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INTRINSIC AND EXTRINSIC FACTORS CONTROLLING BIOFOULING DYNAMICS ON FLOATING SUBSTRATA

FACTORES INTRÍNSECOS Y EXTRÍNSECOS QUE CONTROLAN LA DINÁMICA DEL BIOFOULING EN SUSTRATOS FLOTANTES

Tesis para optar al grado de Doctor en Sistemática y Biodiversidad

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ABSTRACT

The availability of artificial floating substrata in marine ecosystems has significantly increased in recent decades, facilitating the settlement of sessile species, both native and introduced, especially in areas where natural substratum is scarce. This gives plastic a progressively more relevant role in the creation of habitats for these fouling communities. The colonization of these substrata is influenced by intrinsic factors such as surface roughness, the presence of biofilms, and larval selection. On the other hand, extrinsic factors, such as benthic predation, have a significant effect on community structure. In Chapter 1, intrinsic factors were evaluated through larval selection, using two invasive bryozoan species, *Bugulina flabellata* and *Bugula neritina*, commonly found in fouling communities. Energy expenditure during the planktonic and benthic stages, swimming and exploration behaviors prior to settlement, as well as larval selectivity in laboratory conditions on different substrata —plastics, wood, and concrete—were quantified. It was observed that energy expenditure was higher in planktonic larvae than in settled larvae. Larvae of both species swam less and explored more when exposed to plastic surfaces, suggesting a preference for this substratum and lower energy expenditures associated with habitat searching. The larvae actively chose to settle on plastics rather than on wood or concrete substrata. The results suggest that *Bugula* larvae prefer to colonize plastic surfaces over other materials, and that the faster they adhere to artificial substrata, the lower their energy expenditure, which may contribute to greater fitness in these individuals. In Chapter 2, the structure and biomass of the fouling community were evaluated in response to benthic predation during summer and winter. Floating PVC plates were installed during both seasons in central Chile (36˚S) until the growing fouling community caused them to sink. The plates were then moved to the seabed, where they were exposed to benthic predation, while control plates were kept in a mesh cage to prevent predator access. It was found that in summer all plates refloated, while in winter only 60% did so, with differences observed in dominant species according to their buoyancy and resistance to predation. Therefore, the structure of the fouling community influences how predation facilitates the recovery of buoyancy, as predators cannot consume all epibionts. Although previous studies had shown how fouling organisms cause the sinking of floating debris, this is the first study to provide experimental evidence that predation can reverse this process and allow debris to resurface, making it available again as vectors for the dispersal of native and invasive species. These two chapters help us understand the importance of larval selection and how settlement timing could favor the growth of certain species within the fouling community, which could potentially lead to the sinking of these floating substrata. Additionally, they demonstrate how benthic predation could recover lost buoyancy by removing epibionts.

RESUMEN

La disponibilidad de sustratos flotantes artificiales en los ecosistemas marinos ha aumentado considerablemente en las últimas décadas, facilitando el asentamiento de especies sésiles, tanto nativas como introducidas, especialmente en áreas donde el sustrato natural es escaso. Esto otorga al plástico un papel cada vez más relevante en la creación de hábitats para estas comunidades de fouling. La colonización de estos sustratos está influenciada por factores intrínsecos, como la rugosidad del sustrato, la presencia de biopelículas y la selección larval. Por otro lado, factores extrínsecos, como la depredación bentónica, tienen un efecto significativo en las estructuras comunitarias. En el capítulo 1, se evaluaron los factores intrínsecos mediante la selección larval, utilizando dos especies invasoras de briozoos, *Bugula flabellata* y *Bugula neritina*, comúnmente encontradas en comunidades de fouling. Se cuantificó el gasto energético durante las etapas planctónica y bentónica, los comportamientos de natación y exploración previos al asentamiento, así como la selectividad larval en condiciones de laboratorio sobre diferentes sustratos: plásticos, madera y concreto. Se observó que el gasto energético fue mayor en las larvas en fase planctónica que en las larvas asentadas. Las larvas de ambas especies nadaban menos y exploraban más cuando se exponían a superficies de plástico, lo que sugiere una preferencia por este sustrato y menores gastos energéticos asociados con la búsqueda de hábitat. Las larvas eligieron activamente establecerse en plásticos en lugar de en sustratos de madera o concreto. Los resultados sugieren que las larvas de *Bugula* prefieren colonizar superficies plásticas en lugar de otros materiales, y que cuanto más rápidamente se adhieren a los sustratos artificiales, menor es su gasto energético, lo que puede contribuir a una mayor aptitud física en estos individuos. En el capítulo 2, se evaluó la estructura y biomasa de la comunidad de fouling en respuesta a la depredación bentónica durante el verano y el invierno. Se instalaron placas flotantes de PVC durante ambas estaciones en el centro de Chile (36˚S) hasta que la creciente comunidad de fouling provocó que se hundieran. Luego, las placas se trasladaron al fondo marino, donde quedaron expuestas a la depredación bentónica, mientras que las placas de control se mantuvieron en una jaula de malla para impedir el acceso de los depredadores. Se encontró que en verano todas las placas reflotaron, mientras que en invierno solo el 60% lo hizo, observándose diferencias en las especies dominantes según su flotabilidad y resistencia a la depredación. Por lo tanto, la estructura de la comunidad de fouling influye en cómo la depredación facilita la recuperación de la flotabilidad, ya que los depredadores no pueden consumir todos los epibiontes. Aunque estudios anteriores habían demostrado cómo los organismos incrustantes provocan el hundimiento de escombros flotantes, este es el primer estudio que proporciona evidencia experimental de que la depredación puede revertir este proceso y permitir que

los desechos resurjan, volviendo a estar disponibles como vectores para la dispersión de especies nativas e invasoras. Estos dos capítulos nos ayudan a comprender la importancia de la selección larval y cómo el tiempo de asentamiento podría favorecer el crecimiento de ciertas especies dentro de la comunidad de fouling, lo que potencialmente podría llevar al hundimiento de estos sustratos flotantes. Además, demuestran cómo la depredación bentónica podría recuperar la flotabilidad perdida al remover los epibiontes.

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GENERAL INTRODUCTION

Poor waste management has led to an increasing presence of floating plastic debris in the world's oceans, posing a significant environmental problem (Barnes et al., 2009; Andrady, 2011). The amount of plastic in the oceans has risen dramatically and continues to grow (Jambeck et al., 2015; Borrelle et al., 2020), reaching levels that profoundly impact marine ecosystems in various functions (Derraik, 2002; Ryan et al., 2009). A large proportion of this plastic debris floats and is highly resistant to degradation due to its physicochemical properties (Andrady, 2011; Eriksen et al., 2020). This buoyancy makes them prone to being colonized by numerous organisms that can disperse through ocean currents, in a phenomenon known as rafting (Thiel and Gutow, 2005). Rafting on floating objects is considered the most likely mechanism to explain the wide geographic range, discontinuous population distribution, and molecular patterns of organisms with direct development (Johannesson, 1988; Castilla and Guiñez, 2000; Thiel and Haye, 2006).

Regions presenting high concentrations of floating plastics include the central areas of the North Atlantic and the Pacific Ocean (Barnes et al., 2009). Plastics fragments disperse in the ocean (Barnes et al., 2009) until they sink or converge in subtropical gyres (van Sebille et al., 2020). Other plastic accumulation zones include enclosed bays, seas surrounded by coasts, and densely populated watersheds (Thiel and Gutow, 2005). Currently, plastics play a complementary role to natural rafting in geographical areas lacking the presence of natural substrata such as wood and algae (e.g., Thiel and Gutow, 2005; Goldstein et al., 2012).

An example of the effect of plastic is found in the North Pacific Gyre, where the increased availability of habitat due to floating plastics led to a rise in the density of eggs of the pelagic insect *Halobates sericeus* on these floating structures (Goldstein et al., 2012), demonstrating positive population effects of this new artificial habitats. The historical lack of available substrata might have been limiting the colonization of the open ocean by coastal species, which are now increasing their presence due to the rise in plastic debris (Haram et al., 2023).

Among the main factors influencing the colonization and species composition of communities on floating substrata are the intrinsic factors of the substratum (e.g., area, buoyancy, roughness, color) and extrinsic factors such as food availability in water masses (oligotrophic and eutrophic) (Rech et al., 2021) and predation (Leclerc et al., 2018; Rech et al., 2024). Among the intrinsic factors, substratum roughness is important because it promotes the formation of biofilms and larval settlement, which is essential in the early stages of community succession (Devakie and Ali, 2002). In general, plastic materials have a higher density of biofilms, mainly bacteria, compared to cement-based materials (Momba and Makala 2004). PET (polyethylene terephthalate) substrata also host higher densities of bacteria compared to wood (Muthukrishnan et al., 2019). These characteristics may facilitate the settlement of fouling in the early stages of community succession on plastic substrata.

The likelihood of finding a suitable floating substratum (habitat) will directly influence the dispersal of organisms and their pre-settlement behavior (Raimondi and Keough, 1990; Lagos et al., 2015). In particular, the ability of larvae to recognize and select the optimal substratum for settlement and growth (pre-settlement behavior) determines the success of individuals and their subsequent reproduction (Toonen and Pawlik, 2001; Cancino and Gallardo, 2004). Typical settlement behaviors include swimming, which involves movement through the water column, as well as exploration, which involves larvae adopting a stationary position or slow movement on the substratum, including characteristic behaviors of turning and crawling (Walters et al., 1999).

All these active behaviors during settlement involve an energetic cost for the larvae, as they expend energy to ascend and descend in the water column while assessing the viability of the available substrata for settlement (Pires and Woollacott, 1983). In lecithotrophic larvae (with energy reserves in their yolk sacs), the time required to find an optimal settlement substratum is inversely proportional to the amount of energy they will have available for initial growth (Woollacott et al., 1989). If too much energy is used during the habitat selection process, lecithotrophic larvae will have an energy deficit for their post-settlement stages, which could affect the reproductive success of individuals and, therefore, their fitness (probability of reproductive success and/or survival of colony organisms; Sober, 1984b). For example, in the bryozoan *Bugula stolonifera,* longer swimming times in larvae directly affected their growth compared to larvae that swam for less time; this growth deficiency could have effects on the fitness of individuals (Pires and Woollacott, 1983).

In addition to the larval selection, mediated by intrinsic factors, predation represents an external factor that affects the rafting communities on floating substrata. Dense fish assemblages can be associated with floating substrata, which has been proposed as a possible explanation for the low abundance of barnacles on abandoned fishing nets (Castro et al., 2002; Nelson, 2003; Goldstein et al., 2014). Rech et al. (2021) also suggested that fishes and other predators contributed to the low species richness on floating litter in the open ocean, underlining the importance of predation in driving rafting community composition.

Although there is a large abundance of floating plastics at the sea surface, many items have also sunk to the seafloor (Kaandorp et al., 2020, 2023). According to predictions, the amount of plastic waste entering the ocean is significantly greater than the amount of plastic observed floating on the sea surface (Cozar et al., 2014; Eriksen et al., 2014), which raises concerns about the fate of this litter. Sinking could partly explain this difference, as weight gain due to fouling growth causes many plastic objects to become negatively buoyant, causing them to sink (Ye and Andrady, 1991; Lobelle and Cunliffe, 2011; Ryan et al., 2020).

While fouling-induced sinking of floating litter may explain a portion of the "missing" plastics, it has also been hypothesized that litter sunk to the seafloor may resurface when benthic predators remove part of the fouling community. In benthic systems, the impact of predation on communities growing in natural habitats on the seafloor is greater than on those developing on artificial floating or suspended litter (Dumont et al., 2011a; Giachetti et al., 2019; Rech et al., 2024). These habitat-related differences are mediated by the group of predators to which the prey organisms are exposed (Connell, 2001; Dumont et al., 2011a; Giachetti et al., 2020). Predation drives the distribution, abundance, and species composition within communities (Paine 1966; Sih et al., 1985; Chase et al., 2009; Wesner et al., 2012). This can drive changes in community structure in a wide range of ways, such as by eliminating prey species with less effective predator avoidance strategies or selectively removing competitively superior species, thereby facilitating the expansion of other, competitively inferior colonizers (Karlson, 1978; Nydam and Stachowicz 2007; Oricchio et al., 2020). Thus, benthic predation is expected to cause important shifts in the composition of the rafting community, if these litter refloat after being exposed to predators on the seafloor. However, while it is well known that floating substrata can sink due to the accumulation of encrusting biomass (Fazey and Ryan 2016), it is unknown whether benthic predation can be effective enough to remove the necessary epibionts for the substratum to regain buoyancy.

This thesis explored the effects that determine the various stages of fouling communities, such as larval settlement and pre-settlement behavior (Chapter 1), as well as the impact of benthic predation on the recovery of buoyancy in substrata submerged by fouling (Chapter 2). Additionally, we investigated whether there was greater buoyancy recovery during the warmer summer months compared to the winter season. We also determined how the structure of the encrusting community affected its susceptibility to predation (Chapter 2). This study thereby contributes to a deeper understanding of the dynamics of fouling communities on floating substrata.

HYPOTHESES AND OBJECTIVES

General hypothesis:

Fouling communities on floating substrata are determined by the intrinsic characteristics of the substrata to which they adhere, but also by extrinsic factors such as the environment and predation.

