muddy shores. The environmental features and life ways among these two types of shores differ so much that there is little overlap between the species inhabiting them (Knox, 2001). Rocky shores, of interest in this study, usually occur on recently uplifted or geologically young coasts. They can also occur where the effect of waves is largely erosive, resulting in softer and unstable materials wearing down and leaving the hardest rocks exposed (Castro and Huber, 2003). Through their durable physical structure, rocky shores provide protection to surrounding ecosystems (Levin et al., 2001; Gutiérrez et al., 2011; Barbier et al., 2011) and form secure surfaces and crevices upon which a variety of organisms requiring attachment can develop (Tait and Dipper, 1998; Denny, 1999).

Rocky shores also provide shelter, feeding, settling, and nursery areas for a variety of organisms such as birds, fish, a plethora of invertebrates, and other coast-associated organisms. They host a remarkable

biodiversity, thus, resulting in popular recreational and commercial sites important for fisheries around the world (Connell, 1972). Because inland and ocean events strongly influence these ecosystems, they are subject to extreme fluctuations in environmental conditions, such as salinity, irradiance, heat, bulk water flow, and desiccation periods. Additionally, rocky shores are subject to continuous and/or periodic external forcing, such as tidal cycles, storms, freshwater discharges, and anthropogenic disturbances (Nicholls et al., 2009; Valiela 2006). All of which add stress to shoreline organism. Vertical and horizontal physical stress gradients, in addition to biological interaction such as predation and competition, contribute to make rocky shores a harsh environment, which inhabiting organisms must endure.

Ecosystem engineers, which can ameliorate the environment can often have a significant influence on coastal dynamics and on the spatial distribution of the biological component (Barbier et al., 2011; Sueiro et al., 2011). By providing attenuated physical conditions, ecosystem engineers from the rocky shore, including seagrasses, macroalgae, reef-forming bivalves, and burrowing crustaceans, can often contribute to increase diversity in their environment. They can facilitate suitable habitats for a variety of associated organisms allowing them to inhabit areas where they normally couldn't thrive (Figure 3), therefore, potentially increasing their realized niche.

Although we now recognize the significance of ecosystem engineers inhabiting rocky shores, there are well-recorded declines and losses of crucial functions fulfilled by bioengineers worldwide. Extended and magnified disturbance periods driven by human pressures and climate change pressures are prompting an overall deterioration in ecosystem services provided to mankind, such as coastal protection, habitat provisioning, and diversity conservation (Gutiérrez et al., 2011; Gedan et al., 2009; Steneck et al., 2003).

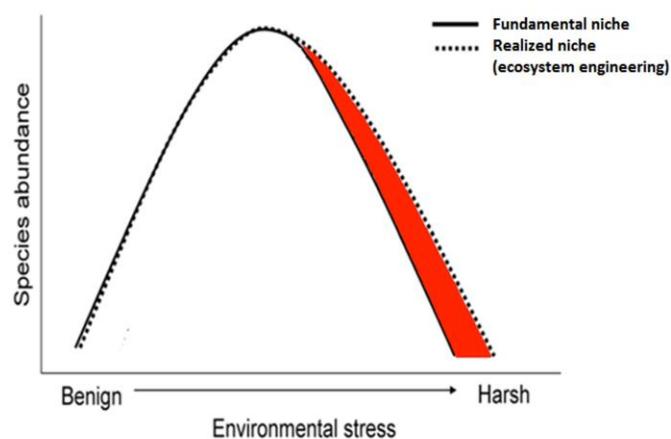


Figure 3. Difference in the abundance of species between their fundamental niche and their realized niche in the presence of ecosystem engineers (red shaded area). Modified from Crain and Bertness (2006).

Therefore, quantifying and assessing bioengineering outcomes by organisms such as macroalgae, typical of rocky shores, is critical for understanding the ecology of biodiversity in the rocky intertidal. This in turn can help in management and conservation efforts, particularly considering the threat caused by extreme changes in the environment. It can also aid understanding the implication that microscale changes could trigger on the macro-environment and the impacts that invasive species with given forms could have on the environment.

1.2 Macroalgae

Macroalgae usually develop between the upper intertidal and the maximum depth where enough irradiance for growth can penetrate (Hurd et al., 2014). They are conspicuous organisms in the intertidal that often form mixed assemblages including turf or small -size, canopy-forming individuals of only a few centimeters to canopy-forming individuals such as *Egregia*, several meters tall. Most macroalgae live attached to the benthos or other substrates, although some can also live on floatation (Radulovich et al., 2013). Because macroalgae respond to the changing abiotic and biotic conditions of their environment, their communities constantly change in space and time.

In the rocky intertidal, macroalgae are influenced by rapid changes in light, temperature and desiccation regimes (Bertness et al., 1999) due to tidal cycles. They are also subject to competition for space and nutrients (Aquilino et al., 2009), herbivory (Wright and Jones, 2004) and symbiotic interactions (Azzini et al., 2008). All of which influence macroalgal distribution, diversity and development. On the other hand, by buffering the effects of rapid and extreme changes in environmental factors caused by daily tidal cycles (Bates, 2009; Meadows et al., 2012; Wright et al., 2014), macroalgae facilitate refuge and habitat for a numerous species. Consequently, shifts in macroalgal communities may have a major influence on the abundance and distribution of other intertidal organisms (Wilson et al., 2014).

To generalize the understanding of how macroalgae could respond to environmental pressures, Littler and Littler (1980) and Steneck and Watling (1982) placed macroalgae into several functional-form groups (Figure 4). They hypothesized that macroalgal growth arrangement and mineralization will predict how they will respond to the biophysical environment. This grouping was based on morphological traits shared by macroalgae, confounding structural properties with their overall morphology (Padilla and Allen, 2000). Because the structural properties of organisms define their mechanical properties, macroalgae within the

same functional-form group could respond differently to physical factors such as water flow (Koehl et al, 2003; Denny and Gaylord, 2002; Koehl, 1984). Therefore, it could be expected that the structural and mechanical properties will also dictate differences in the ability of macroalgae to ameliorate stressful physical conditions. These differences could in turn, result in habitat and refuge provisioning to differ, even if various species of macroalgae share the same morphology.

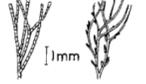
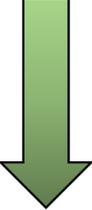
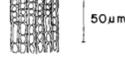
Functional-form groups	Representatives	External morphology	Internal anatomy	Anatomical complexity	Functional-forms in this study	Spatial complexity using fractal dimension	
Microalgae	Diatoms, cyanobacteria						
Filamentous	<i>Cladophora</i> , <i>Ectocarpus</i>						
Foliose	<i>Ulva</i> , <i>Pyropia</i>					Foliose	
Corticated	<i>Caulerpa</i> , <i>Chondracanthus</i>					Corticated	
Leathery	<i>Fucus</i> , <i>Silvetia</i> , <i>Laminaria</i>					Leathery	
Calcareous coralline	<i>Halimeda</i> , <i>Corallina</i>						
Crustose	<i>Ralfia</i>						

Figure 4. Functional-form groups of macroalgae *sensu* Steneck and Watling (1982). Relative measure of the spatial complexity of macroalgae species within given functional-form groups. The increase in the spatial complexity of macroalgae is subjected to the increase of ramifications per unit area.

In fact, Hendriks and colleagues (2010) evaluated the ability of three *Caulerpa* species (corticated functional form) to retain particles and concluded that particle retention was mediated by the surface area, height, and anatomical traits particular to each species, supporting this expectation. Nonetheless, the spatial complexity (three-dimensional external intricacy) specific to every functional form could be used as a more generalized and effective predictor of macroalgal ameliorating ability. By increasing the number of blocking points (*e.g.* branches) that are exposed to physical variables, the complexity added by macroalgae (see Corbit and Garbary; and Veiga et al. 2014 for further explanation on using fractal dimension to measure spatial complexity of macroalgae) may determine their ability to ameliorate stressful conditions (Figure 5).

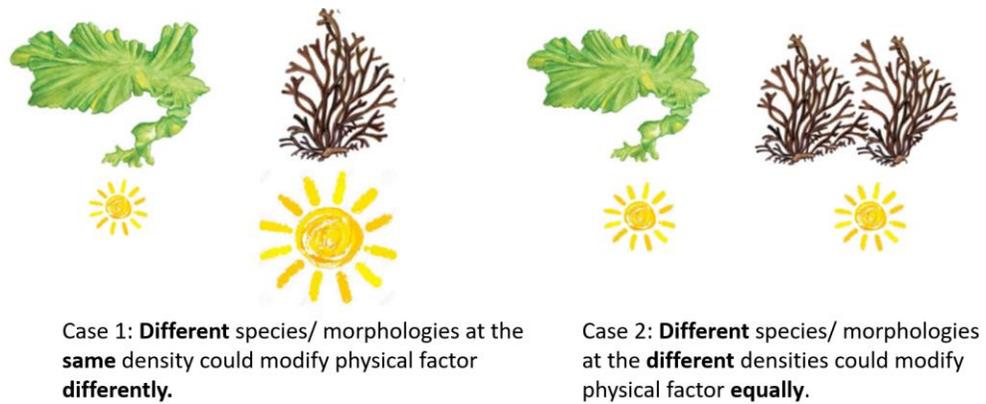


Figure 5. Different species and their functional forms at different densities create distinct understory conditions.

Thus, assuming that both, the density (Kelaheer, 2003) and morphology (Dawes, 1998) of macroalgae contribute to the spatial complexity of macroalgal aggregations, particular functional forms at given densities will create a mosaic of understory conditions with distinct physical properties (Figure 6). Consecutively, this could promote temporal and spatial patchiness of associated organisms below the canopy, such as those in the microphytobenthos or macroinvertebrates communities.

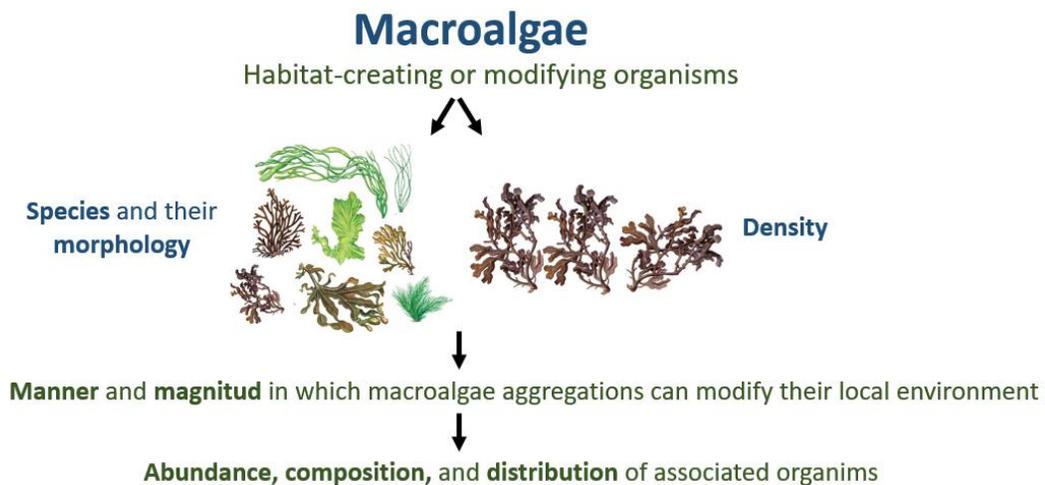


Figure 6. Conceptual model of macroalgae as ecosystem engineers.

This thesis describes how the species (*Silvetia compressa*, J. Agardh, *Halydris dioica*, N.L. Gardner, *Chondracanthus canaliculatus*, Harvey and *Pyropia perforata*, J. Agardh) with different functional forms (leathery, corticated, foliose and a mixed tri-culture of the former three) and density (high, mid and low) influence the ability of macroalgae to ameliorate physical conditions in a stressful environment. This thesis also summarizes the measured effects that the four species of macroalgae at different densities have on light, temperature, particle retention, bulk water flow, and desiccation regimes and the effects that the amelioration of these physical factors have on the abundance, distribution and composition of the microphytobenthos and macroinvertebrates community.

2. Modification of physical variables by macroalgae as ecosystem engineers

2.1 Introduction

Macroalgae play a key role in intertidal environments as primary producers and competitors (Hawkins, 1983; Williams et al., 2000; Sundbäck and McGathery, 2005), and by providing shelter and food for associated organisms (Barry and Ehret, 1993; Stachowicz and Hay, 1996; Jenkins et al., 1999; Phillippi et al., 2014). As they add roughness to the landscape and are semi-permeable to water, light, and particles, macroalgae can modify their local environment by acting as ecosystem engineers. Moreover, they provide refuge and habitat for a myriad of species by ameliorating the effects of rapid and extreme changes in environmental factors caused by daily tidal cycles (Bertness et al., 1999; Bates, 2009; Meadows et al., 2012; Wright et al., 2014). As a result, changes in macroalgal communities may have a significant influence on the diversity and abundance of macroalgae-associated organisms (Wilson et al., 2014).

Intertidal biodiversity is often positively affected by ecosystem engineers (habitat-creating or habitat-modifying organisms) such as macroalgae (Thompson et al., 1996; Stachowicz, 2001; Matias et al., 2015). However, macroalgal structural composition and ecophysiological properties, such as, gross morphology (Padilla and Allen, 2000), surface area (Thomas and Jiang, 1986), functional form (Steneck and Watling, 1982) and even chemical deterrents (Steinberg, 1985) can all contribute to the abundance and distribution of associated species. Therefore, it is challenging to tease apart the extent to which macroalgae can modulate the ecology of associated species. Nevertheless, some generalizations can be made by understanding how macroalgae of different species, at different densities, and within different functional-form groups modify the local environment by ameliorating stressful conditions, and how associated species respond to this amelioration.

