1. Introduction

Antarctic marine ecosystems are immersed in an isolated, relatively constant environment where the organisms inhabiting their benthos are mainly sessile suspension feeders. For these reasons, physical and chemical biotic interactions play an essential role in structuring these marine benthic communities (Dayton et al., 1974; Orejas et al., 2000). These interactions may include diverse strategies to avoid predation (e.g. Iken et al., 2002), competition for space or food (e.g. Bowden et al., 2006) and avoiding fouling (e.g. Rittschof, 2001; Peters et al., 2010). For instance, in the marine benthos, one of the most extended effective strategies among sessile soft-bodied organisms is chemical defense, mediated by several bioactive natural products mostly considered secondary metabolites (e.g. Paul et al., 2011). The study of the “chemical network” (chemical ecology interactions) structuring the communities provides information about the ecology and biology of the involved species, the function and the structure of the community and, simultaneously, it may lead to the discovery of new compounds useful to humans for their pharmacological potential (e.g. Avila, 1995; Bhakuni, 1998; Munro et al., 1999; Faulkner, 2000; Lebar et al., 2007; Avila et al., 2008). In the last three decades, the study of marine chemical ecology has experienced great progress, thanks to the new technological advances for collecting and studying marine samples, and the possibility of identification of molecules with smaller amounts of compounds (e.g. Paul et al., 2006, 2011; Blunt et al., 2011).

Polar organisms have been less studied, compared with their temperate and tropical counterparts (Paul, 1992; Blunt et al., 2009). However, recent studies report that Antarctic benthic invertebrates are a rich and diverse source of natural products, with great interest from both the ecological and the pharmacological point of view (e.g. Avila et al., 2000, 2008; Amsler et al., 2001; Iken et al., 2002; Lebar et al., 2007; Reyes et al., 2008; Taboada et al., 2010; Paul et al., 2011). Moreover, several researches have demonstrated that some Antarctic species inhabiting shallow areas from McMurdo Sound and the Antarctic Peninsula possess chemical defenses (for review see Avila et al., 2008; McClintock et al., 2010), even if only in few cases the chemistry of the metabolites involved has been fully described and/or their ecological role has been established (e.g. Núñez-Pons et al., 2010; Núñez-Pons et al., in prep).
In the last years our research group has been studying the ecological activity of marine natural products obtained from Antarctic benthic organisms by using *in situ* experiments. Furthermore, as part of our investigations, previously unknown species for science have been described (Ballesteros & Avila, 2006; Ríos & Cristobo, 2006; Figuerola et al., in press), and new compounds have been isolated and described too (e.g. Antonov et al., 2008, 2009, 2011; Reyes et al., 2008; Carbone et al., 2009; Carbone et al., in prep). Also, we have extended the range of species from our previous analysis by studying Antarctic macroalgae, which are known to be prolific producers of secondary metabolites with pharmaceutical applications (e.g. Hoyer et al., 2002; Ankisetty et al., 2004). As a general objective our aim here is to integrate all the experimental data obtained from the assays conducted with different taxonomical groups in order to establish a preliminary ecological model of the chemically-mediated interactions in the Antarctic benthos. This model will, for the first time, consider the mechanisms that regulate the chemical interactions among the different Antarctic benthic organisms studied. Our specific objectives are trying to determine the a) feeding-deterrence activities towards sympatric predators, including a macropredator (*Odontaster validus* Koehler) and a mesograzier, *Cheirimedon femoratus* Pfeffer, b) toxicity potential against a copepod, *Metridia gerlachei* Giesbrecht, c) cytotoxicity against embryos and sperm of the Antarctic sea urchin *Sterechinus neumayeri* Meissner and d) antifouling activity against microbial biofilms.