Specific hypotheses:

- Larvae of *Bugula neritina* and *Bugulina flabellata* will show a higher preference, adhere more quickly, and, therefore, having lower energy expenditures on plastic-type floating substrata compared to wood and concrete substrata.
- Plastic litter sunk by fouling recovers its buoyancy when benthic predators manage to remove the epibionts adhered to the sunken rafts.
- Plastic litter sunk by fouling recovers buoyancy more quickly in summer than in winter due to higher consumption rates.

General objective:

To evaluate how the intrinsic and extrinsic characteristics of floating substrata determine the fouling communities that adhere to them.

Specific objectives:

- Estimate substratum preferences, settlement time, and metabolic expenditure in larvae of sessile benthic species, between plastic, wood, and concrete substratum.
- Evaluate whether benthic predation can recover the lost buoyancy of floating plastic litter due to fouling.
- Evaluate whether plastic litter sunk by fouling recovers buoyancy more quickly in summer than in winter.

CHAPTER 1: MARINE INVERTEBRATE LARVAE LOVE PLASTICS: HABITAT SELECTION AND SETTLEMENT ON ARTIFICIAL SUBSTRATA

CHAPTER 1. INTRODUCTION

With 67% of the human population currently living close to the coast, marine environments have been drastically modified by anthropogenic activities. An increasing demand for urban infrastructure such as ports, marinas and piers has resulted in the extensive modification of coastal areas over the last few decades (Hammond 1992). For example, in Europe the urbanization has been so intense that in some areas almost 50% of the shoreline has been modified by construction or overlaid by concrete or asphalt (Duarte, 2002).

Marinas, docks, ports and other hard artificial structures are often built in soft-bottom environments where hard surfaces are scarce, sparsely distributed or absent. Changes to the substratum composition caused by human activity can dramatically change habitat structure (Reise, 2005; Wetzel et al., 2014) and increase connectivity between sessile populations as they provide artificial habitats that sessile animals can attach to and act as marine corridors (Airoldi et al., 2015; Carlton et al., 2017).

Such major modifications on natural environments can benefit invasive species (Dafforn et al., 2009; Lagos et al., 2017a); whilst local species are jeopardised by reduced fitness in the changed environment, non-indigenous species (NIS) are favourably selected and can often efficiently colonise modified environments (MacIsaac and Johansson, 2017). In fact, there is a strong association between artificial structures and NIS, which can lead to drastic changes in the diversity and composition of local communities (Connell, 2000; Glasby et al., 2007; Ruiz et al., 2009). In some cases, the richness of invasive species attached to artificial surfaces can be double that of local species on natural hard substrata. In marine urbanized areas of Australia, for example, NIS can be the dominant group, making up 40% of species, while 28% are cryptic species and only the remaining 32% are native organisms (Glasby, 1999; Piola and Johnston, 2008).

In addition to the increase of artificial structures in marine habitats, anthropogenic marine debris is also on the rise and has been recognized as one of the biggest causes of pollution globally. Some of the most common examples of marine debris are plastics and wood (Duhec et al., 2015; Lebreton et al., 2018; Zhou et al., 2011). This type of drifting garbage is an important factor in the mortality of marine animals, as it can be confused with food or cause entanglement, particularly if the items are large (Bugoni et al., 2001; Miranda and de Carvalho-Souza, 2016; Watts et al., 2015). However, these hard floating substrata are also colonized by diverse species, allowing the debris to act as a vector for the dispersion of animals that live attached to them (Carlton et al., 2017). Microorganisms, hydrozoans and bryozoans are commonly found living on marine debris, which can be transported long distances across oceans through the actions of currents and the wind (Aliani and Molcard, 2003). In the areas where they are introduced, these invasive species can reproduce at a faster rate than native species. The invasive species also compete with native species for food and space. As the invasive species are often more successful than endemic species, they can ultimately become a plague (Aliani and Molcard, 2003; Kiessling et al., 2015; Li et al., 2016).

While the association between invasive species and artificial structures is widely studied, settlement and pre-settlement behaviors, both crucial steps involved in the colonization of artificial and natural structures and paramount in marine invertebrate life cycles, have not been extensively studied to date. Factors such as dispersal, habitat selection, and larvae recruitment can have profound implications upon both populations and communities (Tolimieri, 1995). For sessile benthic species, both their pre-settlement behavior and their ability to choose the habitat and substratum on which to settle are especially important, as the larval stage is the only mobile stage of their life (Burgess et al., 2012). Therefore, the ability to categorize and select where to settle is crucial for success in post-settlement and adult life (Cancino and Gallardo, 2004, Toonen and Pawlik, 2001). So far, one study has suggested that the larvae of invasive species are more likely to settle on plastic than on other anthropogenic materials (Li et al., 2016); however, the ability of the larvae to choose between different types of anthropogenically derived materials remains unknown.

For bryozoan larvae and other lecithotrophic larvae the swimming/exploring pre-settlement behaviours are the first critical stage of the habitat selection process. Whether the larvae settle or not can limit the dispersal of the population and determine the probability of finding a suitable habitat (Lagos et al., 2015, Marshall and Keough, 2003, Raimondi and Keough, 1990). If larvae do not find a suitable place to settle, they will stop exploring and continue to swim until a suitable substratum is found. As many lecithotrophic larvae are negatively-buoyant, swimming and delaying settlement may represent a higher metabolic cost than exploring the bottom substratum (Pires and Woollacott, 1983).

Therefore, the longer the time spent swimming in search for a suitable substratum, the lower the energy available for initial growth post-settlement, compromising the fitness of the future colony. In this research, we used the invasive bryozoans *Bugula neritina* (Linnaeus, 1758) and *Bugula flabellata* (Gray, 1848) as the model species, as they are commonly associated with submerged hard structures around the globe (www.obis.org). Both species release fully competent larvae, which make them good model species for larvae behavior experiments (Doyle, 1974; Keough and Chernoff, 1987; Walters, 1992). The aim of this research is to study energy expenditures before and after settlement in Bugula neritina and Bugula flabellata, the pre-settlement and settlement behaviors, and determine the selection and preference of larvae for different materials commonly found in coastal habitats. We hypothesise that Bugula larvae will have a higher preference for plastic substrata rather than wood or concrete. We also hypothesize that as the preference for plastic is higher, the larvae will spend less time swimming or in the search for alternative substratum and therefore will achieve higher fitness when plastic surfaces are available.

MATERIALS AND METHODS

Study area and experimental design

Mature colonies of *Bugula flabellata* and *Bugula neritina* were collected by scuba diving from the port of Coliumo, Chile (36°33'09.2"S 72°57'24.3"W) in February 2018 (Austral summer). Colonies were then transported to the Comparative Animal Physiology Laboratory at the University of Concepcion, with seawater from the site of collection with gentle and constant aeration. The colonies were kept in darkness for 2 days in aquariums, to avoid larval release (Burgess et al., 2009; Lagos et al., 2015, 2016) with aerated seawater in a temperature controlled room at 14 ± 0.5 ºC.

Four experiments were carried out: 1) a swimming and exploring behavior study, 2) a study on larval settlement, 3) a study on substratum selection and, 4) measurements of metabolic rate of larvae in planktonic (swimming + exploring) and benthonic (early settled) stages in both species. For experiments 1, 2 and 3, six materials commonly found as debris or used in the urbanization of coastal areas were selected and provided as a settlement substratum for the larvae. These materials are also commonly used as settlement surfaces by sessile species. The materials were: concrete, wood (*Eucalyptus globulus*), Polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polycarbonate (PC) (Duhec et al., 2015; Geyer et al., 2017; Lebreton et al., 2018). Cuboid boxes of 5 × 5 × 2 cm (L

× W × H) were built with acetate sheets, each with a volume of 50 ml. The bottom internal surface of each acetate box was lined with 3 mm of one of the above mentioned materials. We used transparent Agorex glue to attach the materials to the acetate. Once we had built the experimental surfaces, they were left to soak in fresh water for one week, with the water changed every two days, to ensure the release of any potential toxic chemical compounds that could affect the experiments. Each experimental surface was then immersed in seawater with constant air bubbling for further 7 days to allow biofilm growth (Wieczorek & Todd 1996, Ruiz et al. 2009). To run the experiments, freshly hatched larvae were obtained by spawning 30 colonies by exposing them to bright light for 30 min. All experiments and biofilm generation were conducted at 14 ± 0.5 ºC.

Pre-settlement behavior

We studied the pre-settlement behavior of *B. flabellata* and *B. neritina larvae* by measuring the swimming and exploring time. Swimming behavior was defined as erratic and rapid movement through the water column, while the exploratory behavior was defined as when the larvae adopted a stationary position or a slow movement on the substratum itself, which includes a characteristic turn and drag behavior (Walters et al., 1999). We randomly selected 10 larvae from the pool and pipetted them into one of the six experimental substrata (10 replicates per treatment). Individual larvae were observed under a microscope Kruss Optronic (30x) for 5 minutes, to measure amount of time each of the ten larvae spent exploring and swimming (Burgess et al., 2009; Lagos et al., 2015, 2016).

Settlement and substratum selection

To measure the proportion of larvae settled on the six experimental surfaces provided, 10 larvae were pipetted into each of the ten experimental replicates for each substratum containing seawater (10 replicates per treatment). To measure if the larvae were settled, each experimental surface was observed under the microscope while gently rinsing the substratum/larvae with seawater. The larvae were considered settled only if they remained attached to the substratum after rinsing (Lagos et al., 2015, 2016). This procedure was done 5, 10, 15, 30, 45- and 60-minutes post exposure to the substratum. Results were presented as percentage of larvae settled.

To measure the preference of larvae for a specific substratum, the larvae were given the chance to choose where to settle. Each box used in this experiment was divided into six equal parts, each part was randomly covered by one of six different substrata. Ten larvae were pipetted into each experimental box (10 replicates per treatment) and after 60 min the number of settled larvae in each substratum was counted as described above and presented as the percentage of larvae settled.

Energy expenditure

Oxygen consumption during the planktonic (swimming + exploring) and benthonic (early settled) larval stages, was measured as proxy for the energy expended in such processes. To measure the oxygen consumption of early-settled larvae, 10 larvae were settled on acetate layers of 0.5 × 2 cm and introduced into 2 ml vial (n = 10 per species). For the planktonic larvae, 10 larvae were pipetted into the vials, which had been rinsed with HCl (1%) to prevent larval settlement on the glass surfaces (Cancino & Gallardo 2004). Vials contained filtered and sterilized seawater. Dissolved oxygen levels were measured in each vial using fiber optic sensors connected to an oxygen meter (Firesting, Pyro Sciences, Aachen Germany). The sensors were calibrated using seawater saturated with air (100% saturation) and seawater containing 2% Sodium sulfite (0% saturation). The oxygen measurements were made at the beginning and end of a 60 min incubation period, and the oxygen consumption was calculated per larva, accounting for incubation volume and the oxygen dissolution coefficient for that temperature and salinity. Metabolic rates were further converted to energy equivalents in joules (J) to obtain the rate of energy expenditure per minute ($J\ m^{-1}$) (Urbina et al., 2010), based on the conversion factor proposed by Gnaiger (1983) where 1 mg O_2 equals to 14.06 J.

The total amount of energy expended by a larva during the 60 minutes of exposure to each material used in the settlement trails was calculated as follows:

1) TEE = (PE X EEp) + ((TTe-Pt) x EEb))

Where TEE is the total energy expenditure (J), EEP is the energy expenditure rate for planktonic larvae stage (J min^{-1}), TTe is the total time of the experiment trial (min), EEb is the energy expenditure rate during benthonic larvae stage (J min^{-1}) and Pt is the planktonic life time (min), as was calculated in the 50 for each replicate (min) (see results section). When 50 was not achieved during the duration of the experiments, a conservative approach was taken, and 60 minutes was used instead.

Statistical analyses

Percentages were arcsine transformed before running statistical analysis (Sokal & Rohlf, 1994). Data normality was evaluated with Shapiro-Wilk tests and homogeneity of the variances with Levene's tests. The swimming-exploration behaviors and the settlement preferences were evaluated using a two-way ANOVA with exploration time (%) and proportion of larvae settled (%) as dependent variables and the type of substratum and species as independent variables. For the evaluation of substratum selection, a Kruskal–Wallis test was used, with the proportion of settled larvae as the dependent variable and the material as the independent variable, followed by a pairwise comparison Mann–Whitney U comparison test. All the analyses were run in the R software (R Core Team 2017). Additionally, settlement % values were fitted using the Parameter Logistic Equation (Sigmoidal) fit approach, to find the time at which 50% of larvae settled at each material (LS50). The energy expenditure rate of larvae during planktonic and benthonic stages larvae was evaluated with a one-way ANOVA with Metabolic rate as the dependent variable and the type of species the independent variable. Then the calculated energy expenditure of larvae exposed to each material was analyzed with a two-way ANOVA. When any ANOVAs showed a significant effect, a posteriori Tukey HSD test was run.

RESULTS

Pre-settlement behavior

The analysis indicates a significant difference between the exploration time among materials ($F_{5, 108} = 22.98$, $p < 0.001$) and between the two species ($F_{1, 108} = 10.03$, $p < 0.002$). There is no significant effect in the interaction “species - materials” ($F_{5, 108} = 0.81$, $p = 0.54$). Larvae of both species explored significantly less on concrete (~ 41%) than all the other materials (with a range of 60-70%) (Tukey < 0.05 for all cases) (Fig.1). *B. neritina* larvae, however, explore more on PP and PVC surfaces than wood and concrete (Tukey < 0.05 for both cases).

