



# To change or not to change: drivers of defensive secondary metabolite distribution in the red macroalga *Plocamium* sp.

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## Abstract

Understanding the spatial distribution of intraspecific variation, like defense phenotypes of sessile organisms, is important for identifying drivers of ecosystem structure. Secondary metabolites are produced as chemical defenses and organisms may have varying types and concentrations of different metabolites (i.e., chemogroups). In a small study area, the Antarctic red macroalga *Plocamium* sp. produced 15 chemogroups. *Plocamium* sp. plays an integral role in the ecosystem by supporting numerous mesograzers, providing them protection from predation. None of these grazers, other than the amphipod *Paradexamine fissicauda*, are known to consume their host. However, *P. fissicauda* feeding rates differ among *Plocamium* sp. chemogroups and they carry fewer embryos when maintained on a subset of chemogroups. We collected *Plocamium* sp. along transects between 5 and 29 m depth at sites near Anvers Island (64° 46.5' S, 64° 03.3' W) in 2017 and 2018 to examine previously documented site-specificity in chemogroup distribution. We confirmed that chemogroup assemblages differed significantly among sites. Chemogroups vary among depths within sites, but not laterally among transects. A yearlong reciprocal transplant experiment revealed very few changes in chemogroup expression, suggesting that the environment cannot change the chemogroup produced by a thallus. The few changes which occurred were mainly among chemogroups with the same two major compounds but differing in their relative quantities. Hence, the distribution of chemogroups is likely driven by the selection of beneficial genotypes and gene flow. As chemical defenses can have profound effects on the presence of other species, it is important to determine what drives their spatial distribution.

**Keywords** Chemogroup · Phenotypic diversity · Chemical ecology · Environment · Seaweed · Rhodophyta

## Introduction

Phenotypic diversity among individuals plays an important role in the success of a population within an ecosystem as well as its ability to respond to long-term environmental changes (Norberg et al. 2001). Without diversity there would

be nothing for selection to act on (Féral 2002). Differences in phenotypic traits can be observed within and among populations, but how much of this variation can be attributed to the environment or to genetics? For sessile marine organisms, particularly those with limited dispersal, the relative contributions of the environment, genetics, or both are important in terms of their fitness (Hu et al. 2020) including with respect to competition for space, finding a mate, and predator avoidance.

Herbivore defense in macroalgae includes both structural and chemical defenses (discussed in Hay and Fenical 1988; Amsler 2008). Species may exhibit varying degrees of either or a mixture of both. Generally, a higher degree of defense has been associated with greater grazing pressure (Hay 1984; Paul and Fenical 1986; Hay and Fenical 1988). Chemical defenses typically involve secondary metabolites which were historically believed to play no role in the primary metabolism of the organism (Williams et al. 1989; Delgoda and Murray 2017). However, more recent evidence

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suggests that these molecules can have regulatory functions or serve as precursors to primary metabolites (Böttger et al. 2018; Erb and Kliebenstein 2020). Secondary metabolites may not only deter feeding but also aid in competition for space, anti-fouling activity, or pathogen resistance (Young et al. 2015). Therefore, chemical defenses are important when it comes to the abundance and success of their producers which can have significant effects on the structure of marine ecosystems (Marques et al. 2006; Paul et al. 2007).

Intraspecific diversity in secondary metabolite production has been studied across a variety of marine taxonomic groups, including algae (e.g., Puglisi and Paul 1997; Thornber et al. 2006; Heiser et al. 2020; Shilling et al. 2021), sponges (e.g., Noyer et al. 2011; Rohde et al. 2012; Reverter et al. 2018), cnidarians and bryozoans (e.g., Martí et al. 2005). Often there is a geographic pattern to the distribution of this diversity (e.g., Puglisi and Paul 1997; Martí et al. 2005; Noyer et al. 2011; Rohde et al. 2012; Reverter et al. 2018; Shilling et al. 2021), raising the question as to whether the environment, genetics, or both affect the production of chemical defenses.

It is generally believed to be costly for an organism to produce secondary metabolites. Several hypotheses have been postulated to explain inter- and intraspecific variation in secondary metabolite production with varying degrees of support (reviewed in Pavia and Toth 2008). They all attempt to predict when it would be more beneficial to produce secondary metabolites over investing in growth or reproduction. One obvious example would be induced defenses which are only produced when grazers are present, as observed in the brown macroalga *Dictyota menstrualis* (Cronin and Hay 1996). If resources affect the production of secondary metabolites, temporal or geographic variation in their production is to be expected. In species of the red macroalgal genus *Portieria*, site-specific monoterpene production was occasionally observed (Puglisi and Paul 1997; Payo et al. 2011). However, while there is some support that concentrations of some but not all monoterpenes in *Portieria hornemannii* were affected by nutrient enhancement, shading, and the time at which samples were collected from the field (Puglisi and Paul 1997), other species such as the red macroalga *Laurencia dendroidea* showed no differences in secondary metabolite levels with irradiance or nutrient enhancement (Sudatti et al. 2011). When transplanting *P. hornemannii* for 1 month or the sponge *Stylissa massa* for 3 months between locations at which different secondary metabolites were predominantly produced, no changes were observed (Matlock et al. 1999; Rohde et al. 2012). In addition, intra-individual variation has been found in some species, such as the green macroalga *Halimeda macroloba*, where new and less-calcified growth has higher secondary metabolite levels than the older and calcified parts of the thallus (Paul and Van Alstyne 1988). Thus, despite known

variations in secondary defense production, the drivers of geographic secondary metabolite distribution are still poorly understood.

Phenotypic variation in secondary metabolite production in the red algal genus *Plocamium* has been studied in many locations around the world (Heiser et al. 2020). Individual thalli differ in the type and relative quantity of secondary metabolites they produce, which divides them into chemogroups (also referred to as chemotypes, Shilling et al. 2021). Differences in chemogroup production in various species of *Plocamium* have been attributed to geographic separation as well as seasonality (Capon et al. 1984; Blunt et al. 1985; König et al. 1990; Abreu and Galindro 1996; Young et al. 2013). The Antarctic *Plocamium* sp. has previously been identified as several species based on morphological variation, but molecular data support the more recent hypothesis that they all belong to the same species (Hommersand et al. 2009; Dubrasquet et al. 2018). However, not the species it has historically been placed in (*P. cartilagineum*) or any other named species (Hommersand et al. 2009; Dubrasquet et al. 2018). To date, it has not formerly been described as a species (reviewed in Heiser et al. 2020) but since we know that *P. cartilagineum* is not the correct name for it, we refer to it as *Plocamium* sp. On the western Antarctic Peninsula, *Plocamium* sp. is an abundant and widespread member in the understory of the macroalgal forests which as a whole exhibit high biomass and cover up to 80% of the hard substratum (Amsler et al. 1995; Brouwer et al. 1995; Heiser et al. 2020; Ko et al. 2020). Despite its unpalatability to grazers other than the amphipod *Paradexamine fissicauda*, it supports a very high number of mesograzers and provides them with an associational refuge from predation, making it an important member of the macroalgal forest (Amsler et al. 2005, 2013, 2022; Aumack et al. 2010; Zamzow et al. 2010). To date, 15 different chemogroups have been described in the Antarctic *Plocamium* sp. (Young et al. 2013; Shilling et al. 2021). They have site-specific distributions and an underlying genetic structure based on two main haplotypes, identified through the *cox1* gene, that are not distinct enough to be considered different species (Young et al. 2013; Shilling et al. 2021). The chemogroups, designated as A through O, are composed of over 60 different secondary metabolites of which eleven have been identified to date (Shilling et al. 2021). Not all metabolites are produced by all chemogroups and chemogroups also differ in the relative quantities of individual compounds (Shilling et al. 2021). Some chemogroups are consumed at a slower rate than others by their only known grazer, *P. fissicauda* (Heiser et al. 2022), which in nature occurs in densities on *Plocamium* sp. that are one to two orders of magnitude higher than on other common macroalgae (Huang et al. 2007).