### 2. Material and methods

#### 2.1 Samples collection and identification

Marine benthic invertebrates and algal samples were collected in the Southern Ocean in four Antarctic campaigns: two in the Eastern Weddell Sea (Antarctica) and vicinities of Bouvet island (Sub-Antarctica) on board the R/V Polarstern, from the Alfred Wegener Institute for Polar and Marine Research (AWI Bremenhaven, Germany) during the ANT XV/3 (January-March 1998) and ANT XXI/2 cruises (November 2003-January 2004); a third one on board the BIO Hespérides during the ECOQUIM-2 cruise (January 2006) around the South Shetland Islands; and finally, the ACTIQUIM-1 cruise at Deception Island mainly by scuba-diving, although other sampling methods were used as well (December 2008-January 2009). Sample collection took place between 0 m and 1524 m depth by using various trawling devices: bottom trawl, Agassiz trawl, Rauscher dredge and epibenthic sledge, and also, as said, by scuba diving (0-15m). Samples were sorted and photographed on deck, frozen at -20 °C, and a voucher portion of each sample or, in some cases, whole individuals, were fixed in 10% formalin or 70% ethanol and stored at the Dept. of Animal Biology (Invertebrates), University of Barcelona (Spain), for taxonomical identification.

Individuals of the sea star *Odontaster validus*, the sea urchin *Sterechinus neumayeri*, the amphipod *Cheirimedon femoratus* and the copepod *Metridia gerlachei* were collected for *in situ* ecological experiments in Deception Island by scuba diving at Port Foster Bay (Deception Island: 62° 59'36" S, 60° 33'42" W) from 0-15 m depth (December 2008 - January 2009 and January 2010). After experimentation, these invertebrates were brought back alive to the sea.

#### 2.2 Chemical extractions

Chemical extractions were done in the laboratories from the Faculty of Biology (University of Barcelona). Frozen animals were carefully dissected into different sections when possible, in order to locate the compounds within the body of the organisms (although this is not
discussed here). The different sections were made according to the taxonomic group (e.g. internal/external, apical/basal parts in sponges, echinoderms and tunicates; poliparium/axis in cnidarian octocorals; mantle/foot in opistobranch molluscs; gill slits in ascidians; tentacles in holoturian echinoderms...). These body sections were extracted separately, and thus the total number of extracts is larger than the total number of species tested. Samples were extracted with acetone, and sequentially partitioned into diethyl ether and butanol fractions. All steps were repeated three times, except for the butanol which was done once. Organic solvents were then evaporated under reduced pressure, resulting in dry diethyl ether and butanolic extracts, and an aqueous residue. An aliquot of all the diethyl ether extracts (lipophilic fraction) was used for the bioassays at different concentrations for the different experiments. The detailed description of the extraction procedure has been reported elsewhere (Avila et al., 2000; Iken et al., 2002). Butanolic extracts and water residues were kept aside for future investigations.

2.3 Experiments of chemical ecology and statistical treatment

All experiments of chemical ecology took place in the Spanish Antarctic Base “Gabriel de Castilla” in Deception Island (South Shetland Islands, Antarctica) during the Austral Summers of 2008-2009 and 2009-2010.

2.3.1 Feeding experiments with a macropredator, the seastar Odontaster validus, and a mesograzier, the amphipod Cheirimedon femoratus

The omnivorous sea star O. validus occupies the top predator position that fish occupy in temperate and tropical areas (McClintock, 1994). For this reason, this ubiquitous sea star is used as putative macropredator in feeding-deterrence experiments to test the presence of chemical defenses in selected marine invertebrates and algae (e.g. Avila et al., 2000, Iken et al., 2002). The amphipod Cheirimedon femoratus was chosen as mesograzier consumer in feeding-prefrence assays because this voracious, omnivorous-scavanger crustacean is found in notably high densities in Antarctica exerting remarkable, localized ecological pressures, often underestimated (Huang et al., 2007).