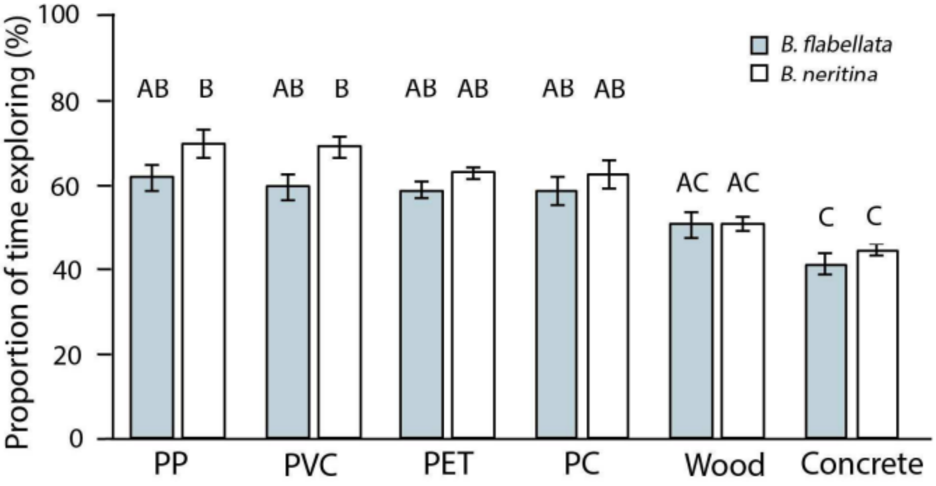

Fig. 1. Swimming/exploring behavior experiment: The bars represent the proportion of time (means ± SE) that Bugula flabellata and Bugula neritina larvae spent exploring different substrata (PP= Polypropylene, PVC= Polyvinyl chloride, PET =

Polyethylene Terephthalate, PC= Polycarbonate, Wood and Concrete). Means not sharing the same letter are significantly different.

Settlement and substratum selection

No differences were found on settlement between both species ($F_{1,\ 108} = 0.75$, $p > 0.38$), nor on the interaction between species and substratum ($F_{5,\ 108} = 0.23$, $p > 0.94$). However, regardless of the species, settlement was affected by the type of substratum ($F_{5,\ 108} = 30.05$, $p < 0.001$). Higher settlement was found on plastics surfaces, with no significant difference among the different types of them (Tukey < 0.05 for all cases). Larvae settled twice as much on plastic surfaces than on concrete and around 15% more than on wood surfaces (Tukey < 0.05 for both cases) (Fig. 2). The concrete was the substratum with lowest settlement, not reaching even 50% of settlement, and significantly lower than wood (Tukey < 0.05 for both species).

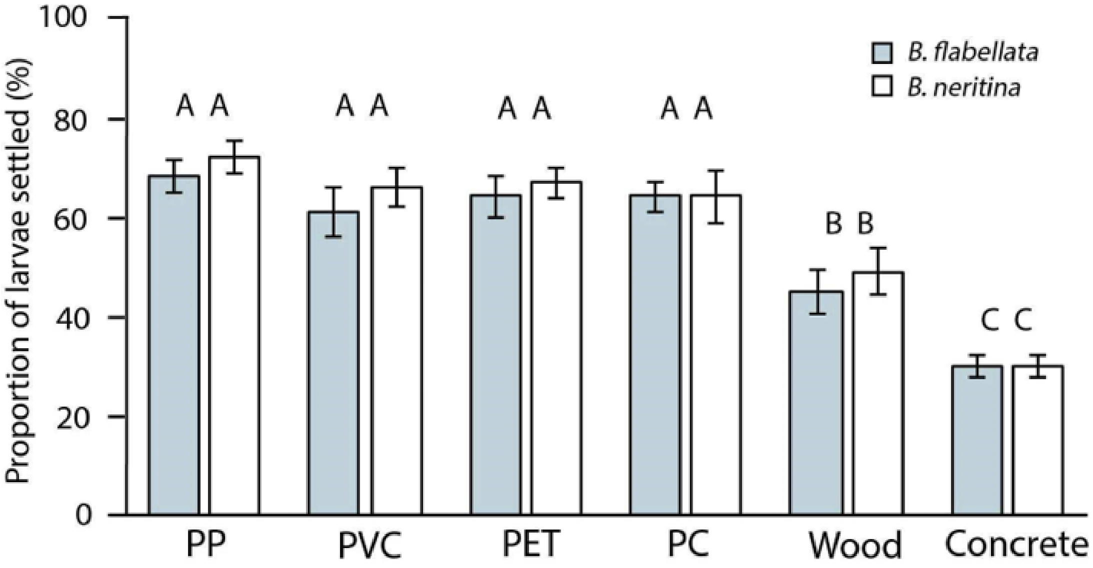

Fig. 2. Settlement experiment: Proportion (means ± SE) of B. flabellate and B. neritina 425 settled larvae on different substrata (PP= Polypropylene, PVC= Polyvinyl chloride, PET = Polyethylene Terephthalate, PC= Polycarbonate, Wood and Concrete). Means not sharing the same letter are significantly different.

Settlement on time followed a sigmoidal fashion in the plastic materials (PP, PVC, PET, PC, $p < 0.01$ for all cases). Larvae reached 50% of settlement (LS50) during the first 7 minutes when any of the plastics substrata was available (Fig. 3 A-D), contrary to wood and concrete surfaces, where it did not reach 50% of settlement after 60 min of the experimental time (Fig. 3 E,F). Therefore, no sigmoidal model was found ($p > 0.05$ for both wood and concrete). There was a significant effect of materials on the LS50 ($F_{3, 72} = 4.20$, $p < 0.01$). The lowest value was found in PVC, reaching 50% of settlement at 5.4 minutes; a value not different to that found in PP and PC (Tukey > 0.05 for all cases) (Fig.3 G). In PET, however, 50% settlement took a few extra minutes, 6.9 min, significantly longer that in PVC (Tukey = 0.01). There was found to be no significant effect for species ($F_{1, 72} = 3.27$, $p = 0.07$) and for the interaction species-materials ($F_{3, 72} = 0.55$, $p = 0.64$). When the larvae were given the choice of where to settle, both species showed preferences for different settlement substrata (Fig. 4A-B) ($p < 0.01$ for both species). *B. flabellate* larvae preferred PP over all other materials ($p < 0.05$ for all cases), but a similar preference to PC ($p = 0.16$). PVC and PET did not differ significantly from concrete and wood ($p > 0.05$ for all cases). No statistical significance was found on larval selection between wood and concrete ($p = 0.83$). PET, wood and concrete were the substrata least selected by *B. flabellate* larvae, with values of ~ 7-11% of settlement (Fig. 4A). *B. neritina* preferred PP over other all the other materials ($p < 0.05$ for all of them). Wood was the least selected material with values of 7.0 ± 2.6 % (Fig. 4B).

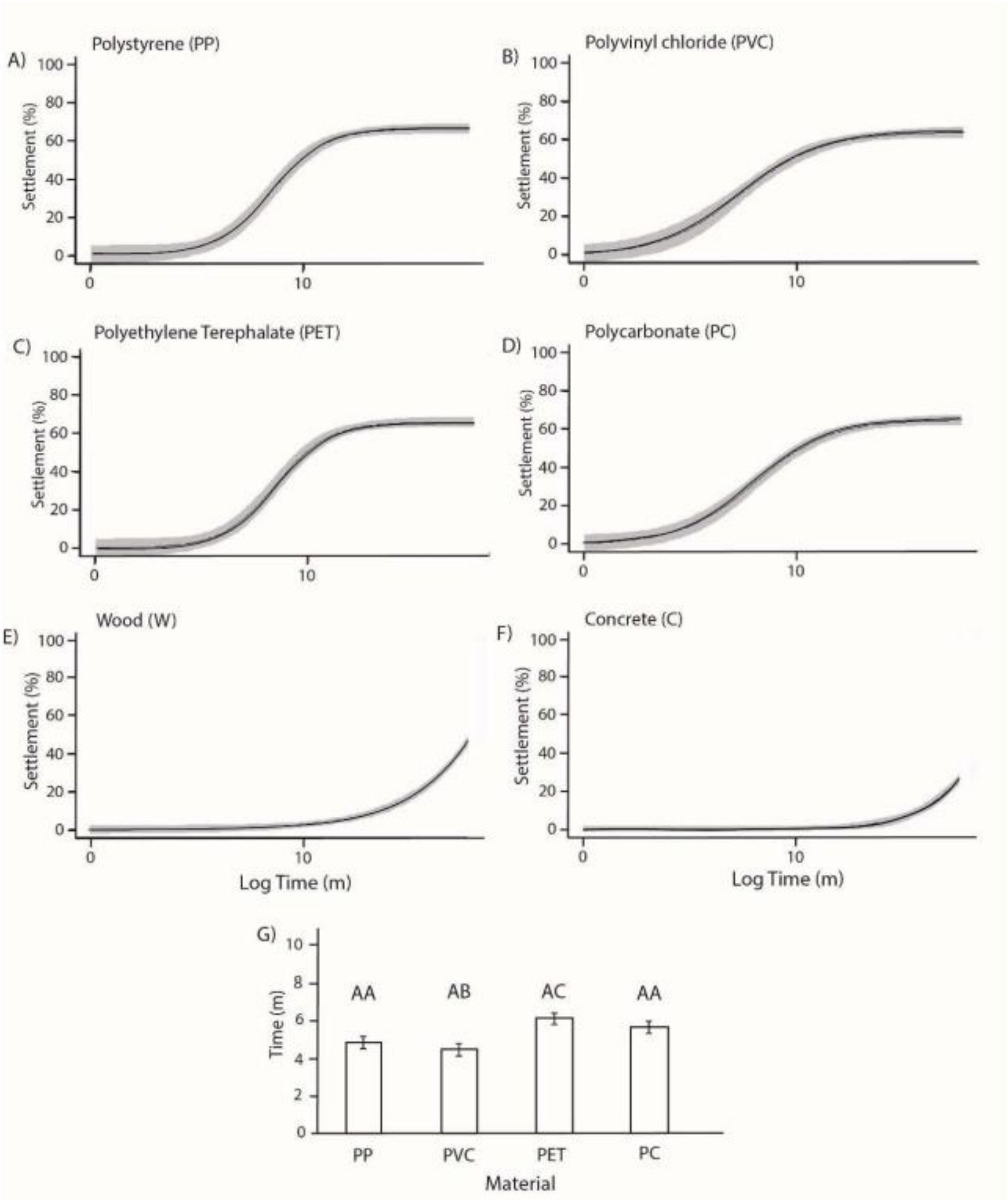

Fig. 3. Proportion of settled larvae (%) during 1 h (Log time) of exposure to different substrata A) Polypropylene, B) Polyvinyl chloride, C) Polyethylene Terephthalate, D) Polycarbonate, E) Wood, F) Concrete, G) Time required to achieve 50% settlement (means 435 ±SE), determined by adjusting a sigmoidal model (LS50). Due to settlements lower than 50% over the 1h experiment, LS50 values for Wood

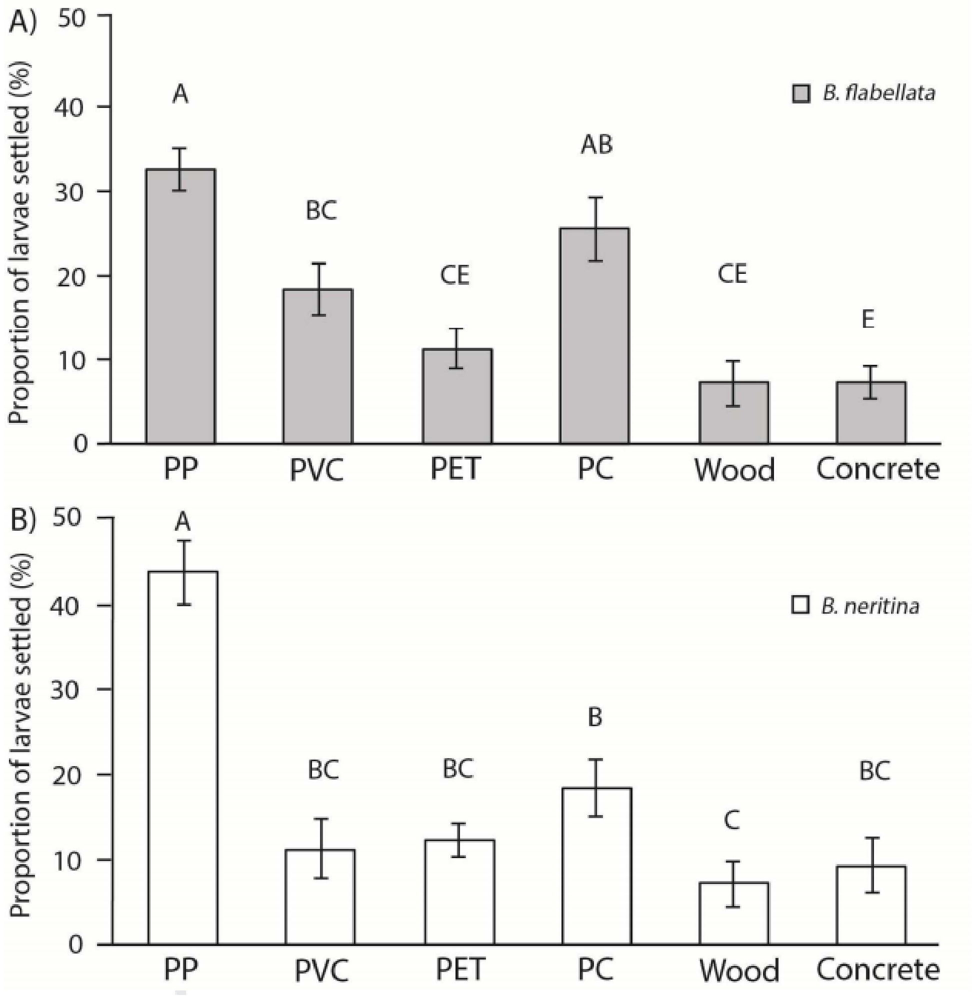

Fig. 4. Substrata selection experiment: Proportion of settled larvae of A) B. flabellate and B) *B. neritina* in different substrata (PP= Polypropylene, PVC= Polyvinyl chloride, PET = Polyethylene Terephthalate, PC= Polycarbonate, Wood and Concrete). Bars represent

Energy expenditures

Energy expenditures were significantly different depending on larval stage (planktonic or benthic ($F_{1, <0.001} = 86.01$, $p < 0.01$). During their planktonic stage, larvae spent 7.3 mJ min^{-1} , while only 3.3 mJ min after settlement. No differences between species were found ($F_{1,} <0.001 = 0.004$, $p = 0.376$), nor on the interaction species-larval stage ($F_{1, <0.001} = 0.313$, $p = 0.579$).

