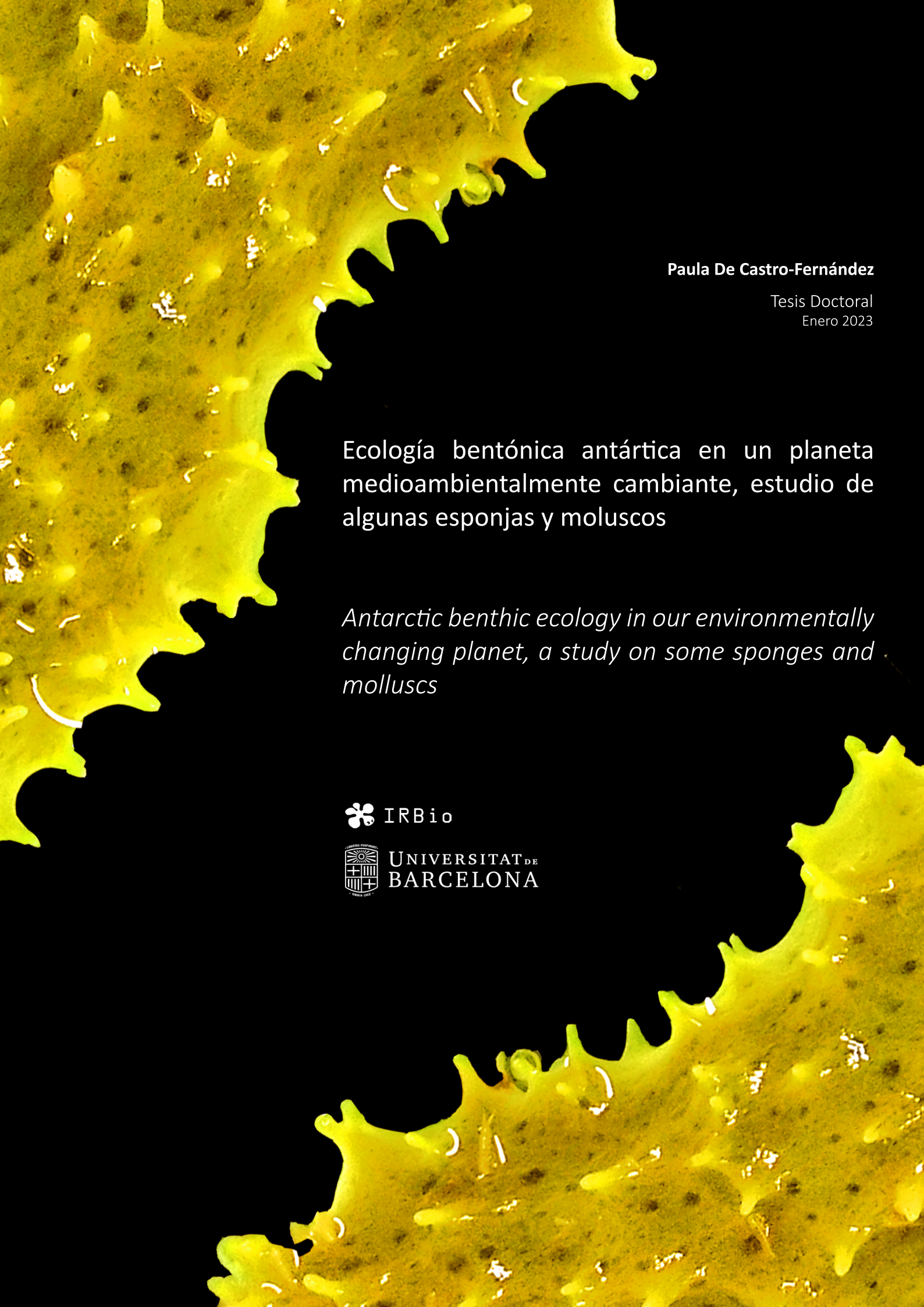
UNIVERSITAT DE BARCELONA

Ecología bentónica antártica en un planeta medioambientalmente cambiante, estudio de algunas esponjas y moluscos

Antarctic benthic ecology in our environmentally changing planet, a study on some sponges and molluscs

Paula De Castro-Fernández



Paula De Castro-Fernández

Tesis Doctoral
Enero 2023

Ecología bentónica antártica en un planeta medioambientalmente cambiante, estudio de algunas esponjas y moluscos

Antarctic benthic ecology in our environmentally changing planet, a study on some sponges and molluscs

IRBio

Universitat de Barcelona

Programa de Doctorado en Biodiversidad

Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona

Institut de Recerca en Biodiversitat (IRBio), Facultat de Biologia, Universitat de Barcelona

Ecología bentónica antártica en un planeta medioambientalmente cambiante, estudio de algunas esponjas y moluscos

Antarctic benthic ecology in our environmentally changing planet, a study on some sponges and molluscs

Memoria presentada por **Paula De Castro-Fernández** para optar al grado de doctora por la Universitat de Barcelona

La directora y tutora,	La directora,	La doctoranda,
		
Dra. Conxita Avila Escartín	Dra. Cristina García-Aljaro	Paula De Castro-Fernández

Barcelona, enero de 2023

A Marifé,

Mar y

Chus

AGRADECIMIENTOS

Parecía que nunca iba a llegar el día, pero aquí está. La verdad es que llevo días pensando en escribir estas líneas, por un lado para poder dar las gracias a todas las personas con las que he compartido estos últimos años, y por otro porque estar escribiéndolas significaría que ha llegado el final de esta etapa. Los últimos meses de tesis se me han hecho especialmente complicados, y ha sido justamente la ayuda de muchas personas la que me ha llevado hasta este momento. Pero ahora que estoy aquí, además de agradecimiento siento un poco de mareo después de tanto viaje. Espero no dejarme a nadie, pero es muy probable que si no estás aquí, has sido una persona afortunada a la que no he dado la tabarra con la tesis últimamente ;)

Volia començar agraint a les dues persones que han fet possible que arribés fins aquí, les meves directores de tesi. Moltes gràcies per l'oportunitat que em vau donar, la paciència i el suport, especialment en els darrers mesos d'escriptura. Conxita, gràcies per haver-me fet part del teu grup i projectes. Durant aquest temps he après molt i he viscut experiències que portaré sempre al record. Cristina, gràcies per tot el suport i l'ajuda, i per la perseverança amb les interminables anàlisis bioinformàtiques.

Quiero también recordar a las compis y profes que me han acompañado a lo largo de mi vida académica. De la etapa de colegio, quería agradecer sobre todo a Carmina, que confió en mí desde el principio; del instituto, especialmente a Belén y Moyano y a los profes que potenciaron mi amor por la biología, Miguel y Añoto; y de la universidad, agradecer especialmente a Juan, que me guio y aconsejó el último año de carrera.

Querría agradecer a todas las personas con las que he colaborado en los trabajos de esta tesis, que los han hecho posibles. Luis, gracias por darme la oportunidad de aprender tanto, por tu paciencia y tu forma de explicar la ciencia. Joan, gràcies pel teu suport i la teva eficiència A Jason (y Abe) por abrirnos las puertas de su maravillosa isla y sus laboratorios. A Lella por darme la oportunidad de aprender unos meses en el ICB y ayudarme a sacar un trabajo que no habría imaginado. Gracias también a Carmine i Pina por su amabilidad. Por supuesto, muchas gracias a todas las personas de los CCiT-UB que me han enseñado y ayudado.

Gracias a todas las personas con las que he coincidido en Conxita's Lab. A Carlos y Themo, que me acogisteis cuando llegué. Gracias Carlitos, por tus aportaciones a los trabajos, por las discusiones científicas y correcciones. Ah y que sepas que ya casi he terminado los ácidos grasos. Gracias Themillo por enseñarme los pasadizos secretos de la facultad, darme apoyo moral con la tesis y ser mi compañero descubriendo la Antártida. Gràcies a Blanque, espero haver sigut una digna

successora teva. Gràcies als “nous conxitos”, Mariona, Andrea, Pere, Tomás, Miguel (i Sara), i Àlex, per fer-me més divertits aquests últims mesos al despatx, pels cafès, els cereals i el cocodrilo sacamuelas. Aprofiteu molt a la campanya i molts d’ànims amb les vostres tesis! Gràcies a totes les persones amb les quals he coincidit mentre feien el TFG i TFM al grup, Gemma, Iga, Ade, Eleonora, Sílvia.

A los compañeros de campañas antárticas, por ser parte de una de las mejores cosas que me han pasado. Vivir la experiencia con todos vosotros fue increíble. Especial agradecimiento a los participantes de los proyectos DISTANTCOM y BLUEBIO, Guille, Joana, Yara, Marc, por todo vuestro trabajo y vuestra amistad. Por supuesto agradecer al personal de la UTM, por el curro que hacen, pero sobre todo por el buen ambiente que se vive allí.

Gràcies Eli, pràcticament ens vam conèixer a l'Antàrtida, i allà em vas ensenyar tot el que havia de saber de microbiologia. T'admiro molt i t'agraeixo tot el suport que m'has donat, però sobretot les mil anècdotes i riures compartits.

Gracias Rafa por todas las aventuras y por tus audiopódcasts que me han animado estas últimas semanas.

Alberto, ¡cómo agradecerte todo lo que me has ayudado! Me has escuchado con paciencia, le has quitado hierro al asunto cuando hacía falta, y me has dado ánimos siempre para seguir. Y sobre todo gracias por tantas risas y por enseñarme a sobrevivir en la selva.

Gracias Jéssica, Dani, y compis de arañas, sois geniales. Agradecer también al resto de personas del departamento con las que he coincidido, al personal de secretaría, del punto de información, a todas las personas que trabajan en la facultad y permiten que funcione.

Gràcies a la comissió de seguiment de la meva tesi, Cristina, Humbert i Eli, a la coordinadora del programa, Dolors, i als membres del tribunal.

Gracias a APECS, y especialmente a las compis de APECS-Spain. Menció especial al Sergi, gràcies per la teva amistat. También agradecer al Comité Polar y a POLAR-CSIC por las actividades en las que hemos participado con APECS-Spain.

Marsit@s, mil gracias a todos por haberme incluido en el grupo desde el principio, a pesar de mi vida nómada entre departamentos. Me habéis ayudado con cualquier cosa que he necesitado y me lo he pasado genial participando en mil historias tanto dentro como fuera del lab. Gracias Dani por

presentarte cuando llegué al lab, por recordarme siempre que no vale la pena estresarse de más y por permitirme desahogarme siempre que paso por el 10. Gracias también a Clara, Ari, Sara y Geno por el club, creo que nunca había disfrutado tanto una serie. Gracias Míriam por ayudarme siempre que lo he necesitado, por los ánimos y consejos. Gracias Raquel por ser la alegría del lab y dominar tantos géneros musicales. Gracias Julia por dejarme ocupar a ratos un trocito del lab12, por tus consejos y por compartir muchas veces horarios "intempestivos". Gracias Anna y Pedro por el apoyo mutuo en la etapa final de la tesis, acompañada siempre se hace mucho más llevadero. Gracias Aiora, Gloria, Lorena, Loles, Laura, Laura, Maite, Anicet, Francisco, Pablo, Hongxia, Irene, Alejandra, Judith, y a todas las personas con las que he coincidido por el departamento.

No em puc deixar a les companyes de Doctorandes en Lluita i la CGT. Moltes gràcies per ensenyar-me altres cares de la universitat.

També vull recordar a les amigues i amics als quals no he pogut veure gaire en els darrers mesos o anys perquè estava amb la tesi, però que m'heu donat suport sempre. Tinc moltes ganes de recuperar el temps "perdut". Gràcies Barbi, Yasmin i Jordina per les teràpies que acaben en molts riures. A Jorge, por el apoyo siempre, mucho mucho ánimo para tu tesis. A Cindy, por ser un ejemplo de trabajo y una gran amiga. A Marta, que me acompañaste desde nuestros inicios en Barcelona y luego en la distancia. A Alejandro, que tienes en parte la "culpa" de que esté yo aquí. A Eva, gracias por estar siempre. A Nieves, que me has regalado tu tiempo, conocimiento y energía siempre, pero sobre todo los últimos meses, porque no sé qué hubiera hecho sin tu ayuda. Gracias Aida, Belén, Maxim, Kistiñe, Vivi, Leti, por acordaros de mí y darme ánimos.

Víctor, al final sembla que sí, que "todo va a salir bien" :) Gràcies per la paciència i el suport sempre.

Muchas gracias a toda mi familia, por su apoyo, pero sobre todo gracias a mi madre y mi hermana, que habéis estado siempre ahí, con paciencia y risas constantes. Gracias a mi padre, que me acompaña siempre. Os quiero.

ABSTRACT

Due to the unique environmental conditions and biological characteristics of Antarctic marine environments, the effects of climate change can be devastating and may reflect future scenarios for other regions. Antarctica is also a sink for some pollutants such as trace elements and the Antarctic Peninsula is considered among the most affected areas by global change on the planet. The main objective of this thesis is to deepen the knowledge of the Antarctic marine benthic ecosystems studying biological interactions and the potential effect of environmental change. In shallow Antarctic benthos, sponges often dominate the communities being a source of carbon for predators like the nudibranch *Doris kerguelenensis*, a generalist spongivore. In Deception Island it was found upon various species of sponges. Analyses of C and N stable isotopes and fatty acids of this mollusc, the most abundant sponges in the area, and some primary producers were performed. The mollusc feeds upon the sponges *Dendrilla antarctica*, *Mycale acerata*, *Haliclona* sp., and *Axinella crinita*, but not exclusively. In Antarctica, a previous study detected a gradient in trace elements, larger to the north (higher human presence) and decreasing towards the south. The presence of Cr, Pb, and Hg was analyzed here in a benthic community along the Antarctic Peninsula, demonstrating that the concentration of these elements was influenced by local conditions rather than by the north-south human activity gradient.

Sponges maintain a very close relationship with their microbiota, which performs vital functions for the animal. To evaluate the effect of heat stress on their microbiota, experiments were carried out exposing sponges from different habitats to heat stress. The microbiota of the Antarctic sponges seems to be more resistant to heat stress than that of temperate and tropical sponges, although the effects on the physiology of the sponge remain to be investigated. Sponges use secondary metabolites as defensive compounds. The chemical profile of *Dendrilla antarctica* showed a high intraspecific variability, but also a differentiation in the predominant metabolites in sponges from different islands. A new diterpene, deceptionin, was characterized. In conclusion, the study of benthic communities from different perspectives has allowed us to build a broader picture of the functioning and conditions of these little-known inhabitants of polar waters.

RESUMEN

RESUMEN

Debido a las condiciones ambientales y características biológicas únicas de los ambientes marinos antárticos, los efectos del cambio climático pueden ser devastadores y pueden reflejar el posible futuro a gran escala de los ecosistemas marinos. La Antártida es, además, un sumidero de algunos contaminantes como los elementos traza y la península Antártica se considera una de las zonas más afectadas por el calentamiento global de todo el planeta. El objetivo principal de esta tesis ha sido profundizar en el conocimiento de los ecosistemas bentónicos marinos antárticos estudiando las interacciones biológicas y el efecto del cambio global. En el bentos somero antártico, las esponjas dominan la comunidad siendo fuente de C para una serie de depredadores como el nudibranquio *Doris kerguelenensis,* un espongívoro generalista. En isla Decepción se observó la presencia de este sobre diversas especies de esponjas. El análisis de isótopos estables de C y N y de ácidos grasos de este molusco y las esponjas más abundantes de la zona, y de algunos productores primarios demostraron que el molusco se alimenta, aunque no exclusivamente, de las esponjas *Dendrilla antarctica*, *Mycale acerata*, *Haliclona* sp. y *Axinella crinita*. En un estudio previo en la Antártida se detectó un gradiente norte-sur en la concentración de elementos traza, siendo mayor al norte (con mayor presencia humana). En nuestro trabajo se detectaron elementos traza (Cr, Pb, Hg) en una comunidad bentónica a lo largo de la Península Antártica, demostrando que la concentración de estos estaba más influenciada por las condiciones locales que por el gradiente norte-sur de la actividad humana.

Las esponjas mantienen una relación muy estrecha con su microbiota, la cual realiza funciones vitales para el animal. Se evaluó el efecto de un estrés térmico en la composición y estructura de la microbiota de las esponjas, siendo las esponjas antárticas más resistentes al estrés térmico que la de esponjas templadas y tropicales, aunque el efecto en la fisiología de la esponja es desconocido. Las esponjas utilizan metabolitos secundarios como compuestos de defensa. El perfil químico de la esponja *Dendrilla antarctica* mostró una alta variabilidad intraespecífica, y variabilidad en esponjas de diferentes islas. Se caracterizó un nuevo diterpeno, la decepcionina. En conclusión, el estudio de las comunidades bentónicas desde diferentes perspectivas nos ha permitido construir una imagen más amplia del funcionamiento y condiciones de estos poco conocidos habitantes de las aguas polares.

ÍNDICE DE CONTENIDOS

INTRODUCCIÓN GENERAL

1. La Antártida y el océano Austral

La Antártida constituye la masa terrestre situada más al sur del planeta, y se encuentra aislada del resto de zonas emergidas y rodeada por el océano Austral. Los movimientos tectónicos que llevaron al aislamiento de este continente y su océano finalizaron hace unos 25-35 millones de años, con la apertura del paso de Drake y la formación de la corriente Circumpolar Antártica (Barker and Burrell, 1977; Livermore et al., 2004; Pfuhl and McCave, 2005; Barker et al., 2007; Lyle et al., 2007) (**Figura 1**). Este aislamiento, junto a la limitada radiación solar recibida debido a su latitud, llevó a la completa glaciación del continente y las aguas que lo rodeaban (Barker and Burrell, 1977; Livermore et al., 2004; Pfuhl and McCave, 2005; Barker et al., 2007).

El continente antártico se divide en dos grandes regiones, separadas por las montañas Transantárticas: la Antártida Oriental (*East Antarctica*), de mayor área y salvo algunas zonas costeras, cubierta permanentemente de un casquete glaciar o *indlandsis* de unos 3 km de grosor medio; y la Antártida Occidental (*West Antarctica*), de terreno más irregular y de la que forma parte una alargada península que alcanza latitudes tan al norte como 63°S, llamada Península Antártica (**Figura 1**) (Ferraccioli et al., 2006). La Antártida Oriental está principalmente dominada por un desierto helado, mientras que la Antártida Occidental comprende una serie de plataformas de hielo y regiones costeras con un clima más suave que el de la Antártida continental.

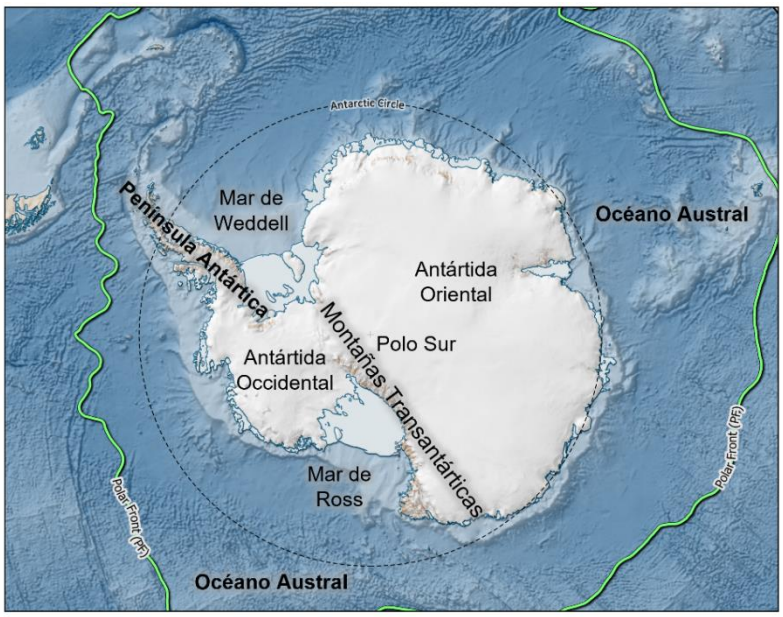


Figura 1. El continente antártico y el océano Austral.

Rodeando el continente, y extendiéndose aproximadamente hasta los 60°S, se encuentra el océano Austral. Esta masa de agua está separada de los océanos con los que limita por una abrupta transición llamada Frente Polar Antártico o Convergencia Antártica, que aísla las frías aguas polares de océanos más cálidos (Bargagli, 2008). Este, junto a la corriente Circumpolar Antártica, forman el mayor sistema de corrientes del planeta, y fluyen en sentido horario impulsados por los fuertes vientos del oeste (*westerlies*) (Lin et al., 2018). Estas corrientes y el océano Austral tienen una gran influencia sobre las condiciones ambientales de la Antártida, especialmente de la Antártida marítima, es decir, las zonas costeras, y especialmente la región de la península Antártica. Las corrientes aislaron térmicamente el continente, lo que favoreció que la temperatura del océano Austral cayera (actualmente entre -2 y 2°C), lo que a su vez llevó al enfriamiento del continente (Francis et al., 2008). Este y otros factores ambientales llevan a considerar estas regiones ambientes extremos, ya que representan límites máximos o mínimos de determinados factores ambientales a nivel del planeta. Las bajas temperaturas, junto a una fuerte estacionalidad en la irradiación solar y la productividad primaria, debido a la latitud y los procesos del hielo marino, son algunas de las condiciones a las que se han adaptado los organismos antárticos a lo largo de la evolución. Esto, además de los periodos de glaciación-deglaciación, han favorecido la aparición de nueva biota, que presenta un alto grado de endemismo (Linse et al., 2007; Griffiths et al., 2008; Downey et al., 2012), además de toda una serie de adaptaciones para lidiar con estas exigentes condiciones (Peck, 2018; Molina et al., 2022; Morley et al., 2022a).

2. La península Antártica y las islas Shetland del Sur

La península Antártica es una de las áreas más accesibles del continente, tanto por su latitud, como por la presencia de zonas libres de hielo, para estudiar las comunidades bentónicas (Clark et al., 2017). Se trata también de una de las regiones del continente con una mayor concentración de bases científicas y actividad humana.

Las costas de la península Antártica están bañadas por el lado oeste por el mar de Bellingshausen, y por el lado este por el mar de Weddell. La zona de estudio en la que se enfoca este trabajo es la península Antártica Occidental y las Islas Shetland del Sur (**Figura 2**). La circulación general en la península Antártica Occidental está influenciada por la corriente Circumpolar Antártica, que supone una entrada de agua relativamente cálida y dulce que viene del mar de Bellingshausen; y por la

circulación del estrecho de Bransfield, que supone una entrada oriental de agua relativamente fría y salada del mar de Weddell (Sangrà et al., 2011). El archipiélago de las islas Shetland del Sur (*South Shetland Islands*) se encuentra al noroeste del extremo de la Península Antártica y separado de esta por el estrecho de Bransfield (**Figura 2**).

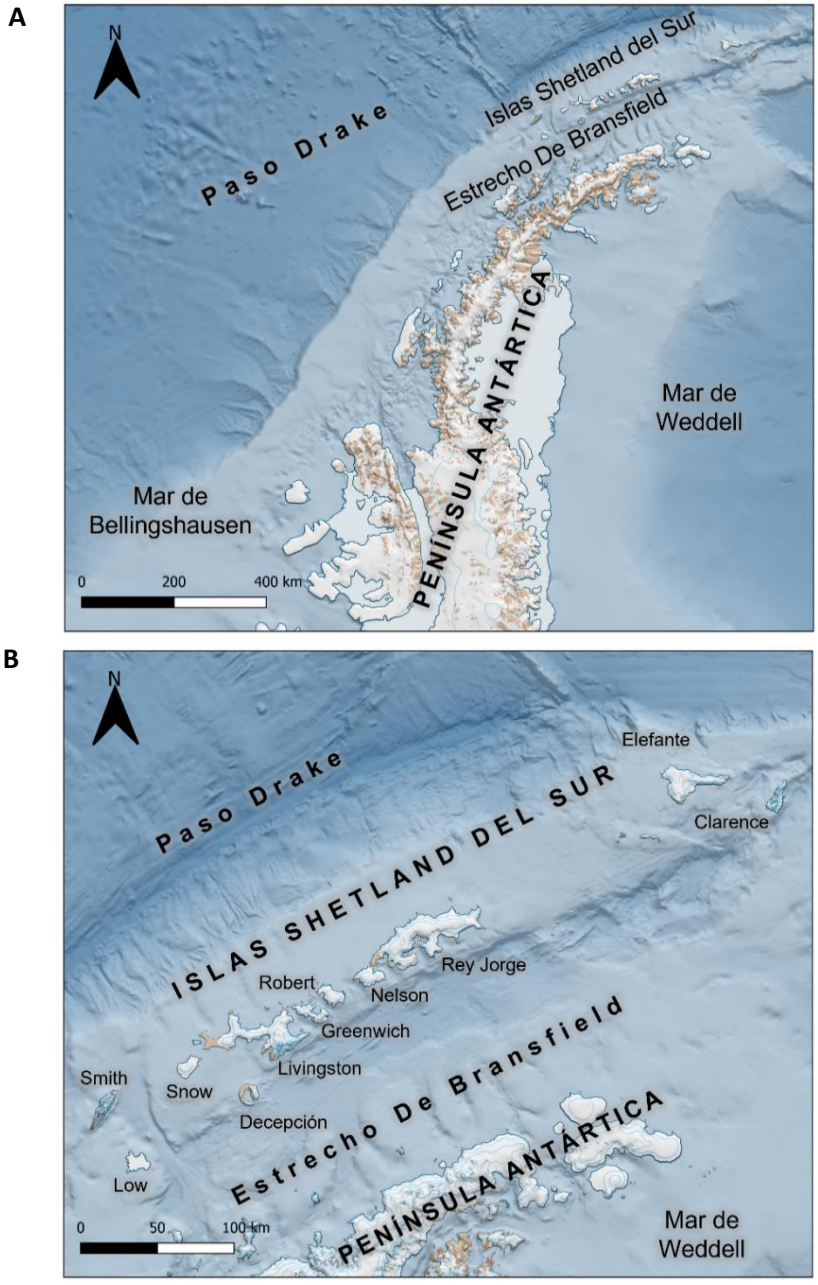


Figura 2. A. Zona de la península Antártica **B.** Islas Shetland del Sur, en el extremo de la península Antártica.

Las islas Shetland del Sur forman parte de la Antártida marítima, que como se ha mencionado anteriormente, engloba las regiones del continente que están más influenciadas por el océano Austral, particularmente las costas y la zona de la península Antártica. Poseen un clima suave, y aunque están en gran parte recubiertas de glaciares y nieve, sus costas tienen más superficies libres de hielo que las costas continentales, lo que favorece la presencia de comunidades bentónicas marinas (Teixidó et al., 2002; Clark et al., 2017). La península Antártica es la región antártica que se ha visto más afectada por el calentamiento global, y una de las áreas que se ha calentado más rápidamente del planeta (Vaughan et al., 2003; IPCC, 2022). Los diferentes estudios recogidos aquí se centran en diversos puntos a lo largo de la costa oeste de la península Antártica, y en dos de las islas Shetland del Sur, Livingston y Decepción (*Deception*). La isla Livingston es la segunda isla de mayor área del archipiélago, después de Rey Jorge, y presenta una geología similar a la del resto del archipiélago. Presenta un relieve montañoso, numerosas bahías (por ejemplo, Sur, Falsa y Hero), y penínsulas estrechas como Hurd, donde se encuentra la BAE Juan Carlos I, o Byers (**Figura 3**). Sus costas son en gran parte frentes glaciares que llegan hasta el mar, algunos acantilados rocosos, playas de arena y playas pedregosas. Los fondos rocosos cercanos a glaciares están erosionados por el hielo que se desprende de los glaciares (*ice scouring*) y el retroceso de los glaciares como consecuencia del cambio climático ha dejado expuestas nuevas zonas rocosas. Estos fondos rocosos son un sustrato adecuado para las comunidades bentónicas, que alcanzan altos niveles de biomasa en estas zonas (Dayton et al., 1974; Echeverría et al., 2005; Mincks et al., 2005; Krymarys, 2011). Isla Decepción, por su parte, es una isla con forma circular situada al sur de Livingston, con una bahía interior (Puerto Foster) que se abre al exterior por un estrecho en el sudeste de la isla (los Fuelles de Neptuno) (**Figura 4**). Se trata de un volcán activo, cuyo cráter hundido se inundó, formando la bahía interior. Isla Decepción presenta por tanto una geomorfología y características diferentes al resto de islas del archipiélago. Algunas zonas tienen propiedades fisicoquímicas especiales, con temperaturas del agua más elevadas, más turbidez por la naturaleza del sustrato, fondos blandos, y química asociada a la actividad volcánica (diferentes niveles de pH, mayores concentraciones de elementos traza, o compuestos sulfurados disueltos) (Barnes et al., 2007). La mayor parte del fondo marino de las costas de Isla Decepción está dominada por fondos blandos, compuestos de ceniza volcánica y lapilli, aunque también hay zonas de fondos rocosos, que es donde se concentran las comunidades bentónicas dominadas por macroalgas y organismos filtradores (Angulo-Preckler et al., 2018a). Debido a sus particularidades, esta isla podría representar un caso de estudio para los potenciales futuros efectos del cambio climático en otras áreas de la Antártida.

Además de servir como modelo del efecto del cambio climático en las comunidades bentónicas del planeta, las comunidades bentónicas de la Península Antártica proveen de servicios ecosistémicos que hasta ahora no habían sido demasiado atendidos. Los océanos juegan un papel clave en el ciclo del carbono, absorbiendo casi el 30% del carbono de origen antrópico (Takahashi et al., 2012), siendo la mitad de este absorbida por el océano Austral. Las comunidades de los fondos someros de la Península Antártica son un importante sumidero de este carbono (Morley et al., 2022b), otra razón más para estudiar su estructura y funcionamiento para poder predecir los cambios que están y sufriendo y sufrirán y gestionar su conservación.

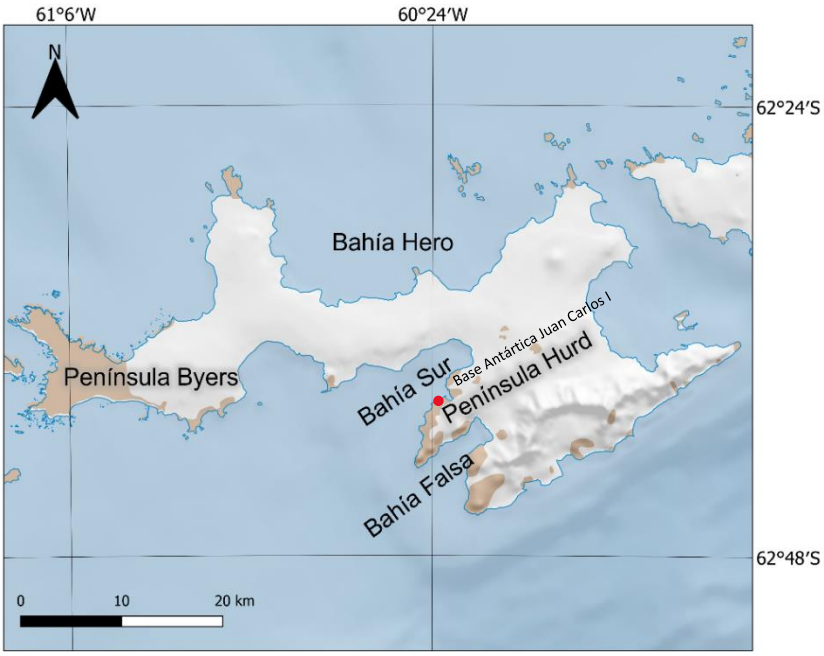


Figura 3. Isla Livingston.

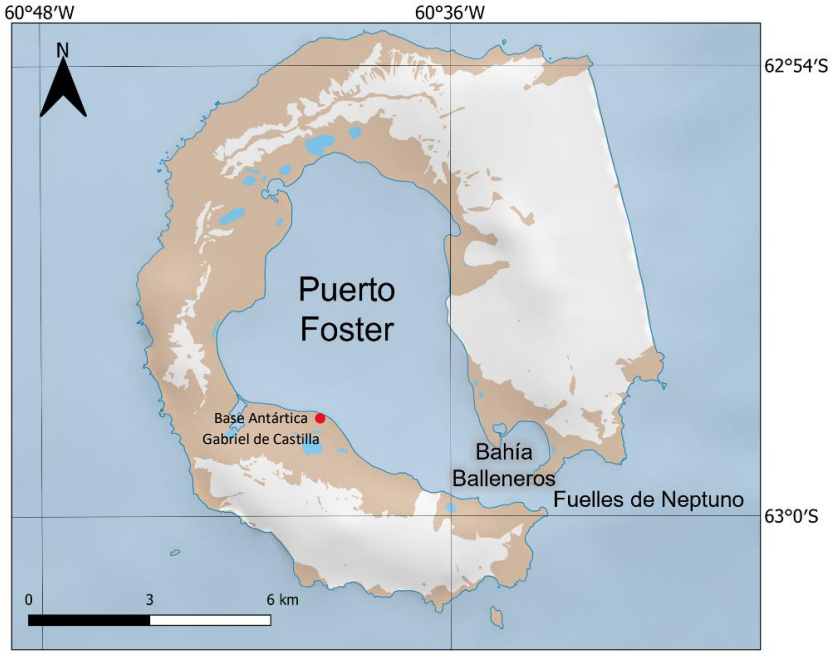


Figura 4. Isla Decepción.

3. Interacciones biológicas en el bentos marino de la Antártida: la red trófica

La estructura de los ecosistemas bentónicos marinos está determinada por factores tanto ambientales como biológicos. Entre los biológicos, la historia filogenética, las relaciones ecológicas (simbiosis, competencia, depredación, entre otras), los ciclos de vida y las tasas de colonización de los organismos son cruciales para la supervivencia (Angulo-Preckler et al., 2020). Además de los factores ambientales que perturban naturalmente las comunidades marinas, como los cambios estacionales, el aumento de la temperatura provocado por el calentamiento global y la acidificación de los océanos están afectando la supervivencia de las especies. Así, varias especies de invertebrados están experimentando un desplazamiento hacia otras áreas, o un aumento en las tasas de mortalidad, lo que está facilitando la introducción y/o nueva colonización de especies invasoras (Vaughan et al., 2003; Barnes et al., 2007; Avila et al., 2020). Dichos efectos se han observado principalmente en las complejas comunidades complejas de los arrecifes de coral y en

zonas templadas (Gordon and Leggat, 2010). En las regiones antárticas, las comunidades también se consideran muy complejas (Dayton et al., 1974), aunque su estructura y su vulnerabilidad a los cambios ambientales están lejos de comprenderse todavía.