The observed geographic distribution of chemogroups could be driven by the environment, genetics, or an interaction between the two. The objective of this study was to

determine the direct influence of the environment on chemogroup production in *Plocamium* sp. To begin to address this, we described fine-scale, within- and among-site distribution patterns of chemogroups in *Plocamium* sp. to expand on their previously known broader-scale geographic distribution. We further tested the hypothesis that the environment affects chemogroup production by conducting reciprocal transplant experiments between sites with observed differences in chemogroup composition. Counts of the grazer *P. fissicauda* on *Plocamium* sp. were taken to attempt to evaluate whether some of the biotic environment could be correlated with chemogroup distribution.

## Materials and methods

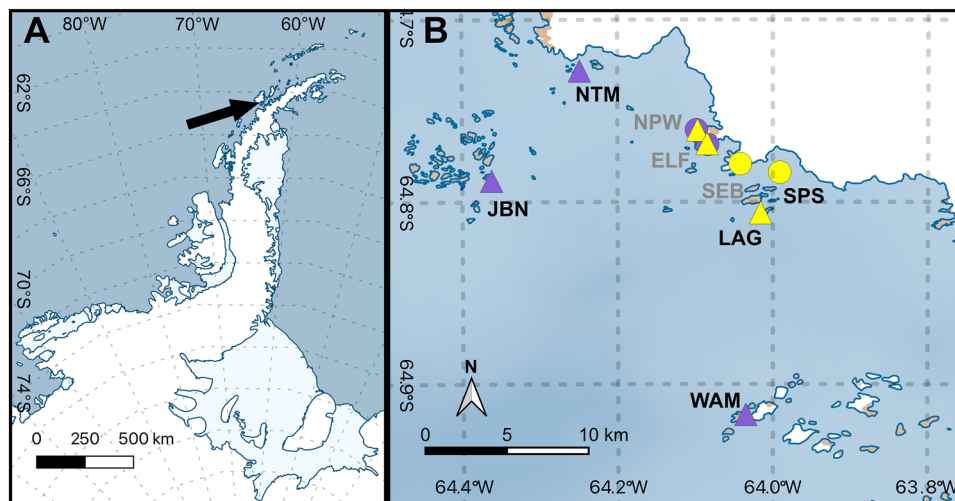
### Transect collections

Subtidal transects using SCUBA were conducted from April to May 2017 and April to June 2018. To assess whether depth, and therefore, potentially light, affects chemogroup production, we collected five *Plocamium* sp. thalli at every 3 m-depth interval between a depth of 5 m and 29 m (i.e., at 5 m, 8 m, 11 m, 14 m, 17 m, 20 m, 23 m, 26 m, and 29 m). At five sites, three parallel transects were run perpendicular to the shore which resulted in the transects going down a slope (Supplemental Fig. 1). The replicated transects within a site were spaced 30 m apart (determined with a tape measure at

a depth near the center of the transect depth range). Two of the five sites were surveyed in 2017 (Southeast Bonaparte and Stepping Stones) and three of the five sites in 2018 (East Litchfield, Laggard Island and Norsel Point). All five sites were within a 4-km radius of the United States Antarctic Program's Palmer Station on Anvers Island ( $64^{\circ} 46.5' S$ ,  $64^{\circ} 03.3' W$ ). For these sites, linear distances between depths were measured using a tape measure to account for differences in slope between and within sites. Three additional sites (Casey Islands, Howard Island, and Wauwermans Islands) which were surveyed with a single transect in 2018 were located between 10 and 16 km from Palmer Station (Fig. 1). Individual transects were usually completed within two dives (two-thirds were completed within a single dive).

All the sites where we collected thalli were away from active glacier faces and, therefore, similar in turbidity (authors' personal observations) which means that light should primarily vary with depth. Nutrients in Antarctic waters, including near Palmer Station, have historically been high (Peters et al. 2005) and only rarely reach levels considered growth-limiting for macroalgae (Wiencke and Amsler 2012). The Southern Ocean generally has a deep mixed layer which results in a homogenous thermal layer down to 50–100 m (Knox 2007) making the possibility of a temperature gradient within depths reached through SCUBA unlikely.

Each algal thallus was bagged separately and the first thallus at each depth in each transect was carefully floated



**Fig. 1** **A** Location of Palmer Station on the western Antarctic Peninsula indicated by the black arrow. **B** Sampling sites for *Plocamium* sp. around Palmer Station (Anvers Island). Circles indicate transects collected in 2017, triangles indicate transects collected in 2018 (NPW and ELF were sampled in both years). Yellow indicates three full transects collected at the site, purple indicates partial transects. JBN—Howard Island in the Joubin Islands archipelago (United States

Antarctic Program (USAP) Joubin Island # 1). NTM—Casey Islands. NPW—Norsel Point. ELF—East Litchfield. SEB—Southeast Bonaparte. SPS—Stepping Stones. LAG—Laggard Island. WAM—Wauwermans Islands (USAP Wauwermans Island # 564). Gray letters indicate sites included in the transplant experiments. Coordinates can be found in Supplemental Table 1. Maps were prepared using QGIS version 3.22 and the Quantarctica package (Matsuoka et al. 2018)

into a fine mesh bag following Huang et al. (2007) for enumeration of associated *Paradexamine fissicauda*. A third member of the dive team recorded qualitative natural history observations by noting the substrate type, and by describing the macroalgal as well as the sessile invertebrate community at each depth. For the five sites closer to Palmer Station, these qualitative observations were made on the same dive, whereas for the more distant Howard and Wauwermans Islands transects, for logistical reasons such observations were made on scouting dives preceding the collection dives. Accordingly, the observations were close to the transects but not exactly on them.

Thalli from all transects were returned to Palmer Station where they were held in flow-through tanks with ambient seawater conditions for no more than 36 h until they were photographed, inspected for reproductive structures, and frozen for chemical analysis at  $-20\text{ }^{\circ}\text{C}$ . In 2017, a subset of the thalli was analyzed at Palmer Station to determine their chemogroups for the transplant experiments. The abundance of *P. fissicauda* on thalli was determined by carefully searching and removing the amphipods individually. In our experience, *P. fissicauda* holds tightly to the branches of *Plocamium* sp. and is not easily dislodged. Afterwards, the thalli were spun dry and their wet weight was determined after removal of other mobile epifauna to standardize the number of *P. fissicauda* by wet weight of *Plocamium* sp.

### Chemogroup determination

The chemogroups were originally described in Shilling et al. (2021) and we followed the same methodology. The wet weight of the thalli was taken before extraction. We aimed to have at least 4 g of the algal thallus (wet), but when the size of the collected thallus was smaller, we used less. After analysis on the GC (described below), ACD/Labs Spectrus Processor 2016.2 (ACD/Labs, Toronto, Ontario, Canada) was used to compare the profiles of each thallus visually to previously established chemogroups A through O (Shilling et al. 2021, Supplemental Figs. 2–3). The relative integrated peak area of metabolites previously determined to be characteristic of each chemogroup was the defining criterion for chemogroup assignment (Shilling et al. 2021).