The calculated total amount of energy expenditure during settlement experiments was significant different between materials ($F_{5, <0.659} = 0.131$, $p < 0.01$). All the plastics were in the same group, presenting lower energy expenditures (Tukey > 0.05 for all combinations), followed by concrete and then wood (Tukey < 0.05 for all combinations). The energy expenditure during settlement was around 50 to 57% higher in concrete and wood compared to the energy expenditure associated with settlement on plastics (Fig. 5). When plastic substrata were available for settlement, the energy expended swimming and exploring (planktonic stage) was around 18% of that when concrete and wood was offered for settlement (Fig.5).

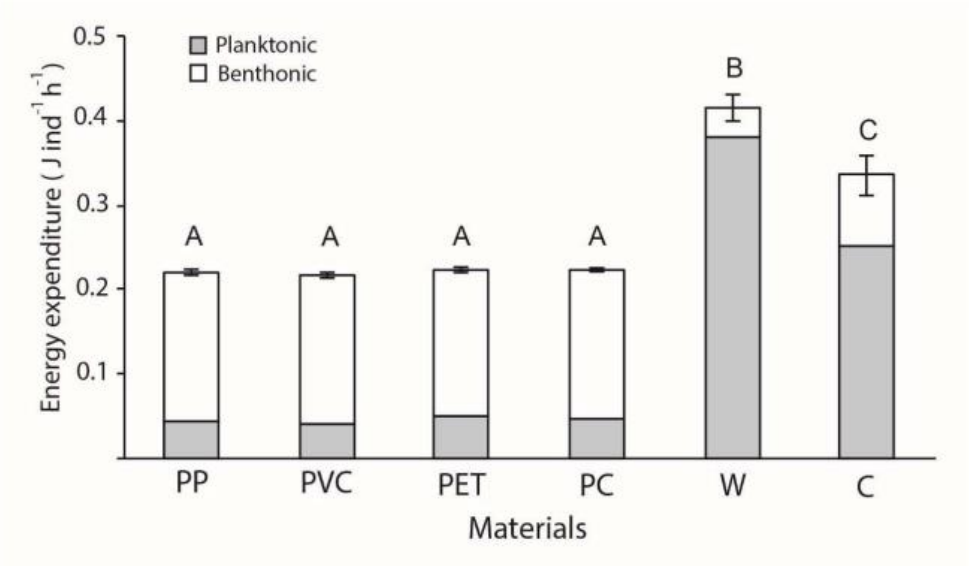

Fig. 5. Larval energy expenditures (means ±SE) during 60 minutes of exposure to different substrata. Grey bars represent energy expenditures during planktonic stage (swimming + exploring) and white bars represent the benthonic stage (early settled larvae). Bars represent means (±SE). Means not sharing the same letter are significantly different.

DISCUSSION

Our results indicate that although *B*. *flabellata* and *B. neritina* larvae settle on all the substrata offered in this study, larvae from both species showed greater preference for plastics over other anthropogenic substrata such as wood and concrete. The preference of this new anthropogenic pollutant may have further implications for the dispersal of sessile colonies, possibly expanding their distribution due to attachment to floating plastic debris. *Bugula* spp. larvae show an enhanced exploratory behaviour and settled both quicker and in higher proportions on plastic surfaces when compared to wood or concrete surfaces. This behaviour and preference led to lower energy expenditures when settling on plastics in comparison to settlement on concrete and wood.

Traditionally, only the final outcome of settlement (number of settled organisms), which is likely biased if the pre-settlement and settlement choices of invertebrate larvae are not considered in the studies. *Bugula* larvae are lecithotrophic and therefore energetically constrained to endogenous reserves and have a limited time-window to find a suitable substrata on which to settle (Jaeckle and Manahan, 1989). We found that *Bugula* larvae spent considerably more energy in their planktonic stage (swimming and exploring) than when settled. Therefore, if the larvae cannot find a suitable substrata, they will expend more of their energy reserves, leaving less energy for the development of the colony when settled. The longer the larvae spend swimming, the lower the energy available for initial growth, which could therefore compromise the fitness of the colony (Cancino and Gallardo, 2004). For example, a 48h delay to settlement reduces the development of the zooid in adult colonies by 40% in *B. flabellate,* while a 24h delay on settlement in *B. neritina* leads to the production of larvae 35% smaller than those which have only a 1h delay (Cancino and Hughes, 1987; Wendt, 1998; Cancino and Gallardo, 2004). Our results suggest that the fitness of colonies growing on plastic, where the swimming time is short, is likely to be higher than those on concrete and wood. Furthermore, given the current abundance and ubiquity of plastics in our oceans (Barnes et al., 2009; Haward, 2018), our results suggest invasive species could benefit from this source of pollution and further strengthen their ability to colonise new environments. Furthermore, native species which are naturally isolated could become a potential exotic species due to the availability of this new plastic vector for dispersal. To test this hypothesis, further research measuring colonial growth and reproductive success is recommended.

For *Bugula* and other lecithotrophic larvae the swimming/exploring pre-settlement behaviours are critical early stages in the settlement process. The point at which the larvae decide to settle or not can limit the dispersal of the population and determines the probability of finding a suitable habitat (Lagos et al., 2015; Marshall and Keough, 2003; Raimondi and Keough, 1990). Larvae of both *Bugula* species invested between 60 to70% of the time exploring on plastic and wood, which is 10% more than in concrete. In both species, concrete was the substrata the least explored (Fig. 1). Low exploration times such as in concrete (45%) means that the remaining 55% of the time was used swimming to find a more suitable environment. For *Bugula* larvae it has been described that exploring times around 40-50 % signals habitat avoidance, while values around 70-80% of exploring time (therefore only 20-30 % of the time swimming) signals favourable conditions and higher affinity to that substrata (Lagos et al., 2016, 2017b). Under favourable conditions, settlement can be as quick as few seconds in *Bugula*, but under non-favourable conditions settlement can be delayed for hours (Lagos et al., 2015).

The results show that the highest proportion of the larvae - around 70% - settled on plastic surfaces. This was followed by wood which hosted around 50% of settled larvae, and finally concrete, which had the lowest percentage of settlement (around 30 %). Furthermore, settlement on plastic substrata was extremely quick, with 50% of the larvae settled after 5 minutes. On wood and concrete, however, settlement did not even reach 50% after 1 hour. Similar results has been described in the field (Li et al., 2016), where settlement on plastic exceeded settlement on other substrata. In these experiments, however, the age of the larvae was unknown, but it can be assumed that larvae were a pool of young and old larvae, with varying abilities to perform habitat selection. Elevated exploration and fast settlement of larvae has advantages but also trade-offs, such as limiting larval dispersion (Krug, 2001;

Marshall and Keough, 2003). When mature colonies grow on fixed substratum such as concrete, the dispersal is limited to the reach of the larvae. However, when they grow on plastic debris, the natural barriers and isolating factors can be overcome as the drifting of this debris across oceans enables dispersal (Bingham and Young, 1991). This is not the case for all plastic waste in the ocean, but certainly for larger items such as ropes, buoys, and bottles which are some of the most found items of marine debris.

When larvae of both species had the chance to select the substratum, Polypropylene and Polycarbonate surfaces were preferred over the other materials, with Polyethylene Terephthalate, concrete and wood being selected the least. substratum selection is a complex process as larvae are capable of sensing and interpreting environmental cues and integrating information from both pelagic and benthonic conditions in a hierarchal way before selecting a substratum (Grosberg, 1981; Lagos et al., 2016). However, the relationship between pre-settlement, settlement and selection behaviours are not always so straightforward. For *B. neritina*, for example, the high selectivity for microhabitats rich in oxygen is not mirrored with high settlement, and only when the biofilm is manipulated with exposure to high oxygen conditions does the selectivity and settlement of the larvae increase (Lagos et al., 2015, 2016). The reason behind this strong association between *Bugula* larvae and plastic surfaces is not clear, though we could hypothesise that the different biofilms generated on different materials and other chemical functionalities may play a role (Lagos et al., 2016; Morohoshi et al., 2018; Shin et al., 2013). Further research exploring this question is needed.

Invasive species are found in higher proportions on artificial structures, whereas native species are found more commonly on natural or wooden surfaces. For example, native algae such as *Sargassum sp*. and *Coralina sp*. are prevalent on natural reefs but not on artificial structures (Glasby et al., 2007). For other invasive bryozoan species, it has been suggested that their prevalence on artificial structures and settlement on plastic panels is explained by their ecology as they are early successional species (Vail and Tranter, 1981). Such characteristics are associated with "r-selected" or "weed" phenotypes: they show faster growth, demonstrate early onset of reproduction and have higher metabolic rates, allowing them to outgrow competitors in the early successional stages of the forming community (Lagos et al., 2017b; Pettersen et al., 2016). However, in this research we provide evidence that the preference for plastics surfaces and the avoidance of surfaces such as wood and concrete begin even earlier, at pre-settlement stages.

The abundance of drifting marine debris has increased dramatically around the world and plastics of diverse sizes, shapes and polymer types (among the many other pollutants) are abundant and dispersed by the wind and oceanic currents. Synthetic polymers are extremely common in the oceans, with materials such as Polypropylene commonly used to make ropes and floating structures for aquacultures and other artificial structures. The combination of the strong preference of invertebrate larvae for plastic substrata, the higher fitness achieved on plastics, and the abundance and durability of plastic materials may increase the probability of dispersion of exotic species, and also the likelihood for native species to extend their distribution beyond its natural limits (Aliani and Molcard, 2003; Kiessling et al., 2015; Rech et al., 2016). The implications of this new anthropogenic vector for marine communities are likely to be more prevalent in the coming years.

CONCLUSION

Our results indicate that, although *B. flabellata* and *B. neritina* larvae settle on various substrata, they show a clear preference for plastics compared to materials like wood and concrete. The behavior of exploration and selection is crucial in this context, as larvae that explore non-ideal substrata , such as concrete, tend to spend more time swimming in search of better options, while those that find plastic as a substratum settle quickly, reducing their energy expenditure. This preference is particularly relevant because *Bugula* larvae are lecithotrophic, meaning they have a limited time to find a suitable substratum before depleting their energy reserves. Our findings show that this process of exploration and selection consumes considerably more energy compared to the post-settlement stage. If the larvae do not find a suitable substratum quickly, they consume more energy, leaving fewer resources for colony development, thereby compromising their fitness. For example, a delay in settlement of just a few hours can lead to a significant reduction in zooid development in the adult colony. Additionally, our results suggest that the fitness of colonies growing on plastic, where swimming time is short, is likely higher than on concrete and wood. The rapid selection and settlement on plastics, combined with the high availability of these materials in the marine environment, could favor species that exhibit this preference, increasing their success in colonizing new habitats.

CHAPTER 2: HOW PLASTIC LITTER SUNK BY BIOFOULING RECOVERS BUOYANCY - THE ROLE OF BENTHIC PREDATION

CHAPTER 2. INTRODUCTION

Poor litter management has led to an increasing presence of floating plastic litter in the world's oceans, which is a major environmental issue (Barnes et al., 2009; Andrady, 2011). The quantities of plastics have increased and continue to increase (Jambeck et al., 2015; Borrelle et al., 2020), reaching levels that impact marine ecosystems in many ways (Derraik, 2002; Ryan et al., 2009). A high proportion of the plastic litter in the oceans is positively buoyant, and therefore becomes floating litter that is resistant to degradation due to their physical-chemical polymeric characteristics (Andrady, 2011; Eriksen et al., 2014). Its buoyancy makes plastic litter suitable for colonization by numerous organisms that can be dispersed via ocean currents, a phenomenon called rafting (Thiel and Gutow, 2005a). Rafting dispersal via floating objects is the most likely mechanism explaining the ample geographic ranges, disjunct population distribution, and phylogeography of organisms that have direct development (Johannesson, 1988; Castilla and Guiñez, 2000; Thiel and Haye, 2006). Floating plastic litter also has been reported as a dispersal vector for a wide range of different species (e.g. Kiessling et al., 2015; Carlton et al., 2017).