Además de la historia evolutiva de las especies, existen otras relaciones entre organismos que son vitales para comprender la diversidad, estructura y distribución de las comunidades bentónicas. En una escala de tiempo corta, la ecología trófica ilustra el flujo de energía que conecta los diferentes compartimentos de una comunidad biológica (ver (Gillies et al., 2013)). El bentos antártico es la sección más representativa en número de especies dentro de la red trófica marina, pero sus funciones e interacciones son poco conocidas. En sistemas con limitación periódica de nutrientes como los ecosistemas polares, se ha propuesto que el suministro de carbono es el principal agente de control de la macrofauna del lecho marino (Piepenburg et al., 2002). Gran parte del *bloom* estacional de plancton se deposita en el lecho marino (Mincks et al., 2005) y su acumulación local parece estar influyendo en la distribución de las especies bentónicas (Kim and Thurber, 2007). Así, los cambios en las fuentes de carbono podrían provocar cambios directos en la distribución y abundancia de la fauna bentónica, alterando significativamente su estructura y función (Massom and Stammerjohn, 2010). Por tanto, comprender la relación entre las fuentes de carbono y los consumidores es fundamental para para evaluar los efectos de la pérdida de cualquier componente de la red trófica (Barbraud and Welmerskirch, 2001). La evaluación del flujo de energía y de las relaciones tróficas dentro de la comunidad es un requisito fundamental para determinar los cambios futuros en la estructura y función en los ecosistemas (Post, 2002). Además, las relaciones de alimentación entre organismos vágiles y sésiles en estas regiones han sido poco exploradas en el océano Austral, y su conocimiento es esencial para identificar las respuestas biológicas a los cambios ambientales en la Antártida (Turner et al., 2014).

La red trófica del bentos somero antártico es en general compleja (Norkko et al., 2007), con predominancia de estrategias generalistas y omnivoría, en respuesta a una disponibilidad de alimento marcada por la fuerte estacionalidad (Barnes and Clarke, 1995). En general, los suspensívoros o filtradores y los detritívoros dominan las aguas poco profundas y profundas (Gutt et al., 2013), pero en determinados contextos, como zonas altamente perturbadas por el hielo, estrategias como los carroñeros juegan papeles importantes en el flujo de energía y materia (Smale, 2007). Las redes tróficas de los ecosistemas antárticos poco profundos son probablemente estables, muy interconectadas y con interacciones plásticas entre los miembros de la comunidad (Gillies et

al., 2013). Sin embargo, el conocimiento existente es demasiado limitado y es necesario realizar más estudios antes de poder generalizar.

4. Interacciones biológicas en el bentos marino: el microbioma de los invertebrados bentónicos

Existe otro tipo de interacciones muy común en el medio marino: las asociaciones existentes entre microorganismos e invertebrados. Las relaciones ecológicas que establecen comunidades microbianas con sus huéspedes pueden ser transitorias, simbióticas o invasoras (patógenas) (Taylor et al., 2007; Ueoka et al., 2015). Hentschel y colaboradores demostraron que las esponjas de diferentes costas contenían microbiomas más estrechamente relacionados entre sí que con los de las aguas circundantes (Hentschel et al., 2003). La naturaleza de la relación entre microbios y macroorganismos todavía no se comprende completamente (Gordon and Leggat, 2010). Se sabe que las relaciones simbióticas son positivas y, en ocasiones, vitales, ya que pueden proporcionar una mayor disponibilidad de nutrientes para los huéspedes, así como protección contra la depredación y el *fouling* a través de metabolitos defensivos (p. ej., (Webster et al., 2004; Gordon and Leggat, 2010). La simbiosis bacteriana en comunidades de esponjas (ej (Sacristán-Soriano et al., 2020; Ruocco et al., 2021; Happel et al., 2022) se encuentran entre las simbiosis estudiadas en la Antártida. Sin embargo, están lejos de ser completamente descritas y aún más lejos de establecer su relación con los huéspedes.

En general, los organismos sésiles como esponjas y corales albergan una rica comunidad bacteriana, que es en general específica de cada especie de invertebrado (Sacristán-Soriano et al., 2020). En el caso de algunas esponjas se ha descrito que las poblaciones microbianas pueden representar hasta el 40% de la biomasa total (Wilkinson, 1978). Hasta el momento, se han encontrado más de 30 filos en estrecha asociación con esponjas en todo el mundo (Fuerst, 2014). Se han asociado distintas comunidades microbianas a diferentes especies de esponjas recolectadas en un solo lugar (Jackson et al., 2012), lo que respalda la importancia de estas comunidades en la historia evolutiva de la esponja. La ruptura de estas asociaciones debido a perturbaciones en los ecosistemas puede resultar en la muerte del huésped (Ramsby et al., 2018).

El estudio de los simbiontes bacterianos es complejo debido a las limitaciones que plantean las diferentes tecnologías disponibles (Ranjan et al., 2016; Callewaert et al., 2018; Pollock et al., 2018).

Los estudios dependientes de cultivo hacen uso de diferentes medios de cultivo ricos o selectivos para grupos particulares de bacterias para cubrir el máximo nivel de diversidad. Se estima que los métodos basados en cultivos pueden identificar o aislar solo del 0.1 al 1% de la diversidad bacteriana esperada (Staley and Konopka, 1985), suponiendo una distribución uniforme de las especies. Con este enfoque ha sido posible aislar miembros de los filos Actinobacteria, Bacteroidota, Cyanobacteria, Firmicutes, Planctomycetota, Proteobacteria y Verrucomicrobiota (Taylor et al., 2007). Las metodologías independientes de cultivo han revelado un número significativo de especies que no habían sido cultivadas hasta el momento. Entre la metodología independiente de cultivo, las técnicas de secuenciación de nueva generación (o *Next Generation Sequencing*) de bibliotecas de amplicones de PCR son ampliamente utilizadas ya que proporcionan una enorme cantidad de datos en un solo experimento, siendo la secuenciación del gen 16S rRNA uno de los más utilizados (Callewaert et al., 2018). Sin embargo, estas técnicas tienen la limitación de que algunas bacterias pueden no ser detectadas (quizás por su baja abundancia relativa o por la baja eficiencia de unión de los cebadores a determinadas especies diana). Por ejemplo, algunas especies que se han aislado mediante métodos dependientes del cultivo y que pueden desempeñar un papel importante en la esponja, no se detectan mediante análisis metagenómicos (Sun et al., 2010; Jackson et al., 2012).

Muy pocos estudios han evaluado el efecto del incremento de la temperatura en el microbioma o lo han comparado en organismos de diferentes latitudes (pero ver (Cárdenas et al., 2018). La vulnerabilidad de las especies antárticas nos podría sugerir que los organismos polares serían más sensibles al incremento de temperatura que aquellos de zonas tropicales y templadas, aunque no hay estudios previos que lo demuestren.

5. Interacciones biológicas en el bentos marino de la Antártida: ecología química

La mayoría de las interacciones biológicas entre organismos marinos, a nivel intra e interespecífico y entre estos y su entorno, están mediadas por metabolitos secundarios (productos naturales). Su estudio proporciona así información sobre la ecología y biología de las especies involucradas, y sobre el funcionamiento y estructura de la comunidad. La red trófica bentónica antártica, con ecosistemas formados principalmente por organismos sésiles, está sujeta a una intensa depredación, principalmente causada por macroinvertebrados vágiles con hábitos oportunistas omnívoros

(Dayton et al., 1974). Algunos de los principales depredadores de estas comunidades bentónicas son la estrella de mar *Odontaster validus* y el nemertino gigante *Parborlasia corrugatus* (Angulo-Preckler et al., 2020). También algunas especies de anfípodos bentónicos son consumidores importantes, ya que forman densas poblaciones asociadas con biosustratos como las esponjas o los briozoos, que representan tanto hábitats o refugios tridimensionales como fuentes potenciales de alimento para estos pequeños crustáceos (Núñez-Pons et al., 2012). La presión localizada ejercida por estos anfípodos sedentarios a veces es más influyente que la causada por depredadores más grandes, como peces o equinodermos. Además de la depredación, la competencia por el espacio y la invasión microbiana (*fouling*) son fuentes de estrés ecológico que afectan a los organismos sésiles bentónicos (Figuerola et al., 2013; Angulo-Preckler et al., 2015, 2018b). La principal estrategia defensiva descrita en los invertebrados bentónicos para evitar la depredación, la competencia o el *fouling* es a través de defensas químicas, a menudo mediante la producción de metabolitos secundarios, considerados así por la falta de una función metabólica primaria. Así, las presiones ecológicas, junto con las estrategias de defensa química estructuran en gran medida las comunidades bentónicas en la Antártida, por lo que su estudio es de vital importancia para comprender la dinámica de estos sistemas (Avila et al., 2008; Núñez-Pons and Avila, 2015; Avila, 2016). De hecho, las moléculas con actividades de protección ecológica han demostrado ser muy comunes en los organismos del océano Austral (**Tabla 1**) (Moles et al., 2015; Núñez-Pons and Avila, 2015; Angulo-Preckler et al., 2020). Sin embargo, hasta el momento, casi nada se sabe acerca de las defensas químicas inducidas (por ejemplo, debido a la presión de depredación), un fenómeno bien conocido en los hábitats terrestres entre insectos y plantas (Chen and Mao, 2020; Pereira et al., 2021). Algunos de estos productos químicos también han demostrado ser útiles para humanos, como precursores de moléculas activas para diseñar nuevos fármacos (e.g., (Avila et al., 2008; Taboada et al., 2010; Avila and Angulo-Preckler, 2021) o con aplicaciones en la industria (Tian et al., 2020).

De hecho, la naturaleza ha proporcionado a la humanidad una amplia variedad de compuestos farmacológicamente activos que se utilizan como fármacos para combatir varias enfermedades mortales y como estructuras principales para nuevas drogas sintéticas (Newman and Cragg, 2016). Los océanos son la fuente de muchos compuestos naturales únicos producidos o acumulados por invertebrados, microorganismos, algas y plantas vasculares (Khalifa et al., 2019a; Tian et al., 2020; Ren et al., 2021). Es probable que estos productos naturales sean el resultado de presiones evolutivas, como la depredación o la competencia (Avila et al., 2008), y se han transformado en

compuestos estructuralmente diversos con actividad biológica específica, que rara vez se encuentran en organismos terrestres (Carroll et al., 2022). El primer descubrimiento de metabolitos marinos biológicamente activos fueron los nucleósidos aislados de una esponja del Caribe en la década de 1950, que luego se usaron como elementos básicos para la elaboración de medicamentos antivirales comerciales (Ara-A (aciclovir) o Ara-C) (Sotomayor et al., 2002; Balzarini et al., 2016). Los principales intentos de utilizar organismos marinos para obtener nuevos medicamentos comenzaron a fines de la década de 1960. Desde entonces, varios grupos han estado activos en el descubrimiento de fármacos marinos, y hasta el momento han informado de más de 39000 productos naturales diferentes, muchos de los cuales tienen actividades farmacológicas (MarinLit). La investigación sobre productos naturales marinos ha tenido éxito hasta ahora en el descubrimiento de fármacos con cinco productos ya en el mercado: Ara-C anticancerígeno, "Prialt" analgésico y "Yondelis" y mesilato de erubilina anticancerosos (Khalifa et al., 2019; Shinde et al., 2019). Varios compuestos (principalmente de invertebrados marinos) se encuentran actualmente en etapas avanzadas de ensayos clínicos, principalmente como medicamentos contra el cáncer (Matulja et al., 2020). Por lo tanto, las posibilidades de encontrar candidatos a fármacos en este campo son especialmente altas. Pero las fuentes marinas están mucho menos exploradas que las fuentes terrestres. Solo se ha analizado la composición química del 1% de las especies marinas registradas (Carroll et al., 2022). Alrededor del 10% de las 145.000 sustancias naturales descritas provienen de organismos marinos (Carroll et al., 2022). Así pues, las posibilidades de descubrimiento de nuevos compuestos marinos y los campos de aplicación siguen abiertos. Las funciones ecológicas de los metabolitos secundarios para los organismos marinos, como la actividad antibacteriana para evitar infecciones, la citotóxica para disuadir a los depredadores o la anti-*fouling* para evitar el crecimiento de organismos en su superficie, suponen una fuente de recursos para avanzar en la investigación de remedios para enfermedades todavía sin cura como el cáncer o enfermedades infecciosas, y también para aportar soluciones a uno de los retos de salud del presente y futuro cercano como son las resistencias a antibióticos.

El papel ecológico sin embargo está muy poco estudiado, aunque se han llevado a cabo experimentos para testar la capacidad de disuadir la depredación, de inhibir microorganismos patógenos, o la citotoxicidad con extractos y moléculas de diversos organismos, como una importante representación de organismos antárticos (**Tabla 2**; Angulo-Preckler et al., 2020).

Tabla 1. Productos naturales de organismos bentónicos antárticos descritos entre 2000-2018. Tabla extraída de Angulo-Preckler et al. 2020.

Natural Products	Phyllum	Taxa	Location	References
Cystosphaerol	Ochrophyta	*Cystosphaera jacquinotii*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Bromoform		*Desmarestia anceps*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Plastoquinones		*Desmarestia menziesii*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Phlorotanins, Acetogenins, Diterpenes		*Desmarestia menziesii, D. anceps, D. antarctica Himantothallus grandifolius, Ascoseira mirabilis, Cystosphaera jacquinotii*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Halogenated furanones, Pulchralides,	Rhodophyta	*Delisea pulchra*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Halogenated organic compounds		*Delisea pulchra, Plocamium cartilagineum*	WAP, South Shetland Islands	Maschek and Baker 2008
Halogenated terpenes		*Delisea pulchra, Plocamium cartilagineum, Pantoneura plocamioides*	WAP, South Shetland Islands	Argandona et al. 2002
Sulfated polysaccharides		*Iridaea cordata*	WAP, South Shetland Islands	Kim et al. 2017
P-hydroxybenzaldehyde,P-methoxyphenol		*Myriogramme smithii*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Epi-plocameneD		*Plocamium cartilagineum*	WAP, South Shetland Islands	Ankisetty et al. 2004b
Norselic acids A-E	Porifera	*Crella sp.*	Anvers island	Ma et al. 2009
Darwinolide, Membranolide B, C, and D, Dihydrogracilin A		*Dendrilla membranosa*	Anvers Island	Ankisetty et al. 2004, Witowski 2015, von Salm et al. 2016, Ciaglia et al. 2017
Antifreeze peptide		*Homaxinella balfourensis*	McMurdo Sound	Wilkins et al. 2002
Erebusinone, 3-Hydroxykyrunenine, Methyl 3-hydroxyanthranilate		*Isodyctia erinacea*	Ross Island (20-30 m)	Moon et al. 2000, Vankalaya et al. 2017
Organohalogens		*Kirkpatrickia variolosa, Artemisina apollinis, Phorbas glaberrima, Halichondria sp., Leucetta antarctica*	King George Island	Vetter and Janussen, 2005
Discorhabdin G		*Latrunculia apicalis*	McMurdo Sound	Furrow et al. 2003
Discorhabdins, Tsitsikammamines		*Latrunculia biformis (deep sea sponge)*	Weddell Sea (303 m)	Li et al. 2018
Discorhabdin R		*Latrunculia sp.*	Prydz Bay (depth of 544 m)	Ford and Capon, 2000
Flabellone		*Lissodendoryx (Lissodendoryx) flabellata*	Terranova Bay	Cutignano et al. 2012
Suberitenones A and B, Oxapyrosuberiterone, Isosuberiterone B, 19-episuberiterone B, Isoxaspirosuberiterone		*Phorbas aerolatus*	Deception Island	Solanki et al. 2018
Caminatal, Oxaspirosuberitenone, 19-episuberiterone B, Suberiterone B		*Suberites caminatus*	King George Island	Díaz-Marrero et al. 2003, 2004
Suberitenones C and D, Suberiphenol		*Suberites sp.*	King George Island	Lee et al. 2004
Linderazulene, ketolactone, C-16-Azulenoid	Cnidaria	*Acanthogorgia laxa*	South Shetland Island	Patiño-Cano et al. 2018
Ainigmaptilone A and B		*Ainigmaptilon antarcticus*	Weddell Sea	Iken and Baker, 2003
Illudalane sesquiterpenes		*Alcyonium antarcticum, A. grandis, A. haddoni, A. paucilobulatum, A. roseum*	Weddell Sea, Deception Is.	Carbone et al. 2009, Núñez-Pons et al. 2013
Paesslerins A and B		*Alcyonium paessleri*	South Georgia Islands	Rodríguez Brasco et al. 2001
Alcyopterosins A-O		*Alcyonium paessleri*	South Georgia Islands	Palermo et al. 2000
Steroids		*Anthomastus bathyproctus*	South Shetland Island	Mellado et al. 2005
Trans-beta-farnesene, Isofuranodiene, Furanoeudesmane		*Dasystenella acanthina*	Terranova Bay (deep water)	Gavagnin et al. 2003
Hodgsonal	Mollusca	*Bathydoris hodgsoni*	Weddell Sea	Iken et al. 1998; Avila et al. 2000
Granuloside		*Charcotia granulosa*	Deception Is., Livingston Is.	Cutignano et al. 2015; Moles et al. 2016
Diterpene glyderides		*Doris kerguelenensis*	Weddell Sea, Ross Sea and Antarctic Peninsula	Iken et al. 2002; Cutignano et al. 2011; Maschek et al. 2012; Wilson et al. 2013
Tambjamine A	Bryozoa	*Bugula longissima*	Antarctica	Lebar et al. 2007
Asterosaponins and steroids	Echinodermata	*Diplasterias brucei*	Terra Nova Bay	Ivanchina et al 2006, 2011
Disulphated polyhydroxysteroids		*Gorgonocephalus chilensis*	Antarctica (unknown locality)	Maier et al. (2000)
Liouvilloside A and B		*Staurocucumis liouvillei*	Antarctica (unknown locality)	Maier et al. (2001)
Triterpene glycosides		*Staurocucumis liouvillei, S. turqueti, Achlionice violaecuspidata*	Weddell Sea	Antonov et al. 2008; 2009; 2011; Silchenko et al. 2013
Rossinones	Tunicata	*A. falklandicum, A. fuegiense, A. meridianum, A. millari, Synoicum adareanum*	Weddell Sea	Núñez-Pons et al. 2010; 2012b
Aplicyanins A-F		*Aplidium cyaneum*	Weddell Sea	Reyes et al. 2008
Meridianins		*Aplidium falklandicum, A. meridianum*	Weddell Sea	Núñez-Pons et al. 2015
Rossinone B		*Aplidium fuegiense*	Weddell Sea	Carbone et al. 2012; Núñez-Pons et al. 2012b
Meridianins		*Aplidium meridianum, Synoicum* sp.	South Georgia Is., Anvers Island	Lebar and Baker 2010
Rossinone A and B		*Aplidium* sp.	Ross Sea	Appleton et al. 2009
Palmerolide A, Hyousterones A-D and Abeohyousterone		*Synoicum adareanum*	Anvers Island	Diyabalanage 2006, Miyata 2007, 2008

Tabla 2. Bioactividad de productos naturales de organismos bentónicos antárticos. Tabla extraída de Angulo-Preckler et al. (2020).

Phyllum	Taxa	Bioactivity							
		Feeding deterrence		Antimicrobial activity	Citotoxicity	Antifouling	Antifreezing	Growth inhibition	Allellopathy
		Macropredator	Mesopredator						
Ochrophyta	*Ascoseira mirabilis*	*	*	*					
	Cystosphaera jacquinotii	*	*	*					
	Desmarestia anceps	*	*	*					
	Desmarestia antarctica	*	*	*					
	Desmarestia mensiezii	*	*	*	*				
	Hymantothallus grandifolius	*	*	*					
Rhodophyta	*Delisea pulchra*		*	*					
	Iridaea cordata	*							
	Myriogramme smithii		*						
	Palmaria decipiens								
	Pantoneura plocamioides		*						
	Plocamium cartilagineum		*	*					
Porifera	*Artemisina apollinis*								*
	Crella sp.		*	*					
	Dendrilla membranosa			*	*	*			
	Halichondria sp.								*
	Homaxinella balfourensis						*		
	Isodictya erinacea	*	*					*	
	Kirkpatrickia variolosa			*	*				*
	Latrunculia apicalis	*			*				
	Latrunculia biformis				*				
	Latrunculia sp.			*					
	Leucetta antarctica								
	Lissodendoryx flabellata				*				
	Phorbas areolatus	*		*	*				
	Phorbas glaberrima								*
	Suberites caminatus				*				
Cnidaria	*Ainigmaptilon antarcticus*	*							
	Alcyonium antarcticum	*							
	Alcyonium grandis	*							
	Alcyonium haddoni	*							
	Alcyonium paessleri								
	Alcyonium paucilobulatum	*							
	Alcyonium roseum	*							
	Dasystenella acanthina	*							
Mollusca	*Bathydoris hodgsoni*	*							
	Doris kerguelenensis	*			*				
Echinodermata	*Achlionice violaecuspidata*								
	Diplasteria brucei								
	Gorgonocephalus chilensis				*				
	Staurocucumis liouvillei			*	*				
	Staurocucumis turqueti								
Tunicata	*Aplidium cyaneum*				*				
	Aplidium falklandicum								
	Aplidium fuegiense								
	Aplidium meridianum								
	Aplidium millari								
	Synoicum adareanum	*			*			*	

6. Impacto antropogénico en las comunidades bentónicas: calentamiento global

El calentamiento global como consecuencia de la actividad humana es uno de los mayores desafíos a los que se enfrenta la sociedad actual y está generando un gran impacto en los ecosistemas. Futuros escenarios de cambio climático prevén un aumento de 1.8-4 °C en la temperatura superficial del mar y un aumento proyectado del doble en la concentración atmosférica de CO_2 para 2100, lo que provocaría la acidificación de los océanos (Bell et al., 2013; IPCC, 2022).

Debido a su aislamiento y evolución independiente, los ambientes marinos antárticos poseen características únicas, pero ello no impide que se vean también afectados por los efectos del cambio global y otros impactos antropogénicos. A pesar de la aparente estabilidad ambiental en el ecosistema marino antártico, existen fuentes de variabilidad y perturbaciones ambientales, como la fuerte estacionalidad, los cambios en la capa de hielo, la acción erosiva producida por los icebergs (Orejas et al., 2000; Barnes et al., 2007). Tanto estos factores ambientales, como las interacciones entre organismos juegan un papel importante en la estructuración de las comunidades (Dayton et al., 1974; Paul et al., 2007), y los cambios en las condiciones ambientales asociados al fenómeno del cambio global pueden afectar a ambos compartimentos. Además de verse afectados los hábitats del continente, los efectos del cambio climático en la Antártida tienen o tendrán consecuencias a nivel global, ya que esta región tiene un papel vital en la regulación del clima del planeta y las corrientes marinas, entre otros.

Algunos estudios han demostrado una adaptación de la comunidad simbiótica de esponjas de los arrecifes de coral, que se vuelve más termotolerante (Oliver and Palumbi, 2011). Sin embargo, los organismos de las regiones polares parecen ser más vulnerables en comparación con otras regiones (Peck, 2005), dado que las especies polares son en general estenotermas y, por lo tanto, menos capaces de soportar cambios de temperatura (Peck and Conway, 2000). Además, una de las regiones antárticas que más se está calentando en los últimos años es la Península Antártica (Vaughan et al., 2003; IPCC, 2022), pero las consecuencias de los cambios térmicos y otras alteraciones sobre los organismos y sus comunidades están todavía poco estudiadas (Peck, 2018).

Las alteraciones provocadas por el cambio climático en las comunidades marinas se reportan cada vez más en todo el mundo. Sin embargo, aunque el aumento gradual proyectado en la temperatura media superficial desde el presente hasta 2100 ha sido una característica de la mayoría de los estudios, es probable que las olas de calor marinas representen una amenaza más inmediata y

potencialmente devastadora (Garrabou et al., 2022). Los impactos de los eventos de temperatura extrema se han reconocido durante mucho tiempo en los hábitats tropicales (por ejemplo, el blanqueamiento generalizado de corales; (Hughes et al., 2017), pero los impactos en los ecosistemas marinos templados y polares solo se han reconocido más recientemente (Pertierra et al., 2017, 2021).

7. Impacto antropogénico en las comunidades bentónicas: contaminación

Otro de los impactos antropogénicos que más está afectando al continente antártico es la contaminación por elementos traza. Esta contaminación tiene origen tanto global, proveniente de otros continentes, como local, producida por la actividad humana, como el turismo o las bases humanas (Bengtson Nash et al., 2011).

La contaminación marina es un problema ambiental creciente en la actualidad (Häder et al., 2020). En la Antártida y el Océano Austral, los efectos de las actividades antropogénicas son visibles y amenazan la sostenibilidad de los delicados ecosistemas de esta región (Gutt et al., 2021). En el pasado considerada una región prístina, la Antártida constituye un sumidero para contaminantes como el mercurio (Hg), que condensan en esta zona fría tras evaporar y ser transportados por el conocido como transporte de largo alcance (Dommergue et al., 2010; Angot et al., 2016). Como consecuencia, el océano Austral presenta una de las mayores concentraciones de metilmercurio (Cossa et al., 2011). En la Antártida se han detectado elementos traza como los llamados metales pesados, compuestos organohalogenados, hidrocarburos y, más recientemente, plástico en la biota, sedimentos, agua de mar, aire, nieve y hielo marino (revisado en da Silva et al., 2023).

El estudio de la biodiversidad y las interacciones ecológicas de las comunidades bentónicas antárticas proporciona la base para identificar y paliar las amenazas a la diversidad de estos ecosistemas únicos, que además constituyen un laboratorio biológico natural sujeto a condiciones muy particulares. Dado que la estructura de las comunidades bentónicas responde a diferentes niveles y factores biológicos, para comprenderlas es fundamental abordarlas desde una perspectiva multidisciplinar, analizando sus interacciones ecológicas, incluidas las relaciones simbióticas, y las relaciones mediadas por moléculas químicas (productos naturales). Esta información es fundamental para prever posibles cambios en la supervivencia de dichas especies en función del

impacto del cambio climático en nuestro planeta. En la **figura 5** se muestran las especies que han sido objeto de esta tesis doctoral.

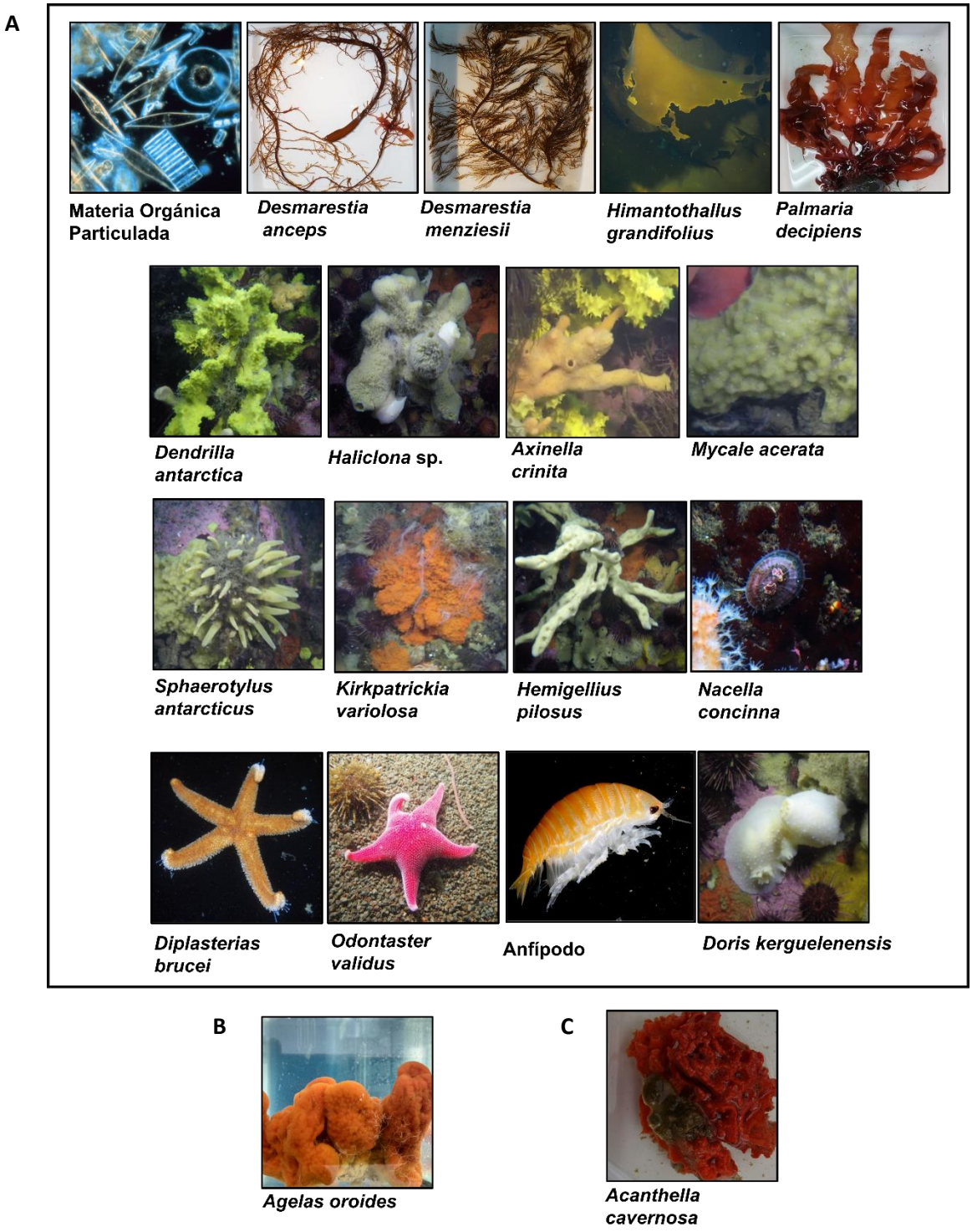


Figura 5. Especies de estudio. **A.** Especies antárticas. **B.** Especie mediterránea. **C.** Especie tropical.

OBJETIVOS

El objectivo principal de esta tesis es el **estudio ecológico del bentos antártico**, incluyendo el análisis de interacciones biológicas como las relaciones tróficas, simbióticas, y las mediadas por moléculas químicas (productos naturales) en diferentes invertebrados marinos bentónicos, y la evaluación de su grado de afectación por el impacto antropogénico (aumento de temperatura y contaminación por elementos traza).

Los objetivos específicos son los siguientes:

1. Estudiar la ecología trófica del molusco heterobranquio *Doris kerguelenensis* y su comunidad bentónica en la Isla Decepción (Islas Shetland del Sur, Antártida) para conocer su dieta utilizando isótopos estables de C y N y ácidos grasos como trazadores tróficos (**Capítulo I**).

2. Estudiar el grado de contaminación por elementos traza presente en organismos bentónicos de diferentes niveles tróficos y evaluar la interconectividad entre estos organismos dentro de la red trófica propuesta (**Capítulo II**).

3. Evaluar la bioacumulación y/o biomagnificación de los diferentes contaminantes a través de la red trófica bentónica y compararlos con lo descrito en otras áreas del Océano Austral (**Capítulo II**).

4. Caracterizar la comunidad de microorganismos simbiontes asociada a especies de esponjas similares de tres latitudes diferentes (Trópico, Mediterráneo y Antártida), y analizar el efecto del aumento de la temperatura del agua sobre la composición de dicha comunidad simbionte (**Capítulo III**).

5. Analizar la variabilidad de los productos naturales de la esponja marina *Dendrilla antarctica* en las islas Decepción y Livingston (Islas Shetland del Sur, Antártida) y los cambios en la composición tanto cualitativa como cuantitativa de su perfil químico producidos al aumentar la temperatura del agua y ante la presión de depredación (**Capítulo IV**).

INFORME DE LAS DIRECTORAS

Como directoras de la tesis doctoral titulada "Ecología bentónica antártica en un planeta medioambientalmente cambiante, estudio de algunas esponjas y moluscos" realizada por Paula De Castro-Fernández, presentamos el siguiente informe sobre la contribución de la doctoranda en los capítulos de resultados en coautoría que forman parte de la tesis:

Capítulo I. P. De Castro-Fernández, J. Giménez, C. Avila y L. Cardona (en preparación) *Insights from stable isotope and fatty acids analysis on the diet of an Antarctic sea slug*. En preparación para ser enviado a publicar.

Contribución de la doctoranda: Realización de los análisis de laboratorio y redacción del primer borrador del manuscrito. Participación en los análisis de datos y en la redacción de la versión final del manuscrito.

Capítulo II. P. De Castro-Fernández, L. Cardona y C. Avila (2021) *Distribution of trace elements in benthic infralittoral organisms from the western Antarctic Peninsula reveals no latitudinal gradient of pollution*. *Scientific Reports* 11:16266. doi: 10.1038/s41598-021-95681-5

Contribución de la doctoranda: Realización de los análisis de laboratorio y los análisis de datos y redacción del primer borrador del manuscrito. Participación en la redacción de la versión final del manuscrito.

Acerca de la revista: *Scientific Reports* tiene un índice de impacto de 4.997 en el *Journal Citation Reports* (*JCR*) de 2021. Se encuentra en el número 19 de 74 en el área de *Multidisciplinary Sciences* (Q2).

Capítulo III. P. De Castro-Fernández, E. Ballesté, C. Angulo-Preckler, J. Biggs, C. Avila y C. García-Aljaro (2023). *How does heat stress affect sponge microbiomes? Structure and resilience of microbial communities of marine sponges from different habitats*. *Frontiers in Marine Science* 9:1072696. doi: 10.3389/fmars.2022.1072696

Contribución de la doctoranda: Realización de los análisis de laboratorio y redacción del primer borrador del manuscrito. Participación en la toma de muestras, análisis de los resultados y redacción de la versión final del manuscrito.

Acerca de la revista: *Frontiers in Marine Science* tiene un índice de impacto de 5.247 en el *Journal Citation Reports* (*JCR*) de 2021. Se encuentra en el número 6 de 113 en el área de *Marine & Freshwater Biology* (Q1).

Capítulo IV. P. De Castro-Fernández, C. Angulo-Preckler, C. García-Aljaro, C. Avila y A. Cutignano (en preparación). *A chemo-ecological investigation on Dendrilla antarctica Topsent, 1905: identification of deceptionin and effects of heat stress and predation pressure on its terpene profiles*. En preparación para enviar a publicar en la revista *Marine Drugs*.

Contribución de la doctoranda: Realización de los análisis de laboratorio y análisis de los resultados. Participación en la redacción de la versión final del manuscrito.

Acerca de la revista: *Marine Drugs* tiene un índice de impacto de 6.085 en el *Journal Citation Reports* (*JCR*) de 2021. Se encuentra en el número 10 de 63 en el área de *Chemistry, Medicinal* (Q1) y en el número 48 de 279 en el área de *Pharmacology & Pharmacy* (Q1).