Thalli were extracted in 3:1 dichloromethane:methanol for metabolomic analysis. The organic layer of each extract was then filtered ( $0.45\text{ }\mu\text{m}$  PTFE membrane), completely dried under streaming  $\text{N}_2$  gas and weighed prior to final preparation at  $25\text{ mg/mL}$  in MeOH for analysis. The thalli from the 2017 transects and the thalli initially included in the transplant experiment were analyzed at Palmer Station using an Agilent 6980A GC with a flame ionization detector and a Zebtron ZB-5HT Inferno column following Shilling et al. (2021) so that we were able to determine where to place the experiments and which thalli to include to ensure equal

representation of chemogroups. Thalli from full transects in 2017 and 2018 as well as those collected at the end of the transplant experiments were (re)analyzed at the University of South Florida (USF) using an Agilent 7890A GC with flame ionization detector and a Zebtron ZB-5HT Inferno column following the same methodology (Shilling et al. 2021).

### Chemogroup community composition

We used the PRIMER version 7 software (Quest Research Limited, Auckland, New Zealand) to analyze the distribution of chemogroups among and within sites. We used statistical methodology following the recommendations of Clarke et al. (2014) and Somerfield et al. (2021). PRIMER is particularly well suited to compare species communities among sites, treatments, or other factors. A sample is usually a representation from within a factor for which the amount of each species is quantified (e.g., a specified volume of a sediment sample, Clarke et al. 2014). In this case, we treated the five thalli from a depth on a transect as a sample and counted the number of times each chemogroup occurred. If no thalli were collected, that specific depth was excluded from the analyses. The samples were standardized to account for differences in number of thalli and then because chemogroup abundances varied greatly between samples, the data were square-root transformed to down-weight the influence of the most abundant chemogroups. The aim was to compare chemogroup community composition among sites and horizontally as well as vertically within sites. To compare the similarity between samples, Bray–Curtis coefficients were calculated which were used to perform a two-way crossed Analysis of Similarities (ANOSIMs). We used the factors site and transect in one analysis where the replicates were the samples collected across depths within a site. We also ran an ANOSIM with the factors of site and depth where samples collected on the three transects within a site were treated as replicates. ANOSIM allows one to determine whether factors are ordered or not. We treated the factors site and transect as unordered, whereas depths were treated as ordered since the same set of depths were sampled across sites, following recommendations by Somerfield et al. (2021). All the sites and transects were included in the ANOSIM analyses, except for partial transects in 2017.

We then performed a variation partition analysis using the *varpart* function in the *vegan* package (Oksanen et al. 2020) in R version 3.6.3 (R Core Team 2020). Data were transformed using the “hellinger” transformation and it was confirmed that the factor transect did not have a significant effect on chemogroup assemblage. Therefore, only site and depth were included in the variation partition analysis.

For the subsequent CLUSTER analysis, transects within sites for each depth were combined since the ANOSIM showed that there were no significant differences among the

transects. A similarity profile permutation test (SIMPROF) was used within CLUSTER analysis to assign and compare chemogroup assemblages within and between sites with a 5% cut off. Combining the transects downweighed the influence of chemogroups occurring once within a depth when assigning SIMPROF groups.

The distributions of the chemogroups were plotted using the cowplot (Wilke 2020), dplyr (Wickham et al. 2021), ggplot2 (Wickham 2016), and ggpubr (Kassambara 2020) packages in R version 3.6.3 (R Core Team 2020).

## Transplant experiments

Reciprocal transplant experiments were conducted at the following three sites: Norsel Point, East Litchfield, and Southeast Bonaparte (Fig. 1). Initial surveys to choose the locations for these experiments were carried out based on a preliminary study that collected thalli at two depths from 19 sites in 2016 (Shilling et al. 2021). We were able to collect three full transects at Stepping Stones and Southeast Bonaparte in 2017 (Fig. 2) and analyze the samples at Palmer Station as described previously. Since we were approaching the end of our field season, we only collected and analyzed partial transects from East Litchfield and Norsel Point in 2017 (Fig. 5). We choose the three transplant sites based on differences in chemogroup abundances. The transplants utilized square cement substrates ( $45 \times 45 \times 6.35 \text{ cm}^3$ ) as anchors with the basal portions of algae inserted into braided ropes spanning the width of the substrates supported by fiberglass racks as described by Fairhead et al. (2006) and Schoenrock et al. (2013). Four substrates per site were placed at 23 m, a depth chosen to reduce the chance of loss due to small iceberg scouring over the course of the year-long deployment. At Norsel Point, two additional substrates were placed at 11 m because initial chemogroup sampling revealed that this site had distinct chemogroup depth distributions (Fig. 5). We only analyzed one substrate from each site (and depth) since each substrate included sufficient replicates but chose to deploy several to increase the likelihood of recovery after a year. Chemogroups A, D, F, L, and M were included since, based on analyses of thalli at Palmer Station, those were the chemogroups present at the three sites. Thalli collected throughout the season from the three sites included in the transplant experiments were kept in individual bags with holes to facilitate water flow in a 3800 L flow-through tank at ambient seawater conditions. The chemogroups of the thalli were determined at Palmer Station so we knew which thalli to include in the transplant experiments. Where possible the same thallus was split among sites to address directly whether, if any changes occurred, chemogroup production would differ among the sites. A subset of the transplanted thalli was returned to the site which they originated from. Five replicates of each chemogroup

originating from different thalli were attached to ropes in the laboratory with the randomly assigned placement of chemogroups. The thalli were blotted dry and weighed using a Mettler Toledo PM4600 (0.01 g readability; Mettler Toledo, Columbus, Ohio, United States) before attachment. Initial weights were between 0.25 and 2.8 g. Seven ropes per concrete substrate with two to four thalli attached to each were deployed using SCUBA in May 2017. In February 2018, the transplant experiments were collected and redeployed after taking portions from thalli where enough tissue was available. The minimum amount required for chemogroup determination was sampled and frozen for analysis in case the experiments could not be recovered at the end of the field season. In May 2018, the transplanted thalli were all successfully recollected and thalli were frozen for chemogroup determination at USF. Chemogroups were determined qualitatively by comparing the profiles to chemogroups A through O in Shilling et al. (2021; also see Supplemental Figs. 2–3) and to the profiles of the same thalli from 2017. Where possible, fresh growth was sampled separately. We attempted to assess the growth rate by weighing thalli before and after transplanting them, but they had often formed secondary attachments to the substrates and many were broken upon retrieval.