The sea star experiments were carried out over 24 h. Extracts, fractions and/or isolated compounds were dissolved in the solvent carrier (diethyl ether) and slowly pipetted at their natural dry weight concentration (mg extract g⁻¹ dry wt tissue) onto shrimp pieces, and the solvent was left to totally evaporate under the hood, resulting in a uniform coating of extract. Normalization of natural concentrations based on biomass using wet or dry weight are appropriate when ingredients are homogeneously distributed, and also when using biting and not-biting predators. Moreover, dry weight has been proven to be the most constant parameter for avoiding the variability caused by the water content. Control shrimp pieces were treated with solvent only. Feeding-deterrence experiments are described in detail in precedent investigations (e.g. Avila et al. 2000). The bioassays consisted on 10 replicates in which the sea stars were individually transferred into 2.5 l-buckets filled with fresh seawater (±0.5°C), and they were offered a treatment or a control diet, respectively, by putting a shrimp piece in the centre of the bucket and the asteroid on top. A food item was considered rejected when Odontaster validus lost physical contact with it, and it was considered eaten when the food was ingested completely after the testing period (Fig. 1). Afterwards, eaten and uneaten shrimp pieces were counted for statistical analysis. Feeding
repellence was evaluated as a contingency table 2x2, and since the number of replicates was small (n=10) by using Fisher’s Exact tests for each experiment using extract-treated shrimp pieces referred to the control run simultaneously (Sokal & Rohlf, 1995).

![Fig. 1. Odontaster validus feeding-repellence experiments. a) Shrimp pieces being prepared with extract coatings for the tests; b) A sea star being offered a shrimp piece.](image)

The generalist amphipod *Cheirimedon femoratus* was used as a potential mesograzer predator. It was presented to a simultaneous choice of two different food types, consisting of a control (extract-free) diet (which the predator readily consumed) and a treatment diet, where the extracts were included at natural concentration according to a dry weight basis (see above). Both diets consisted on alginate-based artificial foods containing a powdered commercial aquarium diet as a food attractant. Control food was prepared with only solvent, which was left to evaporate onto the food powder prior to being gelified into food pellets. For treatment diets, extracts were added into the food mixture dissolved in the carrier solvent (which was similarly evaporated). Groups of 15-20 amphipods were transferred into 1L-bottles filled with sea water, and were presented to a choice of extract-free control and extract-treated diets. The assays ran until either food type had been consumed up to one-half or more. At the end of the experiment, the consumed food was calculated for statistical analysis and determination of feeding preferences of extract-treated foods from the paired simultaneous controls to consequently establish repellent activities. The two food types were presented together, and therefore we measured separately for each replicate container and each food type the quantity of ingested food, and calculated the differences for each experimental unit (replicate). The changes in the two food types held in the same container are not independent and possess correlated errors, making it impossible to analyze them separately. Each replicate is represented by a paired result yielding two sets of data (treatments and controls), which can be compared, since assumption of normality and homogeneity of variances are not met, by non-parametric procedures, that is by applying the Exact Wilcoxon test, which was calculated using R-command software.

### 2.3.2 Toxicity activity against the copepod *Metridia gerlachei*

*Metridia gerlachei* is a common omnivorous copepod frequently found in the waters of Port Foster (King & LaCasella, 2003). For this experiments, we used plates with 2 ml seawater
where 10-15 copepods were placed. Each experiment consisted of 5 replicates with the ethereal extract to be tested at natural concentration, 5 negative control assays (only filtered sea water), and 5 solvent assays (filtered seawater with solvent). During experimentation, copepods were observed over time for survival. Extracts were considered toxic when, considering the 5 replicates for each test, >50% of the copepods died.

### 2.3.3 Cytotoxicity activity against embryo and sperm in the Antarctic sea urchin *Stereochinus neumayeri*

Sessile organisms may prevent the settlement of sympatric organisms by displaying cytotoxic activities that may act against embryos and larvae of other invertebrates, in their attempt to colonize the surface of sessile invertebrates, such as sponges, ascidians, bryozoans and polychaetes (e.g. Heine et al., 1991; McClintock et al., 1990). In the Antarctic marine benthic environment, *Stereochinus neumayeri* is one of the most abundant and common species of sea urchin, and its biology is well known (e.g. Bosch et al., 1987; Brey et al., 1995). For these reasons, this species was chosen for our bioassays.