Las directoras,

Dra. Conxita Avila Escartín

Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
Facultat de Biologia

Dra. Cristina García-Aljaro

Departament de Genètica, Microbiologia i Estadística
Facultat de Biologia

Barcelona, a 2 de enero de 2022

RESULTADOS

CAPÍTULO I

Insights from stable isotope and fatty acids analysis on the diet of an Antarctic sea slug

Paula De Castro-Fernández[1,2], Joan Giménez[3], Conxita Avila[1,2] and Luis Cardona[1,2]

[1]Departament de Biología Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona (UB), Av. Diagonal 645, 08028 Barcelona, Spain

[2]Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Barcelona, Spain

[3]Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (ICM-CSIC), Departament de Recursos Marins Renovables, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

Abstract

Doris kerguelenensis is an Antarctic heterobranch mollusc reported to feed on sponges, according to gut content analysis. We used stable isotopes of carbon and nitrogen, and fatty acids to assess the relative contribution to its diet of several species of demosponges (*Axinella crinita*, *Dendrilla antarctica*, *Hemigellius pilosus*, *Kirkpatrickia variolosa*, *Mycale acerata*, *Sphaerotylus antarcticus,* and *Haliclona* sp.). We also analyzed particulate organic matter (POM) and two macroalgae species (*Desmarestia anceps* and *Himantothallus grandifolius*) to better characterize the diversity of sources fueling the food web. *D. anceps* was the most ^{13}C depleted species in our data set and phytoplankton the most enriched. The $\delta^{13}C$ values of sponges and *D. kerguelenensis* laid in between, thus suggesting that particulate organic matter was not the main source of carbon for sponges. The $\delta^{15}N$ values of all the sponge species were above those of the primary producers, suggesting a trophic position between 2 and 3 for *A. crinita*, *D. antarctica*, *M. acerata* and *Haliclona* sp., and between 3 and 4 for *H. pilosus* and *S. antarcticus*. *K. variolosa* was in between the two groups of sponges. The $\delta^{15}N$ values of *D. kerguelenensis* were also higher than those of primary producers and similar to those of *H. pilosus, K. variolosa* and *S. antarcticus*. Mixing models including as the only sources sponges with $\delta^{15}N$ values lower than those of *D. kerguelenensis* failed to resolve the diet of the latter, because either the sponges were too enriched in ^{13}C or the DTDF values available in the literature for gastropods are unsuitable for this sea slug species. The fatty acid profiles of all the sponge species and *D. kerguelenensis* differed largely from those of macroalgae, because of the very high levels of 15:0 and EPA and low levels of arachidonic acid. However, the fatty acid profile of POM and those of *A. crinita*, *D. antarctica*, *K. variolosa*, *M. acerata*, *S. antarcticus,* and *Haliclona* sp. were rather similar, although the high $\delta^{13}C$ values of POM rule it out as a major dietary source for

sponges. *H. pilosus* differed from the other sponges because of its high levels of 17:0, 20:1n9 and 20:2. *D. kerguelenensis*, *A. crinita*, *D. antarctica*, *K. variolosa*, *Haliclona* sp., and *S. antarcticus* shared a long chain fatty acid absent in phytoplankton and macroalgae. The high abundance of bacterial fatty acid markers in *H. pilosus*, *S. antarcticus* and *K. variolosa*, as well as their high $\delta^{15}N$ values, rule them out as major prey of *D. kerguelenensis*. The overall evidence suggests that *A. crinita*, *D. antarctica*, *M. acerata*, and *Haliclona* sp. are consumed regularly by *D. kerguelenensis*, but do not represent its only dietary sources.

1. Introduction

The analysis of the diet composition of organisms is crucial for understanding the basic ecology of species and their trophic interactions within the food web (Kelly and Scheibling, 2012). This knowledge is also required for predicting how biotic and abiotic changes may affect the community and hence predict the response of ecosystems to anthropogenic change.

Antarctic benthic food webs are characterized by a strong seasonality, due to major changes in day length and further light limitation during most of the year by the ice cover (Knox, 2006; Thomas et al., 2008). As a result, pelagic primary production concentrates in the short ice-free season, and consumers suffer from extreme food shortage during the rest of the year (Dayton et al., 1986; Barnes and Clarke, 1995; Norkko et al., 2007; Thomas et al., 2008; Michel et al., 2019; Rossi et al., 2019). Thus, carbon supply has been proposed as the main driver of seabed macrofauna structure and functioning (Piepenburg et al., 2002; Kim and Thurber, 2007), which is characterized by high levels of trophic redundancy and reliance on diverse carbon sources (Norkko et al., 2007; Mincks et al., 2008; Cardona et al., 2021). On the other hand, western Antarctica is one of the regions of the planet experiencing a more intense warming (Lee et al., 2017; Morley et al., 2020) and massive loss of sea ice and hence there is an urgent need to understand the consequences of such changes on the structure of Antarctic marine food webs, which are currently poorly known.

Suspension feeders often dominate benthic communities in Antarctica, and it is estimated that sponges occupy around 10% of the known Antarctic shelf area (Gutt et al., 2013), where their low metabolic rate contributes to their survival in this harsh ecosystem (reviewed in (McClintock et al., 2005; Morley et al., 2016). Sponges are considered key ecosystem engineers, meaning they create tri-dimensional spatial structure, which serves as habitat, substrate, nursery, or shelter to other

invertebrates, contributing to increase biodiversity and biomass in the ecosystems (Bell, 2008). In fact, the co-evolution of sponges and their epifauna has been proposed as a factor explaining Antarctic benthic species richness (Gutt and Schickan, 1998). In tropical waters, sponges also play a key role in bentho-pelagic coupling through the so-called sponge-loop (de Goeij et al., 2013), but little is known about a similar functional role in Antarctic ecosystems (Rovelli et al., 2019).

Antarctic sponges rely mostly on phytoplankton, but also bacterioplankton or dissolved organic matter (Cerrano et al., 2004; Thurber, 2007) and are consumed by many organisms, although most of their skeletons are hardly digestible (Garrone, 1978; Bjorndal, 1990). Some of their predators are specialist spongivorous, such as the seastars *Perknaster fuscus* and *Acodontaster conspicuous*. Other predators such as *Odontaster validus*, *O. meridionalis* and diverse amphipod species are omnivores that include sponges in their diets (Dayton et al., 1974; Mcclintock, 1994; Gillies et al., 2012). These sponge predators have relevant roles in the benthic community dynamics. For example, in McMurdo Sound, (Dayton et al., 1994) observed that *P. fuscus* specialized on *Mycale acerata*, controlling the populations of this fast-growing and space-dominating demosponge, and thus contributing to higher species diversity in the community (Dayton et al., 1974). The three dominant hexactinellid sponges in McMurdo Sound were preyed by *A. conspicuus* and *D. kerguelenensis*, and the seastar *O. validus* predated on both predator species' larvae and on adult *A. conspicuus*, indirectly contributing to the accumulation of sponge biomass (Dayton et al., 1974).

Spongivory is common in nudibranch molluscs (McDonald and Nybakken, 1997) and each species usually specializes in one or few sponge prey species (Wägele, 2004; Hoover et al., 2012; Goodheart et al., 2017; Mikhlina et al., 2018, 2020; Ekimova et al., 2019; Imbs and Grigorchuk, 2019). However, research on nudibranch trophic ecology is usually hindered by their small size and their scarcity, which dramatically limits the sample size (Todd 1981). Nudibranch diet is usually inferred from observation data and stomach content analysis, and more recently, using molecular markers such as stable isotopes or fatty acids (e.g., Imbs and Grigorchuk, 2019; Camps-Castella et al., 2020; Komisarenko et al., 2021).

Doris kerguelenensis (Bergh, 1884) is a heterobranch mollusk in the Dorididae family, with an Antarctic circumpolar distribution and a broad bathymetric range, from 1 to 1,550 m (Moles et al., 2017). In fact, it has been recently described to be a complex of crytic species, only distinguishable by genetic analysis (Maroni et al., 2022). *Doris kerguelenensis*, as many nudibranchs, is considered a spongivorous specialist, feeding on diverse demosponges and hexactinellid sponges (McDonald

and Nybakken, 1997). Based on direct observations, its diet at McMurdo Sound (Ross Sea) was reported to be based mostly on three species of Hexactinellid sponges (*Anoxycalyx (Scolymastra) joubini*, *Rossella nuda*, and *R. racovitzae*) although smaller amounts of other species (*Polymastia invaginata*, *Haliclona dancoi*, *Calyx arcuarius*, *Sphaerotylus antarcticus*, *Tetilla leptoderma*, *Microxina benedeni*, *Gellius tenella*, *Isodictya setifera*, and *Homaxonella* sp.) were also consumed (Dayton et al., 1970, 1974). *Mycale acerata*, *Kirkpatrickia variolosa* and *Pachychalina pedunculata* were not observed as preys (Dayton et al., 1974). In Terra Nova Bay (Ross Sea), it was observed to feed on various demosponges of the genus *Gellius* (Cattaneo-Vietti, 1991). Analysis of stomach contents from samples collected at different stations at the Scotia Sea revealed feeding on *Ectyodoryx cf. ramilobosa* and *Rossella* sp. (Garcia et al., 1993) and Wägele (1989) and (Iken et al., 2002) observed that small individuals often occur at the central cavity of sponges of the genera *Rossella* and *Anoxycalyx (Scolymastra)* at different locations along the Weddell Sea, Antarctic Peninsula, and Antarctic and subantarctic islands. At Borge Bay (Signy Island, South Orkney Islands) they were observed on the surface of *Dendrilla antarctica* (Barnes and Bullough, 1996). It should be noted, however, that *D. kerguelenensis* was reported to have a low predatory impact on their host sponge, allowing them to feed on the same sponge for many years (Dayton et al., 1974; Barnes and Bullough, 1996). Food-detection experiments using a Y-maze did not show a strong chemodectection of *D. kerguelenensis* to one of its major prey *R. racovitzae* (Iken et al., 2002).

To our knowledge, the information on *D. kerguelenensis* diet composition published so far comes either from direct observation or stomach content analysis and hence could be highly biased. For instance, spongivorous molluscs may only graze the sponge surface, and thus not all the types of spiculae may be found in the stomach, hindering the identification of the sponge species (Wägele, 1989). The use of biochemical markers such as stable isotopes and fatty acids provide a time-integrated observation, and can overcome some biases of gut content analysis Concretely, bulk stable isotope analysis is commonly used to reconstruct the diet because the isotopic ratios of animal tissue reflects the isotopic signature of their diet plus a diet-to-tissue (DTDF) factor (Post, 2002). On the other hand, fatty acid (FA) analyses provide very valuable information in diet studies (Graeve et al., 1994; Phleger et al., 1998), because primary producers have distinct FA profiles, which are conservatively transferred to the consumers because heterotrophic organisms are not capable of synthesizing all of the FAs that they require (Watanabe et al., 1983).

Preliminary analysis using stable isotopes analysis revealed that *Doris kerguelenenesis* from the Antarctic Peninsula had a trophic position similar to that of the starfish *Odontaster validus*, but relied on a highly ^{13}C-depleted sources, likely sponges (Cardona et al., 2021). The main objective of this study is to provide further insights into the feeding ecology of *Doris kerguelenenesis* using chemical markers (i.e., stable isotope and fatty acids analyses) to unravel their role in Antarctic benthic ecosystems.

2. Materials and Methods

2.1. Area of study

Deception Island is an active volcanic island in the South Shetland archipelago. It has a central flooded caldera, Port Foster, of 10x7 km^2 and a maximum depth of 160 m. Most part of the benthos inside the island is characterized by fine-grain sediments. Ash is annually deposited into the bay. The high sedimentation rate inhibits the suspension-feeding taxa, which are the dominant in Antarctic shelf and shallow benthic communities. However, the entrance to Port Foster, Neptune's Bellows, has hard bottoms with communities dominated by macroalgae, sponges and bryozoans (Arnaud et al., 1998; Angulo-Preckler et al., 2018).

Whalers Bay, next to the entrance of Deception Island, has one of the sites with higher species richness of Port Foster (Angulo-Preckler et al., 2018). Macroalgal assemblages there are highly diverse, as well as sponge assemblages, without an apparent dominance of any particular species. The main species are *Dendrilla antarctica*, *Mycale acerata*, *Hemigellius pilosus*, *Axinella crinita*, *Kirkpatrickia variolosa*, the ascidian *Cnemidocarpa verrucosa* and the anemone *Isotealia antarctica*.

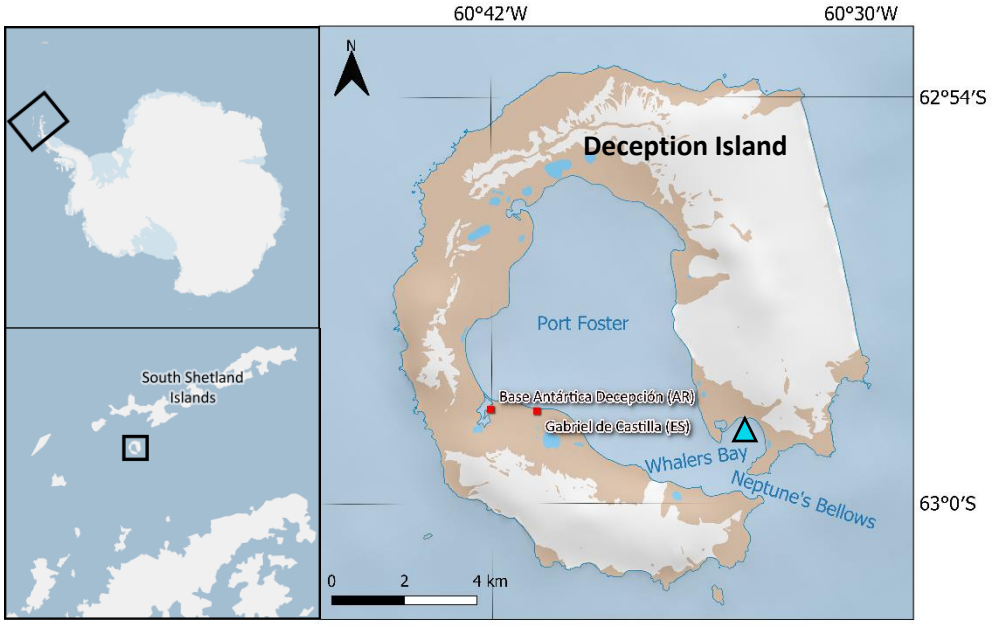


Figure 1. Sampling site is denoted by a triangle. Map generated using Quantarctica (ver. 3.2, see https://www.npolar.no/quantarctica/; (Matsuoka et al., 2018) and QGIS (QGIS Development Team, 2018).

2.2. Species of study

Samples were collected during February and March 2017, by scuba diving at depths between 10 and 15 m, with the exception of phytoplankton, which was collected with 50 µm mesh size plankton net at 5 m depth using a Zodiac boat at 1-2 kn speed. Samples were collected from two different sites in Whalers Bay (**Figure 1**).

The species selected for this study where the sea slug *Doris kerguelenensis*, seven species of demosponges (*Axinella crinita*, *Dendrilla antarctica*, *Hemigellius pilosus*, *Kirkpatrickia variolosa*, *Mycale acerata*, *Sphaerotylus antarcticus*, and *Haliclona* sp.), the kelp-like macroalgae *Desmarestia anceps* and *Himantothallus grandifolius*, and particulate organic matter (POM), dominated by diatoms and dinoflagellates. All the samples were immediately frozen at -20 C after collection.

Table 1. Species analyzed in this study. N= sample size

Phylum	Species	N
Several	Particulate organic matter	5
Ochrophyta	*Desmarestia anceps* Montagne, 1842	5
	Himantothallus grandifolius (A. Gepp & E.S. Gepp) Zinova, 1959	5
Porifera	*Axinella crinita* Thiele, 1905	5
	Dendrilla antarctica Topsent, 1905	5
	Hemigellius pilosus (Kirkpatrick, 1907)	5
	Kirkpatrickia variolosa (Kirkpatrick, 1907)	5
	Mycale (Oxymycale) acerata Kirkpatrick, 1907	5
	Sphaerotylus antarcticus Kirkpatrick, 1907	5
	Haliclona Grant, 1841	6
Mollusca	*Doris kerguelenensis* (Bergh, 1884)	21

Once in the laboratory at the University of Barcelona (UB), samples were thawed on ice and processed prior to analytical determinations. Two ml of concentrated particulate organic matter were collected and a 2 x 2 cm fragment of the macroalgae was selected. Sea slugs were dissected and the gut and its contents removed. We took two aliquots of around 1 ml from each wet sample of sponges and sea slugs.

2.3. Isotopic analysis

Samples were dried for 24 h at 55 °C and then ground to powder using a ceramic mortar and pestle. We split the dry samples into two aliquots. We kept one aliquot for the determination of $\delta^{15}N$ values. We treated the other subsample for lipid removal, adding chloroform/methanol (2:1, v:v) (1 ml) and rotating overnight. The treatment was repeated until the liquid was transparent. To remove inorganic carbonate, which can interfere in the determination of carbon signature, we further treated the samples that contain carbonate (all, except the macroalgae) with 0.5 N HCl and left them rotating overnight. The treatment was repeated until CO_2 bubbles were no longer produced. We rinsed the samples with distilled water and dried them for 24h at 60 °C. After lipid and carbonate removal, these second set of subsamples were used for $\delta^{13}C$ determination. The samples were kept in the desiccator until weighted in tin capsules. We weighted an amount of 0.3 mg for the mollusc samples and 0.7 mg for all other samples.

All tin cups were combusted at 900 °C and analyzed in a continuous-flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA, Thermo Finnigan; www.thermofisher.com) at the

"Centres Científics i Tecnològics de la Universitat de Barcelona" (www.ccit.ub.edu). Stable isotope abundances were expressed in δ notation according to the following expression:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N and R_{sample} and $R_{standard}$ is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and the standard. The reference standards are Vienna Pee Dee Belemnite (VPDB) $CaCO_3$ for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$. However, due to limited supplies of VPDB, we analyzed instead isotopic reference materials, which included known isotopic compositions relative to international measurement standards. For carbon, isotopic reference materials of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA; www.iaea.org) in Vienna (Austria), were used for calibration at a precision of 0.05‰. These included polyethylene (IAEA CH_7, $\delta^{13}C$ = −32.1‰), L-glutamic acid (IAEA $USGS_{40}$, $\delta^{13}C$ = −26.4‰), and sucrose (IAEA CH_6, $\delta^{13}C$ = −10.4‰). For nitrogen, isotopic reference materials of known $^{15}N/^{14}N$ ratios were used to a precision of 0.2‰, and these were namely: $(NH_4)_2SO_4$ (IAEA N_1, $\delta^{15}N$ = +0.4‰ and IAEA N_2, $\delta^{15}N$ = +20.3‰), L-glutamic acid (IAEA $USGS_{40}$, $\delta^{15}N$ = −4.6‰), and KNO_3 (IAEA NO_3, $\delta^{15}N$ = +4.7‰). All these isotopic reference materials were employed to recalibrate the system once every 12 samples and were analyzed to compensate for any measurement drifts over time. The raw data were recalculated taking into account a linear regression previously calculated for isotopic reference materials (Skrzypek, 2013).

2.4. Fatty acids analysis

We homogenized and freeze dried the samples for 24-48h. Total lipids of 50 mg of each sample were extracted with a mixture of CH_2Cl_2/CH_3OH (2:1, v/v). CH_2Cl_2/CH_3OH (5 ml) were added to the sample in a centrifuge tube. We sonicated for 20 min in the sonic bath and centrifuged (5 min x 5000 rpm). The solvent was transferred to a round-bottom flask. We added additional solvent, and repeated sonic extraction and centrifugation 2 more times. We combined all solvents and rotovaped off the excess solvent to ±1 ml. We added 6% KOH/CH_3OH (6 ml) for saponification and left it overnight. The next day, we added ultrapure water (6 ml) and shook for hydrolysis. Then, samples were transferred to a separatory funnel. We rinsed the flask with hexane (3 x 6 ml) and transferred to the separatory funnel. We mixed and allowed to form two phases. We discarded the organic phase (neutral fraction) and transferred the aqueous phase to the original flask. We added new hexane to the separatory funnel after rinsing the original flask and extracted the aqueous phase a second time.

We allowed phases to separate, discarded the organic phase and retrieved the aqueous phase. We repeated extraction a third time and combined the three extracts. We added to the resultant aqueous phase HCl (1:1) until acidification and we extracted the acidic aqueous phase with hexane in a separatory funnel as described above. The organic upper phase (acidic fraction) was retrieved and the aqueous phase was extracted again twice more. We combined the acidic fractions and rotovaped off the excess solvent to ±1 ml. Then we added 20% BF_3/CH_3OH (5 ml) for methylation and left overnight. The next day, we added ultrapure water (6 ml) and shook for hydrolysis. We extracted the aqueous phase with hexane in a separatory funnel as described above. The organic upper phase (acidic fraction II) was retrieved and the aqueous phase was extracted again twice more. We passed the fractions through a filter with Na_2SO_4 to remove any water. We combined the acidic fractions and rotovaped off the excess solvent to ±1 ml. We transferred residue to a 4 ml screw cap vial and rinsed with hexane (3 × 1 ml). We evaporated to full dryness under nitrogen and stored the vials at -20 °C. The analysis was carried out using a GC Network System coupled to an electronic impact-mass spectrometry detector. The capillary column was a BPX70 column (SGE, 30 m l., 0.25 mm i.d., 0.25 µm film thickness). FAME composition was compared with the standard FAME mix (C4–C24, 18919–1AMP, SUPELCO).

2.5. Data analysis

A mixing polygon was built to evaluate if an isotopic mixing model could be constructed based on the potential preys proposed (Smith et al., 2013). As no species-specific diet-to-tissue discrimination factor (DTDF) existed for *Doris kerguelenenesis* or any other sea slug, several DTDFs from the literature were used to calculate the mixing polygon (**Table 2**). We used DTDFs from omnivorous, detritivorous, and carnivorous gastropods in addition to a generalized one.

Differences among species in their fatty acid profiles were assessed through Principal Component Analysis (PCA), considering only fatty acids that had an overall mean of >0.4% of total fatty acids (Iverson et al., 2002). Bacterial fatty acids 15:0 and 17:0 were considered particularly relevant markers of sponge consumption.

3. Results

Desmarestia anceps was the most ^{13}C depleted species in our data set and particulate organic matter the most enriched one. The $\delta^{13}C$ values of sponges and *D. kerguelenensis* laid in between. The $\delta^{15}N$ values of all the sponge species were above those of the primary producers, suggesting a trophic position between 2 and 3 for *A. crinita*, *D. antarctica*, *M. acerata* and *Haliclona* sp., and between 3 and 4 for *H. pilosus* and *S. antarcticus*. *K. variolosa* was in between the two groups of sponges. The $\delta^{15}N$ values of *D. kerguelenensis* were also higher than those of primary producers and similar to those of *H. pilosus, K. variolosa* and *S. antarcticus* (**Figure 2**), hence suggesting that the latter were not relevant diet sources for *D. kerguelenensis*.

Mixing polygons including as the only sources sponges with $\delta^{15}N$ values lower than that of *D. kerguelenensis* (*A. crinita*, *D. antarctica*, *M. acerata* and *Haliclona* sp.) failed to enclose the isotopic values of *D. kerguelenensis* inside, independently of the DTDFs values considered (**Figure 3**). As a result, mixing models could not be run and the diet of *D. kerguelenensis* remained unresolved.

The two first components extracted in the PCA explained 32.2% of the overall variability in the fatty acid profiles. The first component (PCA1) explained 18.3% of the overall variability in the fatty acid profiles and mostly opposed the abundance of 14:00, 15:00, 16:00 and 22:6n-3 (negative values) to that of fatty acids with 18 and 20 C atoms (Table 1). The second component (PCA2) explained 13.9% of the overall variability in the fatty acid profiles and was strongly influenced by the abundance of 18:00, 18:1, 18:1n-9, 20:00 and 20:1n-9 (positive values) and that of odd fatty acids (15:00, 15:01, 17:00 and 21:00) and 20:1n-9 (negative values). The fatty acid profiles of all the sponge species were similar to those of particulate organic matter but differed largely from those of macroalgae, because of the very high levels of 15:0 and other odd fatty acids in sponges and particulate organic matter (**Table 3** and **Figure 4**). *H. pilosus* differed from the other sponges because of the high levels of 17:0, 20:1n9 and 20:2.

The fatty acid profile of *D. kerguelenensis* was distinct from that of macroalgae and sponges, thus suggesting that other food sources were consumed, although the total amount of fatty acids of bacterial origin in *D. kerguelenensis* was similar to that in the sponges *Haliclona* sp., *A. crinita*, *D. antarctica* and *M. acerata*. Furthermore, D. *kerguelenensis* shared with *A. crinita*, *D. antarctica*, *K. variolosa*, *Haliclona* sp., and *S. antarcticus* a long chain fatty acid absent from other sponges, particulate organic matter and macroalgae, thus suggesting that *Haliclona* sp., *A. crinita*, and *D. antarctica* were relevant diet sources for *D. kerguelenensis*, but not the only ones.

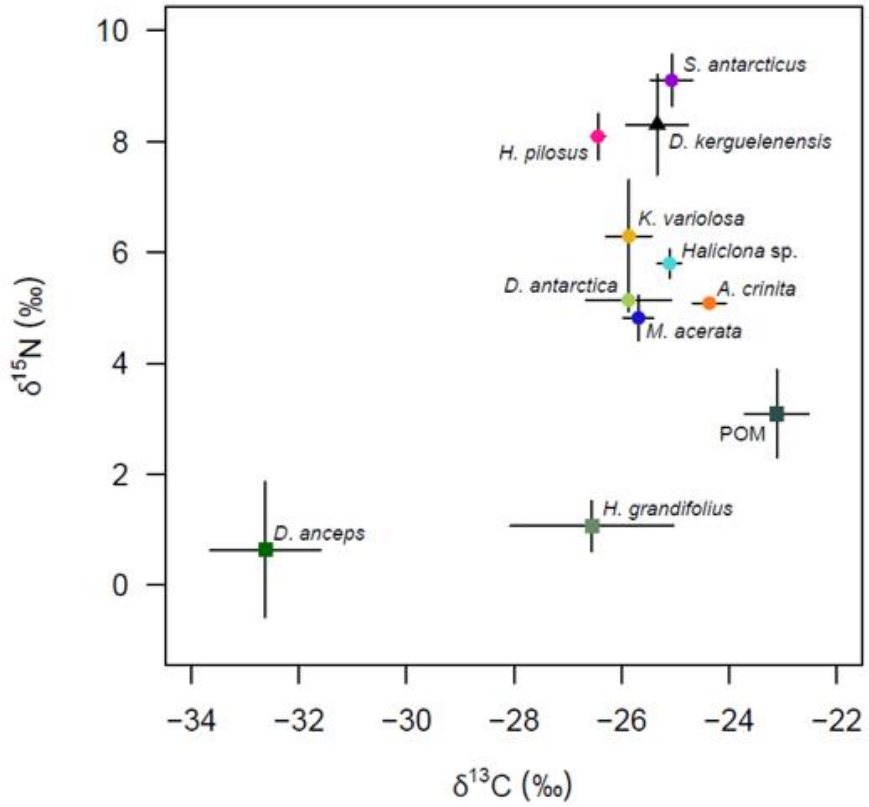


Figure 2. Isoplot of primary producers (*Desmarestia anceps*, *Himantothallus grandifolius* and particulate organic matter), *D. kerguelenensis* and several sponges from Whalers Bay (Deception Island, Western Antarctica).

Table 2: Diet-to-tissue discrimination factors to calculate the different mixing polygons.

	Species	$\Delta^{15}N$	$\Delta^{13}C$	Type of consumer	Reference
A	General	3.4*	1.0*	General consumer	Minagawa and Wada (1984)
B	*Neverita duplicata*	3.58*	1.9*	Omnivorous gastropod	Casey et al. (2016)
C	*Ellobium aurisjudae*	4.2 ±0.2	5.3 ±0.5	Detritivorous gastropod	Teoh et al. (2018)
D	*Ellobium aurisjudae*	6.0 ±0.2	3.2 ±0.5	Detritivorous gastropod	Teoh et al. (2018)
E	*Neptunea heros*	2.3 ±0.2	1.5 ±0.1	Carnivorous gastropod	North et al. (2019)
F	*Neptunea heros*	3.3 ±0.3	1.8 ±0.3	Carnivorous gastropod	North et al. (2019)
G	*Neptunea communis*	1.8 ±0.3	1.3 ±0.3	Carnivorous gastropod	North et al. (2019)

*when standard deviation is not provided a ±0.2 was used to provide certain variability to the discrimination factor

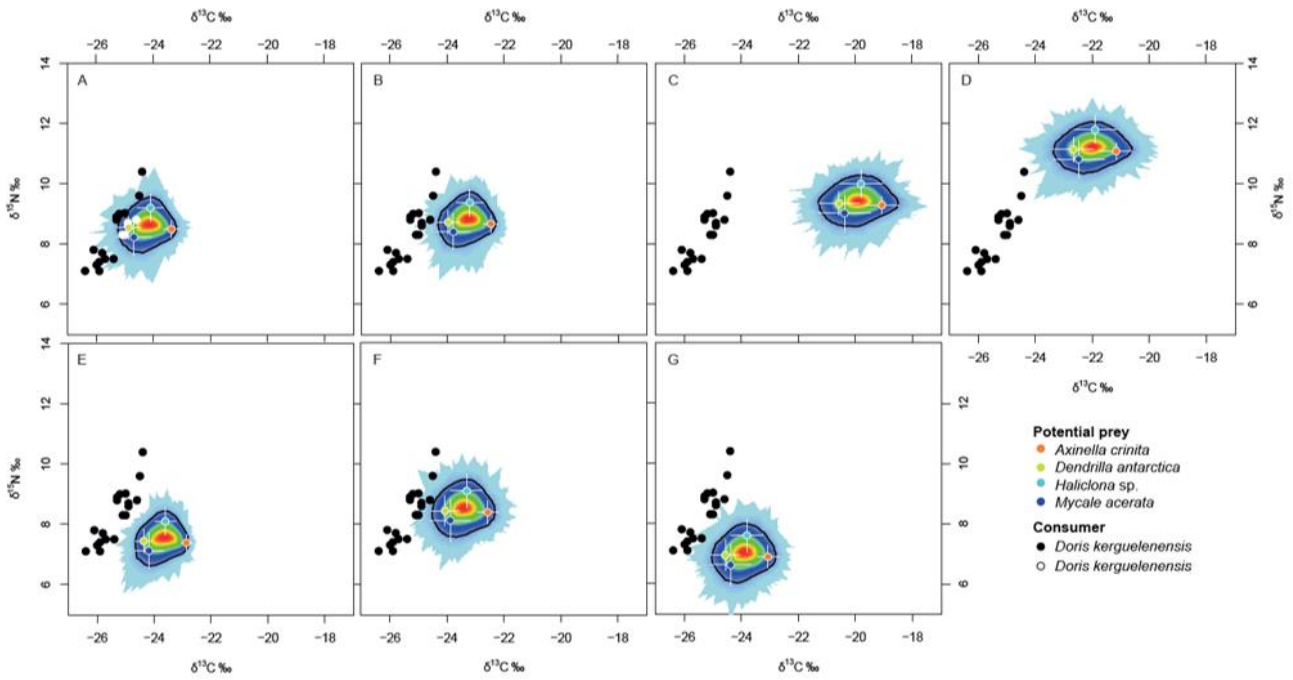


Figure 3. Mixing polygons for *D. kerguelenensis* using different DTDFs. A) 3.4±0.2 for $\Delta^{15}N$ and 1±0.2 for $\Delta^{13}C$ (Minagawa and Wada, 1984); B) 3.58±0.2 for $\Delta^{15}N$ and 1.9±0.2 for $\Delta^{13}C$ (Casey et al., 2016); C) 4.2 ±0.2 for $\Delta^{15}N$ and 5.3 ±0.5 for $\Delta^{13}C$ (Teoh et al., 2018); D) 6.0 ±0.2 for $\Delta^{15}N$ and 3.2 ±0.5 for $\Delta^{13}C$ (Teoh et al., 2018); E) 2.3 ±0.2 for $\Delta^{15}N$ and 1.5 ±0.1 for $\Delta^{13}C$ (North et al., 2019); F) 3.3 ±0.3 for $\Delta^{15}N$ and 1.8 ±0.3 for $\Delta^{13}C$ (North et al., 2019); G) 1.8 ±0.3 for $\Delta^{15}N$ and 1.3 ±0.3 for $\Delta^{13}C$ (North et al., 2019).

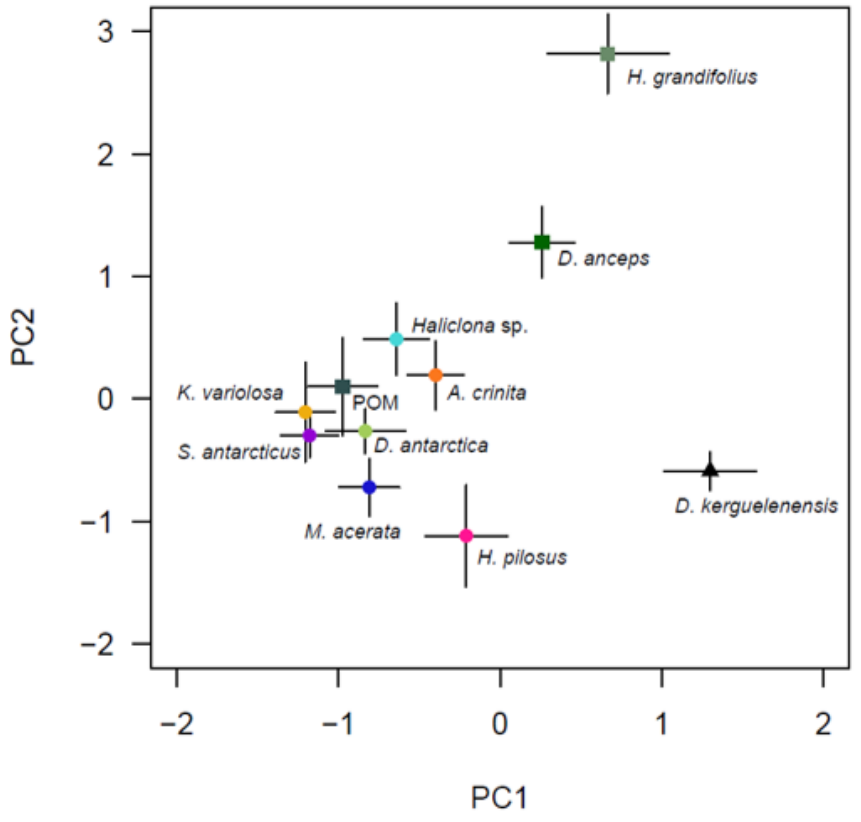


Figure 4. Distribution of *Doris kerguelenensis*, several sponges, two macroalgae (*Himantothallus grandifolius* and *Desmarestia anceps*) and particulate organic matter in the space defined by the two first component extracted by PCA. High levels of fatty acids with 18 and 20 C atoms correlate with positive scores along PC1. High levels of odd fatty acids correlate with low scores along PC2.