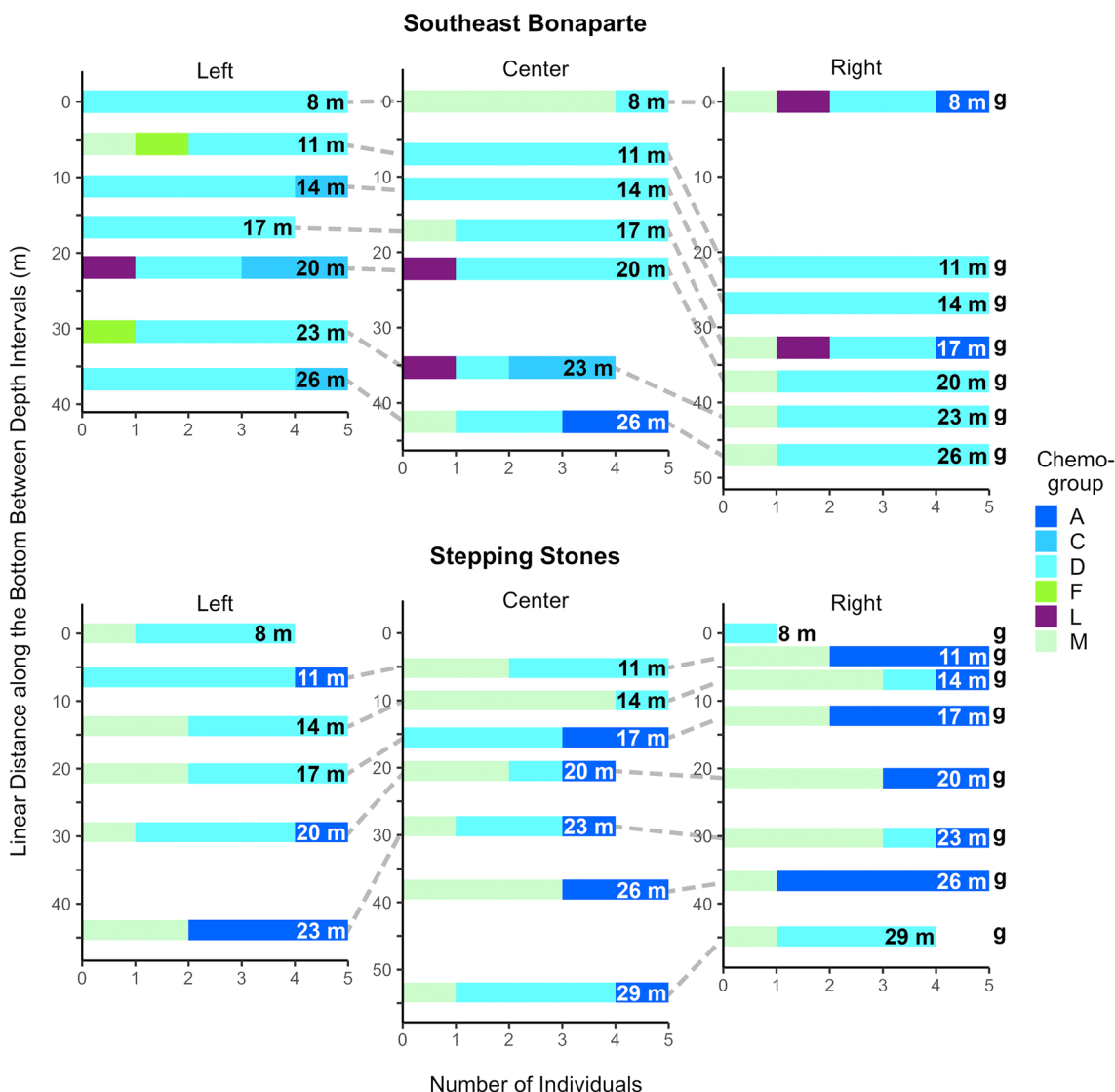
## Statistical analysis of amphipod counts

To test whether the abundance of the only known grazer, *P. fissicauda*, varies among chemogroups, we compared the counts of the amphipod across sites, depths, and chemogroups. We used the Kruskal–Wallis rank sum test in R version 3.6.3 (R Core Team 2020) because there was an unequal sample size between factors.

## Results

### Transect collections

Natural history information for all sites except the Casey Islands can be found in Supplemental Table 1, including data on the linear distances between each depth that was sampled. In brief, at East Litchfield, Norsel Point, Southeast Bonaparte, Stepping Stones, and the Wauwermans Islands site, the deeper depths had sediment present mixed with cobble. At most sites, the substrate changed from cobble to boulders and, finally, bedrock as it was becoming shallower. Generally, the overstory was dominated by the brown macroalga *Himantothallus grandifolius* interspersed with the brown macroalgae *Desmarestia anceps*, *D. antarctica* and *D. menziesii*, some of which often became more dominant at shallower depths. At Norsel Point, the brown macroalga *Cystosphaera jacquinotii* was found in



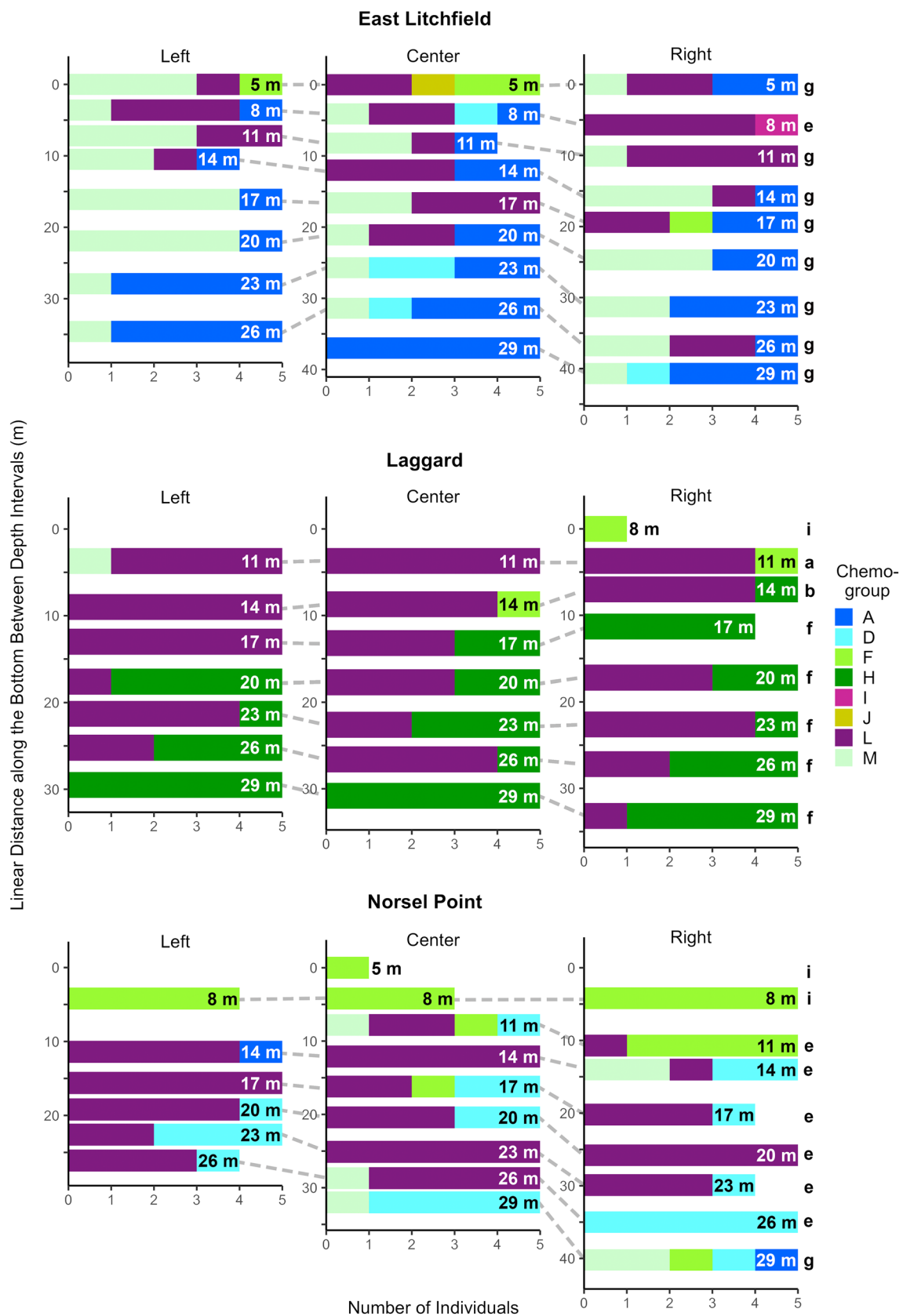
**Fig. 2** Distribution of *Plocamium* sp. chemogroups among three transects and nine depths from two different sites around Palmer Station (Anvers Island) in 2017. Each set of three plots represents one site with the respective name indicated above. Each depth had five thalli collected unless *Plocamium* sp. was not abundant enough or the site was not deep enough (number of thalli are indicated on the horizontal axes). The transects are arranged from left to right when oriented towards the shore and were each 30 m apart. The vertical axes show the linear distances along the bottom between the depths from which

thalli were collected (zero was chosen based on the shallowest sampling depth at a site). Since the slopes sometimes varied among transects within a site, the same sampling depths are connected by gray, dashed lines. The sampling depths are indicated in bold on the right side of each bar. Small, bold letters on the very right indicate the SIMPROF groups assigned based on chemogroup abundances (indicated by colors) of all thalli grouped together across transects for each depth. The same letters for SIMPROF groups mean that chemogroup community assemblages are similar