Although there is a high abundance of floating plastics, many items have also sunk to the seafloor (Kaandorp et al., 2020, 2023; Kvale et al., 2020). According to predictions, the amount of plastic waste entering the ocean is significantly greater than the amount of plastic observed floating at the sea surface (Cozar et al., 2014; Eriksen et al., 2014), raising concerns about the fate of this marine litter. Sinking could partly explain this difference, as the increasing weight due to fouling growth makes many plastic objects negatively buoyant, causing them to sink (Ye and Andrady, 1991; Lobelle and Cunliffe, 2011; Ryan et al., 2020).

Sinking is determined by several factors, which could be intrinsic and extrinsic. Among the intrinsic factors, the inherent buoyancy of the item is crucial, with size and shape being critical, as they affect the surface area-to-volume ratio, which can cause smaller items with coastal origin to sink before dispersing into the ocean (Fazey and Ryan, 2016). Other intrinsic factors such as material type, surface roughness, and color can influence biofilm formation and larval settlement, factors that are particularly important during the early stages of succession (Devakie and Ali, 2002; 2011; Pinochet et al., 2020). On the other hand, extrinsic factors also come into play, encompassing interactions such as competition, predator-prey dynamics, and the environment, seasonally varying temperatures affecting both colonization and growth of the fouling communities, resulting in variable biomass and community composition (Qiu et al., 2003; Satheesh and Wesley, 2011). In addition, for ectotherms, predation rates increase with temperature (Ashton et al. 2022), and thus there might also be seasonal variation in consumer pressure on the fouling communities.

While fouling-induced sinking of floating litter may explain a portion of the "missing" plastics, it has also been hypothesized that objects that have sunken to the seafloor may resurface when benthic predators remove part of the fouling community. Predation drives the distribution, abundance, and species composition within communities (Chase et al., 2009; Wesner et al., 2012). This can drive changes in community structure in a wide range of ways, such as by eliminating prey with less effective predator avoidance strategies or selectively removing competitively superior species, thereby facilitating the expansion of other, competitively inferior colonizers (Karlson, 1978; Nydam and Stachowicz 2007; Oricchio et al., 2020).

In benthic systems, the impact of predation on communities growing in natural habitats on the seafloor is greater than on those developing on floating or suspended artificial litter (Dumont et al., 2011a; Giachetti et al., 2019). These habitat-related differences are mediated by the predator group to which the prey organisms are exposed (Connell, 2001; Dumont et al., 2011b; Giachetti et al., 2020). Within the context of rafting, dense assemblages of fishes can be associated with floating litter, which has been proposed as a potential explanation for the low abundance of barnacles on abandoned fishing nets (Castro et al., 2002; Nelson, 2003; Goldstein et al., 2014).

Although it is well established that floating litter can sink due to the accumulation of fouling biomass (e.g. Fazey and Ryan, 2016), it is not known whether benthic predation can be effective in removing sufficient epibionts from the fouling community causing the substratum to regain buoyancy. Therefore, we conducted a field experiment in central Chile, being the first to test the following hypotheses: (i) the buoyancy of plastic litter will be recovered when benthic predators remove the epibionts adhered to the sunken rafts, and due to higher consumption rates, (ii) once sunk, buoyancy will be recovered faster in summer than in the winter season.

MATERIALS AND METHODS

Study area and experimental design.

To test the effects of benthic predation on the buoyancy recovery of the sunken substrata, a total of 24 foamed PVC floating plates (10 cm x 10 cm x 0.8 cm, with a specific density of approximately 0.43 g/cm^3) were placed in Coliumo Bay, in the Biobío region of Chile (36°32'28.1"S, 72°56'04.3"W). The area is characterized by a sandy bottom, with a nearby artificial reef area. Each system held a total of twelve plates: six arranged vertically with another six placed below them on a floating platform, allowing fouling organisms to attach and grow. One system was placed on each buoy, using a total of two buoys (see Fig. S1). Since at this site there are no or only very few water column predators preying on suspended fouling communities (e.g. Ashton et al., 2022), during the initial colonization phase the plates were not enclosed in cages. In winter, four plates were lost during the colonization phase, and in summer, one plate was damaged, which was not used in the experiment, leaving 20 plates for the predation experiment in winter and 23 in summer.

The weight of the fouling community was recorded once a week until plates lost their buoyancy. The initial weight of the plate was subtracted from the total weight (plate + fouling biomass). The weight was measured on the boat, using a portable balance REIVI (± 0.01 g) after gently draining the excess of water with paper towel. Plates were left to be colonized for 12 weeks in the winter season and 11 weeks in the summer season.

At the end of the colonization phase, when most plates had become negatively buoyant, the sunken plates (20 out of 24 plates in winter and 23 out of 24 plates in summer) were used to evaluate the effects of benthic predation. Each plate was placed in one plastic mesh cage, with each cage measuring 20 cm x 20 cm x 20 cm, with a mesh opening of 5 mm (see Fig. S2A&B). To each cage, a weight (approximately 1 kg) was tied, keeping the cages firmly on the seafloor at a depth of 5 m, with 2 m of spacing between cages. The plates were accessible to predators in open cages (predator exposure), while predators could not enter the closed cages (control). Ten plates were placed in open cages in winter (11 in summer), while another set of plates (10 winter, 12 summer) was placed in closed cages, impeding predators to access these control plates (see Fig. S2B).

In addition, a set of traps with similar dimensions as the experimental cages was built, but with an opening, allowing potential predators to enter (see Fig. S2C). In order not to interfere with the predation experiments, the traps were placed on the seafloor a week after the experiments. Six replicate traps were placed in winter and summer, in order to determine the mass and number of benthic predators that may be feeding on the fouling organisms growing on the plates. These traps were set in the morning and left for 24h; each trap had a piece of mackerel (*Trachurus murphyi*) as bait, to attract predators.

Biomass and buoyancy measurement

During the experiment, the weight of the fouling plates was measured every day, for 8 days, until plates recovered their buoyancy due to predation (preliminary experiments had been carried out to estimate the approximate time needed for buoyancy to be recovered). Buoyancy recovery was defined as the state in which a plate floated at the sea surface. To determine buoyancy, the plate was placed in a container with seawater, where it was determined whether it floated or not. All weight determinations were carried out on a boat using a scale with an accuracy of ± 0.01 g.

Diversity and community structure

Before conducting the seafloor predation experiments (initial day) and after the plates regained buoyancy, they were transported to the Dichato Marine Laboratory, located just 5 minutes from the sampling area, in a container with local seawater. All sessile taxa growing on the plates were identified to the lowest taxonomic level possible, assessing their relative abundance. Quantification and identification were carried out using a 10 cm x 10 cm quadrat, identifying species through 100 intersection points. Organisms located directly beneath each intersection point were counted. To reduce methodological bias, these tasks were consistently performed from a fixed position on the panel, utilizing taxonomic keys (Aldea and Valdovinos, 2005; Häussermann and Försterra, 2009).

Statistical analysis

The recovery of buoyancy was statistically compared between plates inside open and closed cages (representing presence and absence of predation) with a Fisher's exact test. To evaluate variations in plate weight between the initial and final time of each event (recovery of buoyancy or end of measurement), as well as between the winter and summer seasons, a two-way ANOVA was applied. Additionally, a Friedman test was performed (due to the failure to meet parametric assumptions normality and homogeneity of variance) to compare the time it took the plates to recover their buoyancy between treatments (open and closed cages) and seasons (winter and summer). In cases where the plates did not recover buoyancy by the end of the experiment (i.e., after 8 days on the seafloor), they were scored as taken 8 days. A Dunn post-hoc test was performed.

To examine potential differences in community structure between plates with predator access (open cages) and the control plates without benthic predation (closed cages), both at the beginning and end of the experiment and across both seasons, a three-way PERMANOVA was conducted, where time (initial and final) was nested within the treatment, using Bray-Curtis similarity for the analysis. Prior to analysis, coverage data (percentage coverage) were arcsine transformed. In addition, a Non-Metric Multidimensional Scaling (NMDS) analysis was performed using the same variables for the winter and summer seasons. To complement, a SIMPER analysis was conducted to assess the specific contribution of species on the plates at both the initial and final moments for both seasons. All analyses were performed using PAST 2.7 (Hammer et al., 2001) software and primer (Anderson et al., 2008).

RESULTS

Biomass and Buoyancy

The plates needed to reach around 50 grams of total weight of fouling biomass to sink, a value achieved after twelve weeks in winter and eleven weeks in summer. For the plates that recovered buoyancy, the greatest drop in weight was observed between three and five days of exposure to benthic predators in winter (see Fig. 6a), and during the first two days in summer (see Fig. 6b). Control plates had only minor variations in weight, overall decreasing at the beginning after transfer to the seafloor, followed by a small increase in biomass after 8 days.

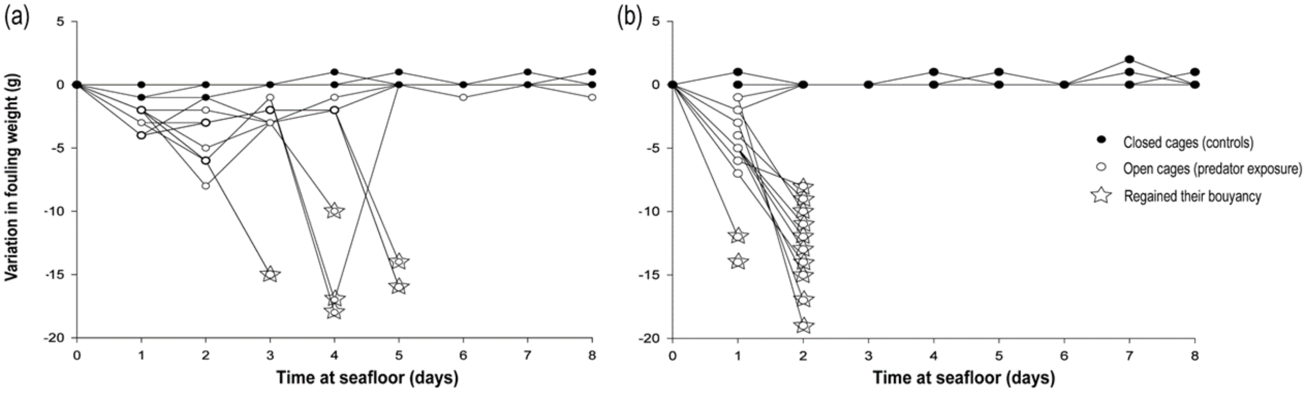

Fig. 6. Variation in fouling weight (weight on final day - weight on initial day) on plates exposed on the seabed (5 m) during the (a) winter season and (b) summer season. In both seasons, plates were exposed to predation (open cages; n = 10 in winter, 12 in summer) and another set of control plates was isolated from predation (closed

cages; n = 10 in winter, 11 in summer). Before exposure on the seabed, fouling communities had developed for 12 weeks in winter and 11 weeks in summer

The plates exposed to predation experienced significant fouling losses (Fig. 6a&b), and consequently many of them regained their buoyancy. In winter, 60% of the plates with predation recovered their buoyancy whereas in summer all plates with predator access refloated (100%) (Fig. 7a). Regardless of season, none of the control plates (without predation) recovered their buoyancy (Fig. 7a). Buoyancy recovery differed significantly between control and experimental plates (Fisher's exact test, p = 0.03251 in winter and p = 0.0001 in summer) and between summer and winter seasons (p = 0.0001).

Regarding the time until plates recovered buoyancy, there was a significant difference between treatments (open cage versus closed cage; p=0.0001), and also between seasons (winter and summer; p=0.0001). In summer, the plates exposed to predators recovered buoyancy significantly faster than in winter (p=0.0001) (Fig. 7b). As for the variation of the final biomass of each event (recovery of buoyancy or end of experiment) between treatments and season (see Fig. 7c), there was a significant difference between treatments ($F_{1,\ 39}$ = 129.9, p = 0.0001), but not between seasons ($F_{1,\ 39}$ = 0.8645, p = 0.3582).