Table 3. Principal Components matrix.

	PC1	PC2
a120	-.231	-.171
a140	-.568	.135
a141	-.346	-.102
a150	-.476	-.381
a151	.039	-.197
a160	-.513	.725

a161	-.536	.244
a170	.487	-.298
a171	-.387	-.188
a180	.002	.652
a181n9t	.326	.558
a181n9c	.830	.138
a182n6t	.010	-.171
a182n6c	.709	-.148
a183n3	-.197	.233
a183n6	.310	.537
a200	.212	.751
a201n9	.762	-.513
a210	.005	-.327
a202	.704	-.523
a203n3	.570	.649
a204n6	.743	.191
a203n6	.311	-.163
a220	-.216	-.038
a221n9	-.112	-.152
a205n6	-.207	-.551
a222	-.002	-.056
a240	-.046	-.081
a241n9	-.034	-.021
a226n3	-.433	-.205

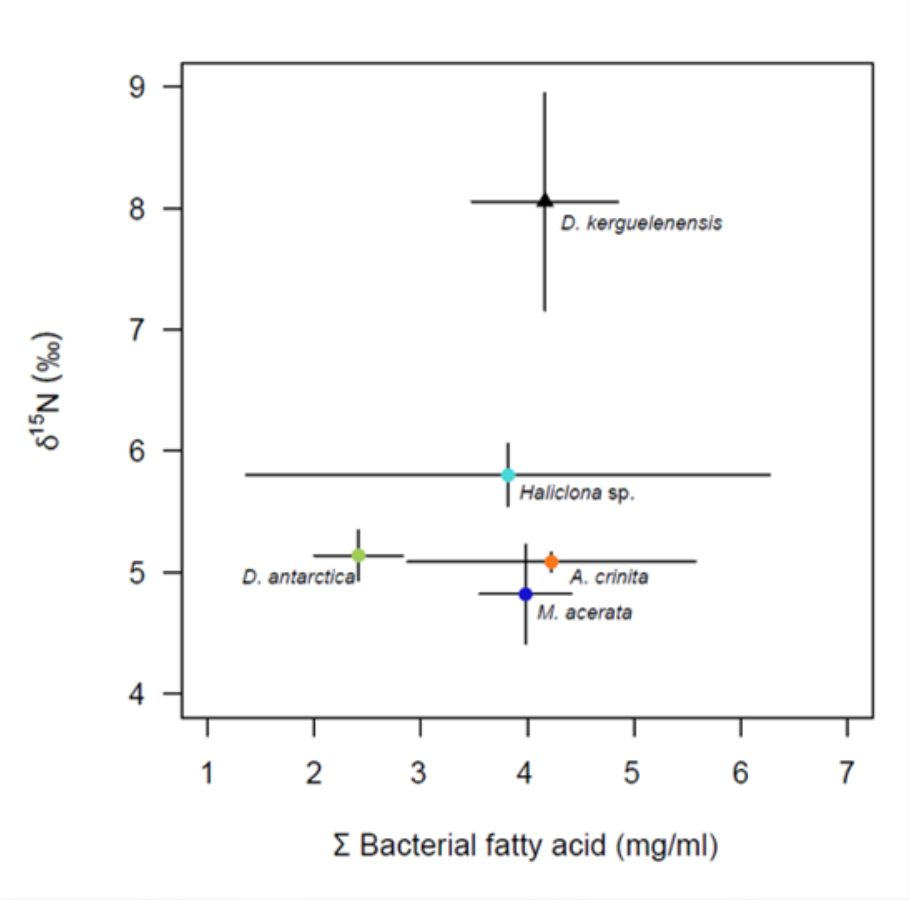


Figure 5. Distribution of *Doris kerguelenensis* and several sponges in the space delimited by the amount of bacterial fatty acids and $\delta^{15}N$, a proxy for trophic position.

4. Discussion

The results reported here suggest that the sponges *Axinella crinita*, *Dendrilla antarctica*, *Haliclona* sp. and *Mycale acerata* play a relevant role in the diet of the nudibranch *D. kerguelenensis,* because all them share several fatty acids of bacterial origin, but are unlikely to be its only food sources, because the C and N stable isotope ratios of *D. kerguelenensis* laid mostly outside the mixing polygon formed by those of the four sponge species.

The uncertainty about species specific DTDF is one of the major limitations when using stable isotope ratios to infer the diet of consumers, because DTDF vary according to diet composition and

consumer metabolism (Kadye et al., 2020). No DTDF has been derived experimentally for gastropods to our best knowledge and none of the values reported in the literature provide meaningful results. This could be because of highly specific DTDF for nudibranchs, the absence of a major prey in the mixing model or both. It should be noted that the mismatch between the stable isotope ratios of *D. kerguelenensis* and its potential food sources is mostly on the $\delta^{13}C$ values, because the DTDF reported in the literature for carnivorous snails provides a good prediction of the $\delta^{15}N$ values. This suggest that the mismatch is because of the absence of a major prey in the model. According to the literature, only macroalgae and some ascidians are more depleted in ^{13}C than sympatric sponges in subtidal Antarctic ecosystems (Gillies et al., 2013; Pasotti et al., 2015; Michel et al., 2019; Cardona et al., 2021; this study). Ascidians are commonly consumed by other nudibranchs (Millar 1971; Paul et al. 1990) and hence they could perhaps be a likely candidate to be the missing prey for *D. kerguelenensis*. Although nudibranchs may feed on many different invertebrates (sponges, hydroids, bryozoans, ascidians, barnacles, anemones, or other nudibranchs; reviewed in McDonald and Nybakken (1997), this would be quite surprising, since these group of dorid nudibranchs has always been considered to rely only on sponges (Belmonte et al., 2015) and therefore should be further investigated with more detail.

Alternatively, the $\delta^{13}C$ values of the spongin fibers that make most of the bulk of sponge samples might differ from that of live sponge cells and endosymbionts, and thus the $\delta^{13}C$ values of *D. kerguelenensis* might reflect those of live cells and not spongin. Although a plausible explanation in the absence of data on the stable isotope ratios of the different components of sponges, this is an unlikely explanation, because the overall fatty acid profile of *D. kerguelenensis* is rather different from that of sponges. Certainly, *D. kerguelenensis* is rich in bacterial fatty acids, but also on fatty acids with 18 and 20 C atoms, which suggests that other prey species are also consumed. This hypothesis is supported by the broad scatter of $\delta^{15}N$ values reported for *D. keguelenensis*, spanning one full trophic level.

The fact that *D. kerguelenensis* has recently been described as a cryptic species complex (Maroni et al., 2022) could be related to the variability in the particular sponge species they prey upon, but it should not affect the isotopes and FA results, since they are all very similar among the sea slug specimens analyzed, and different from all other samples. Furthermore, since it is impossible to distinguish them unless genetic studies are done, a specific study should be designed to analyze in

parallel the genetics and the diet, which could be extremely difficult since many specimens would be needed.

It should be noted also that the low water temperature experienced by Antarctic organisms results in very low turnover rates and hence chemical markers may integrate dietary information over extended periods. This might result in a mismatch between the habitat where an individual is collected and previous foraging habitats during the winter months. The individuals of *D. kerguelenensis* studied here were collected on the surface of sponges, but we ignore for how long they had been foraging there. Stomach contents analysis certainly reveals only the most recent diet and not surprisingly often includes sponge spicules for individuals collected on the surface of sponges, which lead to the conclusion that *D. kerguelenensis* was a specialized spongivore. However, the results reported here do not support such conclusion and point out a more diverse diet exists.

5. References

Angulo-Preckler, C., Figuerola, B., Núñez-Pons, L., Moles, J., Martín-Martín, R., Rull-Lluch, J., et al. (2018). Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica. *Cont Shelf Res* 157, 20–31. doi: 10.1016/J.CSR.2018.02.005.

Arnaud, P. M., López, C. M., Olaso, I., Ramil, F., Ramos-Esplá, A. A., and Ramos, A. (1998). Semi-quantitative study of macrobenthic fauna in the region of the South Shetland Islands and the Antarctic Peninsula. *Polar Biol* 19, 160-166. doi: 10.1007/s003000050229

Barnes, D. K. A., and Bullough, L. W. (1996). Some observations on the diet and distribution of nudibranchs at Signy Island, Antarctica. *J Mollus Stud* 62, 281-287. doi: 10.1093/mollus/62.3.281

Barnes, D. K. A., and Clarke, A. (1995). Seasonality of feeding activity in Antarctic suspension feeders. *Polar Biol* 15, 335-340. doi: 10.1007/BF00238483

Bell, J. J. (2008). The functional roles of marine sponges. *Estuar Coast Shelf Sci* 79, 341–353. doi: 10.1016/j.ecss.2008.05.002.

Belmonte, T., Alvim, J., Padula, V., and Muricy, G. (2015). Spongivory by nudibranchs on the coast of Rio de Janeiro state, southeastern Brazil (Mollusca, Gastropoda). *Spixiana* 38, 187-195.

Bjorndal, K. A. (1990). Digestibility of the sponge C*hondrilu nucuu* in the green turtle, *Chelonia mydas*. *Bull Mar Sci* 47, 67–70.

Camps-Castella, J., Ballesteros, M., Trobajo, R., Pontes, M., and Prado, P. (2020). Not all nudibranchs are carnivorous: trophic ecology of *Polycerella emertoni* in the Ebro Delta. *Mar Ecol Prog Ser* 645, 67–82. doi: 10.3354/MEPS13379.

Cardona, L., Lloret-Lloret, E., Moles, J., and Avila, C. (2021). Latitudinal changes in the trophic structure of benthic coastal food webs along the Antarctic Peninsula. *Mar Environ Res* 167, 105290. doi: 10.1016/J.MARENVRES.2021.105290.

Casey, M. M., Fall, L. M., and Dietl, G. P. (2016). You are what you eat: Stable isotopic evidence indicates that the Naticid gastropod Neverita duplicata is an omnivore. *Front Ecol Evol* 4, 125. doi: 10.3389/FEVO.2016.00125/BIBTEX.

Cattaneo-Vietti, R. (1991). Nudibranch molluscs from the Ross Sea, Antarctica. *J Mollus Stud* 57 (Supplement part 4), 223-228. doi: 10.1093/mollus/57.Supplement_Part_4.223

Cerrano, C., Calcinai, B., Cucchiari, E., Camillo, C. di, Totti, C., and Bavestrello, G. (2004). The diversity of relationships between Antarctic sponges and diatoms: The case of *Mycale acerata* Kirkpatrick, 1907 (Porifera, Demospongiae). *Polar Biol* 27, 231–237. doi: 10.1007/s00300-003-0581-1.

Dayton, P. K., Mordida, B. J., and Bacon, F. (1994). Polar Marine Communities. *Am Zool* 34, 90-99. doi: 10.1093/icb/34.1.90

Dayton, P. K., Robilliard, G. A., Paine, R. T., and Dayton, L. B. (1974). Biological Accommodation in the Benthic Community at McMurdo Sound, Antarctica. *Ecol Monogr* 44, 105–128. doi: 10.2307/1942321.

Dayton, P. K., Watson, D., Palmisano, A., Barry, J. P., Oliver, J. S., and Rivera, D. (1986). Distribution Patterns of Benthic Microalgal Standing Stock at McMurdo Sound, Antarctica. *Polar Biol* 6, 207-213. doi: 10.1007/BF00443397

Dayton, P., Robilliard, G. A., and Paine, R. T. (1970). Benthic Faunal Zonation as a Result of Anchor Ice at McMurdo Sound, Antarctica. In M. W. Holdgate (Ed.), *Antarctic ecology* (Vol. 1, pp. 244–258). Academic Press.

de Goeij, J. M., van Oevelen, D., Vermeij, M. J. A., Osinga, R., Middelburg, J. J., de Goeij, A. F. P. M., et al. (2013). Surviving in a marine desert: The sponge loop retains resources within coral reefs. *Science (1979)* 342, 108–110. doi: 10.1126/SCIENCE.1241981.

Ekimova, I., Valdés, Á., Chichvarkhin, A., Antokhina, T., Lindsay, T., and Schepetov, D. (2019). Diet-driven ecological radiation and allopatric speciation result in high species diversity in a temperate-cold water marine genus *Dendronotus* (Gastropoda: Nudibranchia). *Mol Phylogenet Evol* 141, 106609. doi: 10.1016/J.YMPEV.2019.106609.

García, F. J., Troncoso, J. S., García-Gómez, J. C., and Cervera, J. L. (1993). Anatomical and taxonomical studies of the Antarctic nudibranchs *Austrodoris kerguelenensis* (Bergh, 1884) and *A. georgiensis* n. sp. from the Scotia Sea. *Polar Biol* 13, 417–421. doi: 10.1007/BF01681984

Garrone, Robert. (1978). Phylogenesis of connective tissue: morphological aspects and biosynthesis of sponge intercellular matrix. *In Frontiers of Matrix Biology* vol. 5 (ed. L. Robert), pp. 11-17. Basle: S. Karger.

Gillies, C. L., Stark, J. S., Johnstone, G. J., and Smith, S. D. A. (2012). Carbon flow and trophic structure of an Antarctic coastal benthic community as determined by δ13C and δ15N. *Estuar Coast Shelf Sci* 97, 44–57. doi: 10.1016/J.ECSS.2011.11.003.

Gillies, C. L., Stark, J. S., Johnstone, G. J., and Smith, S. D. A. (2013). Establishing a food web model for coastal Antarctic benthic communities: A case study from the Vestfold Hills. *Mar Ecol Prog Ser* 478, 27–41. doi: 10.3354/meps10214.

Goodheart, J. A., Bazinet, A. L., Valdés, Á., Collins, A. G., and Cummings, M. P. (2017). Prey preference follows phylogeny: Evolutionary dietary patterns within the marine gastropod group Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). *BMC Evol Biol* 17, 1–14. doi: 10.1186/S12862-017-1066-0/TABLES/2.

Graeve, M., Kattner, G., and Hagen, W. (1994). Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. *J Exp Mar Biol Ecol* 182, 97–110. doi: 10.1016/0022-0981(94)90213-5.

Gutt, J., Griffiths, H. J., and Jones, C. D. (2013). Circumpolar overview and spatial heterogeneity of Antarctic macrobenthic communities. *Marine Biodiversity* 43, 481–487. doi: 10.1007/S12526-013-0152-9/FIGURES/5.

Gutt, J., and Schickan, T. (1998). Epibiotic relationships in the Antarctic benthos. *Antarct Sci* 10, 398–405. doi: 10.1017/s0954102098000480.

Hoover, R. A., Armour, R., Dow, I., and Purcell, J. E. (2012). Nudibranch predation and dietary preference for the polyps of Aurelia labiata (Cnidaria: Scyphozoa). *Jellyfish Blooms IV*, 199–213. doi: 10.1007/978-94-007-5316-7_16.

Iken, K., Avila, C., Fontana, A., and Gavagnin, M. (2002). Chemical ecology and origin of defensive compounds in the Antarctic nudibranch *Austrodoris kerguelenensis* (Opisthobranchia: Gastropoda). *Mar Biol* 141, 101–109. doi: 10.1007/s00227-002-0816-7.

Imbs, A. B., and Grigorchuk, V. P. (2019). Lipidomic study of the influence of dietary fatty acids on structural lipids of cold-water nudibranch molluscs. *Scientific Reports 2019 9:1* 9, 1–10. doi: 10.1038/s41598-019-56746-8.

Iverson, S. J., Frost, K. J., and Lang, S. L. C. (2002). Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. *Mar Ecol Prog Ser* 241, 161–181. doi: 10.3354/MEPS241161.

Kadye, W. T., Redelinghuys, S., Parnell, A. C., and Booth, A. J. (2020). Exploring source differences on diet-tissue discrimination factors in the analysis of stable isotope mixing models. *Scientific Reports 2020 10:1* 10, 1–17. doi: 10.1038/s41598-020-73019-x.

Kelly, J. R., and Scheibling, R. E. (2012). Fatty acids as dietary tracers in benthic food webs. *Mar Ecol Prog Ser* 446, 1–22. doi: 10.3354/meps09559.

Kim, S., and Thurber, A. (2007). Comparison of seastar (Asteroidea) fauna across island groups of the Scotia Arc. *Polar Biol* 30, 415–425. doi: 10.1007/s00300-006-0198-2.

Knox, G. A. (2006). The biology of the Southern Ocean, 2nd ed. Boca Raton, FL: CRC Press, 640 pp. doi: 10.1201/9781420005134.

Komisarenko, A., Mordukhovich, V., Ekimova, I., and Imbs, A. (2021). A comparison of food sources of nudibranch mollusks at different depths off the Kuril Islands using fatty acid trophic markers. *PeerJ* 9, e12336. doi: 10.7717/PEERJ.12336/SUPP-4.

Lee, J. R., Raymond, B., Bracegirdle, T. J., Chadès, I., Fuller, R. A., Shaw, J. D., et al. (2017). Climate change drives expansion of Antarctic ice-free habitat. *Nature 2017 547:7661* 547, 49–54. doi: 10.1038/NATURE22996.

Maroni, P. J., Baker, B. J., Moran, A. L., Woods, H. A., Avila, C., Johnstone, G. J., et al. (2022). One Antarctic slug to confuse them all: the underestimated diversity of *Doris kerguelenensis*. *Invertebr Syst*, 1–17. doi: 10.1071/IS21073.

Matsuoka, K., Skoglund, A., and Roth, G. (2018). Quantarctica 3 [Simple and detailed basemaps]. Norwegian Polar Institute. doi: 10.21334/npolar.2018.8516e961.

Mcclintock, J. B. (1994). Trophic biology of Antarctic shallow-water echinoderms. *Mar Ecol Prog Ser* 111, 191–202. doi: 10.3354/meps111191.

McClintock, J. B., Amsler, C. D., Baker, B. J., and van Soest, R. W. M. (2005). Ecology of Antarctic Marine Sponges: An Overview. *Integr Comp Biol* 45, 359–368. doi: 10.1093/ICB/45.2.359.

McDonald, G. R., and Nybakken, J. W. (1997). A List of the Worldwide Food Habits of Nudibranchs. *Veliger* 40(2).

Michel, L. N., Danis, B., Dubois, P., Eleaume, M., Fournier, J., Gallut, C., et al. (2019). Increased sea ice cover alters food web structure in East Antarctica. *Sci Rep* 9. doi: 10.1038/s41598-019-44605-5.

Mikhlina, A., Ekimova, I., and Vortsepneva, E. (2020). Functional morphology and post-larval development of the buccal complex in Eubranchus rupium (Nudibranchia: Aeolidida: Fionidae). *Zoology* 143, 125850. doi: 10.1016/J.ZOOL.2020.125850.

Mikhlina, A., Tzetlin, A., and Vortsepneva, E. (2018). Renewal mechanisms of buccal armature in Flabellina verrucosa (Nudibranchia: Aeolidida: Flabellinidae). *Zoomorphology* 137, 31–50. doi: 10.1007/S00435-017-0370-Y/TABLES/1.

Minagawa, M., and Wada, E. (1984). Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. *Geochim Cosmochim Acta* 48, 1135–1140. doi: 10.1016/0016-7037(84)90204-7.

Mincks, S. L., Smith, C. R., Jeffreys, R. M., and Sumida, P. Y. G. (2008). Trophic structure on the West Antarctic Peninsula shelf: Detritivory and benthic inertia revealed by δ13C and δ15N analysis. *Deep Sea Res 2 Top Stud Oceanogr* 55, 2502–2514. doi: 10.1016/j.dsr2.2008.06.009.

Moles, J., Wägele, H., Cutignano, A., Fontana, A., Ballesteros, M., and Avila, C. (2017). Giant embryos and hatchlings of Antarctic nudibranchs (Mollusca: Gastropoda: Heterobranchia). *Mar Biol* 164. doi: 10.1007/s00227-017-3143-8.

Morley, S. A., Abele, D., Barnes, D. K. A., Cárdenas, C. A., Cotté, C., Gutt, J., et al. (2020). Global Drivers on Southern Ocean Ecosystems: Changing Physical Environments and Anthropogenic Pressures in an Earth System. *Front Mar Sci* 7, 1097. doi: 10.3389/FMARS.2020.547188/BIBTEX.

Morley, S. A., Berman, J., Barnes, D. K. A., Carbonell, C. de J., Downey, R. v., and Peck, L. S. (2016). Extreme phenotypic plasticity in metabolic physiology of Antarctic demosponges. *Front Ecol Evol* 3, 157. doi: 10.3389/FEVO.2015.00157/BIBTEX.

Norkko, A., Thrush, S. F., Cummings, V. J., Gibbs, M. M., Andrew, N. L., Norkko, J., et al. (2007). Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. *Ecology* 88, 2810–2820. doi: 10.1890/06-1396.1.

North, C. A., Lovvorn, J. R., Kolts, J. M., Cooper, L. W., and Grebmeier, J. M. (2019). Discriminating trophic niches of carnivorous benthic macroinvertebrates with gut contents, stable isotopes, and fatty acids. *Mar Ecol Prog Ser* 631, 49–66. doi: 10.3354/MEPS13161.

Pasotti, F., Manini, E., Giovannelli, D., Wölfl, A. C., Monien, D., Verleyen, E., et al. (2015). Antarctic shallow water benthos in an area of recent rapid glacier retreat. *Marine Ecology* 36, 716–733. doi: 10.1111/MAEC.12179.

Phleger, C. F., Nichols, P. D., and Virtue, P. (1998). Lipids and trophodynamics of Antarctic zooplankton. *Comp Biochem Physiol B Biochem Mol Biol* 120, 311–323. doi: 10.1016/S0305-0491(98)10020-2.

Piepenburg, D., Schmid, K., and Gerdes, D. (2002). The benthos off King George Island (South Shetland Islands, Antarctica): further evidence for a lack of a latitudinal biomass cline in the Southern Ocean. 146–158. doi: 10.1007/s003000100322.

Post, D. M. (2002). Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and assumptions. *Ecology*, 83(3), 703-718. doi: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Rossi, L., Sporta Caputi, S., Calizza, E., Careddu, G., Oliverio, M., Schiaparelli, S., et al. (2019). Antarctic food web architecture under varying dynamics of sea ice cover. *Sci Rep* 9. doi: 10.1038/s41598-019-48245-7.

Rovelli, L., Attard, K. M., Cárdenas, C. A., and Glud, R. N. (2019). Benthic primary production and respiration of shallow rocky habitats: a case study from South Bay (Doumer Island, Western Antarctic Peninsula). *Polar Biol* 42, 1459–1474. doi: 10.1007/S00300-019-02533-0.

Smith, J. A., Mazumder, D., Suthers, I. M., and Taylor, M. D. (2013). To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods Ecol Evol* 4, 612–618. doi: 10.1111/2041-210X.12048.

Teoh, H. W., Sasekumar, A., Ismail, M. H., and Chong, V. C. (2018). Trophic discrimination factor and the significance of mangrove litter to benthic detritivorous gastropod, *Ellobium aurisjudae* (Linnaeus). *J Sea Res* 131, 79–84. doi: 10.1016/J.SEARES.2017.11.005.

Thomas, D. N., Fogg, G. E., Convey, P., Fritsen, C. H., Gili, J. M., Gradinger, R., et al. (2008). *The Biology of Polar Regions*. Oxford University Press, Oxford, UK.

Thurber, A. R. (2007). Diets of Antarctic sponges: Links between the pelagic microbial loop and benthic metazoan food web. *Mar Ecol Prog Ser* 351, 77–89. doi: 10.3354/meps07122.

Wägele, H. (1989). Diet of some Antarctic nudibranchs (Gastropoda, Opisthobranchia, Nudibranchia). *Mar Biol* 100, 439–441. doi: 10.1007/BF00394819.

Wägele, H. (2004). Potential key characters in Opisthobranchia (Gastropoda, Mollusca) enhancing adaptive radiation. *Org Divers Evol* 4, 175–188. doi: 10.1016/J.ODE.2004.03.002.

Watanabe, T., Kitajima, C., and Fujita, S. (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture* 34, 115–143. doi: 10.1016/0044-8486(83)90296-X.

CAPÍTULO II

scientific reports

Check for updates

OPEN

Distribution of trace elements in benthic infralittoral organisms from the western Antarctic Peninsula reveals no latitudinal gradient of pollution

Paula De Castro-Fernández✉, Luis Cardona & Conxita Avila

Antarctica is considered one of the most pristine regions on Earth, but evidences of global and local anthropogenic pollution exist. Chromium (Cr), lead (Pb) and mercury (Hg) are bioaccumulated and sometimes biomagnified through the trophic web. We aim to determine whether a latitudinal gradient of these trace elements exists in benthic organisms along the rocky shores of the Antarctic Peninsula and the South Shetland Islands. Levels of Cr, Pb, and Hg were measured by ICP-MS in two macroalgae (*Palmaria decipiens* and *Desmarestia anceps* or *Desmarestia menziesii*), one gastropod (*Nacella concinna*), two starfishes (*Odontaster validus* and *Diplasterias brucei*), and suspended particulate organic matter (SPOM) from five sampling sites ranging in latitude from 62°11′17″S to 67°33′47″S. Levels of trace elements differed among sites and species, but no latitudinal gradient was observed for these pollutants. Levels of Hg and Pb in animals were consistent with biomagnifications along the food web, as were higher is starfish than in limpets. However, macroalgae and SPOM are unlikely to be the main primary producers supporting those consumers, as Hg levels in macroalgae and Pb levels in SPOM were much higher than in animals. The levels of trace elements detected were similar or higher than in other Antarctic places and other regions of the world, thus indicating that the Antarctic Peninsula area is as polluted as the rest of the world.

Trace elements current average concentration is less than about 100 parts per million atoms (ppma) or less than 100 μg g^{-1}[1]. They occur naturally in the earth crust, being present in soil parent materials and in the surface soil in variable proportions[2–4], but human activities, mainly mining, metal processing, fossil fuel combustion, use of pesticides, and waste disposal have introduced high quantities of them in the environment, thus resulting in significant pollution levels[2,3,5–7].

Antarctica is thought to be one of the last untouched and wild areas on Earth, since its remote location and the only recent, scarce, and highly seasonal human presence[8]. Nevertheless, there has been evidence of diverse anthropogenic impacts for a long time[9,10]. Anthropogenic trace element pollution in Antarctica may either be the result of global pollution or be produced locally[11,12]. The arrival with marine currents is largely constrained by the broad belt of the Southern Ocean's water, namely the Antarctic Circumpolar Current, constituting a barrier to this transport[13]. Thus, trace elements from other continents, primarily those in the Southern Hemisphere, are mainly transported with air masses that move towards Antarctica in what is called the long-range atmospheric transport (LRAT)[13–16].

Recently, more attention has been paid to the contaminants emitted locally in Antarctica as a result of increasing human activity[17]. As early as 1987, it was determined that four-fifths of total Pb in Antarctic air at that time had an anthropogenic origin[18]. Later, it was observed that levels of some trace elements (such as Pb and Zn) in the atmosphere over the Antarctic Peninsula were higher than it would be expected due to aerosol contribution[19]. Anthropogenic local pollution could be an explanation and local contamination caused by research stations and their associated activities, such as ship operations, sewage production, fuel consumption, and waste disposal, are the major sources of local contamination, as well as the developing tourism industry[16,20,21]. The Antarctic Peninsula and the South Shetland Islands are the most vulnerable regions to local pollution because they concentrate

Department of Evolutionary Biology, Ecology and Environmental Sciences & Biodiversity Research Institute (IRBio), University of Barcelona, Diagonal Ave. 643, 08028 Barcelona, Catalonia, Spain. ✉email: p.decastro@ub.edu

most of the human activity in the continent[8,12,22–24]. Accordingly, Jerez et al.[25] reported a latitudinal gradient in the levels of Pb, Cr, Al, and Mn in feathers of Adélie penguins *Pygoscelis adeliae* nesting along the Antarctic Peninsula. However, such pattern might not exist for species inhabiting shallow benthic habitats, more influenced by local processes. Currently, there is a wealth of studies reporting the levels of trace elements in Antarctic, benthic organisms[11,26–29], but we are not aware of any study addressing the existence of a latitudinal gradient in the levels of any trace element in benthic species.

Mercury (Hg) and lead (Pb) are non-essential elements that are toxic even at low concentrations[30]. They may displace other elements that act as enzymes cofactors and therefore diminish or block physiological processes[31]. Chromium (Cr) is an essential trace nutrient element that can be toxic at high concentrations[32], for example causing impairment of photosynthetic energetic pathway processes, blocking cell division or inhibiting enzyme activity in microalgal cells[33]. Hg, Pb and Cr are among the most relevant anthropogenic trace pollutants in Antarctica. Indeed, Pb and Hg are considered priority pollutants and Hg is also classified as a priority hazardous substance by the regulation in force[34]. Hence, it is important to monitor the concentration of these—and other—elements at different sites in Antarctica as they are highly related to human activities[25,35]. Furthermore, assessing the concentrations and effects of trace elements on Antarctic marine organisms could contribute to prevent the loss of ecosystem services that Antarctic biodiversity provide[36]. The choice of these elements is further justified since most invertebrates are not capable of regulating their body concentrations[37–39]. Therefore, the levels of these elements in the organisms represent or correlate to the levels in the environment. Hg is particularly interesting because it biomagnifies through the food chain and may be therefore useful as trophic tracer[40].

Sediment conditions usually represent the average state of the system as they have high physicochemical stability[41,42]. They can act as trace elements or pollutants reservoirs, offering a history of pollution of the environment[43–45]. Also, trace elements concentration in water masses could be extrapolated from sediments analysis[23]. Therefore, determining trace element contamination in sediments could provide information about marine environment conditions and represent average water quality[46]. Nevertheless, some studies concluded that measuring the concentration of a chemical in the organism is more useful to predict the effects of the substance in the organism, i.e. toxicity, than analyzing environmental levels such as sediment or water concentration. Marine organisms incorporate trace elements from the environment and accumulate some of them in their soft tissues, where levels of contaminants are several orders of magnitude above the environmental levels[20]. One advantage of using body concentration as an indicator of bioavailability is that environmental chemical conditions, such as salinity, pH, or temperature, as well as the chemical state of the element, which may affect the element toxicity, can be avoided[47]. Besides, this measure integrates the accumulation of the chemical due to exposures that may be intermittent, from different origins and different compartments[48]. However, the element must not be regulated by the organism for body concentration to be a good indicator of toxic effects. Thus, the level of the element in the organism must increase with increasing environmental concentrations[24].

In addition to bioaccumulation, some elements biomagnify trough the food chains, i.e., they are transferred to higher trophic levels via dietary uptake[49]. As a consequence, organisms at higher trophic levels have higher body levels of those elements than their prey, which makes them useful as trophic tracers. It should be noted, however, that Antarctic benthic animals rely on a diversity of primary producers, including sympagic algae, microphytobenthos, macroalgae, and phytoplankton[50–54], although phytoplankton dynamics plays a major role in structuring the benthic marine food web of the region[55].

The main aim of this study is to assess and compare the levels of the three trace elements (Cr, Pb, and Hg) in suspended particulate organic matter and five benthic species from shallow, sheltered rocky bottom ecosystems along a latitudinal gradient along the South Shetland Islands and the western Antarctic Peninsula to test the hypothesis that the pollution levels decrease southward in parallel to human activity. The selected species included three primary producers, one herbivore and two carnivores[55].

Materials and methods

Study area. Five sites along the South Shetland Islands and the Antarctic Peninsula were chosen for the study: Fildes Bay (King George Island), Hope Bay, Cierva Cove, Paradise Bay, and Rothera Point (Fig. 1). These locations cover a latitude from 62°12′7″S in Fildes Bay to 67°34′30″S in Rothera Point, and hence most of the Antarctic Peninsula latitudinal extension. They provide a large enough extension to test the existence of a latitudinal gradient in trace elements pollution. All stations are located in closed and sheltered coastal zones and hence comprise highly sensitive local conditions. Furthermore, all the selected locations are situated near human facilities, mainly research stations[56], which are the most likely sources of local pollution.

Species selection and sampling. Subtidal, sheltered rocky bottoms around Antarctica share a similar community, dominated by the canopy forming brown macroalgae *Desmarestia anceps* and *Desmarestia menziesii* and a dense undergrowth of the red macroalga *Palmaria decipiens*[57]. *Desmarestia menziesii* is usually the dominant species just below the heavy scour area, being eventually replaced at greater depths by *Desmarestia anceps*[57]. However, there are locations where only one of these two *Desmarestia* species are present[57]. The starfish *Odontaster validus* is also widespread around the whole continent[58] and the starfish *Diplasterias brucei* is also thought to be circumantarctic, although detailed information is missing[58]. Both are very abundant in shallow kelp forests off the western Antarctic Peninsula. Finally, the limpet *Nacella concinna* is restricted to the western Antarctic Peninsula[59], where it is one of the most conspicuous gastropods from the shallow kelp forests. Stable isotope analysis has confirmed that *Nacella concinna* is an herbivore and the two starfish species are carnivores[55].