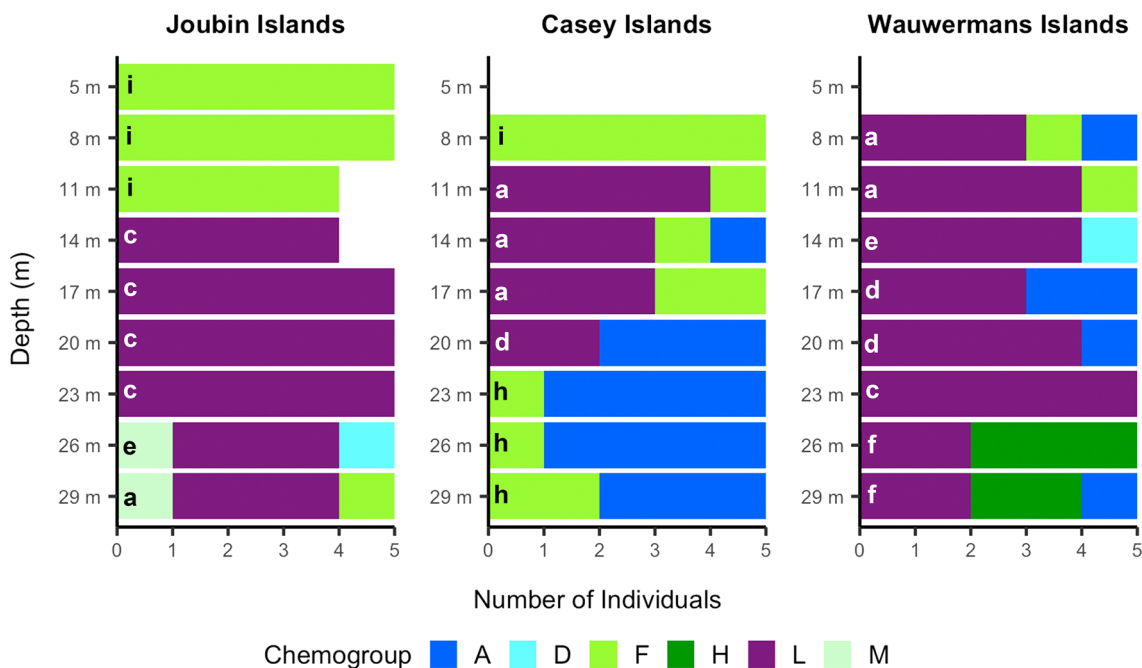
addition at deeper depths. Red macroalgae were generally found in the understory, covered by the brown macroalgae, but at sites such as Southeast Bonaparte they were without overstory cover at deeper depths. At East Litchfield, in addition, much of the bedrock was covered by crustose coralline algae.

**Chemogroup community patterns**

Chemogroups A, C, D, F, H, I, J, L, and M were identified from the sites included in this study (Figs. 2, 3, 4, 5). Chemogroups differ in the type of compounds present as well as the relative quantities they are producing

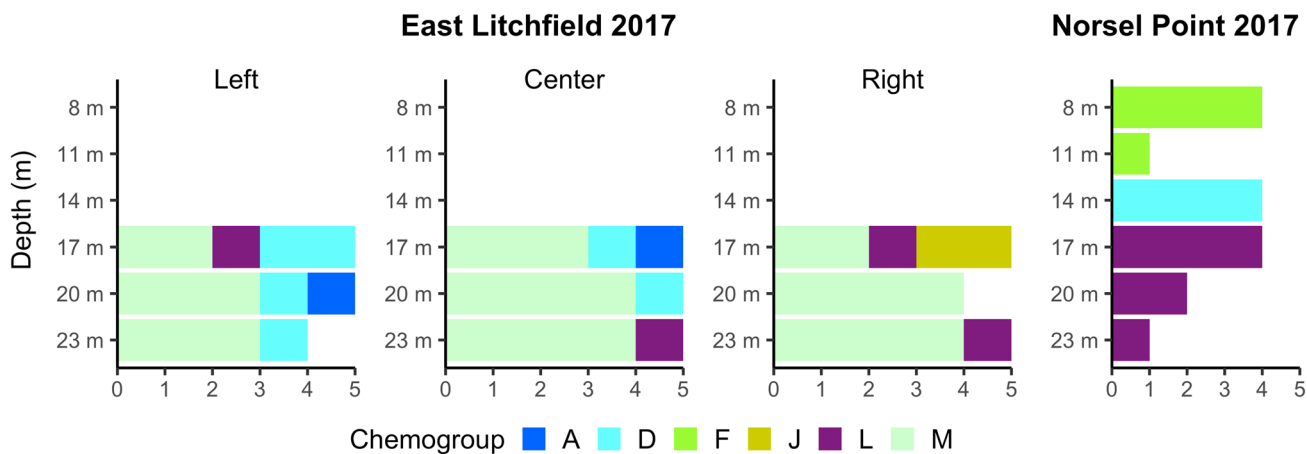


**Fig. 3** Distribution of *Plocamium* sp. chemogroups among three transects and nine depths from two different sites around Palmer Station (Anvers Island) in 2018. Details of the layout are the same as for Fig. 2



**Fig. 4** Distribution of *Plocamium* sp. chemogroups among one transect and nine depths from three different sites around Palmer Station (Anvers Island) in 2018. Each plot represents one site with their respective names indicated above. Each depth had five thalli collected unless *Plocamium* sp. was not abundant enough or the site was not deep enough (number of thalli are indicated on the horizontal axes).

The vertical axes show the depth from which thalli were collected. Linear distances between depths are not available for these sites. Small, bold letters on the left of each plot indicate the SIMPROF groups assigned based on chemogroup abundances (indicated by colors). The same letters for SIMPROF groups mean that chemogroup community assemblages are similar



**Fig. 5** Distribution of *Plocamium* sp. chemogroups at two sites near Palmer Station (Anvers Island) in 2017. At East Litchfield three transects, each 30 m apart, were sampled at three depths (depths are indicated on the vertical axis). At Norsel Point one transect was sampled at six depths. Each depth had five thalli collected unless *Plocamium*

sp. was not abundant enough (number of thalli are indicated on the horizontal axis). The transects are arranged from left to right when oriented towards the shore. Sites were only partially surveyed to determine the placement of reciprocal transplant experiments. Full transects were run in 2018

(Supplemental Figs. 2, 3, and Supplemental Table 2). Chemogroup L is the only chemogroup of those we found that we know to produce a cyclic halogenated monoterpene as its main compound (Supplemental Fig. 4 and

Supplemental Table 2). Chemogroups A, D, and M are very similar since they all produce the same two compounds as their major constituents but vary in the relative quantities they produce (Supplemental Figs. 3, 5).



A total of nine different chemogroups were found at the eight sites surveyed for chemogroup community patterns in 2017 and 2018 (Figs. 2, 3, 4). Chemogroups C, I, and J were only found once each in the transects. Chemogroup H was only found at two sites (Laggard Island and the Wauwermans Islands site). The other chemogroups (A, D, F, L, and M) were found at five to seven of the nine sites, but no chemogroup was found at all of the sites.