After acclimatization, sea urchins were induced to spawn by injecting 1ml of 0.5 M KCl solution into the coelomic cavity through the peristome. The cytotoxicity test was developed according to the protocol proposed by Volpi Ghirardini and collaborators (2005) for the Mediterranean sea urchin *Paracentrotus lividus*. Some modifications were introduced in the original procedure, mainly focused on the volume of sea water used and the time that embryos were exposure to extracts, in order to adapt it to the characteristics of *S. neumayeri*. Details of this modified method are described in a paper that is being prepared (Figuerola et al., in prep) (Fig. 2). The percentage of blastula stage in each treatment was determined for statistical analysis. A S regression model \( Y = \exp (0.702 + 124,928/X) \), \( R^2 = 0.6125 \) was calculated between % of the number of the blastula (Y) and the initial concentration of eggs.

![Fig. 2. Different stages of embryonic development of the sea urchin *Stereochinus neumayeri*. a) Stage of 2 cells; b) Stage of 4 cells; c) Morula stage; d) Dipleurula larva; e) Pluteus larva.](www.intechopen.com)
(X) only using the data from the control group and solvent to obtain a representation of the normal behavior (without the influence of the extracts) of the experimental conditions. Also, confidence intervals (CI) of prediction (upper and lower: UCL and LCL) of 95% coverage were calculated to detect extract samples outside CI.

The sperm test was developed following the procedure of similar experiments conducted in the past using *S. neumayeri* (Heine et al., 1991; McClintock et al., 1992). Previous sperm assays utilized 25-ml volumes of test solutions in 25 x 150 mm glass test tubes. The refined method used here was based on 0.25 ml test volumes. The use of smaller wells also allowed an increase in the number of samples to be tested at one time. *S. neumayeri* sperm was obtained as described above for the cytotoxicity tests. Every day that a sperm toxicity test was conducted, a blank control (sperm with filtered sea water) and a positive control (sperm in filtered sea water with ethereal extract) were run simultaneously. Ten replicates using extracts at different concentrations were tested for each of the samples. Sperm mobility was checked under a light microscope (40x) 20 min after the sperm solution was added to each well. Extracts were considered toxic when <25% of the sperm was active. Moreover, a binomial test of proportions (p<0.001) was calculated in order to evaluate the effects of different concentrations of extracts.

### 2.3.4 Antifouling activity

Different marine organisms such as bacteria, algae and invertebrates colonize surfaces underwater. However, many sessile marine invertebrates possess chemical or physical defenses to prevent the settlement of epibionts (e.g. Kelly et al., 2003; Sivaperumal et al., 2010). The presence of different kinds of compounds may influence the growth of other species which could settle near or over marine invertebrates. We evaluated the antibacterial activity of different extracts using the methods described in the literature with Antarctic bacteria (e.g. Jayatilake et al., 1996; De Marino et al., 1997, Mahon et al., 2003). Selected bacteria from the sea water were collected during the campaign, cultured on marine agar Difco brand (DMA 2216), and later sent to specialists for further identification. Filter paper discs impregnated with 20 μL of solution were placed on the surface of inoculated plates. Each test consisted in one disc without any additive (negative control), one disc with chloramphenicol (positive control), one disc impregnated with the solvent (diethyl ether, negative control) and one disc impregnated with the extract at natural concentration. Each culture of microorganisms was inoculated for triplicate on the surface of marine agar with the paper discs. Diffusion methods were based on the homogeneous distribution of the extract on solid culture media. The amount of the extract, as the number of bacteria (inoculum), was carefully controlled. After incubation, we measured the diameters of the inhibition halos and the results were interpreted using cut points as established internationally. Zones of growth inhibition larger than 2 mm were considered active.