The morphological complexity (three-dimensional internal and external intricacy) particular to every species may be used as an effective predictor of macroalgal ameliorating ability. By increasing surface area and resource availability, the complexity of habitats can enhance local biodiversity (Gutiérrez and Iribarne, 2004; Hastings et al., 2007; Jones et al., 2010; Sueiro et al., 2011). Likewise, by increasing the surface area and/or number of blocking points (*e.g.* branches, thalli) that come in contact with physical factors, the complexity added by particular macroalgae species may determine the degree to which stressful conditions are ameliorated, potentially affecting local biodiversity. For instance, in an experiment assessing the effects of habitat complexity and hydrodynamics on the abundance and diversity of small

invertebrates, water was diverted and flow was significantly ameliorated as the number of blocking points increased, potentially enhancing the presence of invertebrates (Atilla et al., 2005). Assuming that both the density of macroalgae (Kelaheer, 2003) and their morphology (Dawes, 1998) contribute to their spatial complexity, particular species at different densities will create a variety of understory conditions with distinct temperature, light, bulk water flow and particle movement regimes. This, in turn, could promote patchiness of associated organisms below the canopy.

Climate change (Harley et al., 2012) and direct human activities, such as pollution, the introduction of alien species, and overall habitat detriment are altering macroalgal composition around the world (Walker and Kendrick, 1998). Therefore, understanding how the relative influence of macroalgae can vary due to their physical properties is becoming increasingly important for predicting the implications caused by changes in communities, such as the ones in the intertidal. This chapter describes how distinct macroalgae (average 20 cm tall) typical of the intertidal, perform as ecosystem engineers. The attenuation of physical factors, which often govern the dynamics in this ecosystem were measured as a function of the species, density, and diversity of macroalgal aggregations.

2.2 Objectives

Measure how individual-level traits, such as morphology, and group-level traits, such as density and diversity (monocultures vs. mixed cultures) of intertidal macroalgae contribute to the attenuation of bulk water flow, irradiance, temperature particle transport, and water loss underneath their canopies.

Three specific objectives derive:

- Establish manipulative experimental plots including one species (monoculture), and three species (mixed tri-culture) quadrats of macroalgae with different identities (*Silvetia compressa*, *Halidrys dioica*, *Chondracanthus canaliculatus*, and *Pyropia perforata*), at four densities each (high, medium, low and control).
- Assess the physical variables (light, temperature, bulk water flow, desiccation, and particle retention) as a function of the assemblages and the density in each quadrat.

2.3 Hypothesis

Exposed rocky shores are subject to fluctuating physical variables, often resulting in extreme conditions. Because both, the species and their morphology and density contribute to the complexity of macroalgal aggregations, it is expected that more complex aggregations with a greater surface area and/or number of blocking points that come in contact with physical variables will yield a greater amelioration of extreme physical conditions.

2.4 Methodology

2.4.1 Study site

Experiments were carried out at Punta Morro rocky shore (Figure 7) in Ensenada, Baja California ($31^{\circ} 51' 41.6''$ N and $116^{\circ} 39' 58.1''$ W) during spring/summer (2015-2016) when targeted macroalgae (*Silvetia compressa*, *Halidrys dioica*, *Chondracanthus canaliculatus*, and *Pyropia perforata*) were abundant (Figure 8). This intertidal has a south sea view orientation which coastline forms an extended platform slightly inclined until it reaches a man-built protection wall in the upper intertidal. It is characterized by the presence of extrusive igneous rock, mainly basalt, combined with coarse sand and boulders (Tellez-García 2003).

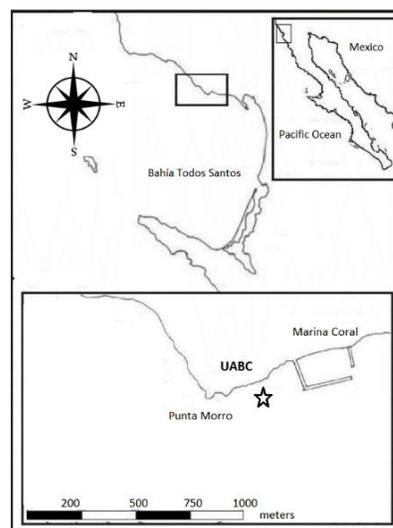


Figure 7. Study site at Playa Punta Morro located in the northern part of Bahía Todos Santos, Ensenada BC. Experimental area indicated with a star. Modified from Ramírez Valdez, 2009.

The study site has a semidiurnal mixed tidal cycle, with two low tides and two high tides of different heights per day, is void of tide pools, and is fully exposed during spring tides (tidal range 0-1500 mm). Several macroalgae species (*Sargassum* sp. *Ceramium* sp. and *Laurencia* s.p among others), molluscs (*Chlorostoma* sp., *Acanthina* sp., *Nucella* sp. *Lottia* sp. and *Littorina* spp.), crustaceans (*Pachygrapsus* sp. and *Balanus* sp.) and cnidarians (*Anthopleura* spp.) can be easily located at site. The experiment plots were installed at the lower end of the mid-intertidal where macroalgae typically abound.

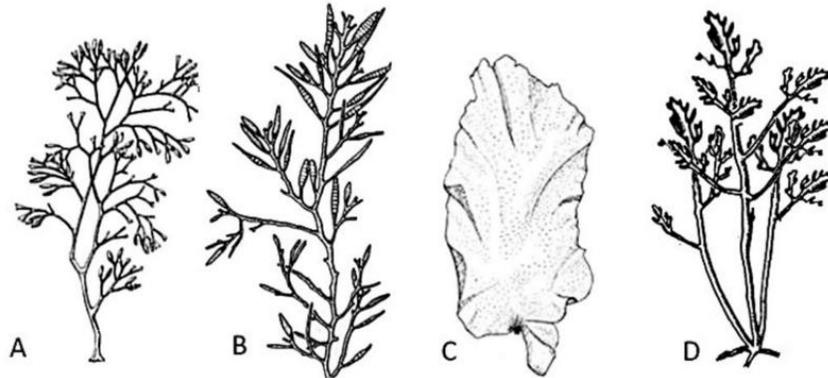


Figure 8. General morphology of the targeted macroalgae a) *Silvetia compressa* (branched-flat morphotype), b) *Halidrys dioica*, c) *Pyropia perforata*, and d) *Chondracanthus canaliculatus*.

2.4.2 Experimental designs

Two experimental designs were developed to compare macroalgal performance in ameliorating stressful environmental conditions. The first experimental design consisted of testing three monoculture treatments (i.e. *S. compressa*, *C. canaliculatus* and *P. perforata*), each one with a distinct functional form (leathery, corticated and foliose), and a mixed tri-culture treatment (including the former three) at four densities (high, mid, low and control) each. Each treatment included four replicates, totaling 64 quadrats in the design (Figure 9). The second experimental design consisted of testing two monoculture treatments (i.e. *S. compressa* and *H. dioica*), both with the same functional form (leathery), at four densities (high, mid, low and control) each. Each treatment included three replicates, totaling 48 quadrats in the design. Both experimental designs followed an orthogonal approach.

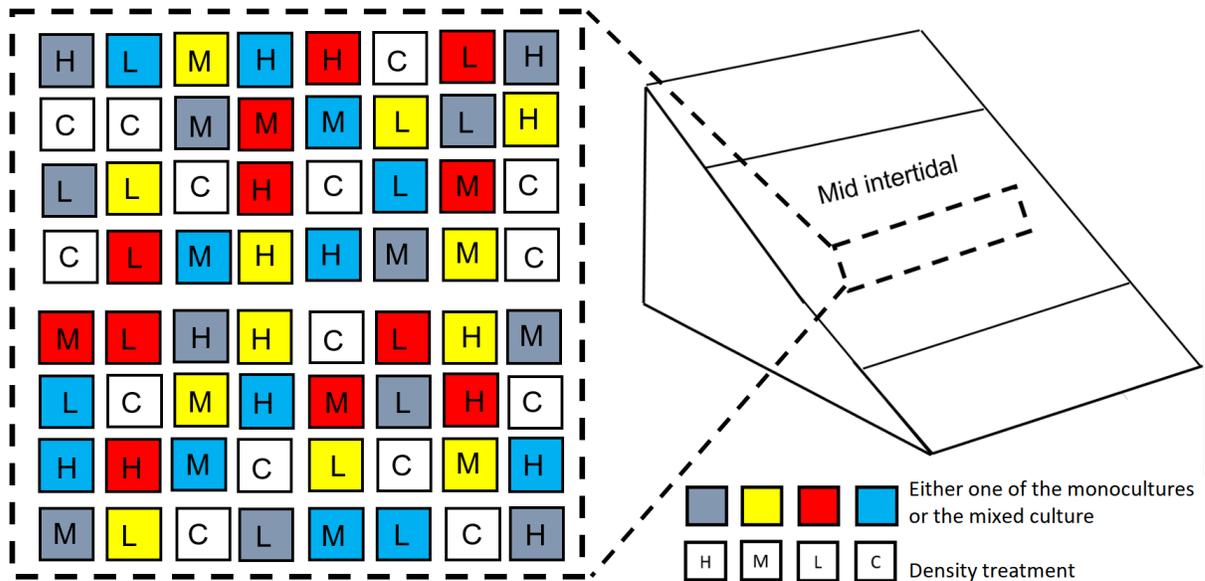


Figure 9. The experimental system consisted of 16 randomly assigned treatments, with four replicates each, placed in the mid-intertidal at the same tidal level. Each quadrat considered either *Silvetia compressa*, *Pyropia perforata*, *Chondracanthus canaliculatus*, or the mixed culture treatment (colored squares), at a given density: high (H), mid (M), low (L) or control (C).

To avoid pseudoreplication of the controls (Hurlbert 1984), one control quadrat was assigned to every species composition treatment. Quadrats consisted of 30 x 30 cm metal frames covered with mesh to which macroalgae and all data collection devices were attached. Quadrats were individually fixed to the intertidal substrate with 2.5 kg diving weights. Biological settlement slides, particle collectors, temperature loggers, light meters, and plaster cylinders were attached below the canopy in each quadrat.

2.4.3 Experimental assemblages

Macroalgae species were selected based on their functional form (Steneck and Watling 1982), ubiquity in the intertidal zone and their cultivability. Macroalgae fragments (10 cm approx.) were collected from the upper, middle and lower intertidal. They were cleaned by shaking vigorously in freshwater and immediately transferred to 1 m³ seawater tanks where they were kept for 15 days under semi-controlled conditions. Daytime maximum net radiation in the tanks was 781 W m⁻², resembling light levels from the intertidal. However, because of the relatively small size of the tanks, water temperature during daytime was up to 9 °C higher than that in the intertidal. Throughout a 24 h period, water temperature in the tanks

oscillated between 16 and 28 °C. Fragments were then outplanted by threading them onto pre-weighed polypropylene ropes (7 mm wide), following the planting methods described in Radulovich et al. (2013).

The densities tested were: high (1246-1152 points m⁻², with an initial biomass of 5 kg m⁻²), mid (768-864 points m⁻², with an initial biomass of 3 kg m⁻²), low (384-432 points m⁻², with an initial biomass of 1 kg m⁻²), and the controls (with no initial biomass) that should resemble the variability of physical factors in the intertidal. High density treatments resembled the most abundant patches of macroalgae per square meter occurring naturally in the local intertidal zones for the spring/summer season. Mid-density and low-density treatments were estimated at approximately half of the mean abundance per square meter.

For equivalence between biomass and density between macroalgae species, fragments were selected based on additional morphological and anatomical traits, including thickness and completeness of the tissue (no holes or missing ends). We included only thin and young fragments of *S. compressa* and *H. dioica*, as older tissue tends to be thicker and heavier, while completeness and thickness were used to select *P. perforata* and *C. canaliculatus* fragments. Macroalgae (at times multiple fragments) were attached to the rope by inserting them through a single point. Because *P. perforata* and *C. canaliculatus* occurred in dense patches where individual thalli often developed from what seems to be the same attachment point, density in our treatments was calculated based on the number of insertion points within the quadrats and not on the number of fragments per insertion point. Therefore, *P. perforata* and *C. canaliculatus* would have more individuals per point but an equivalent number of points per rope when compared to *S. compressa*. Following this approach, the mixed culture was assembled considering the three species at an equal biomass and equivalent density.

Cultured ropes were kept for at least 30 days under the same conditions until each macroalgae fragment grew up to 20 cm. Cultures were fertilized twice a week (0.5 μM KNO₃ and 0.09 μM H₂PO₄), followed by a 100% water exchange 24 hours later. Subsequently, cultured ropes were installed onto the frames and then metal frames into the intertidal quadrats. Every outplanted rope was weighed to standardize initial fresh biomass per assemblage treatment. Once in the intertidal, treatments were checked daily. Planted ropes showing any damage were replaced with new pre-weighed ones.

2.4.4 Variables sampling

Data collection devices were attached to the mesh base, near the midpoint under the canopies of every quadrat. Light and temperature were measured using Hobo thermistors and irradiance meters (ONSET computer corp., Ma, USA) programmed to record every 15 minutes for a 7-day period. Particle retention within the canopy was quantified by dry weight differences of one 25 x 75 mm synthetic polyamide fiber pad (initial weight 1.362 ± 0.003 g) per quadrat which was collected after 48 hr. After collection, pads were oven dried at 70 °C for 48 hours and then weighed three times (Sartorius, Germany ± 0.0001 g) to obtain an average reading per day per quadrat.

A proxy measure of relative bulk water flow was obtained using the dissolution of plaster (Komatsu and Kawai 1992). One cylindrical plaster bar (1 x 8 cm, initial weight 10.422 ± 0.005) was installed per quadrat during four tidal cycles (48 hr period). After collection, the bars were oven dried at 70 °C for 72 hours before weighing them three times to obtain an average reading per quadrat. The difference in dry weight before and after deployment gives an estimate of erosion, and is a rough estimate of bulk water flow in an area over a standardized time when compared to a control with no water motion (Komatsu and Kawai 1992). Preliminary trials showed that due to the hydrodynamic regime during the experimental period, removing the pads and plaster cylinders earlier yielded smaller significant differences between treatments. Contrary, removing them later resulted in over saturation of the pads and total dissolution of the cylinders. Therefore, the 48 and 72 hr period was ideal for detecting differences between treatments.