Five specimens of each of species were collected at five different sites along the South Shetland Islands and the western Antarctic Peninsula during the DISTANTCOM cruise (CTM2013-42667/ANT) from February 12th

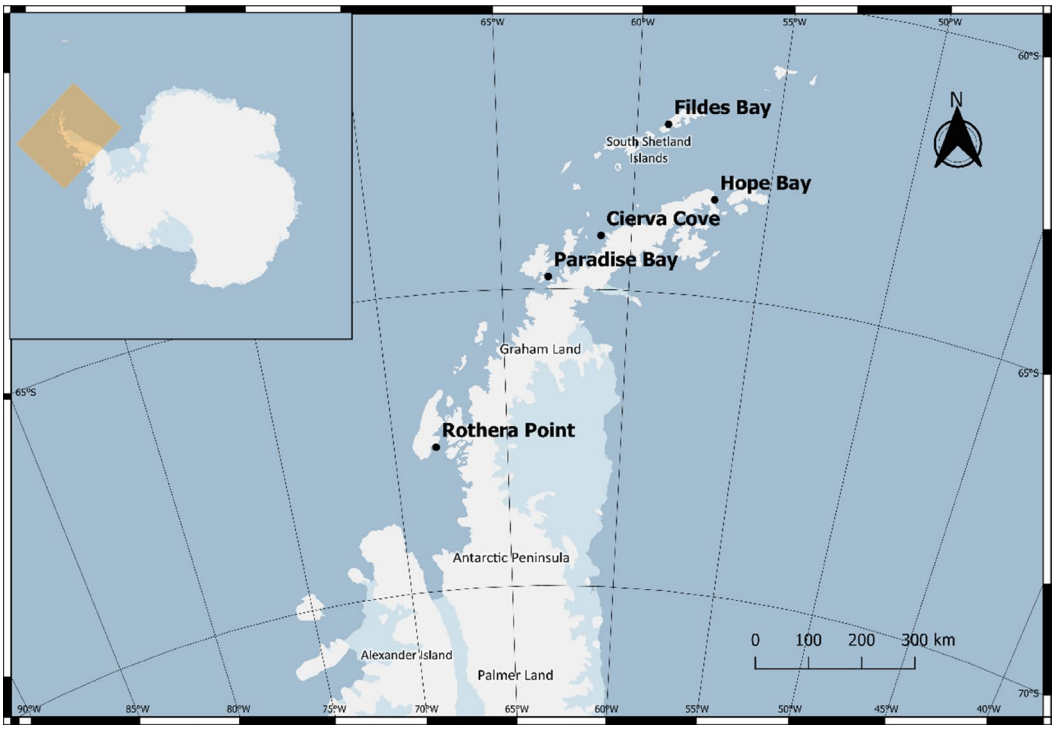


Figure 1. Study area and location of the sampling sites. The map was created using the Free and Open Source QGIS 3.16 software (http://www.qgis.org/) and the Quantarctica 3.2 project (https://www.npolar.no/quantarctica/).

Phylum	Species	Location				
		Fildes Bay	Hope Bay	Cierva Cove	Paradise Bay	Rothera Point
		62°11′17.3″S	63°22′18.4″S	64°05′26.3″S	64°53′43.7″S	67°33′47″S
		58°52′16.8″W	56°58′55.7″W	60°59′06.7″W	62°55′48″W	68°10′01.6″W
Several	(SPOM)	5	5	5	5	5
Ochrophyta	*Desmarestia anceps* Montagne, 1842	0	5	5	5	5
	Desmarestia menziesii J.Agardh, 1848	5	0	0	0	0
Rhodophyta	*Palmaria decipiens* (Reinsch) R.W.Ricker, 1987	5	5	5	5	5
Mollusca	*Nacella concinna* (Strebel, 1908)	5	5	5	5	5
Echinodermata	*Diplasterias brucei* (Koehler, 1907)	5	5	5	5	5
	Odontaster validus Koehler, 1906	5	5	5	5	5

Table 1. Taxa, number and sites of collection of the samples. *SPOM* Suspended Particulate Organic Matter.

to February 22nd, 2016 (Table 1). A sample size of five or less is extensively used to determine the concentration of trace elements in marine communities[20,26,27,29,60].

Benthic organisms were collected by SCUBA diving at depths between 10 and 15 m. Organisms were detached from the rocky bottom by hand or with the aid of a knife and were placed in 1 L clean plastic containers. Suspended particulate organic matter (SPOM) was also collected at the five sampling sites, as sinking phytoplankton plays major structuring the food web of shallow kelp forests[55]. SPOM was collected with a 50 µm mesh size plankton net towed horizontally at 5 m depth at low speed (1.85–3.7 km/h). Each SPOM sample is the result of towing the plankton net for 4–10 min, depending on plankton density at each site.

Sample processing. Subsamples of Suspended Particulate Organic Matter (SPOM) were visually checked under an optical microscope immediately after collection to assess the dominant groups. They consisted in diatoms (Ochrophyta) and dinoflagellates (Myzozoa) in all cases. All samples were frozen at − 20 °C and once in the laboratory at the University of Barcelona (UB), they were thawed on ice and processed prior to analytical

determinations. Two ml of concentrated phytoplankton were collected and a 2 × 2 cm fragment of epibiont-free blade of the macroalgae was selected. Limpets were dissected and the gut and its contents removed. Limpet radulas and shells were also discarded. For starfishes, only 1 to 3 arms were sampled to analyze each specimen, after discarding the gut. For trace elements determination, we followed the protocol established in the Scientific and Technological Centers of the UB (CCiTUB, http://www.ccit.ub.edu/EN/), standardized and validated in previous works[60–65]. In these works, the entire analytical procedure was validated by analysing one or more blanks, replicates and one certified reference material for every batch of samples. Replicates were found to differ below 10% and the recovery percentage fell between 90 and 100%[60–65]. Samples were dried for 24 h at 60 °C and then homogenized to powder using a ceramic mortar and pestle. 100 mg dw of each homogenized sample were digested with a 2:1 HNO_3 (69–70% Baker Instra—Analyzed Reagent) and H_2O_2 (30% Suprapur Merck) solution in Teflon vessels previously cleaned with HNO_3 under pressure at 90 °C for 24 h. For this, 2 mLof HNO_3 and 1 mL of H_2O_2 were used. The digested solution was diluted with 20 mL of ultrapure water (HNO_3:H_2O 1:10). Cr, Pb, and Hg were determined in the diluted digested solution by ICP-MS (PerkinElmer NexION 350D). Three digestion blanks were prepared in each sample digestion series (25 samples) to assess contamination during the analytical procedure.

Data analysis. General linear models (GLM) were used to assess differences in the levels of Cr, Pb, and Hg across species and sites. Previous research has suggested that *Odontaster validus* and *Diplasterias brucei* fed on *Nacella concinna*[55,66]. In order to check this hypothesis and assess the existence of a significant biomagnification pattern, general linear models were performed for each trace element including only these three animal species. A multivariate cluster analysis, using the squared Euclidean distance as a metric and UPGMA as the clustering method, was performed to assess whether the distinct groups of samples recovered according to their levels of Cr, Pb and Hg matched any latitudinal pattern. Furthermore, Principal Component Analysis (PCA) was performed to assess the hypothesis that geographically close sampling sites would lay closer in the space defined by the levels of Cr, Pb and Hg in the five benthic species. The main objective of PCA was to characterize each sampling site by projecting the data in a much smaller sub set of new variables called principal components. To do so, we characterized the benthic community from each sampling site using eighteen variables, corresponding to the levels of each trace element in each species. All the variables were standardized prior to analysis. These new variables extracted by PCA are linear combinations of the initial variables, but highlight the variance within a data set and remove the redundancies, and are orthogonal. Only principal components with an eigenvalue higher than 1 are considered for further analysis[67–69]. General linear models were performed using SPSS Statistics v23 (IBM Corporation) and Principal Component Analysis was performed using PRIMER v7 (PRIMER-e).

Results

Levels of Cr, Hg, and Pb in all the studied species were measured at the five sites (Figs. 2 and 3). The data values represented in Figs. 2 and 3 are provided in Supplementary Table 1. Cr and Pb levels were positively correlated when the whole data set was considered (Pearson correlation; $r = 0.464$, $p < 0.001$), but were uncorrelated with the level of Hg (Pearson correlation; Hg vs. Cr $p = 0.138$; Hg vs. Pb $p = 0.339$).

Species differed significantly in levels of Cr, Pb, and Hg. Site had a significant effect on Cr and Hg and was in the verge of significance for Pb (Suppl. Tables 2–4). The interaction term was always significant, thus revealing idiosyncratic variations among species across sites.

The levels of trace elements in primary producers (SPOM, *Desmarestia* spp. and *Palmaria decipiens*) were usually higher than in the animal species from the same sampling site, with SPOM and *Palmaria decipiens* values being higher than those of *Desmarestia* spp. (Figs. 2 and 3). This difference was even larger in the case of the Hg values of macroalgae and those of animals (Fig. 2B). SPOM was highly enriched in Pb (Fig. 3), with values ranging from 1.85 to 537.08 ng g^{-1}, therefore multiplying the highest value of the other species by a factor of 25. At the same time, SPOM had in general lower Hg values than the sampled species, while both macroalgae (*Palmaria decipiens* and *Desmarestia* spp.) had higher values than the other species, as highlighted before (Fig. 2B).

Regarding the levels of Cr and Pb in consumers, they varied widely across species and zones, as there was a significant (species × site) term in both models (Suppl. Tables 5 and 6). On the contrary, the interaction term of the general linear model for Hg was not significant (Suppl. Table 7), thus revealing that the same pattern was observed in the three animal species across sites, despite differences in the baseline levels among sites. The starfish species had higher levels of Hg than the limpet at all the sites studied. This corroborates that both starfishes are at a higher trophic level than the limpet.

Cluster analysis revealed no latitudinal pattern for any species, as groups always included samples from distant sampling sites (Suppl. Fig. 1–6). For instance, most samples of SPOM, *Desmarestia* spp. and *Nacella concinna* from the five sampling sites clustered together, and samples of *Palmaria decipiens* from Paradise Bay clustered with samples from distant Fildes Bay and Hope Bay, but not with those from nearby Cierva Cove, which in turn clustered with those from distant Rothera Point. Regarding *Diplasterias brucei* and *Odontaster validus*, samples were split in three and four major groups respectively, all them including samples from at least three sites and most including samples from the two most distant sites (Fildes Bay and Rothera Point).

PCA yielded four principal components with eigenvalues higher than 1 and PC 1, PC 2 and PC 3 explained 87.5% of the variance (Fig. 4). High scores of the first component (PC 1) corresponded to high levels of Cr, Pb, and Hg in SPOM and *Palmaria decipiens* and low levels of these trace elements in *Desmarestia* spp. and the animal species (*Nacella concinna*, *Diplasterias brucei,* and *Odontaster validus*) (Table 2). Contrarily, high scores of the second component (PC 2) were associated to high levels of of Cr and Hg in the animal species and low Pb levels in *Nacella concinna*, *Diplasterias brucei* and both macroalgae (Table 2). High scores of the third component (PC 3) corresponded to high levels of Hg in *Desmarestia* spp. (Table 2). Sampling sites were distributed independently

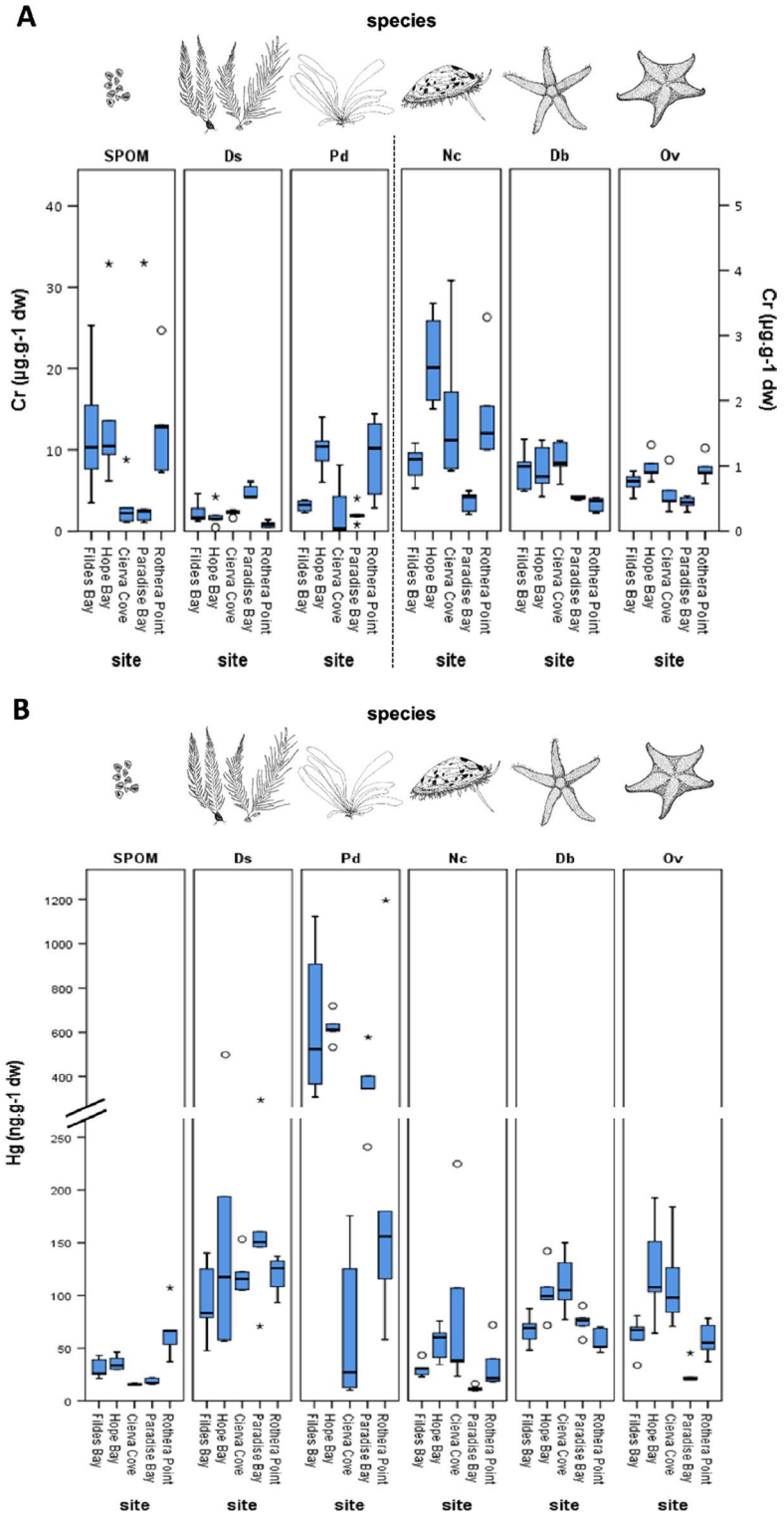


Figure 2. Concentration of Cr and Hg in the different sites and species studied along the Antarctic Peninsula. (**A**) Cr, values expressed as µg g-1 of dry weight. The values of Cr for the primary producers were much higher than the animal species, so they were represented separately and scale changed accordingly. (**B**) Hg, values expressed as ng g^{-1} of dry weight. Discontinuous Y-axis was used to facilitate visualization. Central lines show the mean, boxes extend to the 25th and 75th percentile, whiskers extend to 1.5 × IQR, dots and asterisks are outliers. *SPOM* Suspended Particulate Organic Matter, *Ds Desmarestia* spp., *Pd Palmaria decipiens*, *Nc Nacella concinna*, *Db Diplasterias brucei*, *Ov Odontaster validus*. The images of species were drawn by the authors of this article.

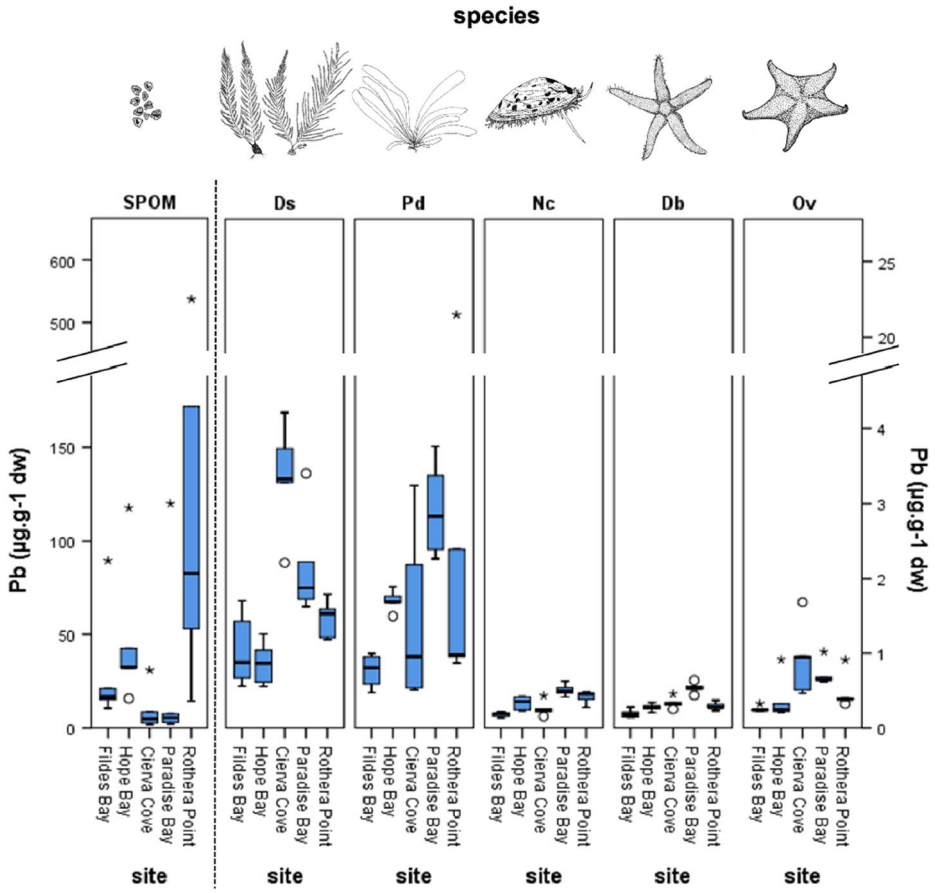


Figure 3. Concentration of Pb in the different sites and species studied. The values of Pb for the SPOM were in different magnitude order than the benthic species, so they were represented separately and scale changed accordingly. Values are expressed as µg g^{-1} of dry weight. Discontinuous Y-axes were used to facilitate visualization. Central lines show the mean, boxes extend to the 25th and 75th percentile, whiskers extend to 1.5 × IQR, dots and asterisks are outliers. *SPOM* Suspended Particulate Organic Matter, *Ds Desmarestia* spp., *Pd Palmaria decipiens*, *Nc Nacella concinna*, *Db Diplasterias brucei*, *Ov Odontaster validus*. The images of species were drawn by the authors of this article.

from latitude in the space delimited by PC 1 and PC 2 axes. Certainly, Rothera Point and Paradise Bay had similar PC 2 scores and the same was true for Cierva Cove and Hope Bay, thus supporting the existence of a latitudinal gradient along that axis. However, the northernmost site, Fildes Bay, had intermediate values.

Cierva Cove and Paradise Bay sampling sites opposed to Rothera Point, Hope Bay and, to a less extent, Fildes Bay along PC 1, and Paradise Bay and Rothera Point opposed to Cierva Cove, Hope Bay and, to a less extent, Fildes Bay in relation to PC 2 (Fig. 4). Fildes Bay and Rothera Point were at opposite ends of PC 3 axis. However, Hope Bay and Paradise Bay had similar PC 3 scores and Cierva Cove was the closest site to Rothera Point along PC 3 axis (Fig. 4). Thus, concentrations of Cr, Pb, and Hg in *Desmarestia* spp. and the three animal species were significantly higher for specimens from Cierva Cove and Paradise Bay, while in Rothera Point, Hope Bay, and Fildes Bay, higher levels of these three trace elements were found in SPOM and *Palmaria decipiens*. In Paradise Bay and Rothera Point, the levels of Pb in *Nacella concinna*, *Diplasterias brucei,* and the two macroalgae were higher than in the other sites. On the other hand, Cierva Cove, Hope Bay, and Fildes Bay showed higher concentrations of Hg and Cr in the animal species than the other sampling zones. Importantly, there was no latitudinal gradient in the ordination of the five sampling sites along any of the two axes.

Discussion

Levels of Cr, Pb, and Hg in primary producers and consumers from five sampling sites along a latitudinal gradient in the South Shetland Islands and the Antarctic Peninsula revealed no clear latitudinal trend. Instead, the levels of each trace element varied idiosyncratically, as revealed by the significant interaction (species × site) in GLMs. As the species analyzed here belong to the same food web[50–55], the most plausible explanation to these idiosyncratic changes in trace element burden is that local processes are more relevant for benthic communities than any latitudinal gradient of human disturbance or natural transport of pollutants from areas at lower latitude. Certainly, a latitudinal gradient has been previously reported for the levels of trace elements in the feathers of penguins[25], but this are pelagic, highly mobile predators, foraging at a much broader scale. On the contrary, the benthic species

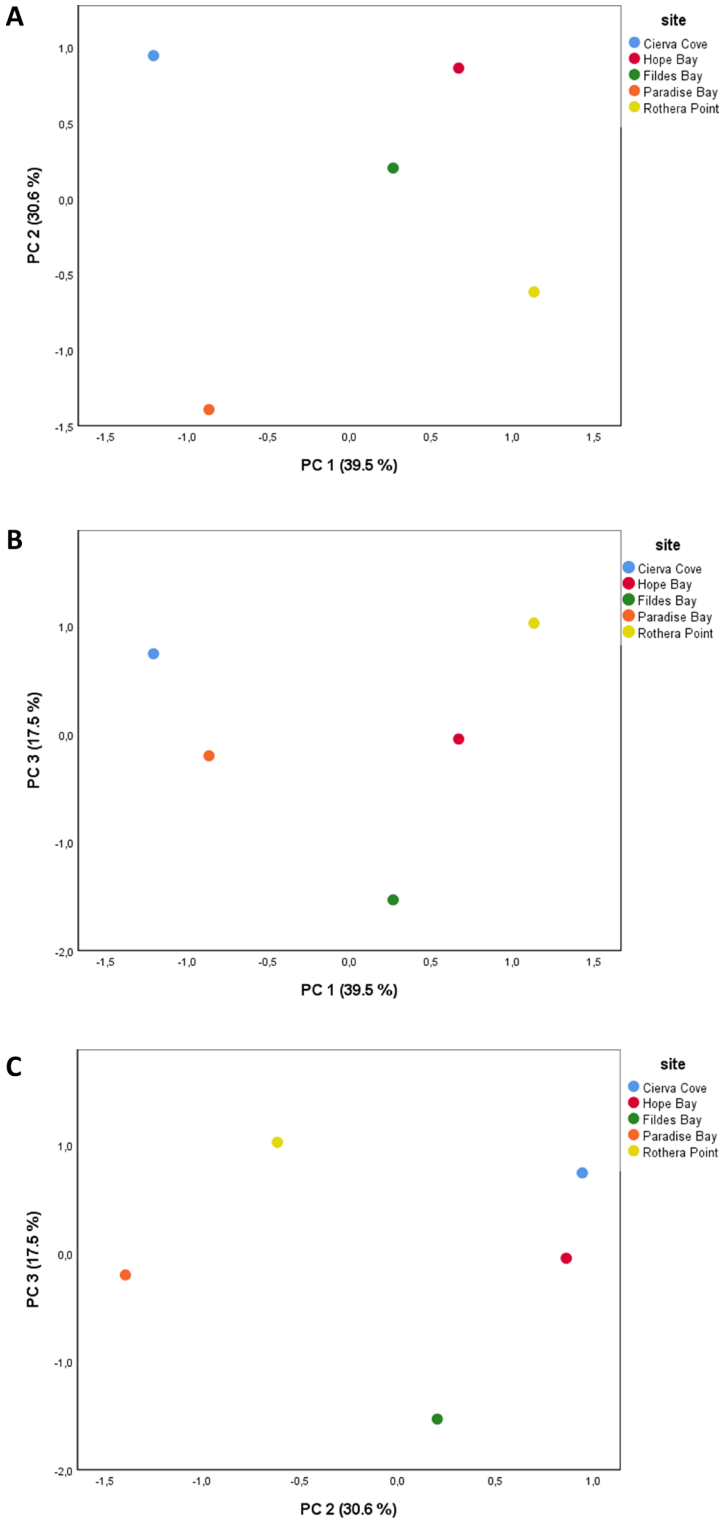


Figure 4. Principal Component Analysis (PCA) score plots. (**A**) PC 1 vs. PC 2. (**B**) PC 1 vs. PC 3. (**C**) PC 2 vs. PC 3.

sampled here were sessile (macroalgae) or had a limited mobility (limpets and starfishes) and hence they seem to be more sensitive to local differences in trace element pollution than to any latitudinal gradient.

Primary producers exhibited the highest levels of trace elements, particularly Cr and Pb, probably because of accumulation mechanisms typical of micro- and macroalgae[70]. Alternatively, Farías et al.[71] argued that high levels of Cr and Pb in some species may be the consequence of not completely removing fine particulate matter during washing of the specimens after collecting them, although we do not believe this is the case in our study. Even

Variable	Component		
	1	2	3
Cr SPOM	0.920	− 0.068	− 0.248
Pb SPOM	0.763	− 0.335	− 0.243
Hg SPOM	0.897	− 0.183	− 0.223
Cr Ds	− 0.690	− 0.510	0.368
Pb Ds	− 0.840	0.050	− 0.256
Hg Ds	− 0.020	− 0.072	0.981
Cr Pd	0.837	0.211	0.346
Pb Pd	0.427	− 0.604	− 0.103
Hg Pd	0.595	− 0.011	0.330
Cr Nc	0.446	0.734	0.253
Pb Nc	− 0.029	− 0.770	0.461
Hg Nc	− 0.268	0.862	− 0.097
Cr Db	− 0.339	0.897	0.001
Pb Db	− 0.585	− 0.651	0.401
Hg Db	− 0.501	0.731	0.412
Cr Ov	0.907	0.380	0.075
Pb Ov	− 0.806	− 0.051	− 0.013
Hg Ov	0.045	0.943	0.230

Table 2. PCA component matrix. Values represent the contribution of each variable to PC 1 and PC 2. *SPOM* Suspended Particulate Organic Matter, *Ds Desmarestia* spp., *Pd Palmaria decipiens*, *Nc Nacella concinna*, *Db Diplasterias brucei*, *Ov Odontaster validus*.

though the trace element content differs among species from the same habitat[72,73], it has been widely reported that macroalgae concentrate trace elements in their tissues, with concentration factors respect to sea water content as high as 10,000 for Ti and with values of up to 500 for Cr in brown algae, as an example[20,72]. Trace elements can be accumulated in different extra- and intracellular compartments[73–77]. Both micro- and macroalgae take up trace elements and other elements basically through two mechanisms: by attaching them to its cellular surface in a process called biosorption, which is reversible, and by irreversibly binding additional elements after biosorption, often through diffusion to the cytoplasm and binding to proteins or other intracellular structures[70]. Regarding SPOM, its enrichment in trace elements had been reported previously[78]. Diatoms silicic frustule turns to be a highly adsorptive surface that is implicated in the removal of trace element ions from the water column[79]. The attachment of Cr and Pb to diatoms surface would explain the high values reported for these trace elements in the SPOM, since diatoms are the dominant taxa in phytoplankton communities of the inshore waters of the Western Antarctic Peninsula during the summer season[80–83].

The range and mean values of concentrations of the three elements in our species, together with levels recorded in the literature for the same species from other places in the Antarctic continent are compared here (Table 3). Cr, Pb and Hg levels obtained in the present study can be also compared to those reported in the literature from other regions of the world (Table 4) showing that trace element pollution in Antarctica is biologically relevant. Taxonomically related and/or ecologically similar species were selected for this comparison. When the same genus was not available, similar feeding strategy or same taxonomic group species were chosen. Particularly, trace element levels in SPOM were quite striking, as highlighted before. The values obtained in the present study were higher than those in the literature, except for mean Hg content compared to other Antarctic sites, similar but slightly lower than that of the SPOM of Terra Nova Bay[84]. Mean Cr content in our SPOM samples was an order of magnitude higher than that reported for the White Sea and the World Ocean. SPOM's Pb mean level (57.57 μg g^{-1} dw) was also an order of magnitude higher than the maximum value reported previously in Antarctica and still higher than the mean value reported for the White Sea (36.05 μg g^{-1} dw), and much higher than the mean Pb value for the world ocean (8.70 μg g^{-1} dw)[85].

Regarding the red algae *Palmaria decipiens*, the mean Cr content doubled the recorded in other works and the range obtained in this study was broad (Table 4). Pb content in the specimens collected in this study are from one to two orders of magnitude higher than samples of edible red algae *Palmaria palmata* cultivated in Asia and in the European Union[86], and with a lower mean value, even though still within the same range of values, than those of *Palmaria palmata* sold in Italy[87]. The mean Pb content in the Antarctic red algae was higher than that of two other different Rodophyceae collected in India and Oman, the latter considered a relatively not impacted place (Table 4).

For Cr, the mean content in *Desmarestia* spp. (2.4 μg g^{-1} dw) fell between the mean value in Runcie and Riddle[88] (1.70 μg g^{-1} dw) and that in Farías et al.[71] (3.25 μg g^{-1} dw), and was slightly lower than that reported in Trevizani et al.[28] near Comandante Ferraz station, in King Geoge Island (South Shetland Islands) (9.33 μg g^{-1} dw). Cr range values in *Desmarestia* spp. and other Phaeophyceae from such different sites as Australia, Svalbard Islands, and Scotland fell within the same range (Table 4). Regarding Pb, the range of values in *Desmarestia*

Sample	Cr (μg g^{-1} dw)		Pb (μg g^{-1} dw)		Hg (ng g^{-1} dw)		Locality	Reference
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD		
Suspended Particulate Organic Matter	1.05–32.98	10.28 ± 9.51	1.85–537.08	57.57 ± 109.48	15.12–107.26	33.49 ± 21.82	South Shetland Islands and Antarctic Peninsula	Present study
Diatom *Phaeodactylum tricornutum*	–	–	–	6.3 ± 0.20	–	–	Bellingshausen Dome, King George Island	102
Phytoplankton	–	–	–	5.7	–	–	Offshore waters, Maxwell Bay, King George Island	102
Nano and micro-phytoplankton	–	–	–	0.13 ± 0.01	–	–	Terra Nova Bay, Ross Sea	103
Particulate Organic Matter	–	–	–	–	28.00–47.00	39.00	Terra Nova Bay, Ross Sea	84
Desmarestia anceps/ D. menziesii	0.46–6.11	2.45 ± 1.63	0.56–4.22	1.78 ± 1.05	47.60–498.08	136.81 ± 90.57	South Shetland Islands and Antarctic Peninsula	Present study
Desmarestia anceps	–	–	–	–	–	32.60	Admiralty Bay, King George Island	11
Desmarestia anceps	–	3.25 ± 0.19	–	< 0.60	–	–	Potter Cove, King George Island	71
Desmarestia anceps	–	1.70 ± 0.80	–	9.40 ± 7.60	–	–	East Antarctica	88
Desmarestia anceps	–	3.25	–	0.82	–	< QLM	Ullman Point, Admiralty Bay, King George Island	28
Desmarestia menziesii	–	9.33 ± 2.69	–	< QLM	–	< QLM	Ferraz, Admiralty Bay, King George Island	28
Desmarestia menziesii	–	3	–	4.41	–	< QLM	Botany Point, Admiralty Bay, King George Island	28
Palmaria decipiens	0.14–14.41	5.39 ± 4.52	0.47–5.34	2.44 ± 4.08	10.17–1194.20	411.89 ± 330.98	South Shetland Islands and Antarctic Peninsula	Present study
Palmaria decipiens	–	–	–	–	–	20.40	Admiralty Bay, King George Island	11
Palmaria decipiens	–	2.05 ± 0.10	–	< 0.60	–	–	Potter Cove, King George Island	71
Palmaria decipiens	–	2.80 ± 0.30	–	2.30 ± 0.70	–	–	East Antarctica	88
Nacella concinna	0.26–3.85	1.56 ± 1.03	0.14–0.62	0.34 ± 0.14	9.66–224.55	43.55 ± 44.62	South Shetland Islands and Antarctic Peninsula	Present study
Nacella concinna	–	2.16 ± 0.58	–	1.42 ± 0.39	–	–	Marian Cove, King George Island	91
Nacella concinna	–	< 0.01	–	0.45 ± 0.06	–	–	Potter Cove, King George Island	90
Nacella concinna	–	–	–	–	–	26.10	Admiralty Bay, King George Island	11
Nacella concinna	–	2.57	–	< QLM	–	< QLM	Ferraz, Admiralty Bay, King George Island	28
Diplasterias brucei	0.28–1.10	0.78 ± 0.36	0.14–0.64	0.33 ± 0.13	45.92–150.13	82.95 ± 28.1	South Shetland Islands and Antarctic Peninsula	Present study
Odontaster validus	0.29–0.98	0.74 ± 0.29	0.21–1.68	0.55 ± 0.36	19.95–192.42	76.47 ± 47.4	South Shetland Islands and Antarctic Peninsula	Present study
Odontaster validus (arms)	–	0.74 ± 0.05	–	0.17 ± 0.03	–	–	Cape Evans, Ross Sea	97
Odontaster validus (arms)	–	0.70 ± 0.05	–	0.13 ± 0.03	–	–	Terra Nova Bay, Ross Sea	97
Odontaster validus (arms)	–	–	–	0.51 ± 0.22	–	–	Terra Nova Bay, Ross Sea	104
Odontaster validus (arms)	–	–	–	–	–	40.00 ± 10.00	Terra Nova Bay, Ross Sea	101
Odontaster validus (arms)	–	–	–	–	60.00–220.00	110.00	Terra Nova Bay, Ross Sea	84
Odontaster validus (integument)	–	–	–	0.60 ± 0.28	–	–	Port Foster, Deception Island	99

Table 3. Trace elements concentrations (Cr, Pb, Hg) of the studied species in Antarctica. Data are given as mean ± SD, when possible. QLM: Quantification Limit of the Method.

anceps and *Desmarestia menziesii* was similar to that of *Desmarestia aculeata* collected in the Svalbard Islands and *Padina tenuis*, another brown algae collected in Australian waters (Table 4). The mean Pb content in *Desmarestia* spp. from the present study was ten times higher than that of the brown algae *Nizamuddinia zanardinii* from a site in southern Oman[89], suggesting the South Shetland Islands and the Antarctic Peninsula are impacted by anthropogenic pollution. On the other hand, the values measured in other brown algae were higher than those presented in this study. The maximum value in *Padina tetrastromatica* from Australia more than doubles the highest concentration in Antarctic *Desmarestia* spp. and *Fucus serratus*. Pb minimum value was similar to the highest value obtained in this study.