### 3. Results

#### 3.1 Feeding experiments with a macropredator, the seastar *Odontaster validus*, and a mesograzier, the amphipod *Cheirimenedon femoratus*

In feeding-deterrence experiments using the seastar *O. validus*, 160 extracts (139 species) were tested belonging to different Phyla: Porifera (43 species), Cnidaria (17), Tunicata (15),
Bryozoa (17), Echinodermata (5), Annelida (7), Algae (8), and other groups (11). A total of 76 deterrent extracts (66 species) were found, revealing significant differences in food consumption between simultaneous control and treatment tests (p<0.05), with control as the preferred food (Fisher’s exact test). The deterrent extracts were thus 48.2% of the tested species, from organisms belonging to the taxa Porifera (22), Cnidaria (10), Tunicata (11), Bryozoa (10), Echinodermata (3), Annelida (4) and Algae (1), and others (5) (Avila et al. in prep) (Fig. 3).

In the experiment of feeding-preference using C. femoratus, 52 extracts were tested from Porifera (15), Cnidaria (14), Tunicata (12), Bryozoa (1), Echinodermata (1) and several extracts from macroalgae (8) (Núñez-Pons et al., in prep) A total of 36 extracts (33 species) out the 52 tested (40 species) were active (88.8% of the tested species) against the amphipod, revealing significant differences in food ingestion (p<0.05), being the control food preferred respect to the paired, unpreferred extract-treated, diet (Exact Wilcoxon test). These extracts corresponded to the taxa Porifera (7), Cnidaria (12), Tunicata (8) and Algae (6) (Fig.4).
3.2 Toxicity activity against the copepod *Metridia gerlachei*

We tested 24 species (32 extracts) belonging to the taxa Porifera (9), Cnidaria (3), Tunicata (1), Bryozoa (6), Echinodermata (4), and Hemichordata (1) and 14 of them (58, 3%) were toxic against copepods. A total of 14 active extracts (12) were detected (50% of the tested species) from organisms belonging to the Phyla Porifera (4), Cnidaria (1), Tunicata (1), Bryozoa (2), Echinodermata (4) (Fig. 5).
Fig. 5. Toxicity activity against *M. gerlachei* in different taxa.

### 3.3 Cytotoxicity against sea urchin embryos

A total of 17 species were tested, belonging to the Phyla Porifera (9), Cnidaria (1), Tunicata (2), Bryozoa (2), Annelida (1), Nemertea (1) and Algae (1). The toxic extracts (extracts outside confidence intervals described above) belong to Porifera (4), Cnidaria (1), Tunicata (2), Annelida (1), Nemertea (1) and Algae (1) (Fig. 6).
Fig. 6. Toxicity against development of *Sterechinus neumayeri* in different Phyla.

### 3.4 Cytotoxicity against sea urchin sperm

A total of 20 species (24 extracts) were tested. All the extracts except one were toxic to sperm (Fig.7) (<25% of the sperm was active) at the maximum concentration (1 mg ml⁻¹) and about 90% of the samples tested were active at the intermediate concentration. These extracts belonged to the taxa Porifera (4), Cnidaria (3), Bryozoa (2), Echinodermata (2), Annelida (6), Nemertea (1) and Hemichordata (3). Finally, 13 (15 extracts) out the 20 tested species (65%) were toxic to sperm at the lowest concentration.
**3.5 Antifouling activity**

We evaluated 130 extracts (70 species) from Porifera (22), Cnidaria (7), Tunicata (4), Bryozoa (14), Echinodermata (3), Annelida (6), Nemertea (1), Hemichordata (4), Algae (8) and others (1). A total of 28 extracts (24) were active (30.8%) from Porifera (5), Cnidaria (3) Tunicata (1), Bryozoa (3), Echinodermata (2), Annelida (3), Hemichordata (1) and Algae (2) (Fig. 8). This means all these active extracts produced zones of growth inhibition larger than 2 mm.
4. Discussion and conclusions