The two-way crossed ANOSIM for sites and transects indicated an effect of site ( $R = 0.494$ ,  $P = 0.0001$ ), but no effect of transect ( $R = -0.034$ ,  $P = 0.893$ ) on chemogroup assemblages. The two-way crossed ANOSIM for sites and depths indicated an effect of site ( $R = 0.645$ ,  $P = 0.0001$ ) and an effect of depth ( $R = 0.368$ ,  $P = 0.0001$ ) on chemogroup assemblages. Since  $R$  was larger and no differences between transects were observed, pairwise ANOSIM comparisons were only reported for factors of site and depth. When looking at the pairwise comparisons between sites, most of them were significantly different from each other with  $R$  values between 0.326 and 0.957 (Supplemental Table 3), except for: the Wauwermans Islands site and Norsel Point ( $R = 0.218$ ,  $P = 0.152$ ); and the Howard Island site and Norsel Point ( $R = -0.17$ ,  $P = 0.843$ ). Each pair of sites was dominated by similar chemogroups and only differed in the presence of additional chemogroups (see Supplemental Figure 6 for an overview of all sites).

Pairwise comparisons between depths showed that 17 m was the only depth that was not significantly different from any other depth in its chemogroup assemblages (Supplemental Table 4). Overall, there were fewer significant differences among the shallower depths (5–17 m) than the deeper depths (17–29 m; see Supplemental Table 4). Between the shallower and deeper depths, there were more significant differences (see Supplemental Table 4). Only 5 m and 29 m, and 14 m and 20 m were not significantly different from each other.

Variation partition analysis revealed that 46.8% of variation can be explained by the factors site and depth (Supplemental Figure 7) with 43.5% of variation explained by site and 4.1% of variation explained by depth.

A total of nine different SIMPROF groups were identified (small letters in Figs. 2, 3, 4, and Supplemental Fig. 8). Since the transects were combined, some among-site differences were masked. East Litchfield, Southeast Bonaparte and Stepping Stones were dominated by the same SIMPROF group g. Since the Howard Island, Casey Islands, and Wauwermans Islands sites all only had one transect each, there was more variation among depths in the SIMPROF groups. Laggard Island was mostly dominated by SIMPROF group f with three other SIMPROF groups in the shallower depths. Norsel Point had three SIMPROF groups which changed from shallow to deep.

The partial transects conducted in 2017 allowed us to qualitatively compare between years and assess inter-annual variation (Fig. 5). All three transects at East Litchfield were dominated by chemogroup M at 17 m, 20 m, and 23 m. In 2018, the same depths at East Litchfield were also dominated by chemogroup M as well as chemogroup A which was also present in 2017 but to a much lesser extent (Figs. 3, 5). One full transect was run in 2017 at Norsel Point which was close to the transect on the furthest right in 2018 where the shallow depth was similarly dominated by chemogroup F, and the deeper depths by chemogroup L (Figs. 3, 5).

## Transplant experiments

Out of 25 thalli on each substrate, seven to eight were missing upon recovery (Table 1). Between eight and thirteen thalli at each site produced the same chemogroups at both deployment and recovery. Between one and four thalli from each site changed slightly in their chemogroup profiles since most changes occurred among chemogroups A, D, and M which share the same two major compounds but vary in their relative quantities (Supplemental Fig. 3, 5). At East Litchfield, one thallus produced chemogroup A initially and chemogroup M upon final recovery. Six thalli that previously produced chemogroup M now produced chemogroup A (one each at East Litchfield, Norsel Point deep, and Southeast Bonaparte; three at Norsel Point shallow). At East Litchfield, one thallus that previously produced chemogroup D now produced chemogroup A in the old thallus part and chemogroup M in the new growth. Another thallus from East Litchfield which previously produced chemogroup M, now produced chemogroup O in the old thallus part and still produced chemogroup M in the newly grown thallus part. In addition, two thalli from Norsel Point deep which originally produced chemogroup D changed. One thallus now made chemogroup M and the other thallus made chemogroup M in the old thallus part and chemogroup D in the new growth. Another thallus changed chemogroup production from F to A which was the only time that a chemogroup other than A, D, or M was involved. The main compound in chemogroup A is a minor constituent among several compounds produced in chemogroup F (Supplemental Fig. 2). At three sites, two to six thalli from the different substrates had no detectable chemistry left at recovery. At East Litchfield, three of the four thalli that changed chemogroup production originated from East Litchfield before the transplant experiments (Table 1). At Norsel Point deep, one thallus that changed from chemogroup F to A originated from Norsel Point but a shallower depth (Table 1).

All thalli which had no chemistry left or changed chemogroup assignment had replicates originating from the same thallus in 2017 across all four analyzed substrates. Fourteen of the 2017 thalli changed their chemogroup designation

**Table 1** Results of transplant experiments of *Plocamium* sp. chemogroups between 2017 and 2018 at Palmer Station (Anvers Island)

2017: Original chemogroup	2018: Thalli missing	2018: Same chemogroup	2018: Different chemogroup	2018: No chemistry
<i>East Litchfield—ELF</i>				
A	3	1	1 M	ELF 0
D	0	4	1 <i>old: A, new: M</i>	SEB 0
F	2	3	0	0
L	1	4	0	0
M	2	1	1 A, 1 <i>old: O, new: M</i>	ELF 0
Total	8	13 (17)	4 (17)	0 (17)
<i>Norsel Point (Shallow)—NPW S</i>				
A	2	3	0	0
D	1	3	1 A	SEB 0
F	2	1	0	2
L	1	4	0	0
M	1	1	3 A	ELF 0
Total	7	12 (18)	4 (18)	2 (18)
<i>Norsel Point (Deep)—NPW D</i>				
A	4	1	0	0
D	0	1	1 M, 1 <i>old: M, new: D</i>	SEB 2
F	0	1	1 A	NPW S 3
L	1	4	0	0
M	2	1	1 A	ELF 1
Total	7	8 (18)	4 (18)	6 (18)
<i>Southeast Bonaparte—SEB</i>				
A	1	3	0	1
D	0	2	0	3
F	1	3	0	1
L	2	3	0	0
M	3	0	1 A	ELF 1
Total	7	11 (18)	1 (18)	6 (18)

Five thalli for each chemogroup were transplanted between four different sites. Where enough material was available old and new thalli pieces were sampled. When chemogroups were different in 2018, the new chemogroups were indicated next to the number of thalli that changed and the sites the thalli originated from were given. Some thalli did not have any detectable chemistry in 2018. The total number of thalli recovered per site are given in parentheses adjusted to the number of thalli missing in 2018

or lost all their chemistry. Only two of them were changes observed at a single transplant location. For all the other locations, replicates from the same original thallus had a different chemogroup assigned, lost all chemistry, or the thalli were completely lost at two or more transplant locations. Five of those replicated thalli had the described changes at all four transplant locations. On the other hand, most thalli that did not change in their chemogroup assignments were represented at two or more sites. Six thalli did not change their chemogroup production at three of the sites they were transplanted to, and two thalli at all four transplant locations.

### Amphipod counts

Many thalli collected during the transect surveys had no associated *P. fissicauda* so the sample size between factors varied considerably. None of the factors, site ( $H_5 = 4.367$ ,  $P = 0.498$ ), depth ( $H_8 = 8.201$ ,  $P = 0.414$ ), or chemogroup ( $H_5 = 5.543$ ,  $P = 0.353$ ) affected the amphipod's abundance. Wet weight of the thalli varied between 0.47 and 58.02 g with a mean  $\pm$  SE of  $5.93 \pm 0.91$  g,  $n = 86$ .