The Cr mean value for *Nacella concinna* fell between the mean values reported in Potter Cove[90] and Admiralty Bay[28], both in King George Island (South Shetland Islands), even though the maximum level obtained in our study exceeded the highest value reported in the literature. Pb mean level in *Nacella concinna* was slightly lower than that reported for the same species in Potter Cove[90] and also fell below that reported in Marian Cove (King George Island)[91] (Table 3). Other limpet species in Southern Oman and the Spanish Mediterranean coast had slightly higher levels of Pb than those reported in the present study (Table 4), although the maximum value here

Sample	Cr (μg g^{-1} dw)		Pb (μg g^{-1} dw)		Hg (ng g^{-1} dw)		Locality	Reference
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD		
Suspended Particulate Organic Matter	1.05–32.98	10.28 ± 9.51	1.85–537.08	57.57 ± 109.48	15.12–107.26	33.49 ± 21.82	South Shetland Islands and Antarctic Peninsula	Present study
Plankton	–	1.38	–	36.05	–	2.50	White Sea	106
Plankton	–	1.80	–	8.70	–	30.00	World Ocean	85
Desmarestia anceps/ D. menziesii	0.46–6.11	2.45 ± 1.63	0.56–4.22	1.78 ± 1.05	47.60–498.08	136.81 ± 90.57	South Shetland Islands and Antarctic Peninsula	Present study
Desmarestia aculeata	0.56–2.40	–	0.08–1.60	––	–	–	Spitsbergen, Svalbard Islands	113
Padina tenuis	1.40–10.00	–	0.10–6.20	–	–	–	Townsville coastal waters, Queensland, Australia	114
Padina tetrastromatica	1.60–9.90	–	1.10–10.20	–	–	–	Townsville coastal waters, Queensland, Australia	114
Nizamuddinia zanardinii	–	–	–	0.17	–	6.00	Dhofar, southern Oman (relatively unspoiled)	89
Fucus serratus	0.70–2.60	–	4.00–21.00	–	–	–	Scotland	72
Stypocaulon scoparium	–	–	–	–	100.00–200.00	–	Port-Cros Bay, France	115
Stypocaulon scoparium	–	–	–	–	–	30.00	Port-Cros National Park, France	115
Palmaria decipiens	0.14–14.41	5.39 ± 4.52	0.47–5.34	2.44 ± 4.08	10.17–1194.20	411.89 ± 330.98	South Shetland Islands and Antarctic Peninsula	Present study
Palmaria palmata	–	0.16 ± 0.15	–	0.04 ± 0.00	–	–	Asia (China, Japan, South Korea)	86
Palmaria palmata	–	0.08 ± 0.05	–	0.05 ± 0.03	–	–	European Union	86
Palmaria palmata	–	–	–	4.40 ± 0.30	–	–	Purchased in Italy	87
Gracilaria longissima		0.90 ± 0.10	–	0.90 ± 0.40	–	–	Gulf of Kutch, India	116
Gelidium sp.	–	–	–	1.41	–	8.00	Dhofar, southern Oman (relatively unspoiled)	89
Nacella concinna	0.26–3.85	1.56 ± 1.03	0.14–0.62	0.34 ± 0.14	9.66–224.55	43.55 ± 44.62	South Shetland Islands and Antarctic Peninsula	Present study
Patella caerulea	–	–	0.67–1.29	0.98	–	–	Spanish Mediterranean coast	117
Cellana rota	–	–	–	0.45	–	21.00	Dhofar, southern Oman (relatively unspoiled)	89
Diplasterias brucei	0.28–1.10	0.78 ± 0.36	0.14–0.64	0.33 ± 0.13	45.92–150.13	82.95 ± 28.1	South Shetland Islands and Antarctic Peninsula	Present study
Odontaster validus	0.29–0.98	0.74 ± 0.29	0.21–1.68	0.55 ± 0.36	19.95–192.42	76.47 ± 47.4	South Shetland Islands and Antarctic Peninsula	Present study
Echinaster sepositus	–	–	–	–	930.00–1620.00	–	Port-Cros Bay, France	115
Echinaster sepositus	–	–	–	–	–	100.00	Port-Cros National Park	115
Echinaster sepositus	–	0.83	–	–	–	–	Saronikos gulf, Greece	98
Marthasterias glacialis	–	–	–	–	–	210.00	Port-Cros Bay, France	115
Marthasterias glacialis	–	1.64	–	–	–	–	Saronikos gulf, Greece	98
Astropecten aurentiacus	–	–	–	–	–	80.00	Port-Cros Bay, France	115
Asterias rubens (oral body wall)	–	–	–	0.85 ± 0.63	–	–	Belgian coast (polluted)	100
Asterias rubens (aboral body wall)	–	–	–	0.36 ± 0.19	–	–	Belgian coast (polluted)	100

Table 4. Trace elements concentrations of studied species and similar species from other regions of the world. Data are given as mean ± SD, when possible.

is higher than the average there. Hg mean value reported here for *Nacella concinna* almost doubles that reported for the same species in King George Island and that reported for the limpet *Cellana rota* from southern Oman.

There were no previous data for Cr, Pb, and Hg levels in *Diplasterias brucei* in the literature. Both asteroids, *Diplasterias brucei* and *Odontaster validus*, are carnivores consuming other invertebrates, such as molluscs, crustaceans, ostracods, and sponges. *Odontaster validus* is known to exploit further feeding modes[92–96]. The concentrations of trace elements were of the same order of magnitude in both starfish species. Both species had similar Cr content than that measured in *Odontaster validus* in other studies from two areas in the Ross Sea[97]. Cr mean content in both starfish was similar to the Cr content in *Echinaster sepositus* from Saronikos gulf, Greece, and half the mean value of *Marthasterias glacialis* collected in the same site[98]. Pb values reported in the present study for the starfishes were more than twice the Pb concentration in *Odontaster validus* reported by Grotti et al.[97] (Table 3) and were very similar to those reported for the same species in Deception Island (South Shetland Islands)[99] and for the common starfish *Asterias rubens* in the Belgian coast[100]. The Hg mean levels reported in our study fell between the levels reported for *Odontaster validus* of Terra Nova Bay in Dalla Riva et al.[101] (40.00 ng g^{-1}) and Bargagli et al.[84] (110.00 ng g^{-1} dw). Hg concentrations were the same as those of *Astropecten aurentiacus*

in Port-Cros Bay (France), but lower than those measured in *Echinaster sepositus* in Port-Cros National Park (France), where low pollution is assumed, and *Marthasterias glacialis* in Port-Cros Bay (France) (Table 4).

In general, Cr, Pb, and Hg levels obtained here fell in the range of or were higher than those measured in other sites around Antarctica, which indicates the reliability of the results in this study. The Cr levels in SPOM and *Palmaria decipiens* levels were around 2 to 10 times higher than those of related species, suggesting trace element levels in Antarctic biota are not negligible, but rather concerning. Regarding Pb, it seems to act as an indicator of the human presence in Antarctica. The mean values obtained in the present study for the primary producers were higher than those recorded for primary producers at three different sites in King George Island, which Curtosi et al.[90] declared practically unaffected by pollution, and Terra Nova Bay in the Ross Sea, more remote and less exposed to human local activity than the Antarctic Peninsula[84]. In Terra Nova Bay, however, there are several research facilities, namely the stations McMurdo, Scott Base, Mario Zucchelli, Gondwana, and Jang Bogo[56] and previous reports of contamination exist[105]. For the three animal species, values of Pb are either similar or slightly lower than those measured in ecologically comparable species from other coastal regions of the world. In general, Hg levels in our studied species were higher than those reported for the same species in the literature and for other species from other regions of the world (Tables 3 and 4). Specifically, all the Hg concentrations measured by Santos et al.[11] in Admiralty Bay, King George Island, were lower than those reported in the present study. Santos et al.[11] reported very low levels of trace elements, i.e. close to natural levels. The Hg value reported for the World Ocean was reported to be doubtful in Demina and Nemirovskaya[106] since it is an order of magnitude higher than that measured in the White Sea in the mentioned study. The authors attributed this to the utilization of different procedures for sample preparation and analyses.

Although there is a large diversity of macroalgae in shallow rocky bottoms around Antarctica[57,107], they are minor contributors to the carbon pool fueling the food web[50,51,54] because most of them are chemically defended from herbivores through phlorotannins and other natural products[96,108–112]. This may explain why the levels of trace elements in limpets and starfish are much lower than in macroalgae, assuming that they lack mechanisms to remove these trace elements, i.e. detoxification. Phytoplankton is often considered to be the primary source of carbon for shallow Antarctic food webs[50–54]. The levels of Hg in SPOM reported in this study are consistent with this hypothesis, as they are lower than those observed in consumers from the same site. However, Pb levels in SPOM were much higher than in limpets and starfish. As Pb is known to biomagnify, these results may suggest limited reliance of the three consumers on SPOM. However, SPOM could be a relevant food source if the Pb trapped in diatom frustules[79] is not absorbed by consumers relying on SPOM. Nevertheless, previous research based on stable isotopes of C has shown that encrusting corallinaceous algae are a major dietary item for limpets[55], but unfortunately, we had not enough material to analyze Hg levels in encrusting algae. In any case, the Hg content of the two starfish was higher than that of the limpet, a result consistent with their higher trophic position[54] and demonstrative of the Hg biomagnification along Antarctic food webs.

Conclusions

A latitudinal Cr, Pb and Hg pollution gradient along the South Shetland Islands and the Antarctic Peninsula was not observed in the studied representative benthic species, possibly because the sampling sites were sheltered coastal zones more influenced by local factors than by processes operating at broader geographic scales. This gradient may still exist, however, and further research is needed to prove it.

Differences in concentration among sites and species were found for the three trace elements studied. Nevertheless, the existence of more globally polluted sites could not be demonstrated, as each species responded independently at each sampling site. The primary producers included in this study have a higher trace element content than the selected animal species, suggesting that neither the particulate organic matter nor the dominant macroalgae in this community are the food web carbon source. This supports our previous results on trophic analysis of this community, revealing encrusting corallinaceous algae as the most likely carbon source for the limpet *Nacella concinna*. In agreement with previous research, the starfishes *Odontaster* validus and *Diplasterias brucei*, top predators in the community, may be feeding on the limpet *Nacella concinna*.

In general, Cr, Pb and Hg levels fell in the range of or were higher than those measured in other Antarctic places. Remarkably, trace element concentrations reported in this study are in general comparable to those of taxonomically or ecologically similar species from other coastal regions of the world, thus indicating that at least the Antarctic Peninsula and South Shetland Islands are not so pristine and unspoiled as it may be generally considered but they are as polluted as the rest of the world. Therefore, monitoring pollution levels in these regions is an important and urgent task to be done.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Received: 3 March 2021; Accepted: 23 July 2021
Published online: 11 August 2021

References

1. IUPAC. *Compendium of Chemical Terminology, 2nd ed. (the 'Gold Book')*. Compiled by McNaught, A. D. & Wilkinson, A. (Blackwell Scientific Publications, 1997).
2. Bolan, N. S., Adriano, D. C. & Naidu, R. Role of phosphorus in (im)mobilization and bioavailability of heavy metals in the soil-plant system. In *Reviews of Environmental Contamination and Toxicology* Vol. 177 (ed. Ware, G. W.) 1–44 (Springer, 2003).
3. Marcovecchio, J., Botté, S., Domini, C. & Freije, R. Heavy metals, major metals, trace elements. In *Handbook of Water Analysis* (eds Nollet, L. M. L. & De Gelder, L. S. P.) 379–428 (CRC Press, 2013).

4. Wedepohl, H. K. The composition of the continental crust. *Geochim. Cosmochim. Acta* **59**, 1217–1232 (1995).
5. Santos, I. R., Silva-Filho, E. V., Schaefer, C. E. G. R., Albuquerque-Filho, M. R. & Campos, L. S. Heavy metal contamination in coastal sediments and soils near the Brazilian Antarctic Station, King George Island. *Mar. Pollut. Bull.* **50**, 185–194 (2005).
6. Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z. & Zhu, Y. G. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Pollut.* **152**, 686–692 (2008).
7. Kabata-Pendias, A. *Trace elements in soils and plants, 4th ed.* (CRC Press, 2010).
8. Waller, C. L. *et al.* Microplastics in the Antarctic marine system: An emerging area of research. *Sci. Total Environ.* **598**, 220–227 (2017).
9. Bargagli, R. Environmental contamination in Antarctic ecosystems. *Sci. Total Environ.* **400**, 212–226 (2008).
10. Lenihan, H. S., Oliver, J. S., Oakden, J. M. & Stephenson, M. D. Intense and localized benthic marine pollution around McMurdo Station, Antarctica. *Mar. Pollut. Bull.* **21**, 422–430 (1990).
11. Santos, I. R. *et al.* Baseline mercury and zinc concentrations in terrestrial and coastal organisms of Admiralty Bay, Antarctica. *Environ. Pollut.* **140**, 304–311 (2006).
12. Tin, T. *et al.* Impacts of local human activities on the Antarctic environment. *Antarct. Sci.* **21**, 3–33 (2009).
13. Corsolini, S. Industrial contaminants in Antarctic biota. *J. Chromatogr. A* **1216**, 598–612 (2009).
14. Bargagli, R., Agnorelli, C., Borghini, F. & Monaci, F. Enhanced deposition and bioaccumulation of mercury in antarctic terrestrial ecosystems facing a coastal polynya. *Environ. Sci. Technol.* **39**, 8150–8155 (2005).
15. Planchon, F. A. M. *et al.* Changes in heavy metals in Antarctic snow from Coats Land since the mid-19th to the late-20th century. *Earth Planet. Sci. Lett.* **200**, 207–222 (2002).
16. Szopińska, M., Namieśnik, J. & Polkowska, Ż How important is research on pollution levels in Antarctica? Historical approach, difficulties and current trends. In *Reviews of Environmental Contamination and Toxicology* Vol. 239 (ed. de Voogt, P.) 79–156 (Springer, 2017).
17. Bengtson Nash, S. *et al.* Contaminant profiles of air and soil around Casey station, Antarctica; discerning local and distant contaminant sources. In *21st Society for Environmental Toxicology and Chemistry (SETAC) Europe Annual Meeting Proceedings* (2011).
18. Boutron, C. F. & Patterson, C. C. Relative levels of natural and anthropogenic lead in recent Antarctic snow. *J. Geophys. Res.* **92**, 8454–8464 (1987).
19. Dick, A. L. Concentrations and sources of metals in the Antarctic Peninsula aerosol. *Geochim. Cosmochim. Acta* **55**, 1827–1836 (1991).
20. de Moreno, J. E. A., Gerpe, M. S., Moreno, V. J. & Vodopivez, C. Heavy metals in Antarctic organisms. *Polar Biol.* **17**, 131–140 (1997).
21. Kennicutt, I. M. C. *et al.* Human contamination of the marine environment-arthur harbor and mcmurdo sound, Antarctica. *Environ. Sci. Technol.* **29**, 1279–1287 (1995).
22. Hughes, K. A. & Ashton, G. V. Breaking the ice: The introduction of biofouling organisms to Antarctica on vessel hulls. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **27**, 158–164 (2017).
23. Aston, S. R. & Thornton, I. Regional geochemical data in relation to seasonal variations in water quality. *Sci. Total Environ.* **7**, 247–260 (1977).
24. Norwood, W. P., Borgmann, U. & Dixon, D. G. Saturation models of arsenic, cobalt, chromium and manganese bioaccumulation by *Hyalella azteca*. *Environ. Pollut.* **143**, 519–528 (2006).
25. Jerez, S. *et al.* Concentration of trace elements in feathers of three Antarctic penguins: Geographical and interspecific differences. *Environ. Pollut.* **159**, 2412–2419 (2011).
26. Negri, A., Burns, K., Boyle, S., Brinkman, D. & Webster, N. Contamination in sediments, bivalves and sponges of McMurdo Sound, Antarctica. *Environ. Pollut.* **143**, 456–467 (2006).
27. Trevizani, T. H. *et al.* Bioaccumulation of heavy metals in marine organisms and sediments from Admiralty Bay, King George Island, Antarctica. *Mar. Pollut. Bull.* **106**, 366–371 (2016).
28. Trevizani, T. H., Petti, M. A. V., Ribeiro, A. P., Corbisier, T. N. & Figueira, R. C. L. Heavy metal concentrations in the benthic trophic web of Martel Inlet, Admiralty Bay (King George Island, Antarctica). *Mar. Pollut. Bull.* **130**, 198–205 (2018).
29. Cipro, C. V. Z., Montone, R. C. & Bustamante, P. Mercury in the ecosystem of Admiralty Bay, King George Island, Antarctica: Occurrence and trophic distribution. *Mar. Pollut. Bull.* **114**, 564–570 (2017).
30. de Oliveira, M. F. *et al.* Evidence of metabolic microevolution of the limpet Nacella concinna to naturally high heavy metal levels in Antarctica. *Ecotoxicol. Environ. Saf.* **135**, 1–9 (2017).
31. Torres, M. A. *et al.* Biochemical biomarkers in algae and marine pollution: A review. *Ecotoxicol. Environ. Saf.* **71**, 1–15 (2008).
32. Neff, J. M. *Bioaccumulation in Marine Organisms. Effect of Contaminants from Oil Well Produced Water. Organic Geochemistry* (Elsevier, 2002).
33. Wong, P. T. & Trevors, J. T. Chromium toxicity to algae and bacteria. In *Chromium in the Natural and Human Environments* (eds Nriagu, J. O. & Nieboer, E.) 305–315 (Wiley, 1988).
34. Community, E. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Parliam.* **L327**, 1–82 (2000).
35. Driscoll, C. T., Mason, R. P., Chan, H. M., Jacob, D. J. & Pirrone, N. Mercury as a global pollutant: Sources, pathways, and effects. *Environ. Sci. Technol.* **47**, 4967–4983 (2013).
36. Pertierra, L. R. *et al.* Ecosystem services in Antarctica: Global assessment of the current state, future challenges and managing opportunities. *Ecosyst. Serv.* **49**, 101299 (2021).
37. Pringle, B. H., Hissong, D. E., Katz, E. L. & Mulawka, S. T. Trace metal accumulation by estuarine mollusks. *J. Sanit. Eng. Div. Proc. Amer. Soc. Civ. Eng.* **94**, 455–475 (1968).
38. Amiard, J. C., Amiard-Triquet, C., Berthet, B. & Metayer, C. Comparative study of the patterns of bioaccumulation of essential (Cu, Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. *J. Exp. Mar. Bio. Ecol.* **106**, 73–89 (1987).
39. Borgmann, U., Norwood, W. P. & Clarke, C. Accumulation, regulation and toxicity of copper, zinc, lead and mercury in Hyalella azteca. *Hydrobiologia* **259**, 79–89 (1993).
40. Windom, H. & Kendall, D. R. Accumulation and biotransformation of mercury in coastal and marine biota. In *The Biogeochemistry of Mercury in the Environment* (ed. Nriagu, J. O.) 303–323 (Elsevier/North-Holland Biomedical Press, 1979).
41. Turner, S. J. *et al.* Are soft-sediment communities stable? An example from a windy harbour. *Mar. Ecol. Prog. Ser.* **120**, 219–230 (1995).
42. Caccia, V. G., Millero, F. J. & Palanques, A. The distribution of trace metals in Florida Bay sediments. *Mar. Pollut. Bull.* **46**, 1420–1433 (2003).
43. Gibbs, R. J. Transport phases of transition metals in the Amazon and Yukon Rivers. *Bull. Geol. Soc. Am.* **88**, 829–843 (1977).
44. Jain, C. K. & Sharma, M. K. Distribution of trace metals in the Hindon River system, India. *J. Hydrol.* **253**, 81–90 (2001).
45. Filgueiras, A. V., Lavilla, I. & Bendicho, C. Chemical sequential extraction for metal partitioning in environmental solid samples. *J. Environ. Monit.* **4**, 823–857 (2002).
46. Salomons, W. & Förstner, U. *Metals in the Hydrocycle* (Springer, 1984).

47. Niimi, A. J. & Kissoon, G. P. Evaluation of the critical body burden concept based on inorganic and organic mercury toxicity to rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.* **26**, 169–178 (1994).
48. Landrum, P. F., Lydy, M. J. & Lee, H. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ. Toxicol. Chem.* **11**, 1709–1725 (1992).
49. Wiener, J. G. *et al.* Monitoring and evaluating trends in methylmercury accumulation in aquatic biota. In *Ecosystem Responses to Mercury Contamination: Indicators of Change* (eds Harris, R. *et al.*) 87–122 (CRC Press & SETAC Press, 2007).
50. Dunton, K. H. $\delta^{15}N$ and $\delta^{13}C$ measurements of Antarctic Peninsula fauna: Trophic relationships and assimilation of benthic seaweeds. *Am. Zool.* **41**, 99–112 (2001).
51. Corbisier, T. N., Petti, M. A. V., Skowronski, R. S. P. & Brito, T. A. S. Trophic relationships in the nearshore zone of Martel Inlet (King George Island, Antarctica): $\delta^{13}C$ stable-isotope analysis. *Polar Biol.* **27**, 75–82 (2004).
52. Norkko, A. *et al.* Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. *Ecology* **88**, 2810–2820 (2007).
53. Michel, L. N. *et al.* Increased sea ice cover alters food web structure in East Antarctica. *Sci. Rep.* **9**, 1–11 (2019).
54. Zenteno, L. *et al.* Unraveling the multiple bottom-up supplies of an Antarctic nearshore benthic community. *Prog. Oceanogr.* **174**, 55–63 (2019).
55. Cardona, L., Lloret-Lloret, E., Moles, J. & Avila, C. Latitudinal changes in the trophic structure of benthic coastal food webs in the Antarctic Peninsula. *Mar. Environ. Res.* **167**, 105290 (2021).
56. COMNAP. *Antarctic Station Catalogue* (COMNAP Secretariat, 2017).
57. Wiencke, C., Amsler, C. & Clayton, M. Macroalgae. In *Biogeographic Atlas of the Southern Ocean* (eds De Broyer, C. *et al.*) 66–73 (Scientific Committee on Antarctic Research, 2014).
58. Danis, B., Griffiths, H. J. & Jangoux, M. Asteroidea. In *Biogeographic Atlas of the Southern Ocean* (eds De Broyer, C. *et al.*) 200–207 (Scientific Committee on Antarctic Research, 2014).
59. Schiaparelli, S. & Linse, K. Gastropoda. In *Biogeographic Atlas of the Southern Ocean* (eds De Broyer, C. *et al.*) 122–125 (Scientific Committee on Antarctic Research, 2014).
60. Borrell, A., Tornero, V., Bhattacharjee, D. & Aguilar, A. Trace element accumulation and trophic relationships in aquatic organisms of the Sundarbans mangrove ecosystem (Bangladesh). *Sci. Total Environ.* **545–546**, 414–423 (2016).
61. Torres, J., Eira, C., Miquel, J. & Feliu, C. Heavy metal accumulation by intestinal helminths of vertebrates. *Recent Adv. Pharm. Sci.* **II**(661), 169–181 (2012).
62. Vighi, M., Borrell, A. & Aguilar, A. Bone as a surrogate tissue to monitor metals in baleen whales. *Chemosphere* **171**, 81–88 (2017).
63. Borrell, A., Aguilar, A., Tornero, V. & Drago, M. Concentrations of mercury in tissues of striped dolphins suggest decline of pollution in Mediterranean open waters. *Chemosphere* **107**, 319–323 (2014).
64. Borrell, A., Clusa, M., Aguilar, A. & Drago, M. Use of epidermis for the monitoring of tissular trace elements in Mediterranean striped dolphins (*Stenella coeruleoalba*). *Chemosphere* **122**, 288–294 (2015).
65. Maceda-Veiga, A., Monroy, M., Navarro, E., Viscor, G. & de Sostoa, A. Metal concentrations and pathological responses of wild native fish exposed to sewage discharge in a Mediterranean river. *Sci. Total Environ.* **449**, 9–19 (2013).
66. Suda, C. N. K. *et al.* The biology and ecology of the Antarctic limpet Nacella concinna. *Polar Biol.* **38**, 1949–1969 (2015).
67. Škrbić, B., Crossed, D. & Signurišić-Mladenović, N. Distribution of heavy elements in urban and rural surface soils: The Novi Sad city and the surrounding settlements. *Serbia. Environ. Monit. Assess.* **185**, 457–471 (2013).
68. Škrbić, B. D., Buljovčić, M., Jovanović, G. & Antić, I. Seasonal, spatial variations and risk assessment of heavy elements in street dust from Novi Sad, Serbia. *Chemosphere* **205**, 452–462 (2018).
69. Škrbić, B., Durišić-Mladenović, N. & Cvejanov, J. Principal component analysis of trace elements in Serbian wheat. *J. Agric. Food Chem.* **53**, 2171–2175 (2005).
70. Wilde, E. W. & Benemann, J. R. Bioremoval of heavy metals by the use of microalgae. *Biotechnol. Adv.* **11**, 781–812 (1993).
71. Farías, S., Arisnabarreta, S. P., Vodopivez, C. & Smichowski, P. Levels of essential and potentially toxic trace metals in Antarctic macro algae. *Spectrochim. Acta B* **57**, 2133–2140 (2002).
72. Black, W. A. P. & Mitchell, R. L. Trace elements in the common algae and in sea water. *J. Mar. Biol. Assoc. UK* **30**, 1–10 (1952).
73. Lignell, A., Roomans, G. M. & Pedersen, M. Localization of absorbed cadmium in *Fucus vesiculosus* L. by X-ray microanalysis. *Z. Pflanzenphysiol.* **105**, 103–109 (1982).
74. Ragan, M. A., Smidsrød, O. & Larsen, B. Chelation of divalent metal ions by brown algal polyphenols. *Mar. Chem.* **7**, 265–271 (1979).
75. Talarico, L. Fine structure and X-ray microanalysis of a red macrophyte cultured under cadmium stress. *Environ. Pollut.* **120**, 813–821 (2002).
76. Vasconcelos, M. T. S. D. & Leal, M. F. C. Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Mar. Chem.* **74**, 65–85 (2001).
77. Pellegrini, L., Delivopoulos, S. G. & Pellegrini, M. Arsenic-induced ultrastructural changes in the vegetative cells of *Cystoseira barbata* forma repens Zinova et Kalugina (Fucophyceae, Fucales). *Bot. Mar.* **33**, 229–234 (1990).
78. Deheyn, D. D., Gendreau, P., Baldwin, R. J. & Latz, M. I. Evidence for enhanced bioavailability of trace elements in the marine ecosystem of Deception Island, a volcano in Antarctica. *Mar. Environ. Res.* **60**, 1–33 (2005).
79. Exley, C. Silicon in life: A bioinorganic solution to bioorganic essentiality. *J. Inorg. Biochem.* **69**, 139–144 (1998).
80. Costa, R. R. *et al.* Dynamics of an intense diatom bloom in the Northern Antarctic Peninsula, February 2016. *Limnol. Oceanogr.* **65**, 2056–2075 (2020).
81. Mendes, C. R. B. *et al.* Dynamics of phytoplankton communities during late summer around the tip of the Antarctic Peninsula. *Deep. Res. I* **65**, 1–14 (2012).
82. Ducklow, H. W. *et al.* Marine pelagic ecosystems: The West Antarctic Peninsula. *Philos. Trans. R. Soc. B.* **362**, 67–94 (2007).
83. Prezelin, B. B., Hofmann, E. E., Mengelt, C. & Klinck, J. M. The linkage between Upper Circumpolar Deep Water (UCDW) and phytoplankton assemblages on the west Antarctic Peninsula continental shelf. *J. Mar. Res.* **58**, 165–202 (2000).
84. Bargagli, R., Monaci, F. & Cateni, D. Marine coastal food web. *Mar. Ecol. Prog. Ser.* **169**, 65–76 (1998).
85. Collier, R. & Edmond, J. The trace element geochemistry of marine biogenic particulate matter. *Prog. Oceanogr.* **13**, 113–199 (1984).
86. Rubio, C. *et al.* Metals in edible seaweed. *Chemosphere* **173**, 572–579 (2017).
87. Desideri, D. *et al.* Essential and toxic elements in seaweeds for human consumption. *J. Toxicol. Environ. Health A* **79**, 112–122 (2016).
88. Runcie, J. W. & Riddle, M. J. Metal concentrations in macroalgae from East Antarctica. *Mar. Pollut. Bull.* **49**, 1114–1119 (2004).
89. Fowler, S. W., Villeneuve, J. P., Wyse, E., Jupp, B. & de Mora, S. Temporal survey of petroleum hydrocarbons, organochlorinated compounds and heavy metals in benthic marine organisms from Dhofar, southern Oman. *Mar. Pollut. Bull.* **54**, 357–367 (2007).
90. Curtosi, A., Pelletier, E., Vodopivez, C., St Louis, R. & MacCormack, W. P. Presence and distribution of persistent toxic substances in sediments and marine organisms of Potter Cove, Antarctica. *Arch. Environ. Contam. Toxicol.* **59**, 582–592 (2010).
91. Ahn, I. Y., Kim, K. W. & Choi, H. J. A baseline study on metal concentrations in the Antarctic limpet *Nacella concinna* (Gastropoda: Patellidae) on King George Island: Variations with sex and body parts. *Mar. Pollut. Bull.* **44**, 424–431 (2002).

92. Dayton, P. K., Robilliard, G. A., Paine, R. T. & Dayton, L. B. Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecol. Monogr.* **44**, 105–128 (1974).
93. Pearse, J. S. Reproductive periodicities in several contrasting populations of Odontaster validus Koehler, a common Antarcticasteroid. *Antarct. Res. Ser.* **5**, 39–85 (1965).
94. Peckham, V. Year-round SCUBA diving in the Antarctic. *Polar Rec.* **12**, 143–146 (1964).
95. Smale, D. A., Barnes, D. K. A., Fraser, K. P. P., Mann, P. J. & Brown, M. P. Scavenging in Antarctica: Intense variation between sites and seasons in shallow benthic necrophagy. *J. Exp. Mar. Bio. Ecol.* **349**, 405–417 (2007).
96. Mcclintock, J. B. Trophic biology of antarctic shallow-water echinoderms. *Mar. Ecol. Prog. Ser.* **111**, 191–202 (1994).
97. Grotti, M. *et al.* Natural variability and distribution of trace elements in marine organisms from Antarctic coastal environments. *Antarct. Sci.* **20**, 39–51 (2008).
98. Papadopoulou, C., Kanias, G. D. & Moraitopoulou-Kassimati, E. Stable elements of radioecological importance in certain echinoderm species. *Mar. Pollut. Bull.* **7**, 143–144 (1976).
99. Di Giglio, S. *et al.* Effects of ocean acidification on acid-base physiology, skeleton properties, and metal contamination in two echinoderms from vent sites in Deception Island, Antarctica. *Sci. Total Environ.* **765**, 142669 (2020).
100. Danis, B. *et al.* Contaminant levels in sediments and asteroids (*Asterias rubens* L., Echinodermata) from the Belgian coast and Scheldt estuary: Polychlorinated biphenyls and heavy metals. *Sci. Total Environ.* **333**, 149–165 (2004).
101. Riva, S. D., Abelmoschi, M. L., Magi, E. & Soggia, F. The utilization of the Antarctic environmental specimen bank (BCAA) in monitoring Cd and Hg in an Antarctic coastal area in Terra Nova Bay (Ross Sea: Northern Victoria Land). *Chemosphere* **56**, 59–69 (2004).
102. Cabrita, M. T. *et al.* Evaluating trace element bioavailability and potential transfer into marine food chains using immobilised diatom model species *Phaeodactylum tricornutum*, on King George Island, Antartica. *Mar. Pollut. Bull.* **121**, 192–200 (2017).
103. Truzzi, C. *et al.* Separation of micro-phytoplankton from inorganic particulate in Antarctic seawater (Ross Sea) for the determination of Cd, Pb and Cu: Optimization of the analytical methodology. *Anal. Methods* **7**, 5490–5496 (2015).
104. Bargagli, R. Trace metals in Antarctic organisms and the development of circumpolar biomonitoring networks. in *Reviews of Environmetal Contamination and Toxicology* (ed. Ware, G. W.) vol. 171, 53–110 (2001).
105. Focardi, S., Bargagli, R. & Corsolini, S. Isomer-specific analysis and toxic potential evaluation of polychlorinated biphenyls in Antarctic fish, seabirds and Weddell seals from Terra Nova Bay (Ross Sea). *Antarct. Sci.* **7**, 31–35 (1995).
106. Demina, L. L. & Nemirovskaya, I. A. Spatial distribution of microelements in the seston of the White Sea. *Oceanology* **47**, 360–372 (2007).
107. Wiencke, C. & Amsler, C. D. Seaweeds and their communities in polar regions. In *Seaweed Biology* (eds Wiencke, C. & Bischof, K.) 265–291 (Springer, 2012).
108. Fairhead, V. A., Amsler, C. D., Mcclintock, J. B. & Baker, B. J. Within-thallus variation in chemical and physical defences in two species of ecologically dominant brown macroalgae from the Antarctic Peninsula. *Oceanology* **322**, 1–12 (2005).
109. Amsler, C. D. *Algal chemical ecology: Algal Chemical Ecology* (Springer, 2008).
110. Amsler, C. D., Mcclintock, J. B. & Baker, B. J. Chemical mediation of mutualistic interactions between macroalgae and mesograzers structure unique coastal communities along the western Antarctic Peninsula. *J. Phycol.* **50**, 1–10 (2014).
111. Aumack, C. F., Amsler, C. D., McClintock, J. B. & Baker, B. J. Chemically mediated resistance to mesoherbivory in finely branched macroalgae along the western Antarctic Peninsula. *Eur. J. Phycol.* **45**, 19–26 (2010).
112. Núñez-Pons, L., Rodríguez-Arias, M., Gómez-Garreta, A., Ribera-Siguán, A. & Avila, C. Feeding deterrency in Antarctic marine organisms: Bioassays with the omnivore amphipod *Cheirimedon femoratus*. *Eur. J. Phycol.* **462**, 163–174 (2012).
113. Ahn, I. Y., Chung, K. H. & Choi, H. J. Influence of glacial runoff on baseline metal accumulation in the Antarctic limpet *Nacella concinna* from King George Island. *Mar. Pollut. Bull.* **49**, 119–127 (2004).
114. Burdon-Jones, C., Denton, G. R. W., Jones, G. B. & McPhie, K. A. Regional and seasonal variations of trace metals in tropical phaeophyceae from North Queensland. *Mar. Environ. Res.* **7**, 13–30 (1982).
115. Augier, H., Gilles, G., Leal Nascimento, M. & Ramonda, G. Évolution de la contamination de la flore et de la faune marines benthiques de la Baie de Port-Cros de 1976 à 1981. *Trav. Sci. Parc Natl. Port-Cros* **10**, 37–50 (1984).
116. Chakraborty, S., Bhattacharya, T., Singh, G. & Maity, J. P. Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: A biomonitoring approach for pollution assessment. *Ecotoxicol. Environ. Saf.* **100**, 61–68 (2014).
117. Pastor, A. *et al.* Levels of heavy metals in some marine organisms from the western Mediterranean area (Spain). *Mar. Pollut. Bull.* **28**, 50–53 (1994).

Acknowledgements

This work was performed under the projects DISTANTCOM (CTM2013-42667/ANT) and BLUEBIO (CTM2016-78901/ANT). We would like to thank the researchers who participated in the DISTANTCOM cruise for their help in collecting samples. Thanks are also due to all the technicians at the Unit of Metal Analysis of the Scientific and Technological Centers (CCiT) of the University of Barcelona, specially P. Menéndez, M. Romero, and R. Martínez. Thanks are also due to A. Gómez-Garreta, J. Rull-Lluch, and R. Martín-Martín for the macroalgae identification, E. Lloret-Lloret for helping with the sample processing, and F. Saporiti for the constant advice and support in the laboratory. This is an AntIcon (SCAR) contribution. P. D. acknowledges financial support from an FPU Grant (2016-02877) of the MEFP (Spain). We acknowledge the QGIS Development Team and the Norwegian Polar Institute's Quantarctica package.