The Antarctic benthos appears to be greatly regulated by chemical interactions, mainly interfering with competence and predation (Fig. 9). Predation seems to be largely driven by the omnivorous sea star *O. validus*, known to have a noteworthy and extensive diet (McClintock et al., 2010). According to the high predation pressure described for this asteroid (Dayton et al., 1974), our results showed that repellence to avoid sea star predation is present in almost all the zoological groups of Antarctic invertebrates. This is demonstrated by the fact that more than 50% of the studied species of the main taxonomical groups exhibited significant deterrent activities. In agreement with these findings, previous experiments already demonstrated strong feeding deterrence towards this sea star in the opisthobrach molluscs *Austrodoris kerguelenensis* (Gavagnin et al., 2000; Iken et al., 2002) and *Bathydoris hodgsoni* (Avila et al., 2000). In the present survey, the tunicates exhibited the highest repellent activity (73%). Not surprisingly, the tunicate *Aplidium falklandicum* was recently found to possess particular alkaloid metabolites, the meridianins (A-G), responsible for this deterrent activity when tested isolated (Núñez-Pons et al., 2010). The phylum Porifera resulted to be also a quite active group (51%) and, in fact, other studies sustain this strong feeding deterrence reported for Antarctic sponges (21 species active out of the 27 species tested; Peters et al., 2009). Our study also found that 60% of the echinoderm samples were unsuitable for *O. validus*, although this species is known to feed on another seastars, namely *Acodontaster conspicuus* (Dayton et al., 1974). Cnidarians have already demonstrated the presence of chemical defenses, like the gorgonian coral *Ainigmaptilon antarcticus* (Iken & Baker 2003), and our results support this with more than 50% of the studied species (58%)
being active. The bryozoans displayed a similar deterrence as the cnidarians (58%), and also the polychaetes (57%), although this group was much less represented in number of samples tested. In fact, bryozoans have also been reported to be part of the diet of *O. validus* (Dayton et al., 1974). Finally, the algae seem also a potential food for this asteroid. Dearborn (1977) found diatoms, as well as red algae, in the stomach contents of *O. validus*. However the lower activity (14% of species) found in our study for this group may indicate a carnivorous preference of the star, despite being described as an opportunistic omnivorous consumer. All these results support the idea that many species from most of the phyla of Antarctic marine benthic invertebrates studied contain chemical defences against this voracious generalist sea star.

![Diagram of the proposed model of chemical ecology interactions according to the results obtained in our experiments](https://www.intechopen.com)

Feeding preferences tested towards the amphipod *C. femoratus* revealed a high repellent activity in both benthic invertebrates and algae. Among the four majorly represented groups, the cnidarians displayed the highest incidence of feeding deterrent activities (85%), followed by the macroalgae (75%), the tunicates (66%), and finally the sponges (46%).

Previous experiments with different species of Antarctic gammarid amphipods suggested that many macroalgae had feeding deterrent properties (Huang et al., 2006). It is worth to note that the amphipod used here, *C. femoratus*, has never been used previously as putative consumer in feeding assays. However, its voracious scavenger-omnivorous habits turn this mesograzer into a very suitable organism to test chemical defense. Actually, Bregazzi (1972) reported a large variety of food items (from algae to copepods and euphausiid larvae) in the stomach contents of this species. The impressive unpalatable activities recorded in our samples could be explained by the fact that, as many benthic amphipods, *C. femoratus* may use sessile organisms (mainly algae and sponges, but also others) both as host (biosubstrata) as well as a potential prey. This exerts a localized, constant pressure, which could be more intense than that caused by wandering mobile predators, such as sea stars or fish (Toth et al., 2007). Another species used as a model in previous Antarctic bioassays is the sympatric omnivorous amphipod *Gondogeneia antarctica*. This species, however, has repeatedly shown the problem of finding artificial foods too attractive, causing a phagostimulatory effect on the crustacean, and hence making the results obtained hard to interpret (Amsler et al., 2005, 2009a and b; Iken et al., 2009; Koplovitz, et al., 2009). Instead, the amphipod *C. femoratus* seems to possess a quite discriminatory potential to detect unpalatabilities, as observed in our results. Only bryozoans and echinoderms did not seem to produce a repellent effect against the mesograzer, but very few species were tested compared to other groups and, therefore, more samples have to be assayed before establishing any general conclusion for these groups.