## Discussion

In this study, we described the fine-scale distribution of chemogroup diversity in the red macroalga *Plocamium* sp. which, to our knowledge, is the first detailed description of intraspecific chemical phenotypic diversity distribution for a subtidal species. We confirmed significant differences among sites in chemogroup assemblages. Within sites, the shallower depths were more homogeneous than the deeper depths. Overall, site explained more of the variation than depth. Reciprocal transplant experiments revealed that the environment is not affecting chemogroup production, at least for the yearlong duration of the experiment.

*Plocamium* sp. plays an important role in the ecosystem. By providing living space and protection from predation for a high number of mesograzers it is an autogenic ecosystem engineer (Amsler et al. 2005, 2013, 2022; Aumack et al. 2010; Zamzow et al. 2010). Autogenic ecosystem engineers, such as macroalgae and other benthic invertebrates like sponges, can have profound effects on the presence of other taxa (Marques et al. 2006; Paul et al. 2007). The prevalence of these autogenic ecosystem engineers is, in part, driven by their defenses and most studies to date have focused on the interspecific diversity of their defenses. Yet, we understand little about the drivers of intraspecific diversity in those defenses and the forces that structure their distribution.

In the Mediterranean sponge *Spongia lamella*, Noyer et al. (2011) found that dissimilarity in secondary metabolite composition increased with geographic distance between populations. However, this was not the case for populations located within 25 km of one another. In contrast, two species of the sponge *Haliclona* had significantly different metabolomes for samples taken within a relatively short distance (4–9 km), but samples from around 170 km away were no more different (Reverter et al. 2018). The same species also showed a marked difference in their metabolome during certain months of the year, suggesting seasonal variation (Reverter et al. 2018). The red alga *Plocamium cartilagineum* from New Zealand showed secondary metabolite variation between samples collected within 2 km of each other (Blunt et al. 1985). In Brazil, *P. brasiliense* collected from two locations about 30 km apart exhibited differences in secondary metabolite profiles between sites which also had marked differences in exposure and turbidity (Pereira and Vasconcelos 2014). In our study, the two sites furthest apart from each other, Casey and Wauwermans Islands, are within about 22 km of each other. Nevertheless, chemogroup assemblages differed significantly. However, within sites, differences among depths were more pronounced than differences between transects even though the distance between depths was always shorter (from 3.3 to 22 m) than between transects (30 m).

Paul and Van Alstyne (1988) found that the green macroalga *Halimeda opuntia* produced a higher concentration of the more deterrent of two metabolites under greater grazing pressure. Within the thalli of *Halimeda macroloba*, newer terminal segments, which mainly produce halimedatrial, were more deterrent than older basal segments (Paul and van Alstyne 1988). In Antarctic *Plocamium* sp., chemogroups D and H are consumed at a significantly slower rate by the amphipod *Paradexamine fissicauda* (Heiser et al. 2022) which is the only known grazer of *Plocamium* sp. among many mesograzers associating with the macroalga (Huang et al. 2007; Amsler et al. 2022). Chemogroup L, on the other hand, did not reduce the feeding rates of *P. fissicauda*, but significantly reduced the reproductive output of the grazer (Heiser et al. 2022). Altogether, 59% of the thalli collected in this study were producing these three chemogroups D, H, and L. In contrast, we did not find any significant differences in *P. fissicauda* abundance between sites, depths, or chemogroups. Overall, 76% of thalli collected did not have any *P. fissicauda* present, although other amphipods and gastropods were present but not identified or enumerated. To increase the likelihood of collecting genetically distinct thalli in the transects, we commonly selected more isolated and coincidentally smaller thalli (this allowed us to identify one single holdfast during collection) which may explain the low numbers of amphipods. Huang et al. (2007) found *Plocamium* sp. supported the second highest density of amphipods compared to seven other macroalgal species with *P. fissicauda* occurring at two to three orders of magnitude higher density on it than any of the other macroalgal species. However, the mean wet weight of thalli collected by Huang et al. (2007) was five times higher than in our study. Huang et al. (2007) also found a significant difference in amphipod densities between the two sites where *Plocamium* sp. was collected. Neither of the two sites was included in this study, but they may have differed in the chemogroups that were present. In addition, it is possible that the smaller and more isolated thalli collected in this study do not provide as much protection from predation as larger thalli which often occur in denser macroalgal assemblages (authors' personal observation). Overall, it is not possible to conclude whether *P. fissicauda* assemblages differ between sites depending on the dominant chemogroups. Even if they did, amphipod abundances might be driving the presence of chemogroups simply by some being consumed at a higher rate, or the presence of certain chemogroups may be driving the abundance of amphipods by reducing its reproductive output.

Environmental factors such as nutrients, light availability, salinity, or temperature have been shown to influence secondary metabolite production in macroalgae (Puglisi and Paul 1997; Palma et al. 2004; Sudatti et al. 2011), but this is not always reflected in a response to being transplanted between sites. When the red macroalga

*Portieria hornemannii* was transplanted between two sites at which the relative concentrations of two metabolites differed, no changes in secondary metabolite production occurred after about a month (Matlock et al. 1999). Effects of environmental factors can also vary between species or locations. For example, secondary metabolite production in *P. hornemannii* on Guam in the Western Pacific was affected by shading (Puglisi and Paul 1997). On the other hand, temperature had a stronger effect compared to light levels on secondary metabolite production in the Chilean *P. cartilagineum* (Palma et al. 2004). The highest concentration of compounds was produced at 15 °C compared to 11 °C and 18 °C (Palma et al. 2004). Seasonal thermal variations in the near-shore marine benthic ecosystem on the western Antarctic Peninsula vary with latitude (Barnes et al. 2006). At Palmer Station, while near-shore temperatures vary by approximately 4 °C over the course of a year, they can vary up to 2 °C within a single day (Schram et al. 2015a, b). The mixed layer depth in the Southern Ocean is generally below the depth where *Plocamium* sp. occurs (Knox 2007) providing a homogenous thermal environment throughout its depth range. In addition, our sampling was done within a few months during which water temperature only varied by 1–2 °C (Schram et al. 2015b). Temperature is, therefore, an unlikely factor explaining the variation in chemogroup assemblages among sites and depths in the present study.

Our results indicate that neither site nor depth affected chemogroup production after thalli had been transplanted for a year between sites. Most of the changes we observed at the end of the transplantation experiment were thalli that were no longer producing secondary metabolites, or which were not producing them at a high enough concentration to chromatographically determine their chemogroup with confidence. We never observed this in freshly collected samples from the field, and although the transplanted thalli looked normal, it is possible that they were in the early stages of senescence since *Plocamium* sp. is a perennial macroalgal species and we do not know how old thalli were when we collected them. The chemically defended brown macroalgae *D. anceps* and *H. grandifolius* are known to become available as a food source once they are dead, indicating that secondary metabolites degrade (Reichardt and Dieckmann 1985; Amsler et al. 2012). When *P. hornemannii* and the sponge *Stylissa massa* were recovered after transplanting for 1 and 3 months respectively, they also showed reduced concentrations of secondary metabolites (Matlock et al. 1999; Rohde et al. 2012). When only taking the recovered thalli into account which either produced the same or different chemistry, 67–92% of thalli per site did not change their chemogroup production. Most changes in chemogroups occurred between A, D, and M. These three chemogroups are composed of the same two main secondary metabolites and primarily differ in the relative concentrations of each