Author contributions

L.C. and C.A. designed the research and conducted the fieldwork. P.D. performed laboratory analyses, analyzed the data and wrote the first draft of the manuscript. L.C. supervised the project, contributed to data analysis, and reviewed and edited the manuscript. C.A. reviewed and edited the manuscript, administrated the project and acquired the funds. All authors contributed to the writing of the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-95681-5.

Correspondence and requests for materials should be addressed to P.D.-F.

SUPPLEMENTARY INFORMATION

Distribution of trace elements in benthic infralittoral organisms from the western Antarctic Peninsula reveals no latitudinal gradient of pollution

Paula de Castro-Fernández*, Luis Cardona & Conxita Avila

Department of Evolutionary Biology, Ecology and Environmental Sciences & Biodiversity Research Institute (IRBio), University of Barcelona, Diagonal Ave. 643, 08028, Barcelona, Catalonia.

*Corresponding author. E-mail address: p.decastro@ub.edu

Supplementary table 1

Range and mean values ± SD of Cr, Pb, and Hg in the studied species at each sampling site.

Species	Cr (µg g^{-1} dw)		Pb (µg g^{-1} dw)		Hg (ng g^{-1} dw)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Suspended Particulate Organic Matter (SPOM)						
Fildes Bay	3.49-25.33	12.46 ± 8.41	10.53-89.61	30.55 ± 33.23	21.43-43.14	30.91 ± 9.53
Hope Bay	6.18-32.87	14.51 ± 10.6	15.73-117.79	48.07 ± 40.13	29.58-46.2	35.93 ± 7.26
Cierva Cove	1.07-8.8	3.25 ± 3.19	1.85-30.81	9.75 ± 12.04	15.12-16.64	15.68 ± 0.71
Paradise Bay	1.05-32.98	8.11 ± 13.92	2.12-120.04	27.66 ± 51.68	15.4-21.96	18.63 ± 3.01
Rothera Point	7.25-24.7	13.07 ± 7.07	14.34-537.08	171.87 ± 212.28	37.23-107.26	66.31 ± 25.88
Desmarestia anceps/ D. menziesii						
Fildes Bay	1.23-4.63	2.36 ± 1.41	0.56-1.71	1.05 ± 0.5	47.6-140.06	94.97 ± 37.36
Hope Bay	0.46-4.23	1.91 ± 1.41	0.56-1.26	0.87 ± 0.29	56.62-498.08	184.69 ± 183.93
Cierva Cove	1.62-2.63	2.28 ± 0.4	2.21-4.22	3.35 ± 0.74	104.97-153.39	120.43 ± 19.78
Paradise Bay	4.22-6.11	4.85 ± 0.9	1.62-3.41	2.17 ± 0.73	70.94-294.34	164.46 ± 80.88
Rothera Point	0.47-1.41	0.85 ± 0.39	1.18-1.79	1.46 ± 0.26	93.24-137.04	119.48 ± 18.3
Palmaria decipiens						
Fildes Bay	2.31-3.81	3.12 ± 0.68	0.47-0.99	0.76 ± 0.23	307.35-1123.31	645.41 ± 354.88
Hope Bay	6.05-14	10.04 ± 2.94	1.50-1.89	1.7 ± 0.14	531.99-718.65	620.83 ± 67.2
Cierva Cove	0.14-8.16	2.63 ± 3.56	0.51-3.24	1.49 ± 1.2	10.17-175.51	70.16 ± 75.63
Paradise Bay	0.8-4.04	2.13 ± 1.18	2.27-3.76	2.93 ± 0.64	240.7-577.29	382.28 ± 123.52
Rothera Point	2.87-14.41	9.05 ± 5.14	0.87-21.49	5.34 ± 9.05	58.1-1194.2	340.79 ± 479.3
Nacella concinna						
Fildes Bay	0.66-1.35	1.03 ± 0.28	0.14-0.22	0.18 ± 0.03	23.09-43.31	30.64 ± 7.88
Hope Bay	1.88-3.5	2.62 ± 0.72	0.23-0.42	0.33 ± 0.09	34.2-75.75	55.08 ± 17.02
Cierva Cove	0.92-3.85	1.85 ± 1.22	0.15-0.43	0.26 ± 0.1	23.57-224.55	86.04 ± 84.03
Paradise Bay	0.26-0.62	0.45 ± 0.16	0.42-0.62	0.51 ± 0.08	9.66-16.23	11.9 ± 2.65
Rothera Point	1.25-3.28	1.84 ± 0.85	0.27-0.48	0.41 ± 0.09	17.89-72.12	34.09 ± 23.05
Diplasterias brucei						
Fildes Bay	0.61-1.41	0.94 ± 0.33	0.14-0.28	0.19 ± 0.06	47.99-87.37	67.26 ± 14.93
Hope Bay	0.53-1.39	0.95 ± 0.37	0.2-0.34	0.27 ± 0.05	71.88-141.91	103.44 ± 25.33
Cierva Cove	0.72-1.39	1.1 ± 0.28	0.25-0.45	0.33 ± 0.07	77.25-150.13	111.84 ± 28.86
Paradise Bay	0.48-0.54	0.51 ± 0.03	0.43-0.64	0.53 ± 0.07	57.84-90.18	74.81 ± 11.73
Rothera Point	0.28-0.51	0.41 ± 0.11	0.23-0.37	0.29 ± 0.05	45.92-70.19	57.39 ± 11.27
Odontaster validus						
Fildes Bay	0.5-0.92	0.74 ± 0.16	0.23-0.32	0.25 ± 0.04	33.74-80.79	61.87 ± 17.76
Hope Bay	0.76-1.31	0.98 ± 0.21	0.21-0.91	0.38 ± 0.3	63.97-192.42	123.79 ± 49.26
Cierva Cove	0.3-1.09	0.59 ± 0.3	0.46-1.68	0.91 ± 0.49	70.79-184.07	112.63 ± 44.9
Paradise Bay	0.29-0.54	0.43 ± 0.1	0.61-1.02	0.72 ± 0.17	19.95-45.28	25.79 ± 10.94
Rothera Point	0.73-1.27	0.95 ± 0.2	0.32-0.91	0.48 ± 0.24	37.19-78.29	58.26 ± 16.75

Supplementary table 2
Results of the general lineal model used to assess the effect of species and site on standardized Cr levels.

Tests of Between-Subjects Effects					
Dependent Variable: **Cr**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	83.294[a]	29	2.872	5.246	< 0.001
Intercept	0.000	1	0.000	0.000	1.000
species	57.583	5	11.517	21.033	< 0.001
site	6.466	4	1.617	2.952	0.023
species * site	19.245	20	0.962	1.757	0.033
Error	65.706	120	0.548		
Total	149.000	150			
Corrected Total	149.000	149			

a. R Squared = 0.559 (Adjusted R Squared = 0.452)

Supplementary table 3
Results of the general lineal model used to assess the effect of species and site on standardized Pb levels.

Tests of Between-Subjects Effects					
Dependent Variable: **Pb**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	63.824[a]	29	2.201	3.101	< 0.001
Intercept	0.000	1	0.000	.000	1.000
species	27.963	5	5.593	7.879	< 0.001
site	6.191	4	1.548	2.180	0.075
species * site	29.670	20	1.483	2.090	0.008
Error	85.176	120	0.710		
Total	149.000	150			
Corrected Total	149.000	149			

a. R Squared = 0.428 (Adjusted R Squared = 0.290)

Supplementary table 4
Results of the general lineal model used to assess the effect of species and site on standardized Hg levels.

Tests of Between-Subjects Effects					
Dependent Variable: **Hg**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	101.303[a]	29	3.493	8.788	< 0.001
Intercept	0.000	1	0.000	0.000	1.000
species	68.752	5	13.750	34.594	< 0.001
site	5.230	4	1.307	3.289	0.013
species * site	27.322	20	1.366	3.437	<0.001
Error	47.697	120	.397		
Total	149.000	150			
Corrected Total	149.000	149			

a. R Squared = 0.680 (Adjusted R Squared = 0.603)

Supplementary table 5
Results of the general lineal model used to assess the effect of species and site on standardized Cr levels for the three animal species (*N. concinna*, *D. brucei*, *O. validus*).

Tests of Between-Subjects Effects					
Dependent Variable: **Cr**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	49.671a	14	3.548	8.750	<0.001
Intercept	0.000	1	0.000	0.000	1.000
species	19.210	2	9.605	23.688	<0.001
site	16.096	4	4.024	9.924	<0.001
species * site	14.365	8	1.796	4.429	<0.001
Error	24.329	60	0.405		
Total	74.000	75			
Corrected Total	74.000	74			

a. R Squared = 0.671 (Adjusted R Squared = 0.595)

Supplementary table 6

Results of the general lineal model used to assess the effect of species and site on standardized Pb levels for the three animal species (*N. concinna*, *D. brucei*, *O. validus*).

Tests of Between-Subjects Effects					
Dependent Variable: **Pb**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CorrectedModel	44.465a	14	3.176	6.452	<0.001
Intercept	0.000	1	0.000	0.000	1.000
species	12.349	2	6.175	12.544	<0.001
site	20.364	4	5.091	10.342	<0.001
species * site	11.752	8	1.469	2.984	0.007
Error	29.535	60	0.492		
Total	74.000	75			
Corrected Total	74.000	74			

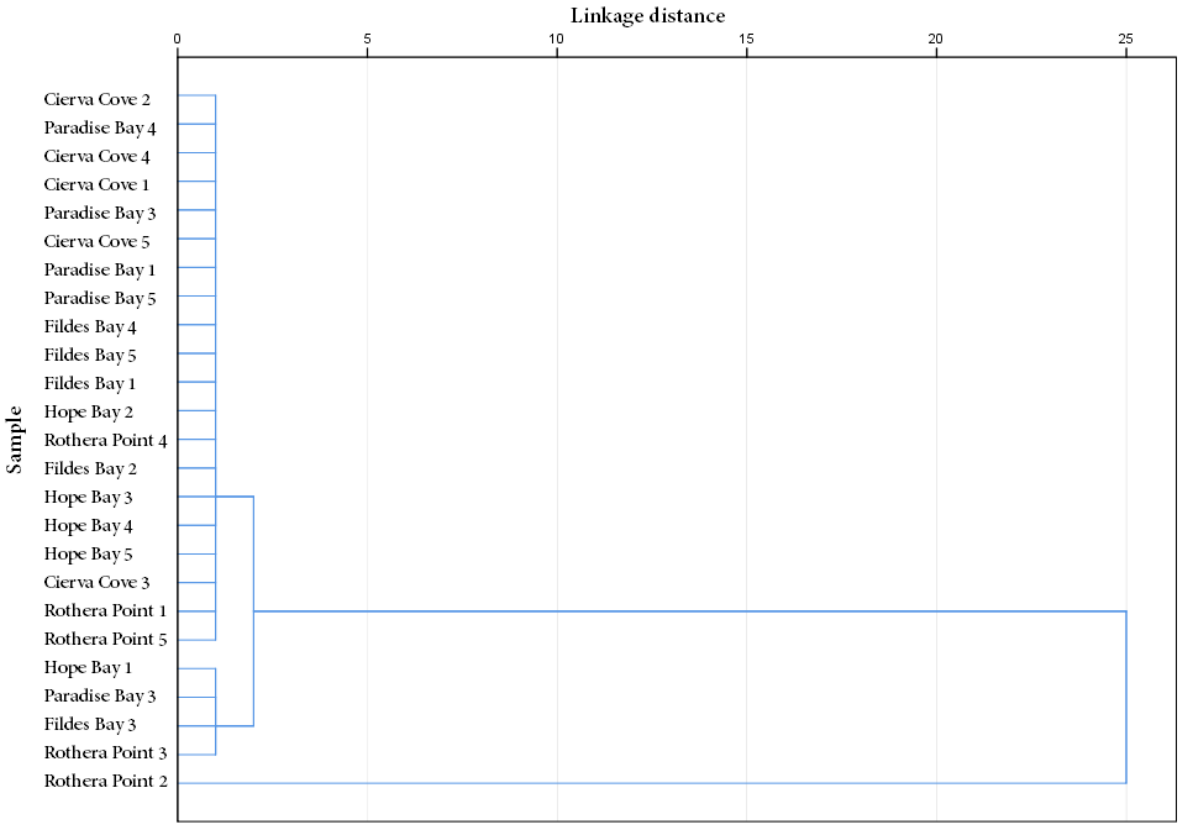
a. R Squared = 0.601 (Adjusted R Squared = 0.508)

Supplementary table 7

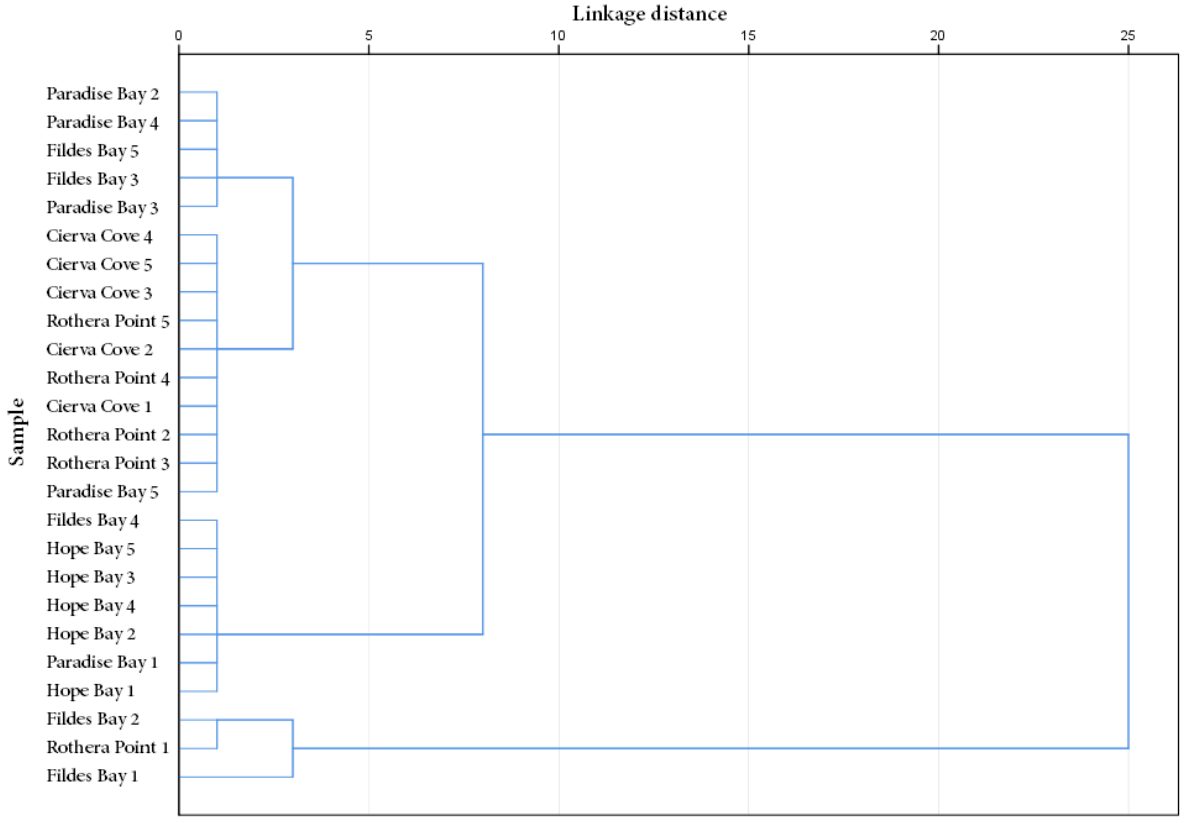
Results of the general lineal model used to assess the effect of species and site on standardized Hg levels for the three animal species (*N. concinna*, *D. brucei*, *O. validus*).

Tests of Between-Subjects Effects					
Dependent Variable: **Hg**					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CorrectedModel	42.799a	14	3.057	5.879	<0.001
Intercept	0.000	1	0.000	.000	1.000
species	11.549	2	5.775	11.105	<0.001
site	26.520	4	6.630	12.749	<0.001
species * site	4.730	8	0.591	1.137	0.352
Error	31.201	60	0.520		
Total	74.000	75			
Corrected Total	74.000	74			

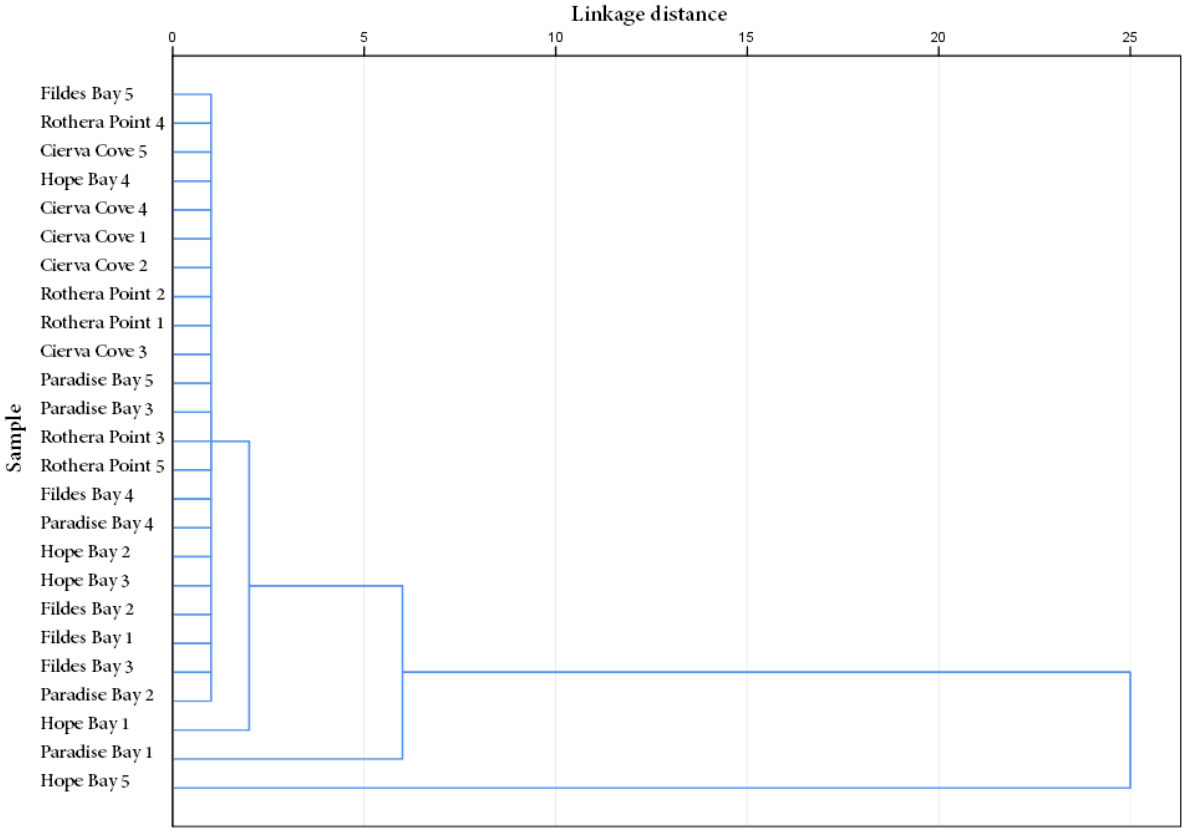
a. R Squared = 0.578 (Adjusted R Squared = 0.480)



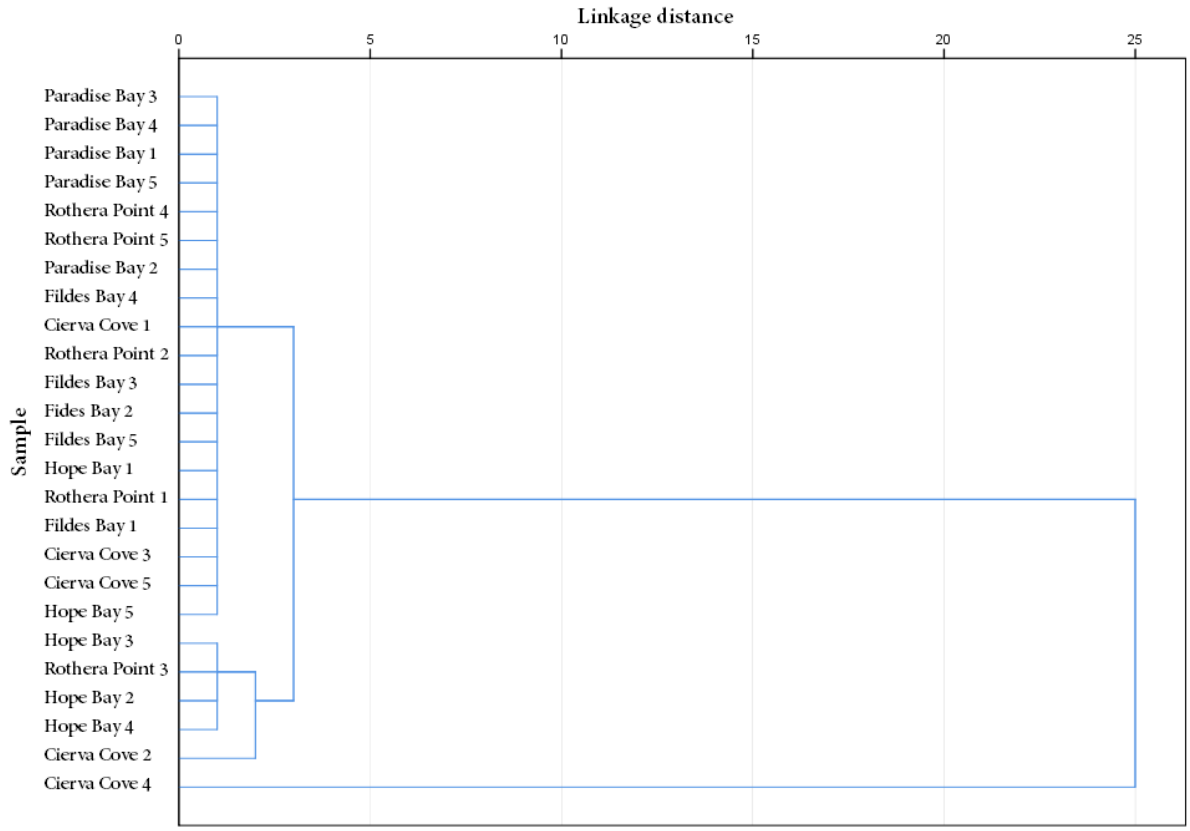
Supplementary figure 1. Dendrogram for SPOM samples obtained using the squared Euclidean distance as a metric and UPGMA as the clustering method.



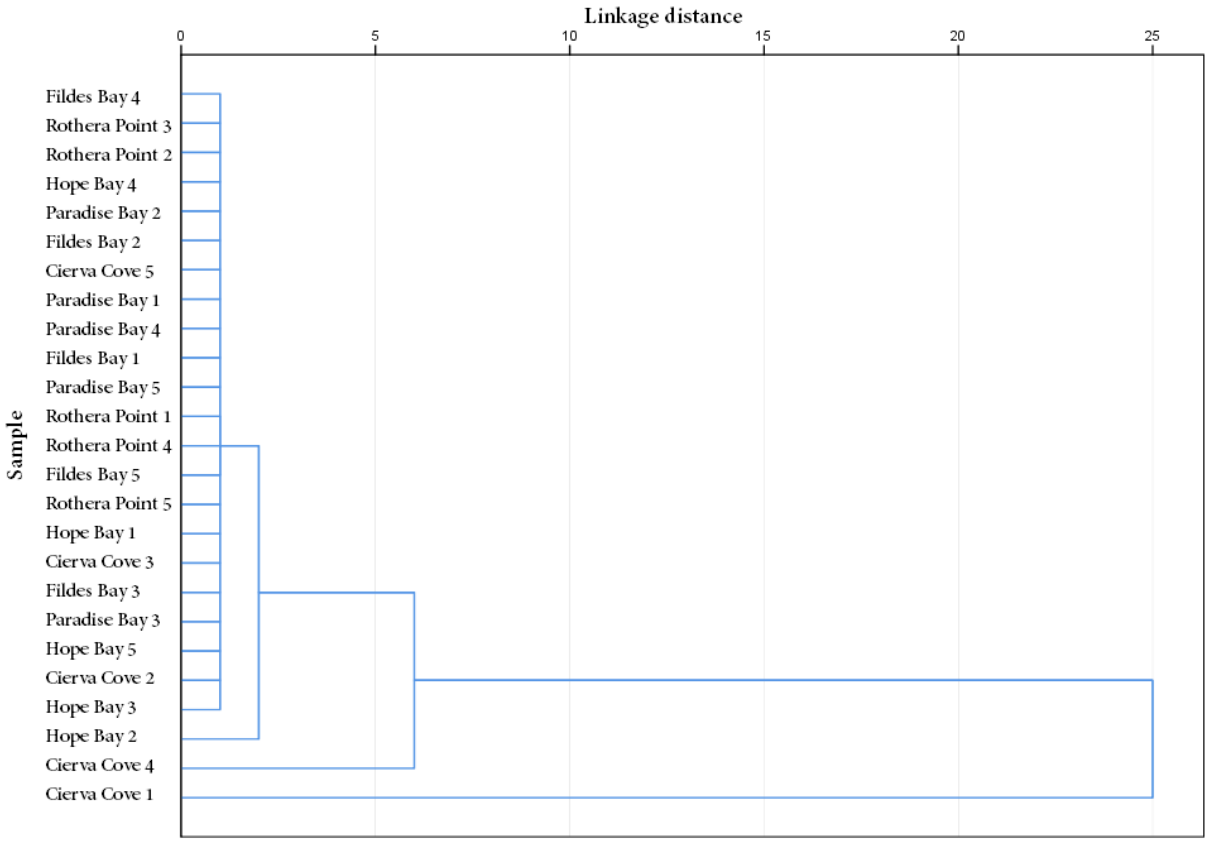
Supplementary figure 2. Dendrogram for *Palmaria decipiens* samples obtained using the squared Euclidean distance as a metric and UPGMA as the clustering method.



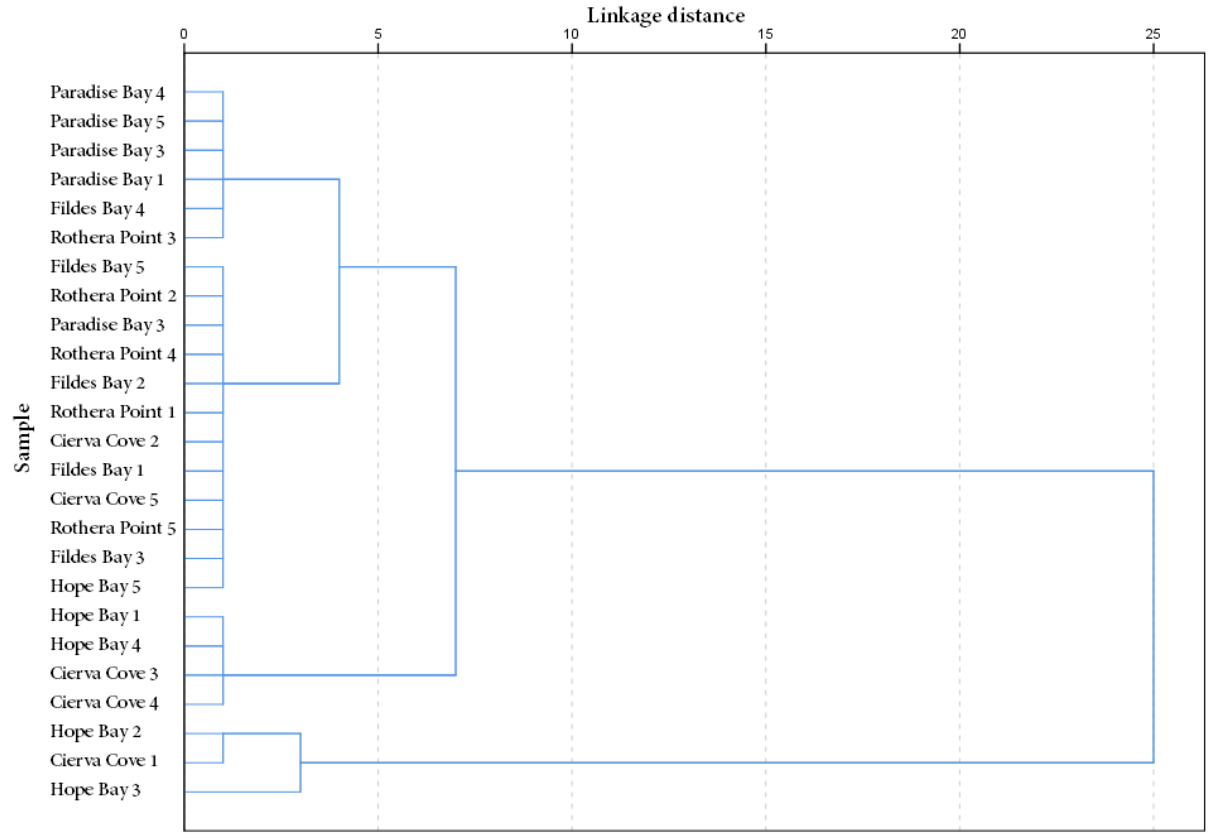
Supplementary figure 3. Dendrogram for *Desmarestia* sp. samples obtained using the squared Euclidean distance as a metric and UPGMA as the clustering method.



Supplementary figure 4. Dendrogram for *Nacella concinna* samples obtained using the squared Euclidean distance as a metric and UPGMA as the clustering method.



Supplementary figure 5. Dendrogram for *Diplasterias brucei* samples obtained using the squared Euclidean distance as a metric and UPGMA as the clustering method.



Supplementary figure 6. Dendrogram for *Odontaster validus* samples obtained using the squared Euclidean distance as a metric and UPGMA as the clustering method.

CAPÍTULO III

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 19 January 2023
DOI 10.3389/fmars.2022.1072696

OPEN ACCESS

EDITED BY
Nicole Trefault,
Universidad Mayor, Chile

REVIEWED BY
Angelina Lo Giudice,
National Research Council (CNR), Italy
Mario Moreno-Pino,
Universidad Mayor, Chile

*CORRESPONDENCE
Cristina García-Aljaro
crgarcia@ub.edu

SPECIALTY SECTION
This article was submitted to Microbial Symbioses, a section of the journal Frontiers in Marine Science

RECEIVED 17 October 2022
ACCEPTED 16 December 2022
PUBLISHED 19 January 2023

CITATION
De Castro-Fernández P, Ballesté E, Angulo-Preckler C, Biggs J, Avila C and García-Aljaro C (2023) How does heat stress affect sponge microbiomes? Structure and resilience of microbial communities of marine sponges from different habitats. *Front. Mar. Sci.* 9:1072696. doi: 10.3389/fmars.2022.1072696

How does heat stress affect sponge microbiomes? Structure and resilience of microbial communities of marine sponges from different habitats

Paula De Castro-Fernández[1,2,3], Elisenda Ballesté[1], Carlos Angulo-Preckler[4], Jason Biggs[5], Conxita Avila[2,3] and Cristina García-Aljaro[1*]

[1]Departament de Genètica, Microbiologia i Estadística, Universitat de Barcelona (UB), Barcelona, Spain, [2]Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Barcelona, Spain, [3]Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona (UB), Barcelona, Spain, [4]Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, [5]Division of Aquatic & Wildlife Resources, Guam Department of Agriculture, Mangilao, GU, United States

Introduction: Sponges are key components of marine benthic communities, providing many ecosystem functions and establishing close relationships with microorganisms, conforming the holobiont. These symbiotic microbiotas seem to be host species-specific and highly diverse, playing key roles in their sponge host. The effects of elevated seawater temperature on sponges and their microbiota are still poorly known, and whether sponges from polar areas are more sensitive to these impacts respect to temperate and tropical species is totally unknown.

Methods: We analyzed the microbiomes of different sponge species in their natural habitat and after exposure to heat stress in aquaria by 16S rRNA amplicon sequencing to (1) characterize the sponge microbiota covering a latitudinal gradient (polar, temperate and tropical environments), and (2) asses the effects of thermal stress on their microbial communities.

Results: Bacterial communities' structure was different in the different sponge species and also respect the surrounding seawater. The core microbiome is maintained in most sponge species after a heat stress, although whether they would recover to the normal conditions previous to the stress remains yet to be further investigated. We observed increased abundances of transient bacteria from unknown origin in sponge species exposed to heat stress.

Discussion: Some of the transient bacteria may be opportunistic bacteria that may benefit from the heat stress-associated dysregulation in the sponge by occupying new niches in the holobiont. According to our results, sponges from Antarctic waters could be more resilient than tropical and temperate sponges. Both the microbiome composition and the changes produced by the heat stress seem to be quite host species-specific, and thus, depend on the sponge species. Under a global change scenario, the microbiomes of the tropical and temperate sponges will probably be those suffering the most the heat stress, and therefore the effects of global change may be dramatic for benthic ecosystems since sponges are a fundamental part of them.

KEYWORDS

holobiont, heat shock, global warming, high microbial abundance, low microbial abundance, metabarcoding

1 Introduction

Sponges (phylum Porifera) are among the most ancient groups of metazoans (Bengtson, 1998; Feuda et al., 2017; Simion et al., 2017), and are widely distributed along tropical, temperate, and polar aquatic environments (Hooper and van Soest, 2002). These organisms are key components of marine benthic communities, providing ecosystem functions such as the creation of three-dimensional habitats to a variety of animals, giving shelter from predators, being source of food for spongivorous species, and contributing to the nutrient cycling (through the so-called benthic-pelagic coupling) due to their filter feeding nature (Bell, 2008; Southwell et al., 2008; de Goeij et al., 2013; Maldonado et al., 2015). Sponges often establish close relationships with diverse groups of microorganisms, including archaea, bacteria, microalgae, fungi, and viruses, that live within them, forming a complex structured ecosystem, called the sponge holobiont (Webster and Taylor, 2012; Thomas et al., 2016; Dittami et al., 2021; Leray et al., 2021). This symbiotic microbiota plays key roles in nutrient assimilation, waste metabolism, vitamin synthesis, or production of compounds with antifouling and defense properties, among many other aspects of the sponge's physiology and ecology (Taylor et al., 2007; Hentschel et al., 2012).