Desiccation was assessed using an agarose bead assay (Brawley and Johnson, 1993) where cylindrical beads (0.7 x 0.8 cm, 0.4 ± 0.0035 g) were deployed and collected while all the quadrats were exposed to air during nighttime and daytime low tides. Six beads were concurrently deployed per quadrat and a pair from each quadrat was collected at minute 45, 90 and 135. Desiccation was determined by the weight difference caused by water loss over time. Environmental conditions during the agarose bead assay were recorded using a weather station at site. Average environmental conditions during the agarose bead assay were determined using a weather station at site to compare average daytime and nighttime conditions.

2.4.5 Data analysis

Density (high, mid, low and control) and species composition were considered categorical independent factors. Normality (Shapiro-Wilk test), independence of variables (Durbin-Watson test) and homogeneity of variances (Cochran's test) were confirmed per factor and level. Transformations (LN and LN+1) were performed as required to fulfill the assumptions. Because the bulk water flow data did not show homoscedasticity with any transformation attempted, a non-parametric Kruskal-Wallis analysis was used to examine the effects of density and species composition on water flow.

A full two-way factorial ANOVA by least mean squares was used to examine the interactive effects of the two categorical factors on water loss, temperature, irradiance, and particle retention at an alpha value of 0.05. Because light and temperature had multiple measurements over time, to summarize the overall effect of the canopy, measurements from a two-hour interval around the higher tidal peak each day were average. Lastly, where differences were found, *post-hoc* (Tukey test) comparisons were conducted.

2.5 Results

2.5.1 Modification of physical variables by macroalgae with different assemblage compositions at different densities (experimental design I)

Overall, the attenuation of physical variables differed in response to the assemblage composition and density per treatment. Furthermore, regardless of the species, higher density treatments consistently attenuated physical variables the most. Surprisingly, there was no significant interaction effect on plaster cylinders as a proxy for bulk water flow. Neither a significant effect by the species identity or the number species per treatment. However, a significant effect of density was found ($H_{(3, N=48)} = 40.18$ $p < 0.001$). Plaster cylinders from the high and mid density quadrats exhibited significantly less erosion than those installed in the low and zero treatments (Tukey $p < 0.001$), suggesting bulk water flow was significantly lower below the canopy in the high and mid density treatments (Figure 10).

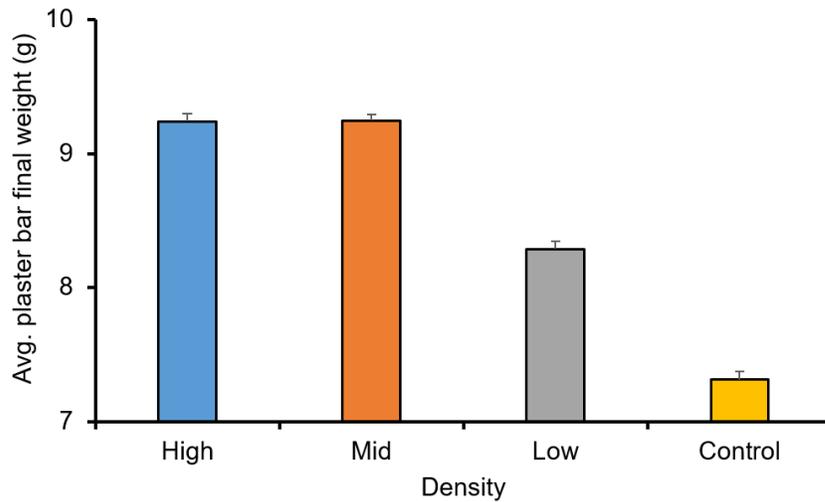


Figure 10. Average final weight of the plaster bars after after four tidal-cycles (48 hr) in the intertidal. Plaster bar initial weight was 10.4219 ± 0.005 . Mean value \pm one standard error.

Similarly, only density had a significant effect on high-tide temperature ($p < 0.001$; Table 1). High and mid density treatments consistently showed slightly cooler high-tide temperatures below the canopies (Tukey $p < 0.05$) than the low and zero treatments when fully exposed to sunlight (days 3-7). However, interestingly, no significant differences in high-tide temperature were found across density treatments after sunset (for example on days 1 and 2, when high tide occurred at night; Figure 11 and Table 1). As expected high-tide water temperature and irradiance showed a positive correlation ($r^2 = 0.94$, $n = 60$, $p < 0.001$).

Table 1. High-tide water temperature and irradiance for a seven-day period based on macroalgae species composition, density and their interaction, using a two-way factorial ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

<i>Factor</i>	<i>Irradiance</i>			<i>Temperature</i>		
	df treatments (df error)	<i>F</i>	<i>p</i>	df treatments (df error)	<i>F</i>	<i>p</i>
Day 1						
Species composition	3 (44)	6.921	**	3 (44)	1	NS
Density	3 (44)	24.900	***	3 (44)	1	NS
Interaction	9 (44)	1.724	NS	9 (44)	1	NS
Day 2						
Species composition	3 (44)	4.163	*	3 (44)	1	NS
Density	3 (44)	26.391	***	3 (44)	0	NS
Interaction	9 (44)	1.085	NS	9 (44)	1	NS
Day 3						
Species composition	3 (44)	5.626	***	3 (44)	3.2	NS
Density	3 (44)	13.595	***	3 (44)	19.7	***
Interaction	9 (44)	0.846	NS	9 (44)	1.2	NS
Day 4						
Species composition	3 (44)	3.777	**	3 (44)	3.9	NS
Density	3 (44)	9.783	***	3 (44)	18.0	***
Interaction	9 (44)	0.810	NS	9 (44)	1.5	NS
Day 5						
Species composition	3 (44)	6.067	***	3 (44)	1.4	NS
Density	3 (44)	16.003	***	3 (44)	9.2	***
Interaction	9 (44)	1.583	NS	9 (44)	1.0	NS
Day 6						
Species composition	3 (44)	5.538	***	3 (44)	1.9	NS
Density	3 (44)	9.914	***	3 (44)	11.7	***
Interaction	9 (44)	1.188	NS	9 (44)	1.0	NS
Day 7						
Species composition	3 (44)	5.837	***	3 (44)	2.3	NS
Density	3 (44)	7.313	***	3 (44)	9.9	***
Interaction	9 (44)	1.064	NS	9 (44)	1.0	NS

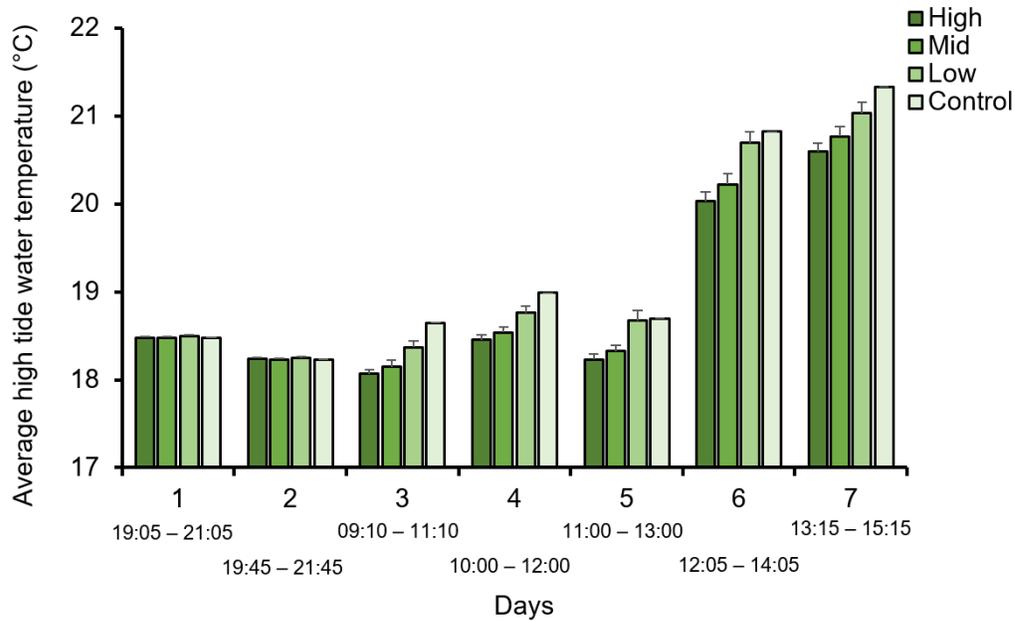


Figure 11. Average high-tide temperature for a seven-day period. A two-hour interval around the highest tidal peak (presented below the x-axis) was used to represent the daily high-tide temperature underneath the canopies. Mean value \pm one standard error.

Moreover, there was no significant interaction effect in the attenuation of irradiance below the canopies. Nevertheless, there was an effect driven by the species identity/functional-form and density treatments ($p < 0.05$; Table 2). The effect on irradiance varied based on the amount of irradiance to which treatments were exposed (Figure 12). As irradiance increased (days 6 and 7), the *Silvetia compressa* (leathery functional-form), and the high and mid density treatments, tended to attenuate irradiance the most (Tukey $p < 0.05$). The variability recorded in the irradiance measures within treatment replicates may be an effect of macroalgae movement due to water motion and thus, can explain the small standard error in the controls.

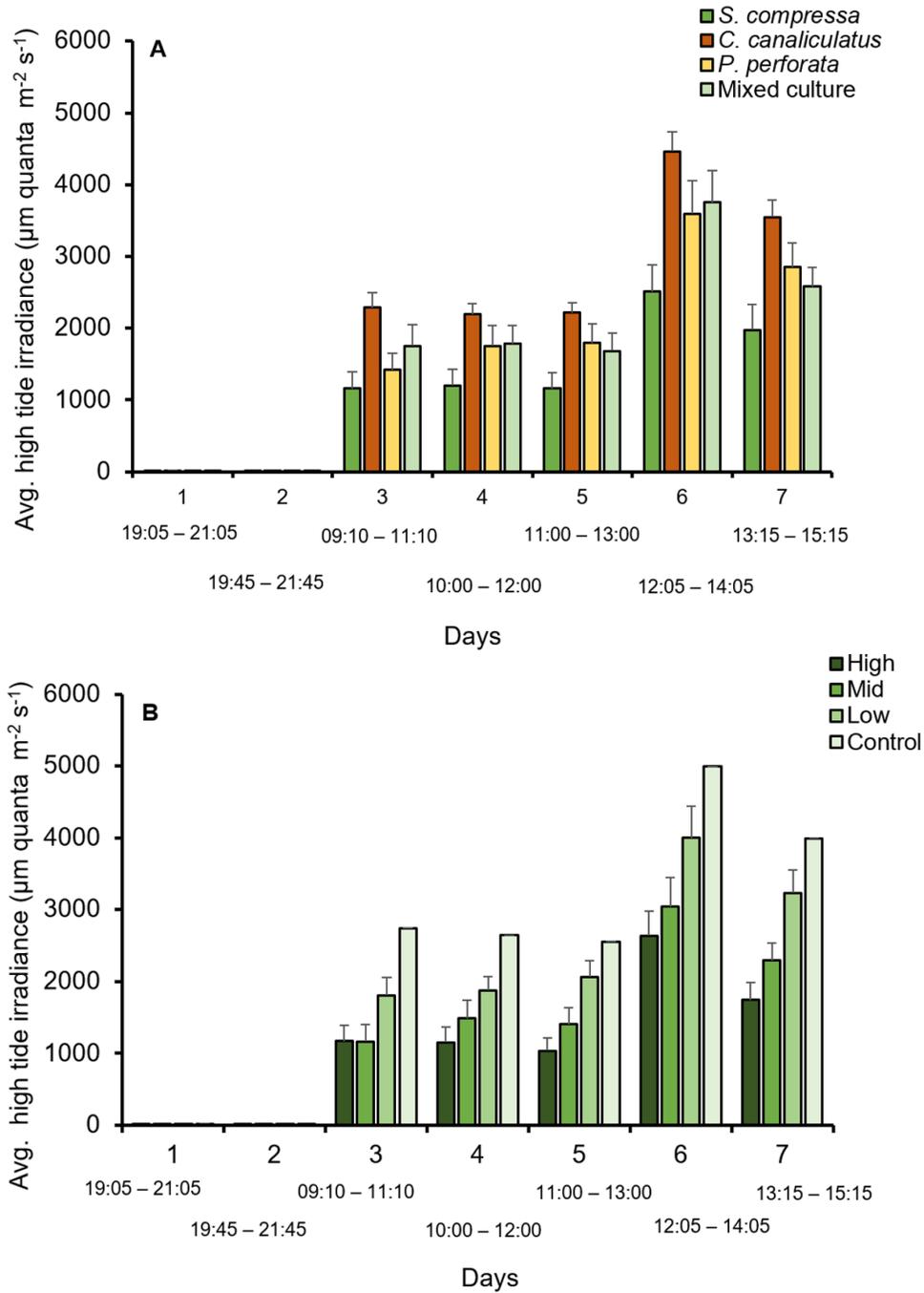


Figure 12. Average high-tide irradiance based on a) species composition and b) density treatments for a seven-day period. A two-hour interval around the highest tidal peak (presented below the x-axis) was used to represent the daily high-tide temperature underneath the canopies. Mean value \pm one standard error.

On the other hand, during daytime low tides, understory temperature (Figure 13) and irradiance (Figure 14) were modulated by the interaction between the macroalgal identity/functional-form and their density

(Table2), with a greater attenuation under the branched canopies (*S. compressa* and *Chondracanthus canaliculatus*) particularly when at high densities (Tukey $p < 0.05$).