Regarding the toxic activity against the copepod *M. gerlachei*, all groups except Hemichordata had some active species. Echinoderms showed, surprisingly, the highest activity (100%). Why this happens remains unknown at the moment, since *M. gerlachei* is omnivorous, feeding on phytoplankton, copepod eggs and small metazoans (Metz & Schnack-Schiel, 1995). Toxicity against the copepod could be a very useful mechanism to avoid the competition for phytoplankton food in benthic filter feeders, such as tunicates, sponges and bryozoans, and this is probably what our results reflect. No comparable results are currently available in the literature.

Few experiments have tested the activity from extracts of Antarctic benthic invertebrates against sperm and early life stages of the common Antarctic echinoid *S. neumayeri* (e.g. McClintock et al., 1990, 1992; Heine et al., 1991; Slattery et al., 1995) and, therefore, our contribution may give a wider idea of these cytotoxic mechanisms. In our tests, the sperm toxicity against *S. neumayeri* was evident in all species (100%) except in the group of Hemichordata (66% of species), similarly to what happened with the copepod test. Cytotoxicity against the development of this sea urchin was 100% in almost all groups, except sponges (44%) and bryozoans (0%), although we tested more samples of sponges than other groups. Pearse & Giese (1966) reported that *S. neumayeri* fed mostly on diatoms. Some studies have reported that the grazing by this sea urchin could be the responsible for significant mortality of settling larvae and juveniles of benthic invertebrates (Bowden, 2005; Bowden et al., 2006). Moreover, the settlement of pelagic larvae on or near their prey is
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frequent in marine predator invertebrates (Pawlik, 1992). Therefore, the presence of cytotoxic compounds found in this study for so many species may play an important role as a mechanism of defence/competence, reducing the recruitment of this sea urchin and, consequently, the grazing pressure and the colonization of the surface (e.g. McClintock et al., 1990; Bowden et al., 2006).

Different marine organisms such as bacteria, algae and invertebrates colonize submerged surfaces. The bacteria are metabolically versatile organisms capable of colonizing multiple surfaces, so it is not surprising that most organisms and, especially, the filter-feeders that are likely to be in trouble if the fouling is intensive, produce defenses against bacterial colonization. In the past, only a few studies were carried out to test the antifouling activity of Antarctic invertebrates against sympatric bacteria. Peters and colleagues (2010) tested the antifouling activity of extracts from Antarctic demosponges isolating bacteria from the surface of them and the majority of extracts did not display an inhibition activity. The reason could be that these bacteria were resistant to the substances from these sponges and, therefore, they were growing on their surface. Contrary to these results, the antifouling activity found in our tests is quite apparent in representatives of most taxa, including sponges (22%). In our case, bacteria were isolated from the water and we tested different species. This could be the cause of a higher activity in our results. Moreover, echinoderms (66%) and polychaetes (50%) were the most actives, followed by the cnidarians (42%). This is in agreement with previous results reporting antimicrobial activity in the soft corals Alcyonium paessleri and Gersemia antarctica (Slattery et al., 1995). Tunicates, pterobranchs and algae had the same percentage of active species (25%). This is quite high if compared to the antimicrobial activity detected in only one (Distaplia colligans) out of 14 tunicate species tested previously (Koplovitz et al., 2011). The fact that the species tested are different could be the reason for these discrepancies. Compared with other taxa, bryozoans exhibited less activity (21%) and nemertins did not show any activity, although the number of species tested in these groups was too low to allow further considerations.