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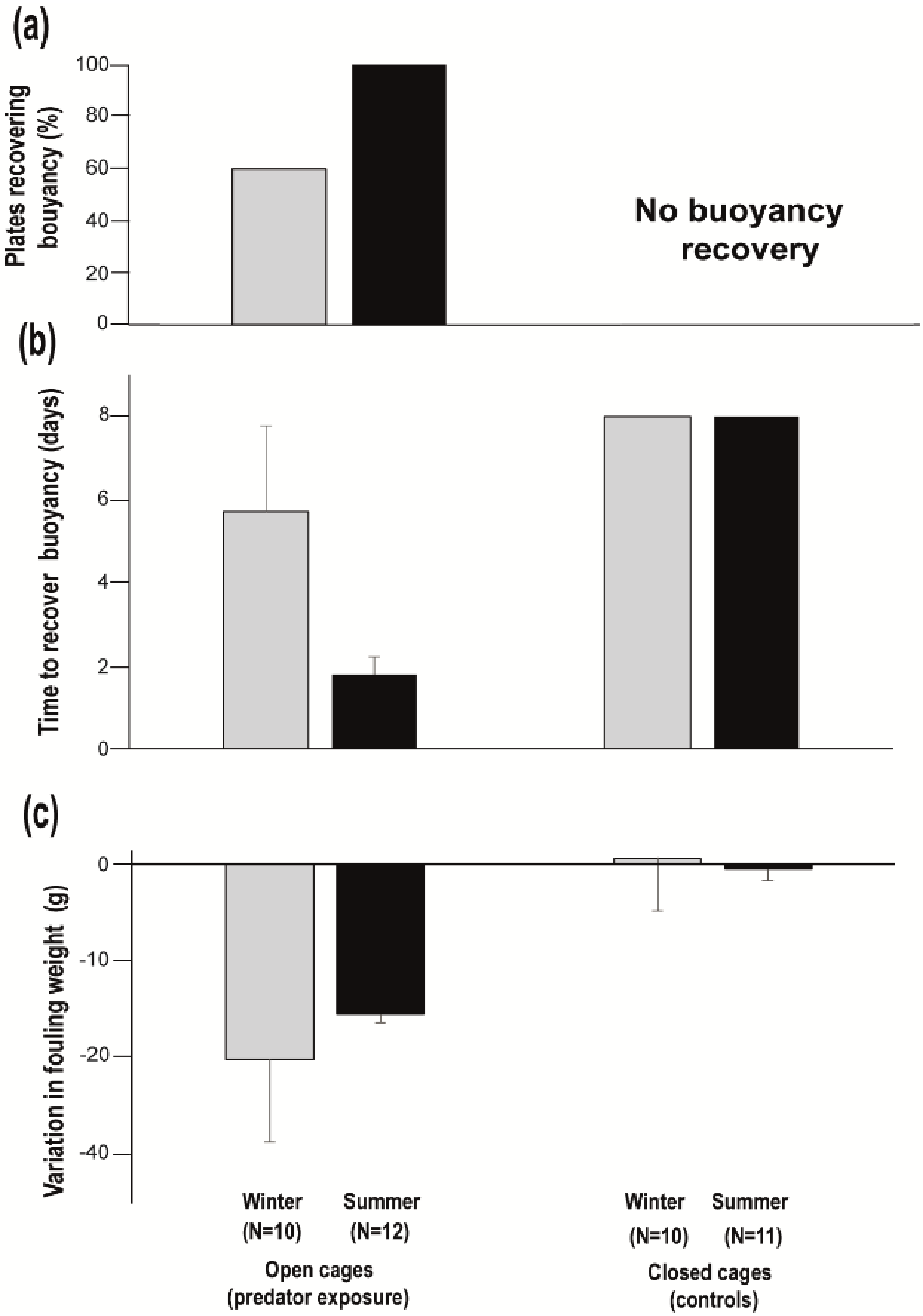
(a)
Plates recovering bouyancy (%)
100
80
60
40
20
0
No buoyancy recovery
(b)
Time to recover buoyancy (days)
8
6
4
2
0
(c)
Variation in fouling weight (g)
0
-10
-20
-40
Winter (N=10)
Summer (N=12)
Open cages (predator exposure)
Winter (N=10)
Summer (N=11)
Closed cages (controls)

Fig. 7. (a) Proportion of plates recovering buoyancy after exposure to benthic predators, in winter (Fisher's exact test. p value = 0.03251) and summer (Fisher's exact test. p value = 0.000). Before exposure to predators on the seabed, fouling communities had developed for 12 weeks in winter and 11 weeks in summer. None of the plates in the control treatment (closed mesh cages without predator access) recovered buoyancy. The number of plates recovering buoyancy differed between summer and winter seasons (p = 0.0001). Variation in (b) the average time (days) needed to regain buoyancy per treatment (open cage and closed cage) and winter and summer, and (c) fouling weight (initial weight – final weight, at last day of predator exposure experiment) of fouling plates exposed on the seabed (5 m) during the winter and summer seasons

Diversity and community structure

During the winter season, a total of eight sessile species settled on the plates, with *Ciona robusta*, *Pyura chilensis*, and *Ulva* sp. emerging as the most predominant fouling organisms. In the subsequent summer season, a total of nine species were identified, with *C. robusta* and *Ulva* sp. being the predominant ones. Across both seasons, the 'softer' species (i.e. without a tough tunica or hard, calcareous exoskeleton) experienced a more significant decrease in coverage due to predation, including *Ulva* sp., *Bugulina flabellata*, *Diplosoma listerianum, C. robusta*, and *Exochella* sp., compared to the 'hardier' species that are more resistant to consumers, such as *P. chilensis*, *Austromegabalanus psittacus*, and *Balanus laevis* (see Table 1; original data on community structure per plate can be found in repository https://zenodo.org/uploads/13376417). According to the SIMPER analysis, *C. robusta*, *P. chilensis*, and *Ulva* sp. exhibited the highest average dissimilarities during the winter season, both at the beginning and the end of the period. In summer, *Ulva* sp., *Semimytilus algosus,* and *C. robusta* showed the highest dissimilarities at the start, while *C. robusta*, *P. chilensis*, and *Ulva* sp. exhibited the highest dissimilarities at the end of the period (see table S1).

Table 1. Average coverage of each taxon on the plates on day 0 (initial) and final in the winter and summer seasons. Non-indigenous species are represented by * and cryptogenic species by **. The highest coverage in each treatment was highlighted in bold.

		Winter						**Summer**					
		Predator Exposure				**Control**		**Predator Exposure**				**Control**	
		Recovered Buoyancy		No Buoyancy Recovery				Recovered Buoyancy		No Buoyancy Recovery			
Phylum	Taxa	Avg. I.T.	Avg. F.T	Avg. I.T.	Avg. F.T	Avg. I.T.	Avg. F.T	Avg. I.T.	Average T8	Avg. I.T.	Avg. F.T	Avg. I.T.	Avg. F.T
Artropoda	*Austromegabalanus psittacus* 	5.6	4.5	10.2	8.6	8.5	8.5	4.7	5.0	-	-	5.1	5.1
Artropoda	*Balanus laevis* 	5.5	1.3	0.8	0.0	1.3	1.3	3.4	2.8	-	-	1.2	1.2
Mollusca	*Semimytilus algosus* 	0.0	0.0	0.0	0.0	0.0	0.0	4.9	4.2	-	-	5.0	5.0
Chordata	*Ciona robusta* 	**55.0**	**32.5**	31.0	24.7	**43.6**	**43.6**	33.4	**16.6**	-	-	32.5	32.4
Chordata	*Diplosoma listerianum** 	6.1	0.0	2.0	0.0	3.1	3.1	3.8	0.3	-	-	4.8	4.7
Chordata	*Pyura chilensis* 	6.5	5.8	**33.7**	**30.7**	17.5	17.5	6.8	5.6	-	-	5.0	5.0
Chordata	*Corella eumyota*** 	0.0	0.0	0.0	0.0	0.0	0.0	3.1	0.7	-	-	3.5	3.5
Clorofita	*Ulva* sp. 	11.0	6.0	10.0	8.0	17.7	17.6	**36.3**	14.6	-	-	**39.4**	**39.4**
Bryozoa	*Bugulina flabellata** 	3.5	1.0	8.2	0.0	1.4	1.3	3.5	0.8	-	-	2.7	2.6
Bryozoa	*Exochella* sp. 	5.6	0.0	2.2	0.0	3.8	3.8	0.0	0.0	-	-	0.0	0.0

The nested PERMANOVA results revealed a significant effect for all three independent variables (season, treatment, and time nested within treatment; p = 0.001). Time nested within treatment showed no significant interactive effect with seasons (p = 0.068). Similarly, the interaction between treatment and season was not significant (Treatment x Season: p = 0.132) (see Table 2 of nested PERMANOVA). The NMDS results from the initial winter period indicated that the community composition of plates that had predator access and recovered buoyancy differed from those that also experienced predation but did not recover buoyancy, with the latter showing similarities to the control communities. Towards the end of the period, a greater differentiation among the three groups began to emerge (see Fig. 8). In summer, while the two groups (experimental and control plates) started off similarly, by the end of the benthic exposure period, differences in their community structures became apparent (see Fig. 9).

The benthic traps caught three species of predators with densities being lower in winter compared to summer (see Fig. 10). During winter, species such as *Cancer plebejus*, *Nassarius gayii* and *Patiria chilensis* were identified. In summer, the only identified species were *C. plebejus* and *P. chilensis*, being substantially more abundant than in winter (see Fig. 10).

Table 2. Nested PERMANOVA results: Season (Winter/Summer), Treatment (Predation/Control), and Time nested within treatment (Initial/Final).

Source	Df	SS	MS	F	P
Treatment	1	5043,2	5043,2	8.4453	0.001
Season	1	175113	17513	29.616	0.001
Time (Treatment)	2	7528.7	3764.3	6.3659	0.001
Treatment x Season	1	1137.6	1137.6	1.9237	0.132
Season x Time (Treatment))	2	3026.9	1513.4	2.5594	0.068
Residuals	78	46124	591.33		
Total	85	809336			

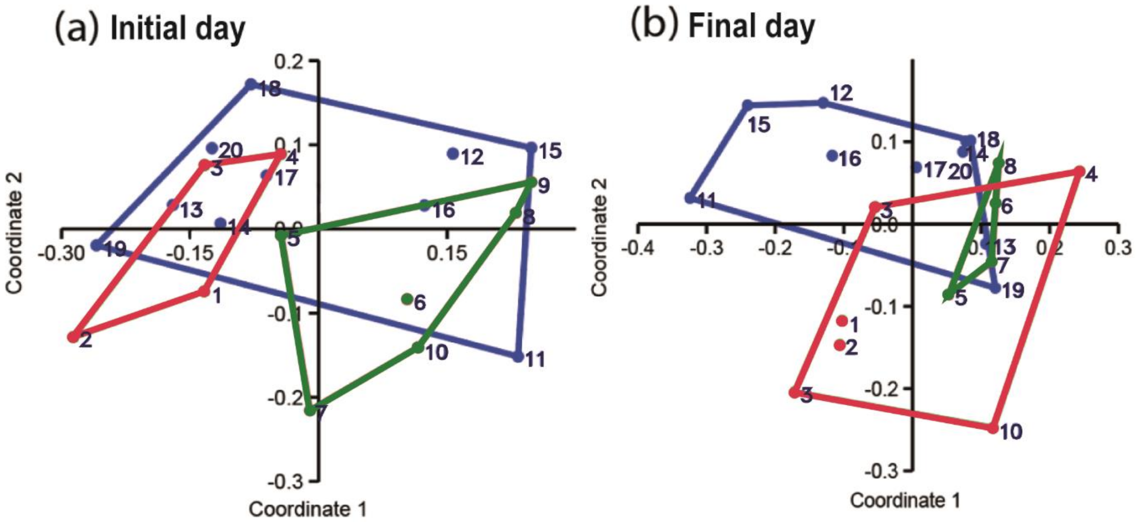

Fig 8. Non-metric Multidimensional Scaling (NMDS) describing the assembly structure after a growing period of 11 weeks during austral winter at (a) the initial day, corresponding to the plates at the beginning of the experiment (before exposure on the bottom), and (b) the final day, corresponding to the plates after having been placed on the seafloor for 8 days or until the recovery of buoyancy; in red and green the plates in open cages exposed to predators (red those that did not recover buoyancy, green those that did recover buoyancy) and in blue the control plates in the closed cages.

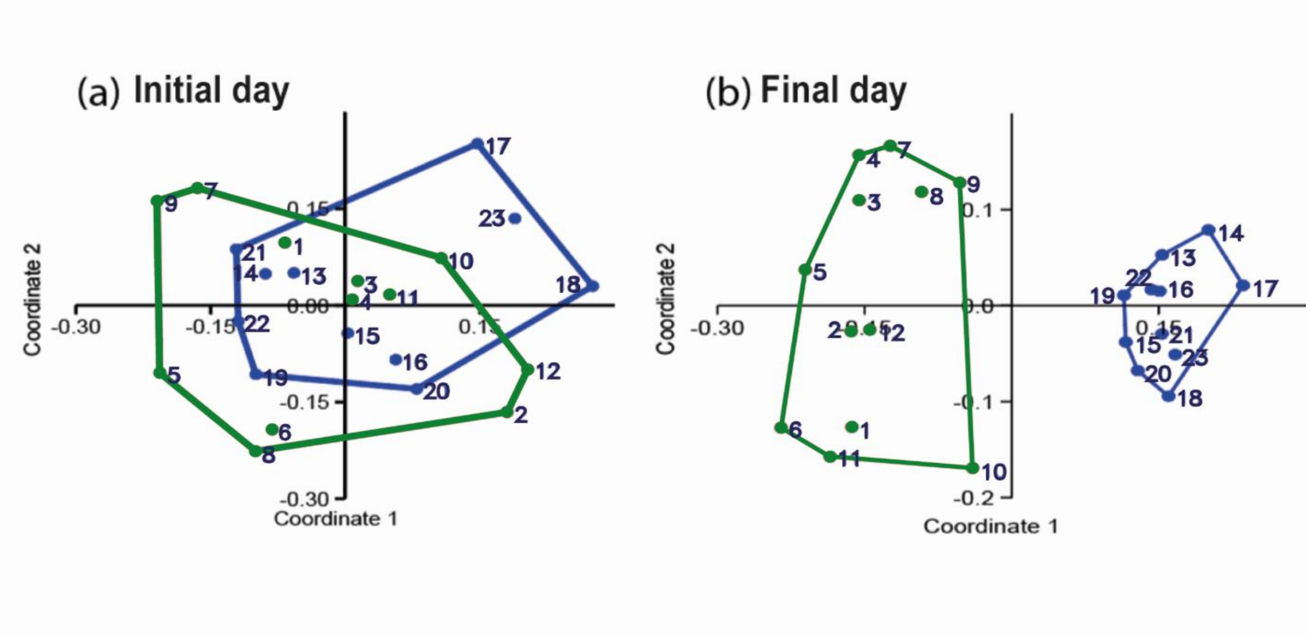

Fig 9. Non-metric Multidimensional Scaling (NMDS) analysis depicting the structure assembly after a 12 week growing period, during austral summer at (a) the initial day, corresponding to the plates at the beginning of the experiment (before exposure on the bottom), and (b) the final day, corresponding to the plates after having been placed on the seafloor for 8 days or until the recovery of buoyancy; in green the plates in open cages exposed to predators and in blue the control plates in the closed cages.