Table2. Daytime low-tide water temperature and irradiance for a five-day period based on species composition, density and their interaction, using a two-factor crossed ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

Factor	Irradiance			Temperature		
	df treatments (df error)	F	p	df treatments (df error)	F	p
Day 1						
Species composition	3 (44)	7.963	***	3 (47)	8.46	***
Density	3 (44)	198.483	***	3 (47)	45.37	***
Interaction	9 (44)	6.150	***	9 (47)	4.12	***
Day 2						
Species composition	3 (44)	23.298	***	3 (47)	23.17	***
Density	3 (44)	52.071	***	3 (47)	80.26	***
Interaction	9 (44)	6.762	***	9 (47)	8.62	***
Day 3						
Species composition	3 (44)	8.61	***	3 (47)	16.22	***
Density	3 (44)	279.95	***	3 (47)	79.42	***
Interaction	9 (44)	3.87	**	9 (47)	6.15	***
Day 4						
Species composition	3 (44)	35.761	***	3 (47)	9.7	***
Density	3 (44)	248.943	***	3 (47)	53.3	***
Interaction	9 (44)	4.648	***	9 (47)	4.9	***
Day 5						
Species composition	3 (44)	3.7923	*	3 (47)	13.8	***
Density	3 (44)	34.6895	***	3 (47)	22.3	***
Interaction	9 (44)	3.7938	**	9 (47)	8.3	***

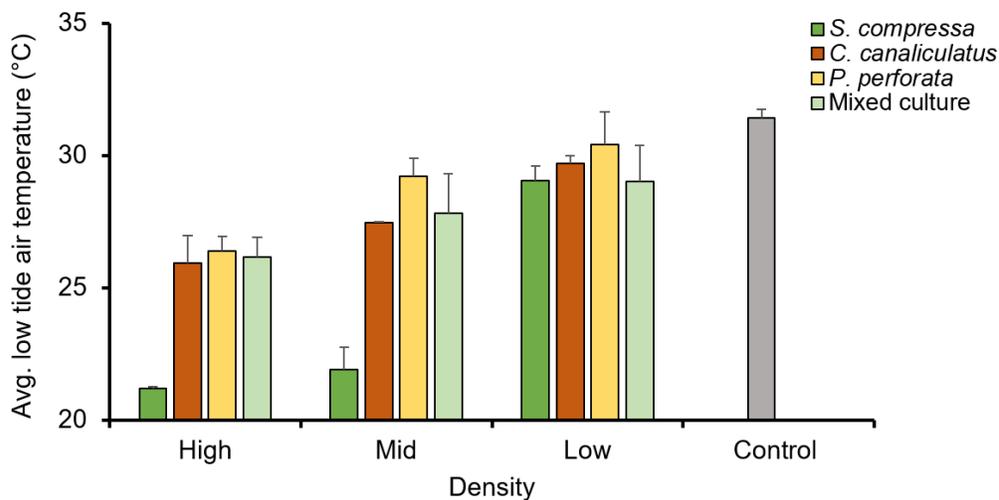


Figure 13. Average low-tide temperature underneath the canopies. Data from a five-day period showed the same trend. Mean value \pm one standard error.

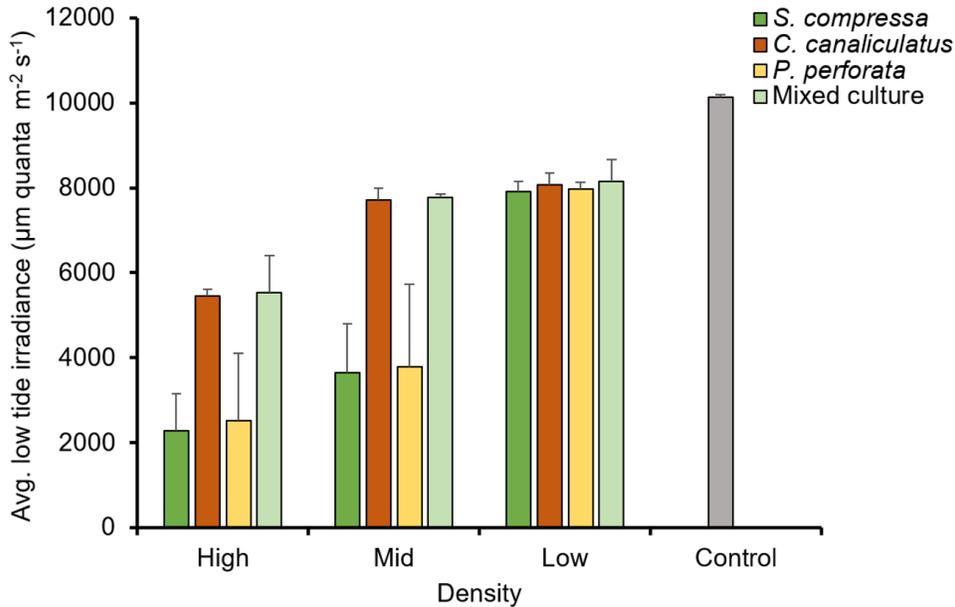


Figure 14. Average low-tide irradiance underneath the canopies. Data from a five-day period showed the same trend. Mean value \pm one standard error.

Likewise, the interaction between the categorical factors also yielded a significant effect on particle retention ($p < 0.001$; table 3). After 48 hours *S. compressa* (leathery functional form) and *C. canaliculatus* (corticated) at high and mid densities retained more particles (Figure 15) than any other treatment (Tukey $p < 0.001$).

Table 3. Particle retention based on species composition, density and their interaction, using a two-way factorial ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

Factor	df treatments (df error)	F	p
Species composition	3 (32)	8.628	***
Density	3 (32)	46.382	***
Interaction	9 (32)	2.292	*

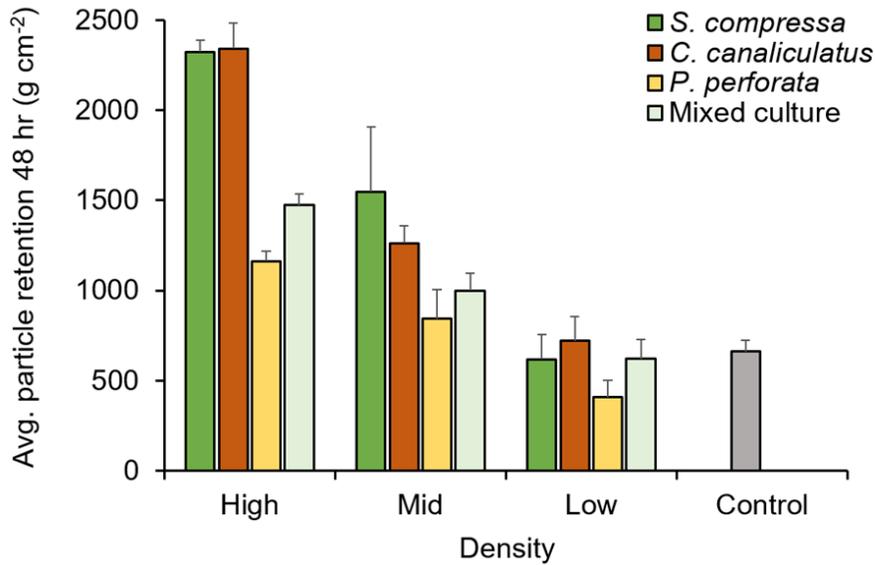


Figure 15. Average particle retention of fiber pads installed underneath the canopy treatments after 48 hours in the intertidal. Mean value \pm one standard error.

Furthermore, the interactive effect of the two categorical factors also seemed to affect the ability of macroalgae to provide protection against desiccation (Table 4), with greater desiccation after longer emersion times and lower macroalgal densities (Figure 16). The *post-hoc* test did not show significant differences in water loss for any of the species composition treatments when present at high density. Neither between *Pyropia perforata*, *S. compressa* and the mixed tri-culture at mid densities.

Table 4. Daytime two-way ANOVA of desiccation based on species composition, density and their interaction, after 45, 90 and 135 minutes of emersion. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

Factor	df treatments (df error)	F	p
45 minutes			
Species composition	3 (32)	3.0	*
Density	3 (32)	119.5	***
Interaction	9 (32)	1.0	NS
90 minutes			
Species composition	3 (32)	5.1	**
Density	3 (32)	793.2	***
Interaction	9 (32)	1.6	NS
135 minutes			
Species composition	3 (32)	24.74	***
Density	3 (32)	1319.07	***
Interaction	9 (32)	8.43	***

C. canaliculatus treatments offered the least amelioration of water loss, particularly after 90 minutes of exposure (Tukey $p < 0.05$). Remarkably, after 135 minutes of emersion and even at lower densities, macroalgae significantly ameliorated understory water loss (Tukey $p < 0.001$) compared to the control treatments.

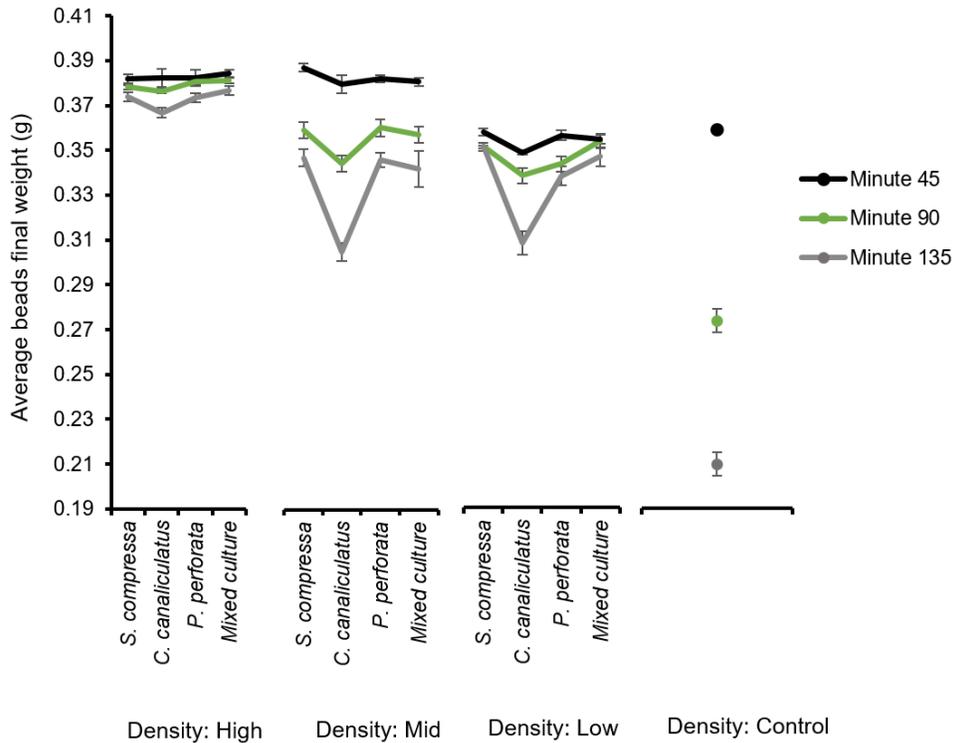


Figure 16. Final weight of agarose beads after 45, 90 and 135 minutes of exposure under emersed macroalgae canopy treatments. Initial weight corresponded to 0.4 ± 0.0035 g. Mean value \pm one standard error.

2.5.2 Modification of physical variables by macroalgae within the same functional-form group at different densities (experimental design II)

As expected, no significant differences in low-tide temperature, irradiance, water movement, or desiccation were detected between the two branched-flat morphotypes (*S. compressa* and *Halidrys dioica*) when compared at the same densities (Table 5). Differences were only detected between density treatments ($p < 0.05$), having higher density treatments attenuating these variables the most (Tukey $p < 0.05$).

Table 5. Average low tide temperature, irradiance and desiccation based on species composition, density and their interaction using a two-way factorial ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

<i>Factor</i>	<i>df treatments (df error)</i>	<i>F</i>	<i>p</i>
Temperature			
Species composition	3 (14)	1.99	NS
Density	9 (34)	4.61	***
Interaction	9 (34)	0.40	NS
Irradiance			
Species composition	3 (9)	0.50930	NS
Density	9 (22)	2.61341	*
Interaction	9 (22)	1.33822	NS
Desiccation			
45 minutes			
Species composition	1 (18)	0.0	NS
Density	3 (18)	479.3	***
Interaction	3 (18)	0.90	NS
90 minutes			
Species composition	1 (18)	0.165	NS
Density	3 (18)	79.079	***
Interaction	3 (18)	0.017	NS
135 minutes			
Species composition	1 (18)	0.258	NS
Density	3 (18)	137.206	***
Interaction	3 (18)	0.100	NS
Particle retention			
Species composition	1 (18)	10.466	**
Density	3 (18)	37.649	***
Interaction	3 (18)	0.986	NS

Nonetheless, there was a significant difference in their ability to retain particles when compared at the same densities. Although there is no interaction, both, the species ($p < 0.05$) and density ($p < 0.001$) are important factors that determine the significance of their bioengineering outcomes regardless of time (Figure 17).

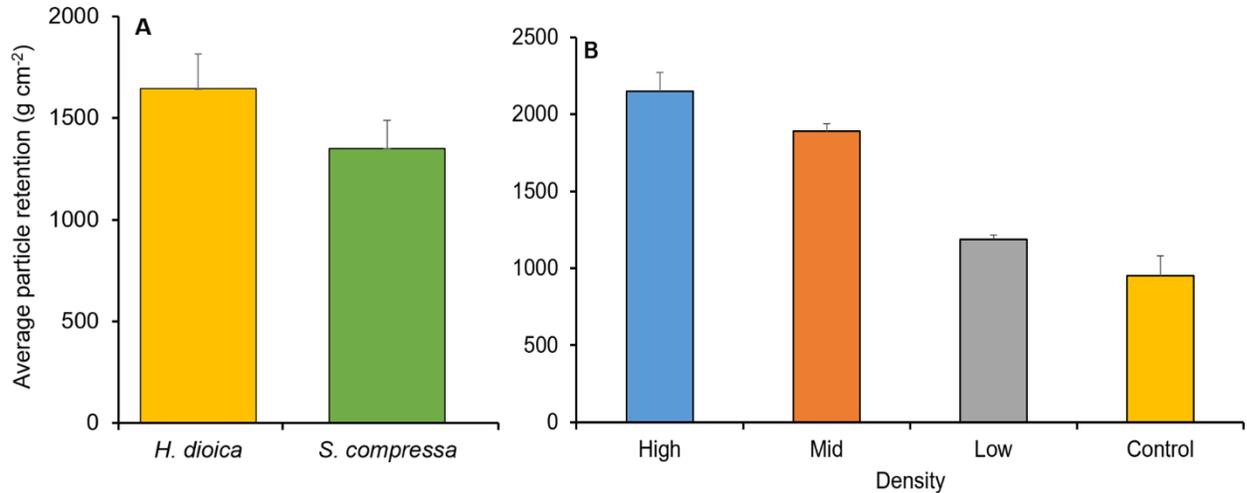


Figure 17. Average particle retention by fiber pads installed underneath the canopy treatments based on a) species composition and b) density after 48 hours in the intertidal. Mean value \pm one standard error.