In summary, our results show that many different benthic organisms showed different strategies of defense, protection and/or competition (Fig. 9). However, it is important to emphasize that, not all phyla were equally tested, and therefore conclusions have to be considered cautiously. In many cases we found different defensive mechanisms in the same organism. This was previously reported for the Antarctic soft corals Alcyonium paessleri and Gersemia antarctica, which possess compounds with feeding-deterrence, antifouling and toxicity properties (e.g. Slattery & McClintock, 1995, 1997; Slattery et al., 1995). In our case, many species of Porifera and Cnidaria were active in all experiments (frequently over 40% or more of the tested species) and most Tunicata species quite as well (over 50% of species). In contrast, the Antarctic tunicates of the genus Aplidium displayed notable repellent activity in the feeding experiments (Núñez-Pons et al., 2010), but not in those of antibacterial nor antifouling.

Not surprisingly, other taxonomical groups were active in just one or a few tests, thus indicating the presence of one or only a few defensive lines (Fig. 9). For example, the phylum Bryozoa exhibited more activity in the experiment of feeding-deterrence against O. validus (58% of species) in relation to the antifouling experiment (21%) and no activity was found in the cytotoxicity experiment. The reason of these differences may be the presence in
species of this phylum of physical defenses, such as avicularia, used for different roles, such as the prevention of the settlement of epibionts or larvae (e.g. Harmer, 1909). Moreover, in some groups, such as Annelida, only some species were active, and possibly, they have other types of defences not tested here, or physical protection strategies, such as living in their own bio-constructed tubes.

To our knowledge, this is the first ecological model proposed for describing the interactions in the Antarctic marine benthos, considering a wide array of possible chemical ecology relationships. We believe that these interactions are mainly generated to prevent the strong pressure of competition for space and/or food, predation and fouling to which Antarctic organisms are exposed. This general model shows an amazingly complex network of interactions between Antarctic organisms (Fig. 9). Further studies with larger number of samples are needed to complete and enrich this model and to bring some light to the existing gaps of knowledge. Nowadays, the research in marine chemical ecology in Antarctica continues to grow and new data will help to further advance in our knowledge on the role of chemical compounds in the Antarctic benthos. In order to successfully accomplish this task, the close collaboration among ecologists, chemists and microbiologists is essential. Also, further studies, such as those regarding antifouling and cytotoxicity activities, are needed to determine the ecological relevance of these mechanisms in Antarctic environments. Moreover, the bulk of the research in chemical ecology has been done on the phylum Porifera (Paul et al., 2011) compared to the few studies carried out in other phyla, such as Bryozoa, Annelida, Nemertea and Hemichordata, which are quite understudied. To fully understand this "chemical network", we will expand our studies to more types of experiments and more organisms during the development of our current project, ACTIQUIM-II.

5. Acknowledgements

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6. References


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Marine ecosystems, a very wide topic, includes many different processes, groups of organisms and geographical peculiarities. The objective of this book is to present various topics of great importance for understanding the marine ecosystems, what they are, how they work and how we can model them in order to forecast their behaviour under changing conditions. They have been thoroughly reviewed and accepted for publication. The chapters cover aspects such as: Threats to ultraoligotrophic marine ecosystems (Ch. 1); Modelling the pelagic ecosystem dynamics: the NW Mediterranean (Ch. 2); The marine ecosystem of the Subantarctic, Prince Edward Islands (Ch. 3); Meiofauna as a tool for marine ecosystem biomonitoring (Ch. 4); Chemical interactions in Antarctic marine benthic ecosystems (Ch. 5); An Interdisciplinary Approach on Erosion Mitigation for Coral Reef Protection- A Case Study from the Eastern Caribbean (Ch. 6); A revisit to the evolution and ecophysiology of the Labyrinthulomycetes (Ch. 7); Seabed mapping and marine spatial planning: a case-study from a Swedish marine protected area (Ch. 8); Management strategies to limit the impact of bottom trawling on VMEs in the High Seas of the SW Atlantic (Ch. 9); Hydrocarbon contamination and the swimming behavior of the estuarine copepod Eurytemora affinis (Ch. 10), and Interactions between marine ecosystems and tourism on the Adriatic and Mediterranean (Ch. 11).

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