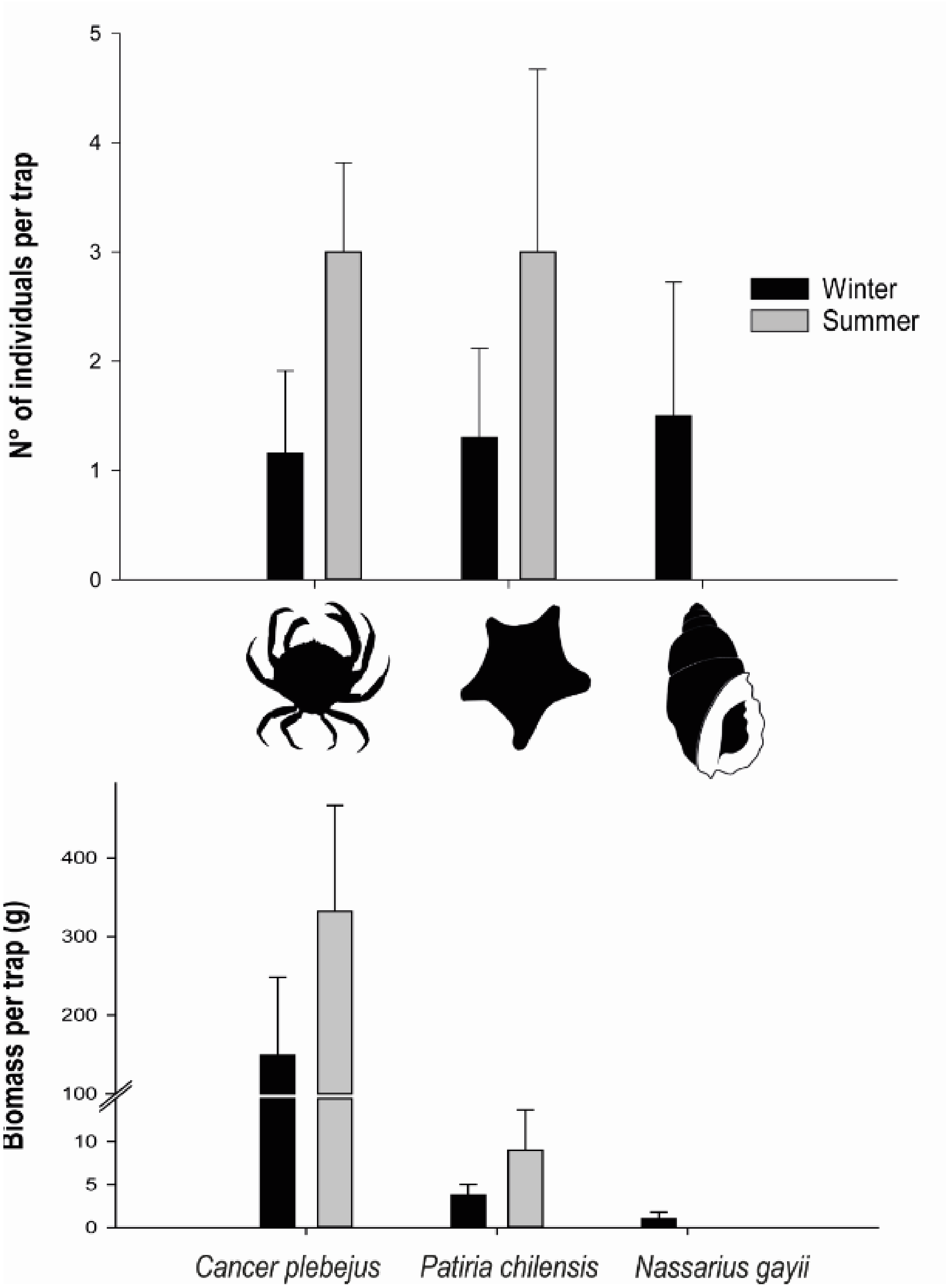

Fig. 10. (a) Average numbers and (b) weight of predators found in the traps (n=6), in summer and winter seasons. Data are presented as average ± SD.

DISCUSSION

Variability in predation effects on floating recovery

This study is the first experiment *in situ* testing the effect of benthic predation on the buoyancy recovery in biofouled litter that had sunk to the seafloor. Our results indicate that when litter objects lose their buoyancy and sink to the seafloor, they become accessible to benthic predators, triggering rapid consumption of fouling organisms and, consequently, the recovery of their floating capability (i.e., within five days in winter and only two in summer). This buoyancy recovery might not only result in a second chance for these litter items to act as floating and dispersal vector for the associated fouling community, but also as a food source for benthic consumers.

The variation in predation leading to some litter objects recovering their buoyancy faster than others can be explained by the differential removal of species in our study. The low predation on *P. chilensis* and *A. psittacus*, and the high consumption of *Ulva* sp., bryozoans, and *C. robusta* can be attributed to the preference and capability of benthic predators to prey upon these species. The effects of predators on different prey taxa are likely dependent on functional traits of the prey, such as those related to defense (Sheppard-Brennand et al., 2017; López and Freestone, 2021, 2022). Indeed, certain functional prey groups, such as solitary tunicates, may be consumed to the point of exclusion in these communities (Freestone et al., 2013; Torchin et al., 2021, Garcia da Silva et al., 2024). This phenomenon can also be observed in the study by Dumont et al. (2011b), where it was found that benthic predators could easily eliminate *C. robusta* due to its fragile tunic, while adult *P. chilensis* with its very tough tunic showed greater resistance to predation. As demonstrated in the study by Young (1986), species from the genus *Pyura* develop physical defenses such as tough, leathery tunics and microscopic spines around the siphons, which offers effective protection against benthic predators.

The variability of communities in marine ecosystems depends not only on the preferences of predators but also on variations in their species abundance and diversity. Factors such as diurnal cycle, tides, seasons, and proximity to submarine reefs play a crucial role. Seafloor communities are particularly vulnerable to benthic predators (Dumont et al., 2011b; Rogers et al., 2016; Leclerc and Viard, 2018), while floating structures tend to attract swimming predators. Several studies (Freestone et al., 2020, 2021; López and Freestone, 2021, 2022) have focused on the impact of predation on fouling communities in pelagic systems. For example, Ye and Andrady (1991) reported a significant loss of fouling biota within a week, when items tethered near the sea surface were transferred to near the seabed at 10 to 15 meters deep. In the study by Rech et al. (2024), the authors highlighted the importance of considering different depth levels, finding that at mid latitudes predation on the seafloor is considerably more important than predation at or near the sea surface. These findings support our conclusions about the relevance of benthic predation in the elimination of epibionts and, therefore, in the buoyancy recovery of sunken litter at mid latitudes.

It is important to highlight the differences in habitat within the benthic zone, as the composition of predators varies significantly depending on the substratum type. On natural rocky bottoms, it is common to find seastars, sea urchins, and gastropods (Dumont et al., 2011b; Bonaviri et al., 2012), while sandy or muddy bottoms often harbor a greater abundance of crabs (Jesse and Stotz, 2003; Pacheco et al., 2013). Additionally, in reef areas, predators can also exert intense predation pressure in the adjacent open habitat areas (Gusmao et al., 2018). The importance of habitat is evidenced in a recent study (Janiak and Branson, 2024), where stronger predation was found on fouling communities in artificial habitats than at seagrass sites where predation effects were relatively minor.

In Chile, subtidal environments range from extensive rocky reefs with seaweed beds, such as *Lessonia trabeculata* or *Macrocystis pyrifera* (Macaya and Zuccarello, 2010; Uribe et al., 2015), to vegetation-free areas dominated by sea urchins (*Tetrapygus niger*) and snails (*Tegula* sp.) (Stotz et al., 2016). Species composition is relatively consistent along the Chilean coast (between 18°S and 42°S), with key predators including fishes like *Cheilodactylus variegatus*, *Pinguipes chilensis*, and invertebrates such as crabs *Romaleon setosum*, *Homalaspis plana* and the mollusk *Concholepas concholepas* (Dye, 1991; Fernández and Castilla, 2000; Pérez-Matus et al., 2007; Fischer and Thatje, 2008; Pacheco et al., 2013; Musrri et al., 2019). This could also explain the findings of our study, carried out in a locality with soft bottoms and a nearby artificial reef area, where seastars *Patiria chilensis* are common and could be moving in from rocky areas, as well as crabs *Cancer plebejus* in sandy areas. The availability of these different predators and their access to epibionts is likely to affect the prey communities, influencing the recovery of biofouled litter that had sunk to the seafloor. While in some regions, strong currents might affect the development of fouling communities, we consider it unlikely to be important in protected coastal zones, such as Coliumo Bay, where biotic interactions such as benthic predation are driving the buoyancy recovery of fouled litter objects.

Variable predation effects

More plates regained buoyancy in summer than in winter. Although there are no statistically significant differences in biomass variation between seasons, a greater and more rapid biomass loss was observed in summer than in winter, where consumptive effects only became noticeable after the fourth and fifth day of exposure to predators. This variation could be related to the composition of predators and their appetite, which are influenced by higher metabolic rates in summer due to the temperature increase (Rall et al., 2010; Doney et al., 2012).

Thermal changes could also be affecting aspects such as colonization timing, reproductive synchronization, settlement, species availability, and interspecific competition. For example, the tunicate *P. chilensis* is more abundant in winter, possibly due to its reproductive peak in cold waters (Cea, 1973; Pérez-Valdés et al., 2017). This could partly explain the observed seasonal differences, as this species is less predated, thereby contributing more to sinking. In summer, the presence of *P. chilensis* decreases, and species such as *C. robusta* and *Ulva* sp. become more common, suggesting that seasonal variability affects the recruitment of different taxa and, consequently, the succession of the fouling community.

In addition to seasonality, latitudinal gradients can be crucial in benthic predation. The variation in consumer impact spans extensive geographical areas, influenced by natural gradients in abiotic factors such as solar radiation and temperature (Reynolds et al., 2018). There is a latitudinal trend in consumer pressure intensity, with peaks observed at lower latitudes (Schemske et al., 2009; Freestone et al., 2021). These trends are more strongly related to water temperature than latitude, suggesting that climate warming may influence top-down control of communities (Ashton et al., 2022). However, Whalen et al. (2020) found a peak in predation intensity at temperate mid latitudes and argued that the presence of specific consumer taxa is more important. Our study was conducted in a temperate zone (~36°S), where a lower predation rate might be expected compared to tropical areas (Freestone and Osman, 2011; Freestone et al., 2020). Nevertheless, it has also been suggested that there is functional redundancy within the predator guild, with benthic crustaceans becoming more important at higher latitudes and replacing fish as important predators at lower latitudes (Musrri et al., 2019).

Implications for sinking/floating dynamics of positively buoyant marine litter

The occurrence of fouling is more prevalent in coastal waters, characterized by higher nutrient concentrations and productivity, in contrast to oligotrophic zones, which typically exhibit lower productivity. This is particularly apparent in the central-eastern South Pacific Subtropical Gyre (SPSG), recognized as the most oligotrophic marine region (Morel et al., 2010; von Dassow and Collado Fabbri, 2014). For example, in the study by Rech et al. (2021), very low fouling coverage was observed on floating objects in the SPSG, suggesting that hyperoligotrophic conditions and intense water column predation strongly limit fouling growth. In contrast, a study on the coast of Chile found a diverse community of floating species (116 species) on aquaculture buoys, where the environment is more eutrophic (Astudillo et al., 2009). This highlights that sinking due to extensive and dense fouling communities is more likely to occur in productive coastal areas than in the open ocean.

It is important to highlight that our study focuses on coastal and shallow waters, as sunken substrata may not return to the surface in deeper waters, even after the fouling has been removed by benthic predators. The hydrostatic pressure at such depths can alter the volume of the object, increasing its density beyond that of seawater, which prevents it from resurfacing. Additionally, surface tension in muddy sediments can trap plastic debris, even when much of the fouling community has been consumed. In deeper waters exceeding 200 meters, multiple reports indicate that when a new resource reaches the seabed, numerous predators and scavengers quickly target it, resulting in a strong predation effect (see Lundsten et al., 2010; Higgs et al., 2014). The present results show that buoyancy recovery can rapidly occur in coastal areas and given that most floating plastics are concentrated in these regions (Onink et al., 2021; Kaandorp et al., 2023), the current findings are relevant to a significant proportion of sunken plastics.