(Shilling et al. 2021). Hence, a change among those three chemogroups results from differing relative proportions of those main metabolites rather than producing completely different compounds. Even for the change of chemogroup F to A, the main compound in A was already present in chemogroup F as a minor constituent. Since we also found discrepancies in chemogroup A, D and M assignments for samples measured at Palmer Station and again at USF, it is possible that the storage and usage of slightly different instruments could have affected the detection of more minute differences in relative quantities of the main compounds. This is further supported by the fact that thalli returned to East Litchfield which originated from the same site also showed similar differences between deployment and recovery. Paul and Van Alstyne (1988) reported that *H. macroloba* had higher concentrations of certain compounds in freshly grown segments of its thalli. In our *Plocamium* sp. transplants, where we could be confident of sampling both original and newly produced parts of the thalli, new growth was often in the same chemogroups as in the older portions. When new growth differed from old growth, two out of three times, the new growth matched the original chemogroup from deployment. Since thalli returned to the same site and those transplanted to different sites lost chemistry or showed minute changes in their chemogroup profiles, it could indicate a stress response of the thallus to being transplanted. However, Heiser et al. (2022) compared thalli of *Plocamium* sp. before and after being held in flow-through aquarium tanks for several weeks, arguably a more stressful environment than the natural environment where the transplant experiments were placed, and they did not change their chemistry. If the environment a thallus grows in determines the chemogroup being produced, we would have expected stronger changes in chemogroup production where most transplanted thalli would change to the pre-dominant chemogroup at the site they were transplanted to. Especially since chemogroup D, dominant at Southeast Bonaparte, is known to be consumed at a slower rate by *P. fissicauda* (Heiser et al. 2022). If this was a grazer-induced response, we would expect at least chemogroups A and M to change their relative secondary metabolite concentrations to resemble chemogroup D after being transplanted. Overall, our results indicate that there is no substantial effect of the site-specific environment on the chemogroup production of *Plocamium* sp. thalli within the yearlong experiment. Therefore, factors, such as the environment selecting for certain chemogroups and/or dispersal capabilities of gametes and spores (neither of which are flagellated) and their inherent chemogroups, play a stronger role.

Several red macroalgal species have been found to vary in the concentration and type of secondary metabolites they produce depending on the life cycle stage (Wright et al. 2000b; Thornber et al. 2006; Vergés et al. 2008; Payo et al. 2011). In *Portiera* sp. for example, female

gametophytes produce a higher number of secondary metabolites, many of which were unique to that stage (Payo et al. 2011). Tetrasporophytes of the red macroalga *Delisea pulchra* differ from male and female gametophytes in secondary metabolite composition, but it is unknown whether these differences have defense benefits (Payo et al. 2011). *Delisea pulchra* tetrasporophytes also produce higher furanone levels during the summer (Wright et al. 2000b). In terms of palatability to herbivores, which often but not always is due to variations in secondary metabolites, there are differences between the life cycle stages of the red macroalga *Mazzaella flaccida* (Thornber et al. 2006). However, two grazers, the snail *Tegula funebris* and the urchin *Strongylocentrotus purpuratus* differed in their feeding response. Feeding preference in *T. funebris* of female reproductive gametophytes is due to morphological differences, whereas *S. purpuratus* preferred reproductive tetrasporophytes due to secondary metabolite production (Thornber et al. 2006). Vergés et al. (2008) found that the red macroalga *Asparagopsis armata* produces a low concentration of secondary metabolites in male gametophytes which are also higher in nutrient content. Like *Portiera* sp., female gametophytes are higher in secondary metabolite concentration which is especially high in the pericarps (outer, gametophytic cell layer of the cystocarps protecting the diploid gonimoblast filaments) (Vergés et al. 2008). Consequently, the sea hare *Aplysia parvula* consumed *A. armata* male gametophytes at the highest rate and female gametophytes lowest (Vergés et al. 2008). When female gametophytes are divided into different thallus parts, the cystocarps which had higher metabolite levels in their walls were consumed less compared to the branches. In our study, 85% of thalli were vegetative for which we require methods like quantitative DAPI staining (Kapraun 2005) or genetic markers, such as microsatellites (e.g., Krueger-Hadfield et al. 2013) or sex-linked markers (e.g., Krueger-Hadfield et al. 2021), to determine life cycle stage. Heiser (2022) developed microsatellite markers to analyze the *Plocamium* sp. samples collected in this study. All but two chemogroups (with only one and three replicates each) consisted of gametophytes as well as tetrasporophytes. Thus, life cycle-dependent chemogroup production is overall unlikely.

It is becoming evident that the geographic distribution of intraspecific chemogroup diversity in *Plocamium* sp. is not driven by environmental conditions. Within-site diversity is higher than had been recorded in the previous study (Young et al. 2013). It is difficult to assess whether a similar diversity exists in other species since commonly only the concentrations of a few selected metabolites are measured. In contrast, we utilized more complete metabolomes for chemogroup determination in *Plocamium* sp. It

is possible that whole metabolome concentrations vary with environmental factors. However, the chemogroup itself that an individual thallus produces appears to be driven by underlying genetic factors. In fact, chemogroups in *Plocamium* sp. are known to fall within two distinct *cox1* haplotypes (Young et al. 2013; Shilling et al. 2021). The red macroalga *D. pulchra* is also known for its geographic, seasonal, and life cycle stage-dependent variation in furanone production (Wright et al. 2000b). Moreover, Wright et al. (2004) found that furanone concentrations were heritable to a certain degree and that the concentrations of the four furanones were linked to each other. Wright et al. (2000a) found genetic differentiation of *D. pulchra* between two sites separated by 2 km and evidence for gene flow within sites. In contrast, furanone concentrations varied significantly within sites rather than between sites (Wright et al. 2004). It would be useful to determine if patterns of genetic differentiation in *Plocamium* sp. correlate with the differences in chemogroup assemblages we observed among sites and among depths within sites. Red macroalgae lack flagellated spores or gametes throughout their life cycle (Searles 1980). Gene flow is likely restricted to prevailing currents, turbulence through swell, and reattachment after becoming detached in a storm or through iceberg scouring. If mostly genetically determined, the distribution of phenotypes, such as chemogroup production, should be driven by the aforementioned factors and mirror that of genetic differentiation among thalli.

*Plocamium* sp. plays an important role in the Antarctic ecosystem as it supports high numbers of amphipod and gastropod mesograzers (Huang et al. 2007; Amsler et al. 2022) which do not consume their hosts (Amsler et al. 2020). The relationship between amphipods and macroalgae in Antarctica has been described as a community-wide mutualism (Amsler et al. 2014). In return for protection from predators, the amphipods keep the macroalgae free from epiphytes by consuming them. This means that the success of *Plocamium* sp. could directly benefit mesograzers and indirectly other macroalgae that those feed on. In addition, *Plocamium* sp. increased over the past 30 years at King George Island indicating that it may continue to do well under future climate change conditions (Ko et al. 2020). Since chemical defenses can have profound effects on associated organisms (Marques et al. 2006; Paul et al. 2007) understanding chemogroup diversity and distribution helps unravel factors involved in ecosystem structure. We found that the environment does not explain the high degree of chemogroup diversity and its distribution, which suggests that genotype differences are probably responsible. Whitham et al. (2003) discussed the concept of the extended phenotype in which heritable genetic variation within dominant species has consequences at the community and ecosystem level. Therefore,

understanding the genetic basis of chemogroup distribution in *Plocamium* sp. is critical. Our detailed description of the chemogroup distribution in *Plocamium* sp. is the first step towards understanding the drivers of phenotype distribution patterns in this ecologically important species with likely relevance to understanding phenotype distributions in other marine communities.

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**Data availability** Data are available at the United States Antarctic Program Data Center: <https://www.usap-dc.org/view/project/p0010016>.

## Declarations

**Conflict of interest** The authors declare no conflict of interests.

**Compliance with ethical standards** All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

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