Overall, no significant difference ($p > 0.05$) was found in the physical parameters measured among the experimental quadrats during night-time low tides. The absence of solar irradiance (0 lum ft^{-2}), reduced wind (0.6 m s^{-1}) and high relative humidity (89-94%) contributed to stable, cool temperatures ($20 \text{ }^{\circ}\text{C}$) that persisted underneath the canopies and in control quadrats. In addition, agarose beads showed no significant differences in water loss even after 135 minutes of emersion (beads final weight 0.3905 ± 0.0031).

2.6 Discussion

As hypothesized, the ability of macroalgae to ameliorate stressful conditions of their physical environment is a consequence of the species and their form and density. Species treatments representing the most complex structures, either by morphology, density or both, showed the highest ameliorating ability. Differences in the ability of ecosystem engineers to ameliorate stressful conditions are associated with a range of physical attributes. For example, macrophytes with distinct morphological characteristics reduce wave heights differentially (Anderson et al., 2011; Ysebaert et al., 2011). Also, seagrasses showing differences in shoot stiffness and densities modify water velocities and friction forces differentially (Paul et al., 2012; Fonseca and Fisher, 1986). And macroalgal species with different forms trap different grain sizes (Morrow and Carpenter, 2008; Churchill, 2009).

The particularities of how ecosystem engineers with given traits can modify physical conditions tend to increase the challenge in predicting the effect they will have on their local environment. Yet, in this study, by considering the functional-form group to which the targeted macroalgae belong, it is possible to explore and predict in greater detail how macroalgae at different densities can modify different physical variables. *P. perforata*, for instance, represents the foliose functional-form group. This group is characterized by having soft texture, thin, sheet-like and tubular forms, with an uncorticated internal structure. On the other hand, the corticated group is composed of macroalgae with coarsely branched, upright oriented and approximately cylindrical thalli, such as *C. canaliculatus*. These thalli can exhibit a fleshy-wiry or tough texture and are morphologically more complex than the foliose group. Moreover, leathery-form macroalgae, such as *S. compressa*, have thick blades and branches, which are deeply corticated, exhibiting a leathery or rubbery texture. This group is the most morphologically complex of them all (Littler and Littler, 1984; Steneck and Dethier, 1994; Steneck and Watling, 1982).

The data in this chapter showed that species with an increased morphological complexity and at higher densities resulted in more complex spatial arrangements, effectively increasing the number of particles retained under the canopies. Although a higher particle retention was expected in the mixed culture treatments, the combination of functional forms did not necessarily always translate into greater spatial complexity (Heck and Wetstone, 1977), as foliose forms might have interrupted or smoothed the complexity added by branched forms when combined in mixed assemblages. In fact, because of their flexibility, macroalgae with foliose forms can obstruct particles captured when compared to branched forms (Morrow and Carpenter, 2008), such as the leathery and corticated forms evaluated herein.

Moreover, macroalgae within the foliose group and leathery group tested in this study effectively attenuated irradiance when compared to the corticated form, probably because their canopies could cover a greater horizontal surface area due to their flexibility (*P. perforata*) or lateral branching (*S. compressa*). Contrary, the upright orientation of the corticated thalli (*C. canaliculatus*) may have limited this horizontal cover, thus reducing the ability of this functional-form group to significantly attenuate light underneath their canopies. Therefore, mixed cultures considering only functional forms with canopies able to cover relatively large horizontal surface areas are foreseen as reducing understory irradiance the most, when compared to mixed cultures like those that were tested here.

Furthermore, different species of macroalgae may have similar hydrodynamic performance (Boller and Carrington, 2007) and physiological responses (Einav et al., 1995). In this study, water movement and temperature were only significantly attenuated when macroalgae were present at high and mid densities.

This suggests that when submerged, the magnitude of these physical variables in the intertidal can only be ameliorated through the abundance of ecosystem engineers (Hendriks et al., 2010; van der Heide et al., 2012; Meadows et al., 2012). These results also highlight the differences in the modulating effect that the physical environment and macroalgae aggregations can have on each other.

There has been increasing recognition of the key role ecosystem engineers play as facilitators. This has help understand the dynamics, community structure and biodiversity in environments such as intertidal environments exposed to different stress levels and factors (Bouma et al., 2009; Bruno et al., 2003). The relevance of the bioengineering outcomes and thus, the importance of ecosystem engineers may vary depending on the associated organism that are influence by the modification of physical variables. These will be explored in the subsequent chapters.

3. Microphytobenthos response to the modification of physical variables

3.1 Introduction

The microphytobenthos, consisting of mats of eukaryotic algae and cyanobacteria (MacIntyre et al., 1996), contributes significantly to the total ecosystem primary production, particularly in areas with sufficient irradiance (Lake and Brush, 2011). When abundant, they can stabilize the sediment surface against sediment resuspension and erosion by binding particles with their mucilaginous secretions (Stal and de Brouwer, 2003; Stal, 2010). They also modulate the benthic geochemistry and associated fauna by impacting the regeneration rates and fluxes of nutrients, relevant to the nitrogen and oxygen budgets, and to the redox processes at the benthos-water interface (Sundbäck and Benno, 1988; Sundbäck and Snoeijs, 1991; Blackford, 2002; Hardison et al., 2013).

The composition of microphytobenthic communities is largely affected by physical factors such as irradiance, temperature, salinity, type of substrate and exposure to tidal currents (Frankovich et al., 2006; Vyverman et al., 2011; Mariotti and Fagherazzi, 2012; Totti et al., 2009). These environmental limitations, along with the large difference in size between macroalgae and the microphytobenthos, enable the microphytobenthos to be used as a proxy to assess physical changes caused by macroalgae and the biological implications these changes may have. However, few studies have focused on the ecosystem engineering aspects of canopy-forming macroalgae (some less than 50 cm in the intertidal) and their effect on the microphytobenthic community.

Some literature has explored the role of intertidal macroalgae as ecosystem engineers (Bertness et al., 1999; Kelaher, 2003). Other studies have described the community of epiphytic microalgae on seaweeds (Worm and Sommer, 2000; Totti et al., 2009; Al-Handal and Wulff, 2008). Yet, very little has been published on the interaction between macroalgae and the microphytobenthos (*e.g.*, Tyler, et al., 2003; Sundbäck and McGlathery, 2005). Although a relationship between macroalgae and associated microphytobenthic organisms has been shown, there is relatively little understanding of how small-size, canopy-forming, intertidal macroalgae may modulate their environment, both physically and biologically, and how these modulations may affect microphytobenthic settlement and distribution.

This chapter evaluates how the microphytobenthos responds to the attenuation of irradiance, temperature, bulk water flow, and particle retention caused by our targeted canopy-forming macroalgae,

which are typical of the intertidal. It was expected to find a higher abundance of microphytobenthic settlers in microhabitats with greater spatial complexity where physical variables are attenuated the most.

3.2 Objective

Assess the response, in terms of abundance and richness, of the microphytobenthos due to the modification of physical variables driven by various species of macroalgae with different functional forms, at different densities and assemblages.

3.3 Hypothesis

The ability of macroalgae to attenuate physical variables is related to their functional form and density. Thus, an increasing abundance of microphytobenthic organisms is expected to be associated with the functional form and density treatments offering the most attenuated conditions. Moreover, as extreme conditions become ameliorated, it is expected to find a greater richness underneath the most ameliorated understories.

One objective was derived:

- Assess microphytobenthos settlement as a function of the macroalgal assemblages in each quadrat.

3.4 Methodology

The experimental site, design and assemblages used for this experiment were formerly described in chapter one. Because microphytobenthic organisms need water to transport and settle, only high-tide environmental scenarios were considered as relevant to assess the response of the microphytobenthos underneath the experimental canopies.

3.4.1 Biological variable sampling

To assess the biological response by the microphytobenthos, a polycarbonate slide (25 x 75 mm) was fixed to the mesh underneath every canopy treatment. Slides were subsequently removed after 14 days. Because the first days after the installation of the slides represent a colonization phase (Hoagland 1983; Hoagland 1981), removing them earlier could have resulted in lower abundances and diversity of microphytobenthic cells. Leaving them throughout the length of the experiment most likely resulted in colonization and growth of a greater number of species. After 14 days, slides were placed individually in plastic petri dishes with filtered and UV irradiated seawater, and fixed with Lugol's solution (1%) for direct counting under an inverted microscope (Zeiss AxioObserver, Germany). A total of 10 optical fields (36 mm²) were photographed per slide for quantification and genus identification (Figure 18). Only cells bigger than 20 µm were considered.

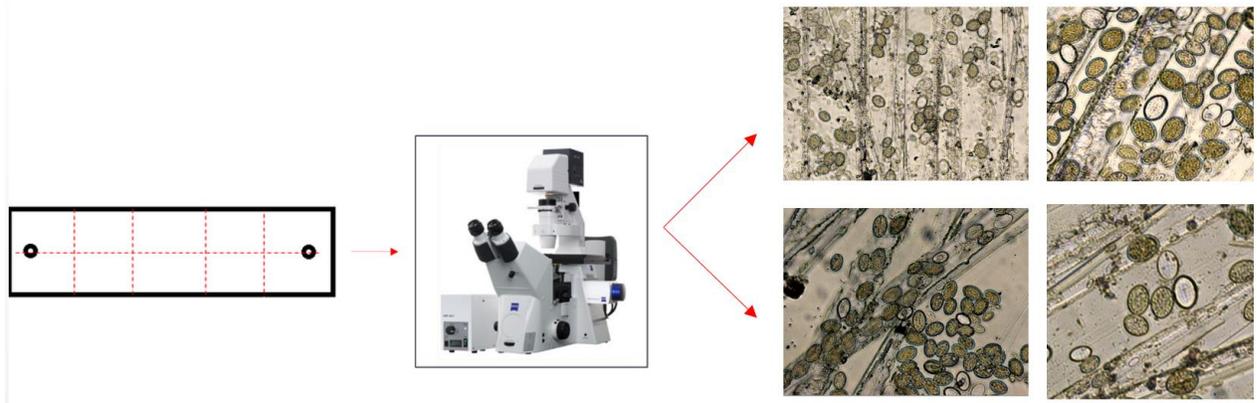


Figure 18. Photographs were taken to identify and quantify the organisms in the microphytobenthic biofilm. Each slide was visually divided in 10 sections from which one randomly located photograph per section was taken.

3.4.2 Data analysis

Density (high, mid, low and control) and species composition were considered categorical independent factors. Natural log transformations were performed as required to fulfill the assumptions (Underwood 1997; see Chapter 1). A full two-way factorial ANOVA by least mean squares was used to examine the interactive effects of the two categorical factors on the microphytobenthos at an alpha value of 0.05. Where differences were found, *post-hoc* (Tukey test) comparisons were conducted. Additionally, multiple regressions were performed to identify which of the environmental factors measured (see Chapter 1) resulted in significant predictors of the abundance in microphytobenthic cells.

Similarities in taxonomic richness were quantified using Jaccard's index based on a presence/absence matrix. The relationship among sites was subjected to a Principal Coordinates Analysis (PCoA) to identify spatial patterns in the composition of the microphytobenthos. Subsequently, a Permanova analysis was used to determine if the density and species identity the quadrats influenced the microphytobenthic community of the understory.

3.5 Results

As expected, a greater abundance of microphytobenthic cells under the canopies with the most ameliorated conditions was found. However, contrary to what was anticipated, microphytobenthic richness decreased on these understories. Furthermore, microphytobenthos settlement responded to the interaction of species composition and density ($p < 0.001$, Table 6). With benthic diatoms and macroalgal spores as the most abundant microphytobenthic settlers.

Table 6. Microphytobenthos abundance using a two-way factorial ANOVA and richness using a PERMANOVA, both based on macroalgal species composition, density and their interaction, using a two-way factorial ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

<i>Abundance</i>	<i>df treatments</i>	<i>df error</i>	<i>F</i>	<i>p</i>
Species composition	3	48	4.995	**
Density	3	48	14.059	***
Interaction	9	48	4.671	**
<i>Richness</i>	<i>df treatments (df error)</i>	<i>Pseudo-F</i>	<i>P</i>	<i>Unique perms</i>
Species composition	3 (48)	2.15	**	999
Density	3 (48)	4.62	***	998
Interaction	9 (48)	1.23	NS	996

Overall, diatoms were the dominant organism. The *C. canaliculatus* and *S. compressa* treatments at high and mid densities had significantly more microphytobenthic settlers than any other treatment (Tukey $p < 0.05$), having at least twice as many cells than the *P. perforata* and the mixed tri-culture quadrats at any density (Figure 19). The multiple regression analyzes indicated that the microphytobenthic abundance of

the understory is modulated by the ability of macroalgal aggregations to particle retention. Therefore, the ability of *C. canaliculatus* and *S. compressa* treatments to ameliorate this factor greatly determines microphytobenthic understory settlement. ($r^2 = 0.7875$, $p < 0.001$).

The PCoA captured 70% of the variation in the microphytobenthos richness data (Figure 20). The richness was not modulated by the interactive effect of species composition and density (Table 6). However, independently, species composition ($P(\text{perm}) < 0.05$) and density ($P(\text{perm}) < 0.001$) had a significant effect on the microphytobenthos community. *Cocconeis* spp. was the most abundant diatom across treatments, often forming dense biofilms and representing 96% of the settlers.