When a substratum sinks with adhered epibionts that are resistant to predators, it is unlikely to regain buoyancy, as suggested in our study. For example, a study conducted off the coast of South Africa revealed that 77% of plastics on the seabed regained positive buoyancy after the authors had removed the epibionts (Ryan et al., 2020). However, it is likely that these epibionts could not be removed by natural benthic predation due to their resistance. This phenomenon is also evident in the study by Harms (1990), where plastics on the seabed had a higher abundance of predation-resistant epibionts such as *Balanus crenatus*, *Mytilus edulis*, and *Elminius modestus*. Similar results were found in the study by Rumbold et al. (2020), where the most abundant species on fouled litter were the barnacle *Amphibalanus improvisus* and the encrusting bryozoan *Membranipora* sp., both belonging to taxa known for their predation resistance. This suggests that the susceptibility of epibionts to benthic predators affects the potential for buoyancy recovery. These sinking-surfacing dynamics have significant ecological implications, as refloated plastic litter can benefit species lacking sufficient natural floating habitats, playing a complementary role to natural rafting in areas where there is a limited presence of natural substrata such as wood and algae. Furthermore, this sinking due to fouling might also represent a significant source of carbon for benthic communities, in some coastal, eutrophic and plastic polluted areas.

In future studies, it would be interesting to evaluate the buoyancy of species, as the amount of fouling necessary for plates to sink depends on the specific density of the attached organisms. The buoyancy of the fouled plate is therefore influenced by the intrinsic buoyancy of the raft combined with the buoyancy of the fouling organisms determined by important species traits, such as presence of shells, mass, shape (exposure to currents) and density. Future studies exploring this combination will likely provide a more accurate approximation of raft buoyancy along the fouling formation/growth period. Furthermore, evaluating the process of buoyancy recovery in different types of benthic habitats, such as rocky reefs or sandy bottoms, will also contribute to a better understanding of the importance of benthic predation on the buoyancy recovery of sunken litter.

CONCLUSION

Although it had been assumed that litter objects that sink due to the fouling-induced loss of buoyancy can regain their floating capacity through benthic predation, our study is the first to demonstrate that benthic predation indeed plays a key role in buoyancy recovery. The recovery of buoyancy in these litter objects depends on the prey trait “susceptibility to predation”, highlighting the important role of community structure in this process. Seasonality generates an important effect on both community structure and predator availability, which are key factors for the possible recovery of buoyancy. The ubiquity of marine litter, especially in coastal environments, calls for future studies to evaluate the effect of predation on buoyancy recovery in different types of benthic habitats, at different depths and latitudes.

Supplementary materials

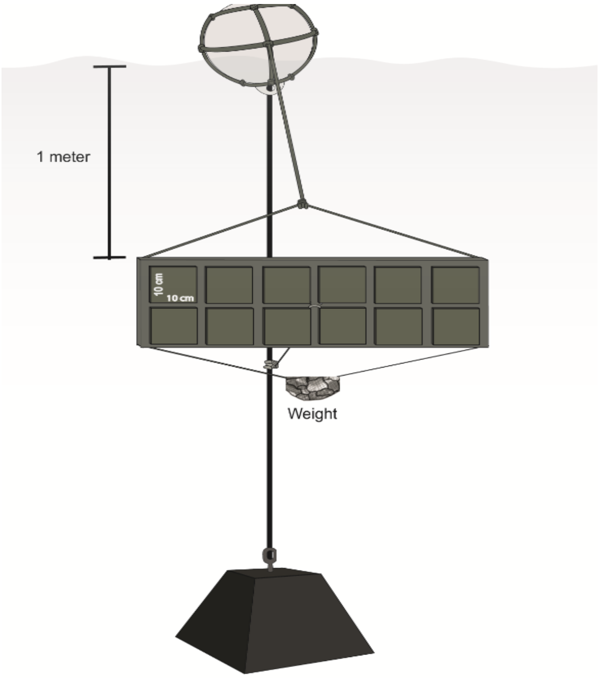

Fig. S1. Experimental setup for the development of fouling communities on foamed PVC plates in Coliumo Bay, Biobío region, Chile during both winter and summer. Each system held a total of twelve plates: six arranged vertically, with another six placed below them on a floating platform, allowing fouling organisms to attach and grow.

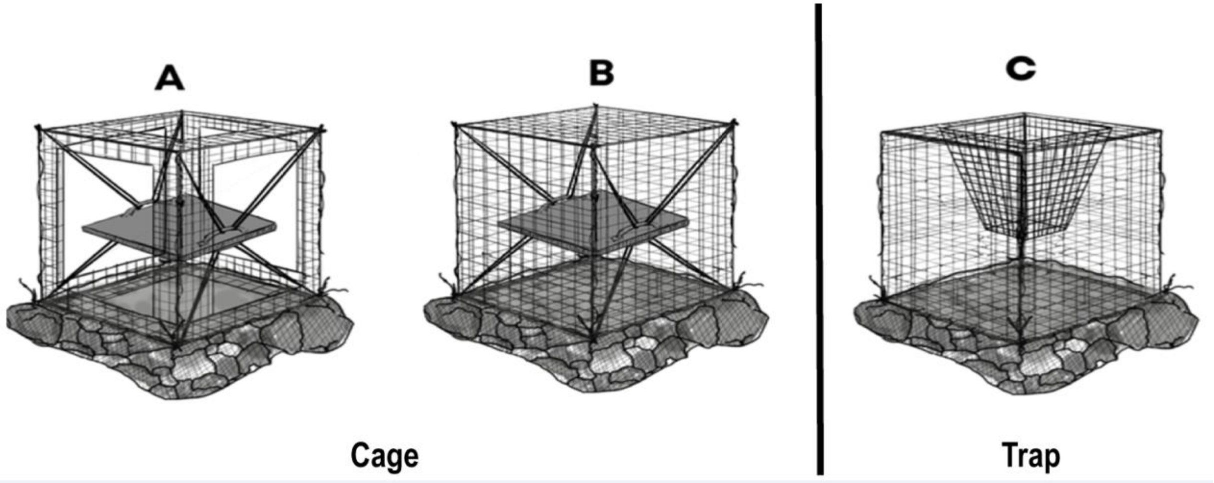

Fig. S2. Design of a 20 cm x 20 cm x 20 cm plastic mesh cage (5 mm mesh size) with open apertures, fully exposing the plates to benthic predation (A). Another cage was designed with the same plastic mesh but fully enclosed, preventing the entry of benthic predators (B). To obtain information about benthic predators that were active in the area where cages with the plates were placed on the bottom, cages (n = 6) of the same dimensions as the experimental ones were used as traps to capture predators, with an opening of 3 cm x 3 cm (C). In all cases, a weight (1 kg) was attached to the bottom of the cages and traps.

Table S1. SIMPER analysis results for Treatment (Predation/Control), considering initial (a) and final (b) states during Winter, as well as initial (c) and final (d) states during Summer.

(a) Taxon	Av. dissim	Contrib. %	Cumulative %	Predator	Control
Ciona robusta	9.501	23.7	23.7	45.3	43.6
Pyura chilensis	9.41	23.48	47.18	17.4	17.5
Ulva sp.	6.645	16.58	63.76	10.1	17.7
Austromegabalanus psittacus	4.579	11.42	75.19	7.5	8.5
Exochella sp.	2.867	7.153	82.34	5.4	1.4
Diplosoma listerianum	2.69	6.711	89.05	4.3	3.8
Balanus laevis	2.427	6.056	95.11	4.2	3.1
Bugulina flabellata	1.962	4.894	100	3.6	1.3
Semimytilus algosus	0	0	100	0	0
Corella eumyota	0	0	100	0	0

(b) Taxon	Av. dissim	Contrib. %	Cumulative %	Predator	Control
Ciona robusta	12.37	27.96	27.96	29.4	43.6
Pyura chilensis	11.3	25.55	53.5	15.8	17.5
Ulva sp.	8.927	20.17	73.68	7.39	17.7
Austromegabalanus psittacus	4.948	11.18	84.86	5.8	8.5
Exochella sp.	2.441	5.516	90.38	0	3.8
Diplosoma listerianum	2.028	4.584	94.96	0	3.1
Balanus laevis	1.178	2.662	97.62	0.8	1.3
Bugulina flabellata	1.052	2.377	100	0.4	1.4
Semimytilus algosus	0	0	100	0	0
Corella eumyota	0	0	100	0	0

(c) Taxon	Av. dissim	Contrib. %	Cumulative %	Predator	Control
Ulva sp.	3.19	14.29	14.29	36.3	39.4
Semimytilus algosus	3.009	13.45	27.74	4.92	5
Ciona robusta	2.727	12.87	39.96	3.83	4.75
Diplosoma listerianum	2.45	11.57	51.53	4.65	5.48
Pyura chilensis	2.443	11.54	63.07	5.7	4.99
Austromegabalanus psittacus	2.177	10.28	73.35	34.2	33.1
Corella eumyota	2.136	10.09	83.44	4.05	2.7
Bugulina flabellata	2.01	9.491	92.93	2.78	3.6
Balanus laevis	1.498	7.074	100	3.23	1.15
Exochella sp.	0	0	100	0	0

(d) Taxon	Av. dissim	Contrib. %	Cumulative %	Predator	Control
Ciona robusta	16.19	34.47	34.47	14.6	39.2
Pyura chilensis	11.08	23.59	58.06	16.4	33.1
Ulva sp.	8.15	66.21	4.95	4.75	4.75
Austromegabalanus psittacus	3.317	7.061	73.27	4.95	5.48
Bugulina flabellata	3.198	6.809	80.08	5.63	4.99
Exochella sp.	6.703	86.78	1.35	4.91	4.91
Diplosoma listerianum	2.414	5.139	91.92	0.75	3.6
Balanus laevis	1.915	4.077	96	0.75	2.7
Semimytilus algosus	1.88	4.003	100	3.08	1.15
Corella eumyota	0	0	100	0	0

GENERAL DISCUSSION

Effect of Intrinsic Factors on the Fouling Community Dynamics

Larval selection, driven by intrinsic characteristic of the substratum, plays a crucial role in surface colonization. We observed that the larvae of both species swam less and explored more when exposed to plastic surfaces. Additionally, they actively chose to settle on plastics rather than on wood or concrete substrata, suggesting a preference for colonizing plastic surfaces in *Bugula* larvae.

This preference for plastics facilitates faster and more efficient settlement with lower energy expenditures, potentially leading to greater fitness of colonies on these substrata compared to wood and concrete (Jaeckle and Manahan, 1989; Cancino and Gallardo, 2004). The abundance of plastics in the oceans could benefit invasive species, allowing them to overcome natural barriers and extend their distribution (Barnes et al., 2009; Haward, 2018). This behavior could facilitate the dispersal of species, including invasive ones, through floating plastic debris, highlighting the need for further research to fully understand the association between *Bugula* larvae and plastic surfaces (Morohoshi et al., 2018; Shin et al., 2013). Additionally, this rapid preference and settlement of larvae on plastic may promote greater biomass growth on these floating plastics, which could eventually lead to their sinking.

Effect of Extrinsic Factors on the Buoyancy Recovery of Floating Substrata

Predation is a crucial extrinsic variable that influences the recovery of buoyancy in substrata sunk by fouling. More plates recovered buoyancy in summer than in winter. Although there were no significant differences in biomass variation between seasons, greater and faster biomass loss was observed in summer. In winter, the effects of predators only became noticeable after the fourth and fifth days of exposure. This difference may be attributed to faster biomass reduction in summer, possibly related to the composition and appetite of predators, influenced by higher metabolic rates due to increased temperatures (Rall et al., 2010; Doney et al., 2012). Thermal changes can also affect aspects such as the timing of colonization, reproductive synchronization, settlement, species availability, and interspecific competition. For example, *P. chilensis* is more abundant in winter, likely due to its reproductive peak in cold waters (Cea, 1973; Pérez-Valdés et al., 2017). This could also explain the observed differences in buoyancy recovery, as this species is more resistant to benthic predators, contributing more to sinking. In summer, the presence of *P. chilensis* decreased, and species like *C. robusta* and *Ulva* sp. become more common, suggesting that seasonal variability affects the recruitment of different taxa and consequently the succession of the fouling community.

Future Perspectives

In this work, although we have contributed to the knowledge of fouling communities on floating plastics, many questions have emerged that need to be addressed in the future. It is essential to evaluate larval selection on different substrata, primarily those of natural origin such as algae but also on the most abundant types of plastic litter, and to test this selection in other taxa besides bryozoans. Additionally, it is crucial to explore the effect of benthic predation on the buoyancy recovery process in different types of benthic habitats, such as rocky reefs or sandy bottoms, and at different depths. This will contribute to a better understanding of the importance of benthic predation in the buoyancy recovery of sunken debris.

GENERAL CONCLUSION

The findings of this research highlight the importance of larval selection, where the strong preference and settlement of larvae on plastic substrata could lead to increased biomass growth on these floating plastics, potentially causing them to sink. Once submerged, the epibionts adhered to the plastics come into contact with benthic predators that remove them. While it had been assumed that substrata sunk due to fouling-induced loss of buoyancy could recover their flotation capacity through benthic predation, our study is the first to demonstrate that benthic predation plays a key role in buoyancy recovery. Additionally, the recovery of buoyancy in these substrata depends on the susceptibility of the fouling to predation, underscoring the critical role of community structure in this process. Seasonality significantly affects both community structure and predator availability, which are key factors in the potential recovery of buoyancy.

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