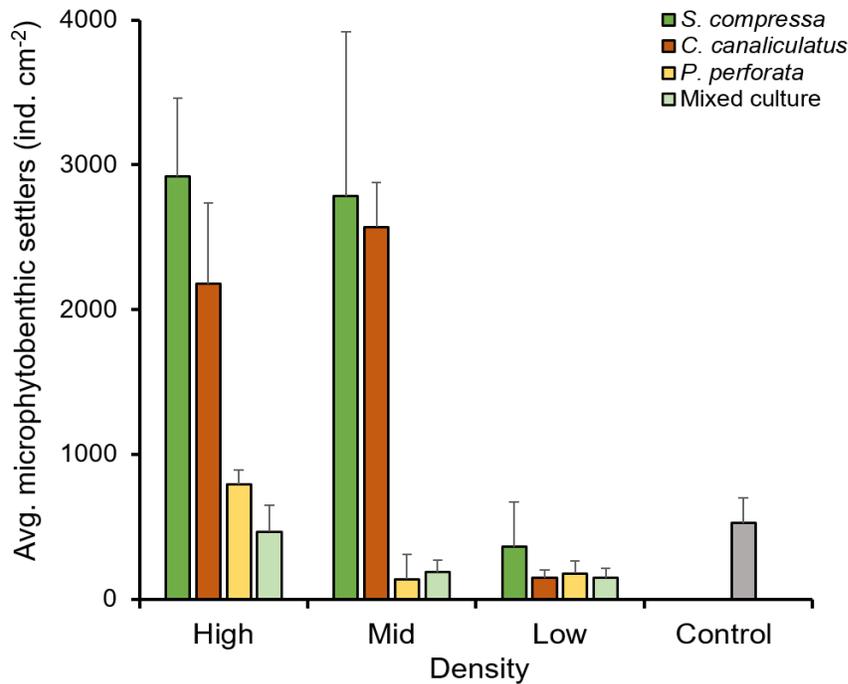


Figure 19. Average number of microphytobenthic organisms per treatment after 14 consecutive days in the intertidal. Mean value \pm one standard error.

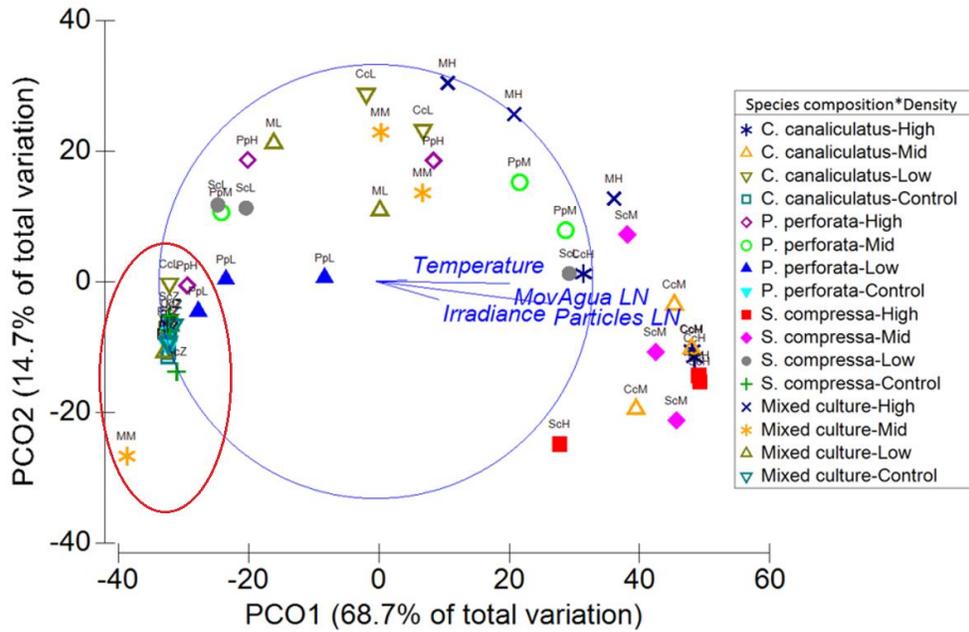


Figure 20. Microphytobenthic cells distributed among two major groups. One by *S. compressa* and *C. canaliculatus* at high and mid densities (red oval) and another one by the controls and most of low density treatments.

Fewer representatives of other benthic diatoms, such as *Climacosphenia* sp., *Achnanthes* sp. and cyanobacteria, such as *Chroococcus* sp., were present at a lower abundance (< 1%). Macroalgae spores, most likely from phaeophytes and rhodophytes (Figure 21), were also identified across treatments (3.5%), particularly settling at low and control density treatments.

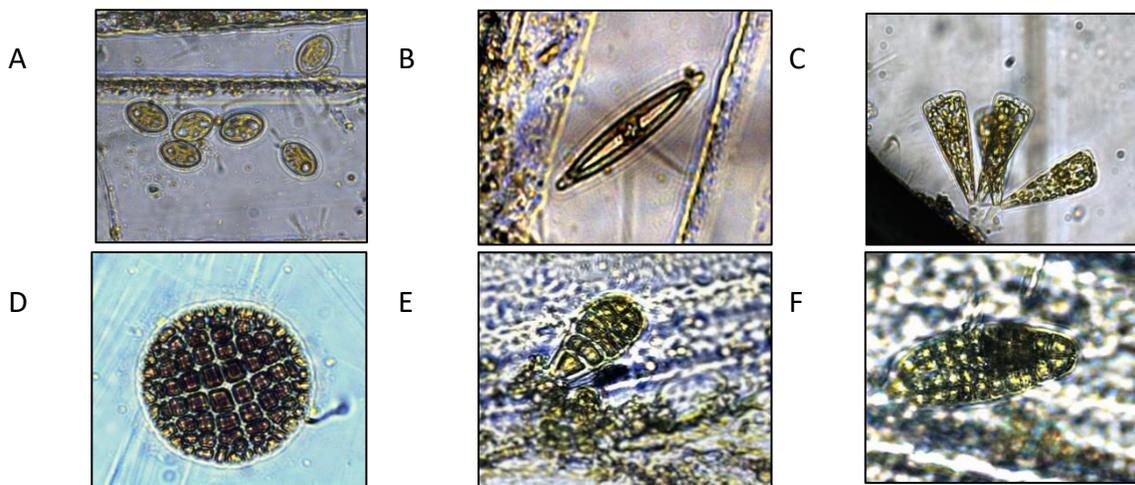


Figure 21. Microphytobenthic organisms settled underneath the canopy treatments. Benthic diatoms a) *Cocconeis* sp., b) *Navicula* sp., c) *Climacosphenia* sp., d) red macroalgae spore and E- F) brown macroalgae sporophytes.

Although the attenuation of physical variables driven by macroalgae influences the response of the microphytobenthos, the effect this attenuation has, differs between microorganisms. Benthic diatoms and macroalgae spores have an opposite response with a higher abundance of diatoms in high density treatments (Figure 22a) and a higher abundance of spores in low density treatments (Figure 22b), regardless of the morphotype treatments.

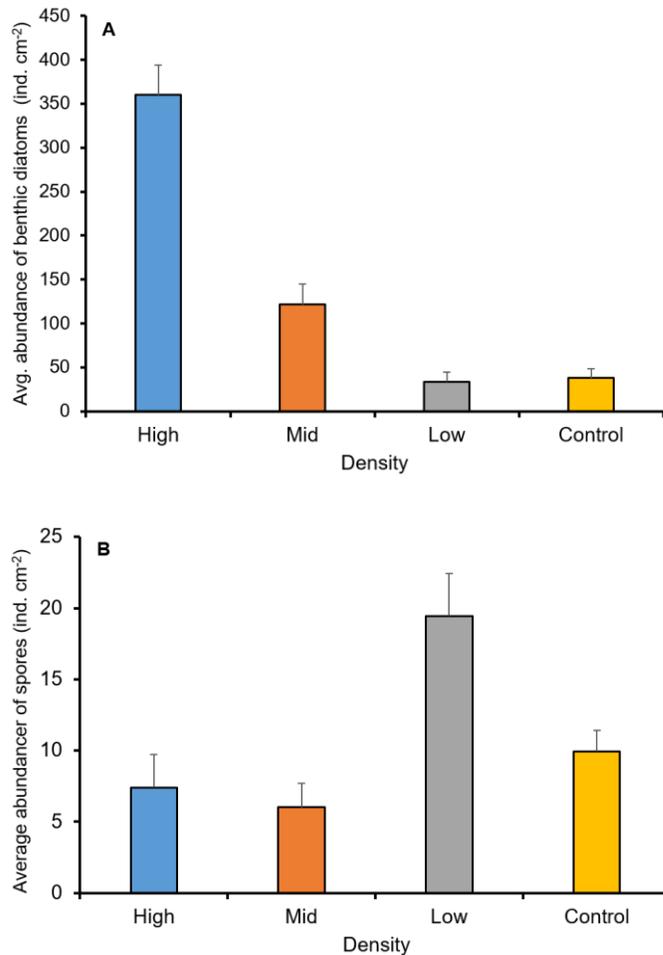


Figure 22. Average number of a) benthic diatoms and b) macroalgal spores per density treatment after 14 consecutive days in the intertidal. Mean value \pm one standard error.

Results also shows that at equal densities, even when two species of macroalgae have a similar morphotype (*i.e.* branched-flat) there is a difference in the response by the microphytobenthos (Table 7; $p < 0.001$). This difference could be driven by either subtle variation in the physical environment or due to chemical cues that were not detected here (Figure 23).

Table 7. Microphytobenthic settlement based on macroalgae species, their density and their interaction, using a two-way factorial ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

Factor	df treatments (df error)	F	p
Species composition	1 (18)	19.1270	***
Density	3 (18)	18.1930	***
Interaction	3 (18)	2.4033	NS

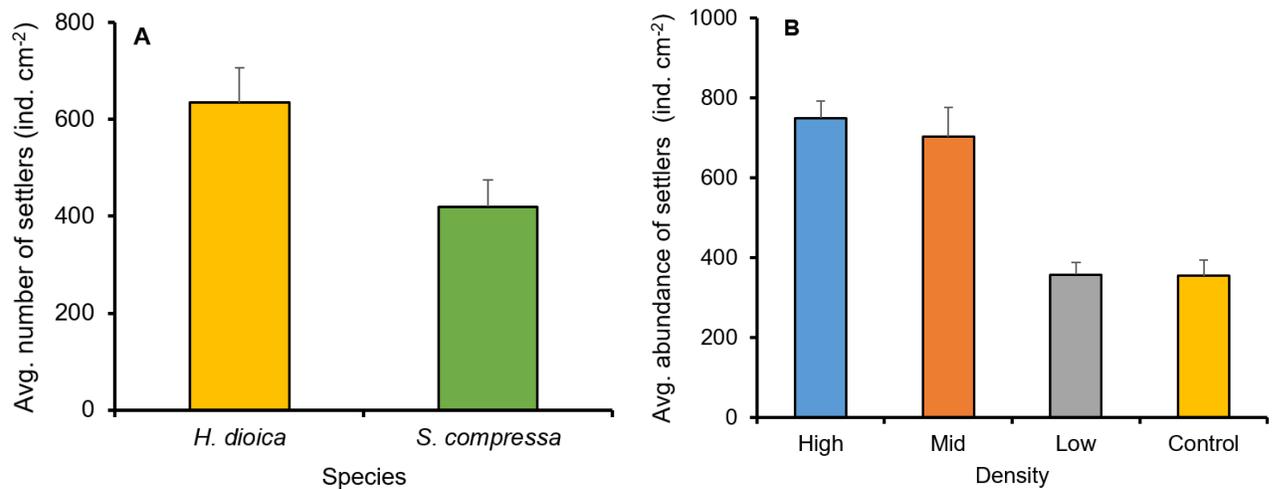


Figure 23. Average abundance of microphytobenthic cells underneath the canopies based on a) species composition and b) density treatments after 14 consecutive days in the intertidal. Mean value \pm one standard error.

3.6 Discussion

The hypothesis, that macroalgal aggregations with the most attenuated conditions would result in more microphytobenthic settlers, was supported. However, contrary to what was predicted, microphytobenthic richness did not follow the same trend. Overall, only one genera dominated settlement underneath the treatments, particularly explicit in the high density treatments. Here, *Cocconeis* spp. dominated the microphytobenthic community. As a result, any evidence that microphytobenthic settlement under the canopies was a host-specific driven response was found, contrary to the trends found in studies that have analyzed epiphytic diatoms on macroalgae, where substratum preferences were identified (Al-Handal and Wulff, 2008, Costa et al., 2016).

Although *Cocconeis* spp. diatoms are frequent epiphytes of macroalgae (Suzuki et al., 2001), here macroalgae was not evaluated as a substrate, but rather only settlement underneath the canopy was considered as a response to the bioengineering of the macroalgae treatments tested. Results show that the environmental modifications driven by the macroalgae appear to play a significant role in promoting distinct spatial patchiness in the microphytobenthos. However, a biological response based on species specificity cannot be disregarded, as other factors such as chemical cues or deterrents may also influence microphytobenthic settlement and development (Bucolo et al., 2012; Lam et al., 2008).

Results show a higher abundance of microphytobenthic organisms under the *Condracanthus canaliculatus* and *Silvetia compressa* treatments when compared to the *Pyropia perforata* and mixed culture treatments at the same densities. The high abundance of *Cocconeis* spp. underneath these canopies most likely results, at least in part, from their effect on particle retention. Moreover, the relatively lower abundance in the *P. perforata* aggregations may be linked to foliose forms obstructing particle capture, thus preventing microphytobenthic organisms from settling. Although a higher settlement response was expected in the mixed culture treatments, there appears to be a similar effect as that described for particle retention, where particle trapping may be reduced by the addition of a foliose species. This may have occurred potentially due to the gaps formed by the foliose species, through which particles could escape, or to a blocking effect, preventing particles from entering the understory.

Overall, results suggest that for this particular scenario the presence of species of macroalgae within given functional-form groups may be more relevant in modulating microphytobenthos abundance than having a mix of species exhibiting less complexity. In addition, the spatial complexity added by macroalgae may also have an indirect effect on the richness of the microphytobenthic community. Besides ameliorating the physical environment, the complexity of a habitat may contribute to reducing predation and competition stress by providing spaces suitable for organisms with particular traits (Bertness and Callaway, 1994; Grabowski, 2004).

Here, *Cocconeis* diatoms almost exclusively colonized the understories of the high and mid density treatments. Contrary to other diatoms, the *Cocconeis* genus is not inhibited by low light availability (Watson et al., 2004; Raniello et al., 2007), thus increasing their probability of development under canopies where irradiance was attenuated the most. Because more light was available at the lower densities, other organisms may have been able to develop under these treatments. Yet, this trend may also be associated to *Cocconeis* spp. rapidly colonizing new substrates during the first steps of succession (Siqueiros-Beltrones et al., 2004; Majewska et al., 2014) and should be explored further.

While considering the complexity of intertidal dynamics and the difficulty of obtaining in situ measurements of the interactions between microphytobenthic communities and short macroalgae, isolating a key factor driving the biological response by the microphytobenthos is challenging. Complex interactions driven by specific adaptations of organisms to the particular conditions under the canopies could have significant effects on the microphytobenthic community. However, results show that the ability of macroalgal aggregations to reduce bulk water flow and retain particles underneath their canopies plays a key role in microphytobenthic settlement, and that this ability is density-dependent.

Similar to other bioengineers, the modifications of physical conditions driven by these macroalgae appear to modulate the dynamics, community structure, and biodiversity of the intertidal (Bruno et al., 2003; Wright and Jones, 2004; Bouma et al., 2009; Gutiérrez et al., 2011). The spatial and temporal heterogeneity added by short intertidal macroalgae as ecosystem engineers significantly influenced the abundance and richness of the microphytobenthic community in the rocky intertidal. Nevertheless, it cannot be disregarded that ecophysiological traits particular to every macroalgal species could also have an effect on the microphytobenthic community of the understory.

In conclusion, how different macroalgae aggregations can modify their environment with differential microphytobenthic responses can greatly affect the dynamics of the intertidal with possible effects for higher trophic levels through a “magnification” effect (Thomsen et al., 2010). For instance, it could be expected a higher number of grazing macroinvertebrates to be associated with macroalgal aggregations enhancing microphytobenthic abundance. Moreover, although the functional-form model approach is debated (Hay, 1994; Phillips et al., 1997), results described herein suggest that functional-form groups could be useful in predicting specific physical changes in the intertidal due to changes in macroalgal aggregations.

4. Macroinvertebrates response to the modification of physical variables

4.1 Introduction

An important challenge in marine ecology is to understand the structuring processes that determine species distributions and community composition. Recently, several authors have focused on understanding the mechanisms by which habitat-forming or habitat-modifying organisms (i.e. ecosystem engineers; Jones et al., 1994) influence marine communities. In marine coastal systems, macroalgal canopies (Wright et al., 2014), mollusks, such as mussels (Gutiérrez et al., 2003), and seagrasses (Holmquist, 1998) are known to influence community structure by modifying biophysical conditions. In the intertidal, positive ecosystem engineer interactions are a major community structuring factor influencing the abundance and distribution of associated organisms, such as macroinvertebrates, primarily because many ecosystem engineers are dominant space holders with the ability to ameliorate stressful forces acting within these habitats (Bertness and Callaway, 1994).

Ecosystem engineers from the intertidal, such as small-size canopy-forming macroalgae and mussels, can ameliorate extreme physical conditions in their environment by modifying local hydrodynamic regimes, by stabilizing sediments and by providing spaces free from competition and predation. For instance, *Ascophyllum* sp., a branched brown seaweed, significantly reduces physical pressure in the intertidal zone and positively affects the recruitment, survival, and growth of a range of macroinvertebrates (Bertness et al., 1999). And the red alga, *Gracilaria vermiculophylla*, provides milder biophysical conditions increasing the survival of the amphipod, *Gammarus mucronatus* (Wright et al., 2014).

Although there is a general understanding of the effects that ecosystem engineers may have on their environment, the manner and magnitude in which they ameliorate conditions may differ based on the species of the engineer, their density, and the baseline conditions of the environment. Consequently, differences in ecosystem engineering could trigger differences in the abundance and distribution of associated organisms. For instance, blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) beds can modify sediment characteristics in the intertidal, however, their ability to modify sediments differs as mussel beds can result in finer sediment grain-size, which in turn affects the abundance and distribution of associated infaunal and epibenthic mobile organisms (Kochmann et al., 2008). Also, canopy-forming macroalgae can potentially influence particle transport, however, differences in macroalgal morphologies

can redirect particles differently, potentially affecting the abundance and distribution of suspension feeders (Morrow and Carpenter, 2008).

Intertidal zones are among the most popular sites to study ecosystem engineers. Several authors have examined ecosystem engineering processes, which structure local communities (e.g., Bertness et al., 1999; Borthagaray and Carranza, 2007; Kochmann et al., 2008; Byers et al., 2010; Sueiro et al., 2011). Rocky intertidal zones are physically extreme marine environments (Tomanek and Helmuth, 2002), in part because their vertical and horizontal stress gradients occur in a relatively small area (Crowe et al., 2000; Valdivia et al., 2011). As a result, intertidal invertebrates and macroalgae must deal with strong selective pressures related to physical stress, in addition to competition and predation (Connell, 1972; Denny, 1985; Denny and Wethey, 2001), in their zones. Because of the environmental heterogeneity in the intertidal zone and the multitude of biological interactions present, the rocky shore is an ideal study system to examine the effects that different ecosystem engineers at different densities could have on the abundance and distribution of associated organisms.

In this chapter, we quantified the relative importance of macroalgae as ecosystem engineers by examining the abundance and distribution of macroinvertebrates. The data collected was then used to test the hypothesis that under harsh environmental conditions (i.e., daytime low tides), the provision of ameliorated conditions under the canopies would vary with the species composition and the density of the macroalgal assemblages. We predicted that macroinvertebrates would associate most with the macroalgal species and densities showing the most ameliorated environments. Furthermore, for relatively milder environmental conditions (i.e., nighttime low tides), macroalgal aggregations were not expected to provide any significant ecosystem engineering and macroinvertebrates were expected to be similarly distributed among the macroalgal treatments.

Differences in the environmental baseline conditions of the rocky intertidal, in addition to differences in the physical attributes of macroalgae, could result in small-scale differences within the habitats provided below the canopies, reflected in the structure of the macroinvertebrate communities present. Therefore, understanding how differences related to form, species or density of macroalgae could affect the abundance and distribution of macroinvertebrates will provide a better understanding of the biodiversity and community stability controls present.

4.2 Objective

Assess the biological response of macroinvertebrates, in terms of abundance of macroinvertebrates, as a function of the modification of physical factors driven by macroalgal aggregations with various functional forms, at different densities and within various levels of environmental stress.

4.3 Hypothesis

Because the main community-structuring process varies based on the level of environmental stress, under harsh environmental conditions (*i.e.*, daytime low tides), the provision of ameliorated conditions under the canopies would vary with the species composition and the density of the macroalgal assemblages. It is expected that macroinvertebrates would associate most with the macroalgal species and densities showing the most ameliorated environments. On the other hand, for relatively milder environmental conditions (*i.e.*, nighttime low tides), macroalgal aggregations will provide any significant ecosystem engineering, therefore macroinvertebrates are expected to be similarly distributed among the macroalgal treatments.

Two objectives were derived:

- Assess the physical variables (light, temperature, and particle retention) as a function of the assemblages and the density in each quadrat.
- Quantify the abundance and distribution of macroinvertebrates (response) in each stress scenario as a function of the attenuation the physical variables.

4.4 Methodology

To assess the biological response by macroinvertebrates, experiments were carried out in the study site and following the same experimental assemblages described in chapter one. Daily visual surveys were used to quantify the effect of the canopies on small mobile macroinvertebrates during daytime and nighttime low tides when all quadrats were fully exposed to air. Macroinvertebrates associated to every treatment were counted and identified *in situ*. In addition, in order to help identify possible processes

driving macroinvertebrate patterns in abundance and distribution, we also logged information indicating whether macroinvertebrates were sheltering (e.g., mollusks with closed operculums) or feeding (e.g., actively scraping or grazing on macroalgal tissue).

4.4.1 Experimental design one

This experiment consisted of assessing the effect the different canopy treatments (see Chapter 1, Figure 7) on the physical conditions during daytime and nighttime low tides and their effect on macroinvertebrates abundance and distribution as a function of macroalgal assemblages and baseline conditions of the environment.

4.4.2 Experimental design two

This experiment consisted of testing the effect that two macroalgal species, *S. compressa* and *H. dioica*, within the leathery functional-form groups and install at different tidal levels have on macroinvertebrates. Quadrats were assembled as monocultures at the four densities described (see Chapter 1), each one with three replicates.

4.4.3 Data analysis

Density (high, mid, low and control) and species composition (*S. compressa*, *C. canaliculatus*, *P. perforata*, and the mixed culture) for the first experiment, and density and species identity (*S. compressa* and *H. dioica*) for the second experiment were taken as categorical and independent factors. Natural log and natural log +1 transformations were performed as required to fulfill the assumptions (Underwood, 1997; see Chapter 1). A full two-way ANOVA with interaction was used to examine the interactive effects of the categorical factors on the abundance of macroinvertebrates. Where differences were found, *post-hoc* (Tukey test) comparisons were conducted.

4.5 Results

4.5.1 Physical factors

As expected, the ability of macroalgae to provide ameliorated understories varied with the environmental conditions (*i.e.* extreme vs. mild). The ameliorated conditions differed based on the species and density of macroalgae per treatment. However, regardless of the species identity or functional form, higher density treatments consistently attenuated physical variables the most. Moreover, differences found in the amelioration of understory light, temperature and desiccation appeared to have a significant effect on the macroinvertebrate composition of the understory. Nonetheless, it was also found that under milder scenarios the abundance of macroinvertebrates was species-specific.

In the harsh conditions of daytime low tides, the interaction between species composition and density seemed to modulating understory temperature and irradiance with *S. compressa* (leathery functional form) and *P. perforata* (foliose functional form) at higher densities attenuating these factors the most. Similarly, the ability of macroalgae to provide protection against desiccation also resulted from the interaction between species composition and density, with greater desiccation after longer emersion times and lower macroalgal densities. As expected, no significant differences were found in the attenuation of the physical variables during nighttime low tides (see chapter one).

4.5.2 Biological response

4.5.2.1 Experiment one: Macroinvertebrate response during daytime and nighttime low tides

The effect of macroalgal canopies on macroinvertebrate abundance varied with the baseline conditions of the environment. The two-way full factorial ANOVA showed that during daytime low tides, only density treatments appeared to modulate the abundance of macroinvertebrates ($p < 0.001$; Table 8). *Chrorostoma* (formerly *Tegula*) *funnebralis* was the most abundant of these macroinvertebrates, and was often found motionless and grouped in compact aggregations below the canopies during the day (Figure 24).

Table 8. Macroinvertebrate abundance based on species composition, density and their interaction, using a two-way factorial ANOVA. NS = no significant differences, *indicates $0.05 > p > 0.01$, **indicates $0.01 > p > 0.001$; ***indicates $p < 0.001$.

Factor	df treatments (df error)	F	p
Daytime low tide			
Species morphotype	3 (48)	1.97	NS
Density	3 (48)	17.36	***
Interaction	9 (48)	2.01	NS
Nighttime low tide			
Species morphotype	3 (48)	25.04	***
Density	3 (48)	20.19	***
Interaction	9 (48)	10.11	***

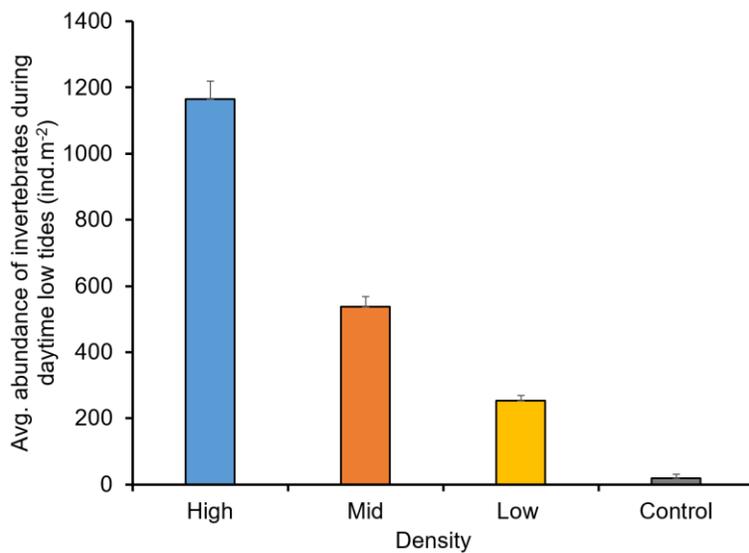


Figure 24. Average abundance of macroinvertebrates during nighttime low tides. Plots were installed at the same tidal level in the mid intertidal. Mean value \pm one standard error.

On the other hand, during nighttime low tides, the abundance of macroinvertebrates was modulated by the interaction between species composition and density ($p < 0.05$; Table 9). *Silvetia compressa* at high and mid densities showed the greatest abundance of associated macroinvertebrates (Figure 25), mostly littorinids and *Chlorostoma funebris*. Nonetheless, *Pagurus samuelis*, *Lottia gigantea*, and *Pachygrapsus crassipes* were also distributed among the experimental quadrats. In contrast to daytime low times, most macroinvertebrates were actively grazing or scraping on top of the canopies at night.

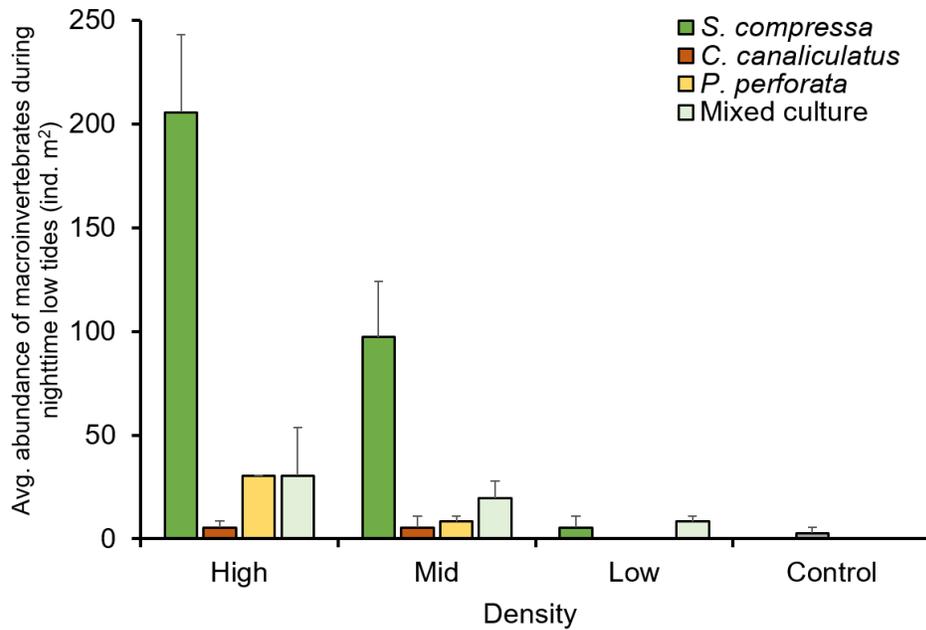


Figure 25. Average abundance of macroinvertebrates during nighttime low tides. Plots were installed at the same tidal level in the mid intertidal. Mean value \pm one standard error.

4.5.2.2 Experiment two: Macroinvertebrate response towards macroalgal species within the same functional-form group.

Similarly to what was formerly described, when comparing the response of macroinvertebrates towards *H. dioica* and *S. compressa* treatments during daytime low tides, results revealed a response modulated by macroalgal density only ($p < 0.001$). Contrarily, during nighttime low tides, data showed that more macroinvertebrates associated with the *H. dioica* treatment and the higher density treatments ($p < 0.001$; Figure 26).

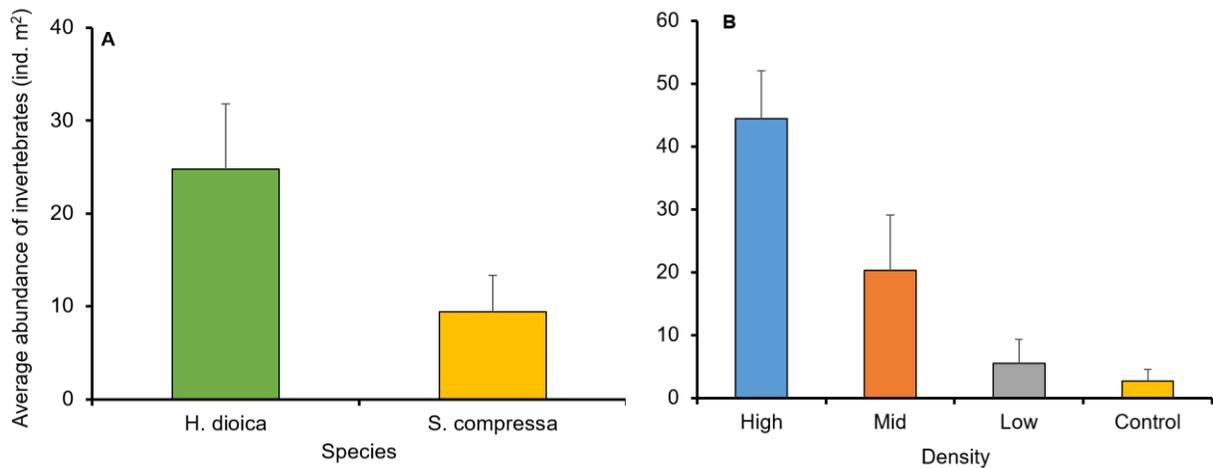


Figure 26. Average abundance of macroinvertebrates towards a) species within the leathery function group and b) different density treatments. Mean value \pm one standard error.

4.5 Discussion

As expected, our results showed that macroalgal canopies can have a major influence on the abundance and distribution of intertidal macroinvertebrates. The reasons for this relationship appear to differ between harsh, daytime low tides, and milder environments, such as nighttime low tides. By reducing heat, irradiance, and water loss, high-density canopies positively affected the abundance and distribution of macroinvertebrates during daytime low tides. Yet at nighttime low tides, despite the fact that no significant differences in the physical parameters measured were found between experimental quadrats, macroinvertebrates still showed greater abundances at higher densities of *Silvetia compressa*.

Many authors have studied the macroinvertebrate communities of the rocky intertidal (Pereira et al., 2006; Cardoso et al., 2004; Sueiro et al., 2011; Londoño-Cruz et al., 2014). Similar to what they have reported, we also found a relationship between intertidal macroinvertebrates and macroalgal aggregations. However, the abundance and distribution of macroinvertebrates as a function of macroalgal canopies was not always as expected. During daytime low tides, high temperature, irradiance and desiccation can limit the mobility of macroinvertebrates. Correspondingly to what was previously described by Connell (1961), comparing the distribution of *Chthamalus stellatus* and *Balanus balanoides* in the intertidal. Under these harsh scenarios, macroinvertebrates showed greater abundance underneath high-density canopies, regardless of species composition. These results suggest that under extreme

environmental conditions, density might be the main factor driving the abundance and distribution of small and mobile macroinvertebrates.

In this study, *Chlorostoma funebris* was the most abundant organism, possibly because this invertebrate is highly tolerant to heat stress (Gleason and Burton, 2013). However, because high levels of irradiance can greatly restrict the mobility of *C. funebris* (Doering and Phillips, 1983), they were found sheltering underneath our high-density treatments. Other facilitation outcomes are also strongly density-dependent, regardless of the species involved. For instance, high densities of macroalgae and oysters positively affect epifaunal richness and abundance by providing greater surface area for colonization (Bishop et al., 2012). Additionally, when abundant, suspension feeders appear to facilitate growth and recruitment of seagrasses by clearing the water column and allowing for increased light penetration (Wall et al., 2008).

Conversely, at nighttime low tides, although we expected no pattern in the abundance and distribution of macroinvertebrates as a function of our macroalgal treatments at night, a significantly higher abundance of macroinvertebrates did associate with the higher densities of *S. compressa* at first and *Halidrys dioica*, second. Theoretical models summarize that because under relatively milder environmental conditions, competition and predation become the most relevant community structuring processes, organisms would tend to associate with ecosystem engineers providing competition and/or predation-free spaces (Menge and Sutherland, 1987; Crain and Bertness, 2006). As an example, in a relatively low-stress seagrass meadow with mild physical stressors, blades provide clinging surfaces for shrimp where they can hold on and be protected from predators (Main, 1987). Because nighttime low tides showed relatively milder environmental conditions than daytime low tides, we might have expected a similar protective interaction between macroinvertebrates and macroalgae.

However, we did not find any indication of this interaction that could explain the abundance and distribution patterns found at night. It is possible that the relatively short canopy of the macroalgae assessed did not provide macroinvertebrates with effective shelter, or that local predators did not represent an immediate threat during low tides. Although we did not assess any process that occurred at high tides, it is also probable that predation and competition could have been greater when predators such as octopi and fish had access to the mid-intertidal. Under this scenario, it is likely, that macroalgae could have provided some shelter; although, this merits further testing.

Our observations indicate that during nighttime low tides, the relationship between macroalgae and macroinvertebrates is trophically driven, rather than macroinvertebrates actively searching shelter. In fact,

the most abundant macroinvertebrate, *C. funebris*, as well as littorinids, would choose to feed on brown seaweeds, such as *S. compressa* or *H. dioica* before feeding on red macroalgae, such as *Pyropia perforata* and *Chondracanthus canaliculatus* (Steinberg, 1985; Granado and Caballerom 1991; Kubanek et al. 2004). In addition, some authors have found that branched-articulated macroalgae such as *S. compressa* and *H. dioica* support high densities of epiphytic diatoms (Al-Handal and Wulff, 2008; Totti et al., 2009) and understory biofilms (Umanzor et al., 2017). Because several of the macroinvertebrates found, for instance, *C. funebris*, littorinids and *Lottia gigantea*, are grazers (Mak and Williams, 1999; Shanks, 2002), it is plausible that the greater abundances of diatoms associated with experimental macroalgae canopies could have had an effect on the abundance and distribution of macroinvertebrates at nighttime low tides.

Although the former hypothesis needs further testing, it suggests that the presence of particular macroalgal species can strengthen trophic connections between a variety of primary producers and macroinvertebrates (Bulleri et al., 2013). Direct, but also indirect positive effects via facilitation and/or trophic cascades (Thomsen et al., 2010; Borer et al., 2005) can also affect species abundances and distributions. For instance, the intertidal macroalgae *Hormosira banksii* is the host for its obligate epiphyte, *Notheia anomala*. Although invertebrates directly associate to *H. banksii* alone, this epiphyte seems to diminish desiccation stress, which in turn, increases the abundance and richness of associated invertebrates (Thomsen et al., 2015). Likewise, in this study, limpets and snails found within the quadrats could have attracted the predatory crab *Pachygrapsus crassipes* (Barry and Ehret, 1993).

Our results highlight the key role played by relatively small-sized ecosystem engineers inhabiting variable environments. The differences found in the abundance and distribution of macroinvertebrates associated to our experimental macroalgae show the varying outcomes that can be driven by the presence of ecosystem engineers. Still, further experimental fieldwork is required to fully comprehend different feedbacks across the intertidal system. Nevertheless, we show that environmental conditions and the physical attributes of bioengineers contribute to modulating ecosystem engineering and the strength and nature of interspecific interactions (Wootton and Emmerson, 2005; Boström-Einarsson et al., 2014). In addition, we suggest that it is essential to evaluate the scale at which these interactions occur to properly determine the relative effectiveness and importance of organisms as ecosystem engineers.

5. General remarks and conclusions

Ecosystem engineers play central roles in coastal environmental including rocky shores. They have a remarkable impact on the structure and function of this ecosystem. However, due to human activities and the substantial threats by rapid changes in environmental conditions, there are uncertainties surrounding the factual outcomes following the deterioration of ecosystem engineers (Walker and Kendrick, 1998; Gedan et al., 2009). Repeatedly the importance of bioengineering outcomes are recognize after the engineer becomes deteriorated, reduced in density or missing. For example, the accelerated loss of seagrasses is affecting nutrient cycling, substrate stability and fish production around the globe (Waycott et al., 2009). Moreover, the decline of coral reefs intensifies the effects by waves impacts (Fernando et al., 2005), and the deterioration of macroalgal communities is causing significant biodiversity loss (Schermer et al., 2013).

Although these negative outcomes are stressed by the decline of ecosystem engineers providing protection and structural stability, we are now more prepared to identify in advance the relevance of their bioengineering outcomes. Due to their functional properties, ecosystem engineers exert a strong effect on ecosystems, exceeding what may be expected based on their relative abundance only (Hooper et al., 2005). Theoretically, bioengineering pathways and outcomes may be easily understood. Nonetheless, comprehending the underlying mechanism by which bioengineers influence the ecosystem, is more intricate.

A general description of ecosystem engineering outcomes aids revealing commonalities among the predominating groups of rocky shore ecosystem engineers. For instance, all intertidal reef-forming bivalves, seagrasses and macroalgae create structures that initiate dense, and sometimes, extensive aggregations. Such outstanding changes in the physical structure of the environment significantly affect the characteristics of physical space and the occurrence of the different forms of kinetic energy. Thus, for example, leading to variations in sedimentary process, hydrodynamic regimes and availability of habitats for other organisms (Gutiérrez et al., 2011). However, as shown through the set of experiments described here and as well- stated in Jones et al. (1994) “the devil is in the details”. Particular traits of species and local ecological conditions often make a real difference that should be assessed closely.

Intertidal canopy-forming macroalgae have a noticeable role as ecosystem engineers. Even though relatively small-sized, their presence have a significant effect on the attenuation of extremes of physical

factors such as bulk water flow, particle retention, heat and water loss driven by high temperatures and exposure to air for extended periods. The attenuation of extreme conditions caused by macroalgae bioengineering also has a significant effect on the abundance, distribution and composition of intertidal communities such as the microphytobenthos and macroinvertebrates.

Nevertheless, the effectiveness and relative importance that these small-sized organisms can have as ecosystem engineers will depend on their morphological characteristics, abundance in the intertidal, and the baseline conditions of the environment. Macroalgal aggregations exhibiting a greater spatial complexity tend to attenuate extreme physical conditions the most. Moreover, the relative importance of the engineering outcomes will also depend on the scale and relative size ratio between the bioengineer and the associated organisms.

The spatial and temporal heterogeneity added by intertidal macroalgae as ecosystem engineers enhanced particle retention and reduced bulk water flow underneath the canopies influencing the abundance of the microphytobenthic community in the rocky intertidal. Additionally, under harsh environmental conditions, macroalgae provided shelter against heat, water loss and irradiance, allowing macroinvertebrates to remain in the upper mid-intertidal for longer periods.

Similar to other bioengineers, the modifications of habitat conditions driven by these short-canopy forming macroalgae appeared to play a key role in the dynamics, community structure and biodiversity of the intertidal (Bouma et al., 2009; Bruno et al., 2003; Gutiérrez et al., 2011; Wright and Jones, 2004). Nonetheless, although macroalgal assemblage composition, their functional forms, and density modulated physical factors and influenced the abundance and distribution of directly and indirectly associated organisms, biological responses based on species specificity cannot be disregarded. Other factors such as chemical cues or deterrents that were not measured in these experiments and may also have an influence on the response by associated organisms.

Through this study, it has been shown that the environmental baseline conditions and physical attributes of bioengineers contribute in modulating ecosystem engineering pathways, strength and nature of interspecific interactions (Boström-Einarsson et al., 2014; Wootton and Emmerson, 2005). It also becomes clear that it is key to evaluate the scale at which these interactions occur to properly determine the relative effectiveness and importance of organisms as ecosystem engineers.

Still, further experimental fieldwork is needed to fully comprehend the array of pathways and feedbacks due to ecosystem engineering at different scales and across various levels of environmental stress. However, general conclusions can already be highlighted. Further efforts should continue assessing density-dependent and functional-form impacts on the physical environment, as slight differences between ameliorating primary producers can result in magnified changes in the abundance and community structure of directly and indirectly associated organisms.

Finally, due to the complexity of intertidal dynamics and the difficulty of obtaining *in situ* measurements of the interactions between short-canopy forming macroalgae and associated communities, isolating a single factor driving biological responses is challenging. However, the ability of macroalgae to enhance particle capture, reduce bulk water flow, irradiance, temperature and water loss underneath their canopies, play a significant role in promoting distinct temporal and spatial patchiness in the intertidal communities which could have significant effect on the overall productivity dynamics and ecosystem services essential to the intertidal and mankind.

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