Marine sponge-associated microbial community has been reported to be host species-specific and highly diverse, harboring over 60 bacterial and four archaeal phyla (Lee et al., 2011; Jackson et al., 2012; Schmitt et al., 2012a; Cleary et al., 2013; Kennedy et al., 2014; Reveillaud et al., 2014; Thomas et al., 2016; Moitinho-Silva et al., 2017a; Freeman et al., 2020). The sponge microbiome generally consists of a core microbiome (microbial species or amplicon sequence variants (ASVs) present in all specimens of the sponge species) and a variable part (ASVs not present in all specimens or present but at very different abundances) (Schmitt et al., 2012b; Blanquer et al., 2013; Astudillo-García et al., 2017). Despite the continuous flow-through of seawater within sponges, they manage to maintain a specific microbiome that is different and often more diverse than the surrounding seawater (Taylor et al., 2004; Taylor et al., 2007; Webster et al., 2010; Schmitt et al., 2012b; Taylor et al., 2013; Horn et al., 2016; Thomas et al., 2016). Although how the sponge selects and keeps certain microbial populations remains still unclear, some studies suggest that a very specific recognition system to discriminate symbiotic microbes may exist, with the host's immune system being involved (Hentschel et al., 2012; Riesgo et al., 2014; Pita et al., 2018).

In terms of microbiota's contribution to the sponge biomass, sponges have been classified as either high microbial abundance (HMA) or low microbial abundance (LMA) (Hentschel et al., 2003; Gloeckner et al., 2014). HMA sponges harbor microbes' densities 2-4 orders to magnitude higher than LMA sponges (Webster et al., 2001; Hentschel et al., 2006; Hentschel et al., 2012). The microbiome of HMA sponges is richer and more diverse than that of LMA sponges, which is usually dominated by a few taxa (Björk et al., 2013; Erwin et al., 2015; Moitinho-Silva et al., 2017b). The dominant bacterial groups are also different for LMA and HMA sponges. While LMA sponges are usually dominated by Proteobacteria or Cyanobacteria, HMA sponges are enriched in Chloroflexi, Actinobacteria, or Acidobacteria, and usually harbor members of the phylum Poribacteria (Giles et al., 2013; Simister et al., 2013; Moitinho-Silva et al., 2017b). Despite the differences between HMA and LMA sponges' microbiomes, the core microbial functions in the holobiont seem to be conserved independently of the HMA or LMA nature of the sponge (Thomas et al., 2010; Fan et al., 2012).

Since the development of next generation sequencing technologies in the late 1990s and early 2000s, the

characterization of the microbiome diversity has grown exponentially (Taylor et al., 2007; Thomas et al., 2016; Webster and Thomas, 2016; Moitinho-Silva et al., 2017a; Stevens et al., 2017; Pita et al., 2018) The most common technique used to characterize microbial communities' composition (e.g., Earth Microbiome Project) is 16S rRNA gene amplicon sequencing. Even though sponges' microbiomes have been studied worldwide, most studies have focused on tropical and temperate sponges, and less attention has been paid to polar species. This is reflected in "The sponge microbiome project" (Moitinho-Silva et al., 2017a), where the microbiomes of 268 temperate and tropical sponge species were analyzed, but no polar species were included. However, in recent years, the microbiota of 31 Antarctic sponge species (or probably less since some of them were only identified to genus level) have been studied using 16S rRNA amplicon sequencing analysis (Webster et al., 2004; Rodríguez-Marconi et al., 2015; Cárdenas et al., 2018; Cárdenas et al., 2019; Lo Giudice et al., 2019; Steinert et al., 2019; Díez-Vives et al., 2020; Moreno-Pino et al., 2020; Papale et al., 2020; Sacristán-Soriano et al., 2020; Ruocco et al., 2021; Cristi et al., 2022; Happel et al., 2022). This is still a very small amount, considering that the most recent estimates of sponge species richness in the Southern Ocean and neighboring oceanographic regions were of 400 species as published in the Biogeographic Atlas of the Southern Ocean (Janussen and Downey, 2014).

Anthropogenic climate change has for long been known to have detrimental effects on marine environments (Smale et al., 2019; Cooley et al., 2022). For the last decades, along gradual ocean warming, ocean acidification, deoxygenation, and sea level rise, extreme events like heat waves have increased in frequency, duration, intensity, and extension (Oliver et al., 2018; Collins et al., 2019; Cooley et al., 2022). There is evidence that these heat waves have caused massive mortality events on marine benthic environments (Garrabou et al., 2009; Hereu and Kersting, 2016; Rubio-Portillo et al., 2016; Hughes et al., 2017; Ereskovsky et al., 2019; Garrabou et al., 2022). Although the sponge microbiome is in general stable across geographical and temporal scales (Erwin et al., 2012; Erwin et al., 2015; Cárdenas et al., 2019; Happel et al., 2022), it can also be influenced by environmental perturbations (*e.g.* Webster et al., 2008; Lesser et al., 2016; McDevitt-Irwin et al., 2017). Some studies have addressed the effect of elevated seawater temperature particularly on sponges, both on their physiology and on their microbiota (Simister et al., 2012b; Simister et al., 2012a; Vargas et al., 2021). While some sponges exposed to thermal stress suffered from tissue necrosis and bleaching (*e.g.* Bennett et al., 2017; Perkins et al., 2022), others seem to be less vulnerable (*e.g.* González-Aravena et al., 2019). Similarly, while warming produced changes and disruption on the microbiota of some sponge species (*e.g.* Simister et al., 2012a; Fan et al., 2013; Blanquer et al., 2016; Ramsby et al., 2018; Rondon et al., 2020), others remained unaffected (Webster et al., 2008; Erwin et al., 2012; Simister et al., 2012b; Strand et al., 2017). Mostly, however, the effect of the temperature increase in the microbiota of the sponges remains to be further investigated. Moreover, whether sponges from polar areas are more sensitive to these impacts respect to temperate and tropical species is totally unknown. The water temperature in Antarctic environments is very stable all year round, whereas temperate and tropical regions experience larger variations in seawater temperature (Gonzalez-Acosta et al., 2006; Thébault et al., 2007; Clarke et al., 2008; Lie and Lee, 2010). The adaptation of organisms to the particular environmental conditions of their region have led to organisms in the Antarctic regions to survive in a narrow temperature range (stenothermal organisms), compared to temperate and tropical organisms (Peck and Conway, 2000).

Here, the microbiomes of different sponge species from their natural habitat along with that of sponge specimens exposed to heat stress experiments in aquaria were assessed by 16S rRNA amplicon sequencing to (1) characterize the sponge species microbiota covering a latitudinal gradient (polar, temperate and tropical environments), and (2) asses the effects of thermal stress on their microbial communities.

2 Materials and methods

2.1 Sample collection

A total of 88 specimens belonging to four demosponge species living in similar shallow rocky benthic environments were collected at three regions representative of polar, temperate, and tropical coastal environments. *Mycale acerata* (LMA) (Sacristán-Soriano et al., 2020; Happel et al., 2022) and *Dendrilla antarctica* (LMA) (Koutsouveli et al., 2018; Díez-Vives et al., 2020), common yellow sponges in hard bottom benthic environments from polar (Antarctic) waters; and *Agelas oroides* (HMA) (Vacelet and Donadey, 1977; Blanquer et al., 2013), a lobed orange sponge present in temperate (Mediterranean Sea and Eastern Atlantic) waters; and *Acanthella cavernosa* (LMA) (Coelho et al., 2018; Cleary et al., 2019), a red spiky sponge present in tropical (Pacific Ocean) waters. Both Antarctic sponges, *M. acerata* (n=28) and *D. antarctica* (n=28) were collected at four different stations on Deception and Livingston Islands (South Shetland Islands) (Figure 1 and Table 1). The temperate species, *A. oroides* (n=25) was collected at two different stations from the Western Mediterranean coast (Costa Brava, Catalonia), while the Indo-pacific sponge, *A. cavernosa* (n=25) was collected at two different stations from Guam (Mariana Islands). Whole healthy sponge specimens and 2 L of seawater adjacent to the animals were collected manually by scuba diving at 10-25 m depths. Sponge specimens were kept in independent plastic zip bags or screw cap plastic containers filled with seawater and transported to the lab in liquid N_2 or ice, within 1-2 h. Sampling took place in Antarctica (polar site) during the austral Summer of 2018, and 2019, while sampling in the Mediterranean Sea (temperate site) took place in

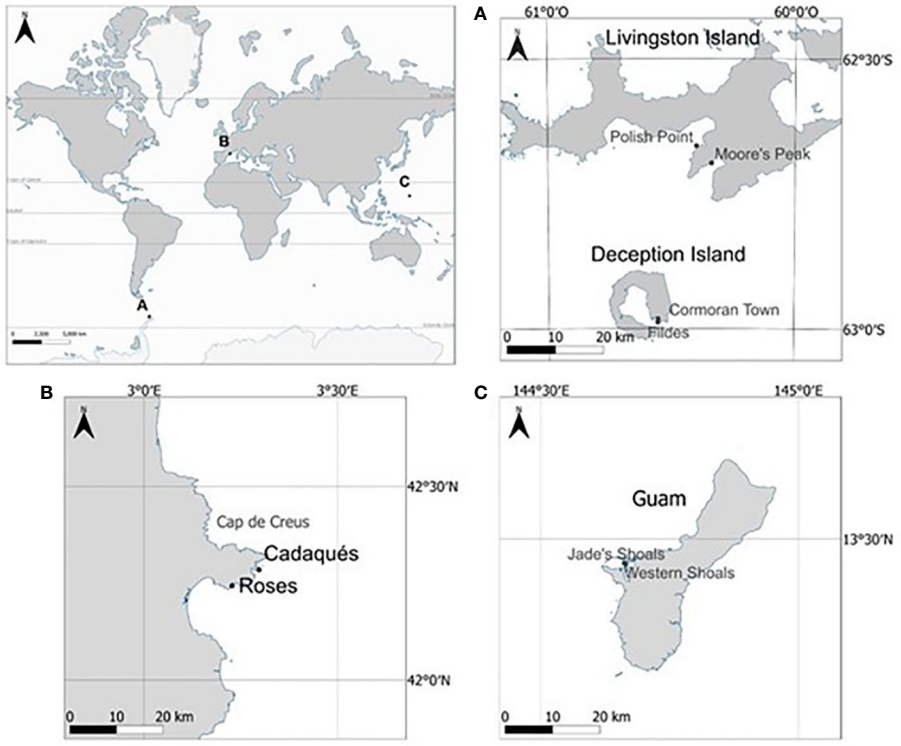


FIGURE 1
Sampling sites of the different regions. **(A)** Antarctic region (South Shetland Islands). **(B)** Mediterranean region. **(C)** sampling sites in Guam Island (Mariana Islands). Sampling locations are marked with black dots at each sampling region.

May 2018 and sampling in Guam (tropical site) took place in April 2019 (Figure 1). Seawater temperatures at the moment of collection were -1 to 4°C in the South Shetland Islands, 14-15°C in the Mediterranean coast, and 28-29°C in Guam Island. A total of 29 specimens were used to assess the bacterial composition of the holobiont and 59 were used to simulate the effect of a heatwave in the microbiome. A summary of the number of samples used in the different experiments are shown in Table 1.

2.2 Heat stress experimental design

For the temperature experiments, at each locality, 20 different individuals of each species (*M. acerata*, *D. antarctica*, *A. cavernosa*, and *A. oroides*) were placed in aquaria at three different temperatures, including local seawater temperature, to be used as control temperature (CT), and at two higher temperatures [heat stress temperature (HST) and extreme heat stress temperature (EHST), around 4-5°C and 6-10°C higher than the CT, respectively]. Temperatures were chosen on each site according to the increase predicted by the IPCC and other reports, by duplicating the expected values and higher, and to the aquarium possibilities available for us to carry out the experiments on each place. Five more specimens of each species were placed in the aquaria at the beginning of the experiment and processed after 24 h to control for changes in the microbiota due to the manipulation, transport, and placement in the aquaria (aquaria adaptation control group). The experimental temperatures are summarized in Table 1. Water temperature was measured and controlled using a digital controller (Aqua Medic T controller twin) connected to heating (Sera 50 W or 150 W) and/or cooling (Aqua Medic Titan 150) units. The system was kept steady all along the experiment. Sponge specimens were kept in compartmented tanks (volumes ranging between 24-112.5 and 480 L according to the organism sizes), with seawater circulating through all the compartments, and were incubated for approximately three weeks for the Antarctic, two weeks for Mediterranean and one week for tropical sponge species. Samples were incubated at different times because temperature affects metabolism, and this is much fastest in the tropics and slowest in Antarctica. Therefore, the experimental times were adjusted to these different metabolisms.

TABLE 1 Samples, number of sponge specimens, experimental conditions and sampling sites coordinates of the different specimens.

Host species	Natural habitat	Aquaria Experiments				Location	Coordinates
	N	CT	HST	EHST	N		
Mycale (Oxymycale) acerata Kirkpatrick, 1907	5	0.7 ± 1.2	5.7 ± 0.7	9.8 ± 0.8	20	Cormoran Town, Whaler's Bay, Deception Island	-62.981666, -60.554979
	2					Polish Point, Livingston Island	-62.661183, -60.398817
	1					Moore's Peak, False Bay, Livingston Island	-62.693056, -60.338056
Dendrilla antarctica Topsent, 1905	3	0.5 ± 0.3	5.4 ± 0.4	9.7 ± 0.8	16	Fildes, Whaler's Bay, Deception Island	-62.987531, -60.556302
					4	Cormoran Town, Whaler's Bay, Deception Island	-62.981666, -60.554979
	5					Moore's Peak, False Bay, Livingston Island	-62.693056, -60.338056
Agelas oroides Schmidt, 1864	3	15.6 ± 0.2	20.1 ± 0.2	24.7 ± 0.3	12	Rostella Cove, Roses, Catalonia	42.243307, 3.227282
	2				8	Es Caials Cove, Cadaqués, Catalonia	42.284554, 3.297230
Acanthella cavernosa Dendy, 1922	5	29.2 ± 0.6	32.6 ± 1.2	33.1 ± 1.2	11	Jade's Shoals, Guam Island	13.453844, 144.661835
					9	Western Shoals, Guam Island	13.450547, 144.664566

N, number of samples; CT, control temperature; HST, heat stress temperature; EHST, extreme heat stress temperature. Temperature indicated in Celsius degrees. Temperature indicated as mean ± SD.

2.3 Total DNA extraction and PCR amplification of 16S rRNA

All sponge specimens were washed with sterile seawater. An amount of 250 mg of each sponge specimen was homogenized to small pieces in a glass Petri dish with sterile scissors and blade. DNA was extracted using FastDNA™ SPIN Kit for Soil (MP Biomedicals, Illkirch, France). Extraction was performed following manufacturer's instructions. Isolation of bacterial DNA from seawater collected from the surrounding environment was performed using a concentration-filtration method. One liter of each sample was filtered by vacuum filtration through a 0.22 µm pore-size mixed ester cellulose membrane. DNA from filter-retained bacteria was extracted using the same DNA extraction kit. Each filter was fitted in a 5 ml flask so that the bacteria retaining side was exposed to buffers and the beads in the first step of the kit's protocol. The DNA concentration was quantified by Qubit fluorometer (Invitrogen). A negative control for all the extractions were performed.

2.4 Illumina 16S rRNA amplicon sequencing

Negative controls (blanks from the DNA extraction process, as well as from the DNA amplification, and sterile seawater used to wash the animals) as well as two different positive controls (ABRF-MGRG 10 Strain Staggered Mix Genomic Material (ATCC MSA-3002), and ZymoBIOMICS Microbial Community DNA Standard (D6306) were included in the 16S rRNA amplicon sequencing. Sample sequencing was performed in three runs using the Illumina MiSeq platform at the Genomics Unit of Centre for Genomic Regulation Core Facilities (CRG, Barcelona). The data is available at Mendely Data public repository (DOI: 10.17632/9c6y62nv9z.1, DOI: 10.17632/d67pjpc47g.1, DOI: 10.17632/fnjhzsybxj.1).

The V4 region was amplified from DNA sample extracts using the primers from the Earth Microbiome Project [515F (Parada et al., 2016) (5'- GTGYCAGCMGCCGCGGTAA-3') and 806R (Apprill et al., 2015) (5'-GGACTACNVGGGTWTCTAAT-3')] The PCR was performed in 25 µl volume with 0.2 µM primer

concentration and KAPA HiFi HotStart ReadyMix (Roche). Cycling conditions were initial denaturation of 3 min at 95°C followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, ending with a final elongation step of 5 min at 72°C. After this first PCR step, water was added to a total volume of 50 µl and reactions were purified using AgenCourt AMPure XP beads (Beckman Coulter). The first PCR primers contain overhangs allowing the addition of full-length Nextera adapters with barcodes for multiplex sequencing in a second PCR step, resulting in sequencing ready libraries with approximately 450 bp insert sizes. To do so, five µl of the first amplification were used as template for the second PCR with Nextera XT v2 adaptor primers in a final volume of 50 µl using the same PCR mix and thermal profile as for the first PCR but only 8 cycles. After the second PCR, 25 µl of the final product was used for purification and normalization with SequalPrep normalization kit (ThermoFisher Scientific), according to manufacturer's protocol. Libraries were eluted and pooled for sequencing. Final pool libraries were analyzed using Agilent Bioanalyzer or Fragment analyzer High Sensitivity assay to estimate the quantity and check size distribution, and were then quantified by qPCR using the KAPA Library Quantification Kit (KapaBiosystems) prior to sequencing with Illumina's Miseq 2x300bp.

2.5 Bioinformatic analyses

Cutadapt was used to trim adapters, primers, barcodes and leading Ns from sequencing reads. Sequences were processed to amplicon sequence variants (ASV) using the default parameters of the Dada2 workflow (Callahan et al., 2016). Firstly, quality filtering and the trimming of sequences was set to 180 bp (for forward reads) and 150 bp (for reverse reads) with a maximum number of expected errors allowed per read set at two (EE = 2). This parameter has been shown to be a better filter than simply averaging quality scores (Edgar and Flyvbjerg, 2015). Filtered sequences were dereplicated, the forward and reverse reads were aligned and merged. The sequences from the three runs were merged, chimeras were removed and an amplicon sequence variant (ASV) table was obtained. Taxonomy was assigned to the resulting ASVs using the SILVA SSU 138 reference database and was imported to the phyloseq R package for microbiome analyses. To obtain more accurate profiling of microbial communities, the *decontam* (Davis et al., 2018) R package was used to remove sequences derived from contaminating DNA present in extraction or sequencing reagents. In addition, chloroplast and mitochondrial reads were removed. The sequences of most abundant ASV that could not be identified at genus level were searched against GenBank database (rRNA database for Archaea and Bacteria) using Blast tool from NCBI (https://blast.ncbi.nlm.nih.gov/).

2.6 Data analyses

Alpha and beta diversity were analysed using the Phyloseq (McMurdie and Holmes, 2013) and *vegan* (Oksanen et al., 2022) R packages. For alpha diversity analysis, estimates of richness (namely Chao1 index, which is an estimate based on the abundance but affected by the number of ASVs appearing few times), and diversity indices (Shannon, which indicates both the richness but also considers the abundance of ASV, and Inverse Simpson, which is mostly affected by the dominance of certain ASVs in the sample) were calculated after rarefying the ASV table. In temperature experiments, rarefaction was performed to the different experimental groups ASV table for each target species. One-way analyses of variance (ANOVA) were used to assess differences in the alpha diversity indices across species (*Mycale acerata*, *Dendrilla antarctica*, *Acanthella cavernosa*, *Agelas oroides*), followed by HSD Tukey *post-hoc* test for significant ANOVAs. Student's t-test was used to detect differences in the alpha diversity indices between the sponge specimens of the two Antarctic locations (Deception Island and Livingston Island) for *M. acerata* and *D. antarctica*, and between the sponge specimens of the two Mediterranean locations (Roses and Cadaqués) for *A. oroides*. One-way analyses of variance (ANOVA) were used to assess differences in the alpha diversity indices across aquaria experimental groups (natural habitat, adaptation group, control temperature, heat stress temperature, and high heat stress temperature) within each sponge species, followed by HSD Tukey *post-hoc* test for significant ANOVAs. The univariate statistics were performed using SPSS Statistics v27 (IBM Corporation). For beta diversity analysis, the number of reads of each ASV was previously transformed to relative abundance, the Bray Curtis distance was calculated, and samples were ordinated by non-linear multidimensional scaling (nMDS). Microbial core communities were determined at the sponge species level, defined as taxa shared (ASVs) by 100% of the sponge specimens. Additionally, for those sponges collected at different locations a separate core community was also determined for each location.

Furthermore, SourceTracker2 package (Knights et al., 2011) was used to determine the source of ASVs of sponge specimens from different experimental groups. For this, the natural habitat group, seawater, and aquarium water from the end of the aquaria experiments were treated as "source", whereas the aquaria adaptation control group, control temperature (CT), heat

stress temperature (HST) and extreme heat stress temperature (EHST) groups from each sponge species were treated as "sink" samples.

3 Results

3.1 Diversity of the sponge-associated bacterial communities in their natural habitat

A total of 2,947,270 reads were obtained after denoising and quality filtering of the raw sequencing data of sponge specimens collected from their natural habitat and their surrounding water. Among these, after chloroplast and mitochondria removal, 2,583,832 reads (88%) affiliated to Bacteria, representing 5,183 ASVs, with an average of 80,744 reads per sample (ranging from 9,681 to 180,387 reads) (Table 2). A total of 689 ASVs were recovered from *M. acerata*, 936 ASVs from *D. antarctica*, 2,071 ASVs from *A. cavernosa*, and 550 ASVs from *A. oroides*. Samples of seawater recovered in the same area as the sponge specimens had 480 ASVs on average. The sponge species were in all cases enriched for ASVs in relation to the surrounding seawater with the exception of the Mediterranean environment, from where 755 ASVs were recovered from the water whereas 550 ASVs were recovered from *A. oroides*.

Around 3% of the reads affiliated to domain Archaea (ranging from 3 to 25,329 reads per sample), showing an uneven distribution between the different sponge species. In fact, only two of the studied species (*A. oroides* and *D. antarctica*) and their corresponding seawater samples harbored Archaea at an abundance higher than 1% of the total reads [*A. oroides* (8.08-18.84% and *D. antarctica* from Livingston (1.12-2.22%)]. In both cases, the archaeal community was dominated with 99-100% abundance by a unique genus, *Cenarchaeum* in *A. oroides* and "*Candidatus* Nitrosopumilus" in *D. antarctica*, respectively. Both genera belong to the Nitrososphaeria class in the Crenarchaeota phylum. Seawater associated to *A. oroides* were dominated by Marine Group II archaea (52%), "*Candidatus* Nitrosopumilus" (28%), "*Candidatus* Nitrosopelagicus" (11%) and *Cenarchaeum* (6%) genus, and Marine Group III (4%). On the other hand, seawater

TABLE 2 Summary of reads, ASVs, and diversity of sponge species and water of the different geographic areas.

Sponge species	N	Average reads	Total ASVs	Core ASVs	Chao1	Shannon	InvSimpson
Merged by species							
M. acerata	8	51,014 ± 9,459	689	14	170 ± 66	1.8 ± 0.4	2.4 ± 0.7
D. antarctica	8	41,250 ± 261,23	936	9	179 ± 47	3.5 ± 0.3	14.5 ± 5.7
A. oroides	5	130,001 ± 34,336	550	94	212 ± 8	3.9 ± 0.2	29.5 ± 9.3
A. cavernosa	5	133,296 ± 29,838	2071	65	500 ± 179	2.4 ± 0.4	5.0 ± 1.4
Merged by location							
M. acerata DEC	5	44,905 ± 2,236	438	35	153 ± 60	1.7 ± 0.3	2.2 ± 0.3
M. acerata LIV	3	61,195 ± 7,374	434	43	199 ± 78	2.0 ± 0.6	2.8 ± 1.1
D. antarctica DEC	3	65,840 ± 14,814	478	36	205 ± 56	3.6 ± 0.3	16.7 ± 4.8
D. antarctica LIV	5	26,497 ± 18,943	573	19	163 ± 39	3.5 ± 0.4	13.1 ± 6.3
A. oroides ROS	3	142,586 ± 32,815	458	120	217 ± 4	3.8 ± 0.0	23.5 ± 1.9
A. oroides CAD	2	111,124 ± 37,075	294	147	203 ± 2	4.1 ± 0.2	38.5 ± 8.2
Seawater							
M. acerata/ *D. antarctica* DEC SW	1	94,819	374	NA	369	3.73	15.06
M. acerata LIV SW	2	76,731 ± 30,708	399	NA	282 ± 29	3.8 ± 0.1	15.7 ± 0.4
D. antarctica LIV SW	1	78,026	412	NA	377	3.70	13.00
A. oroides CAD SW	1	113,801	755	NA	698	4.88	57.54
A. cavernosa SW	1	89,115	456	NA	410	4.44	28.32

SW, seawater. The surrounding seawater of M. acerata DEC and D. antarctica DEC was the same. No seawater for A. oroides ROS specimens was available. Number of reads, as well as Chao1, Shannon, and InvSimpson indices indicated as mean ± SD. NA, not aplicable; N, number of samples.

10.3389/fmars.2022.1072696

associated to *A. cavernosa was* dominated by the Marine Group II (98%). Archaea were not included in further analyses.

The richness was significantly higher in the Tropical sponge *A. cavernosa* (observed ASVs, 2071; and Chao 1 index, 500) than in the other sponge species ($p<0.001$) (Table 2). It has to be noted that more than 50% of these ASVs were found less than 10 times. A lower number of ASVs was observed for *D. antarctica* (936), followed by *M.* (689), and *A. oroides* (550). *A. oroides* and *D. antarctica* had the highest Shannon diversity index (3.9 and 3.4, respectively), followed by *A. cavernosa* (2.4) and *M. acerata* (1.8) (Table 2). Statistically significant differences were observed between *M. acerata* and the sponges *D. antarctica*, *A. cavernosa* and *A.* oroides, and between *A. cavernosa* and the sponges *M. acerata*, *D. antarctica* and *A. oroides* ($p<0.001$). This index indicates both the richness but also considers the proportion of ASV. In the case of the Inverse Simpson index, which is mostly affected by the dominance of certain ASVs in the sample, a similar trend was observed with the highest average value Obtained for *A. oroides* (29.5), followed by *D. antarctica* (14.5), *A. cavernosa* (5.0) and *M. acerata* (2.4) (Table 2). Statistically significant differences were observed between *M. acerata* and the sponges *D. antarctica* and *A. oroides*, between *D. antarctica* and the sponge *A. oroides*, and between *A. cavernosa* and the sponges *D. antarctica* and *A. oroides.* The effect of the sampling location on the richness and diversity indices was also investigated showing no statistically significant differences among the different sampling locations of the same species. ($p>0.05$). The exception was the Chao1 index in *A. oroides*, which was higher in the sponge specimens from Roses than in the sponge specimens from Cadaqués ($p=0.025$) (Table 2).

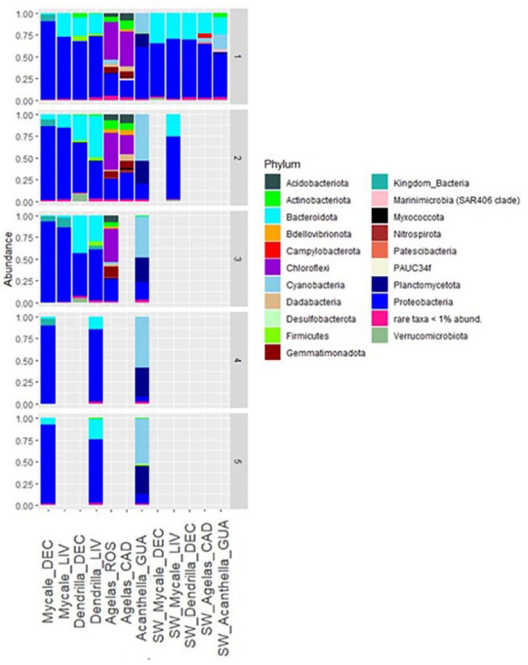


FIGURE 2
Taxonomic affiliation of ASVs detected in sponge species and seawater in their natural habitat at Phylum level. DEC, Deception Island; LIV, Livingston Island; ROS, Roses; CAD, Cadaqués; GUA, Guam; SW, seawater. Numbers on the right axis indicate sample replicates.

3.2 Taxonomic composition and structure of the sponge-associated bacterial communities in their natural habitat

The taxonomic composition of the bacterial communities at phylum level is shown in Figure 2 and Supplementary Table 1. The bacterial communities of *M. acerata* were dominated by Proteobacteria (86±7%), specifically class Gammaproteobacteria (79±9%), independently of the sampling location. *D. antarctica* was also dominated by Proteobacteria (62±13%), with Alphaproteobacteria constituting 35±13% of the bacterial community, followed by Bacteroidota (29±11%) and Gammaproteobacteria (27±15%), also independently of the sampling location. *A. oroides* was dominated by the phylum Chloroflexi (37±9%), which was practically absent in the other sponge species and the surrounding seawater, followed by Gammaproteobacteria (22±4%), in both sampling locations. Finally, *A. cavernosa* was dominated by Cyanobacteria (47 ±14%), followed by Planctomycetota (27±7%), which was present at <1% on average in the other sponge species and seawater, and Gammaproteobacteria (20±22%).

The bacterial community of the seawater from the different locations where the sponges were collected was dominated by Proteobacteria (63±8%), followed by Bacteroidota (27±6%) (Figure 2). Class Alphaproteobacteria was in all seawater samples equal or more abundant than class Gammaproteobacteria. Specifically, seawater surrounding *M. acerata* hosted a bacterial community dominated by Proteobacteria (67±6%) and Bacteroidota (30±5%), independently of the sampling location. The seawater surrounding *D. antarctica* in Livingston Island had a community dominated by Proteobacteria (66%) and Bacteroidota (31%). Seawater surrounding *A. cavernosa* specimens hosted a bacterial community dominated by Proteobacteria (50%) (with Alphaproteobacteria as the most abundant (37%) versus Bacteroidota (20%), Cyanobacteria (17%), and Gammaproteobacteria (12%). Finally, seawater around *A. oroides* (temperate sampling region), was enriched in Proteobacteria (61%), Bacteroidota (24%), Cyanobacteria (5%) and Campylobacterota (5%).

The majority of ASVs could not be affiliated to the genus taxonomic rank, and therefore they were affiliated at a higher taxonomic rank. The complete taxonomic affiliation from genus to class is shown in Supplementary Figures 3. Notably, the ten

10.3389/fmars.2022.1072696

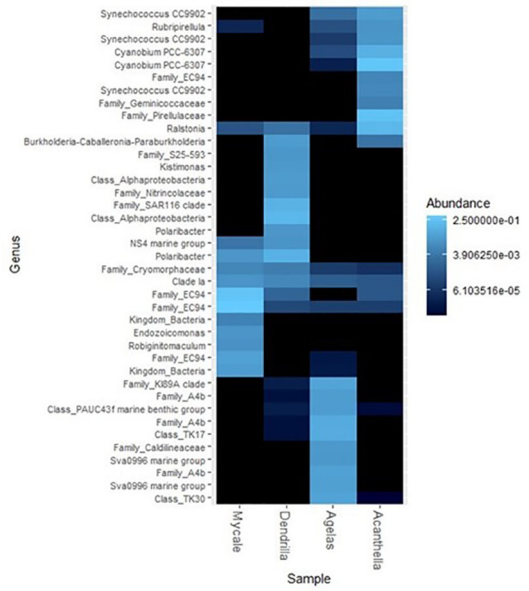


FIGURE 3
Heatmap representation of the abundance of the 10 most abundant ASVs of the different sponge species with its taxonomic affiliation at the lowest taxonomic rank. Their presence in all sponge species is shown.

most abundant ASVs from each sponge species were distributed unevenly in the other sponges (Figure 3). For example, *M. acerata* most abundant ASVs were affiliated to the family EC94 with an abundance of 69% altogether. In this species, 4% of the reads could not be affiliated at a lower taxonomic rank below Bacteria kingdom. In *D. antarctica*, the most abundant ASVs were an unidentified ASV affiliated to an unidentified genus of class Alphaproteobacteria (>13%), *Polaribacter* (>12%), an unidentified ASV affiliated to the family SAR116 clade (10%), followed by *Burkholderia-Caballeronia-Paraburkholderia* (3%), two unidentified ASVs affiliated to family Nitrincolaceae and family S25-593, respectively, *Kistimonas* and the NS4 marine group (all of them at relative abundance <3%). Among the ten most abundant ASVs detected in *A. cavernosa Cyanobium* was the most abundant (40%), followed by an unidentified ASVs affiliated to family Pirellulaceae (23%), and ASVs affiliated to *Ralstonia* (13%) and *Synechococcus* (6%). In *A. oroides*, again the most abundant ASVs at a relative abundance higher than 5% were not shared with the other species or were practically absent. These included mostly unidentified genera from different families or classes (family A4b (>14%), Sva0996 marine group (6%), clade KI89A (5%), class TK17 (7%), PAUC43f marine benthic group (3%), class TK30 (5%), and family Caldilineaceae (2.5%)).

This distinct bacterial communities' structure observed between the different sponge species and between the surrounding water and the corresponding sponges was also visualized by non-metric multidimensional scaling (nMDS) of Beta-diversity (Bray-Curtis) coefficients (Figure 4). The samples clustered together according to the sponge species. The Antarctic sponges *M. acerata* and *D. antarctica* were closer between them than the other sponge species, but without overlapping each other. All sponge species were distant from seawater in the multidimensional space, except from *M. acerata* specimens from Livingston, that exhibited overlap with the group formed by Antarctic seawater samples.

3.3 Core bacterial communities of the sponges in their natural habitat

The core bacterial community of *M. acerata* from Deception Island was composed of 35 ASVs, and that from Livingston Island of 43 ASVs (Table 2). Considering all replicates from both locations together, the core community of *M. acerata* was restricted to 14 ASVs. It must be noted that although the most abundant bacterial taxa in all *M. acerata* replicates was a unidentified genus from the EC94 family, with relative abundance ranging 68-78%, this genus was represented by a total of 27 ASVs, none of which was shared by 100% of the replicates. ASVs 1 and 3 (both affiliated to the EC94 family) were the two most abundant ASVs in *M. acerata* specimens in average. These ASVs showed the highest identity to different *Nitrosomonas* spp using Blast against GenBank rRNA database [Supplementary Table 3 (Table 1)]. All *M. acerata* replicates had at least one of these ASVs. ASVs 1 and 3 were also present in seawater samples, whereas ASV47 (also affiliated to the EC94 family) was only present in one seawater sample. Nevertheless, they were up to 1000-2000 times more abundant in the sponge specimens than in seawater. The most abundant ASVs shared by

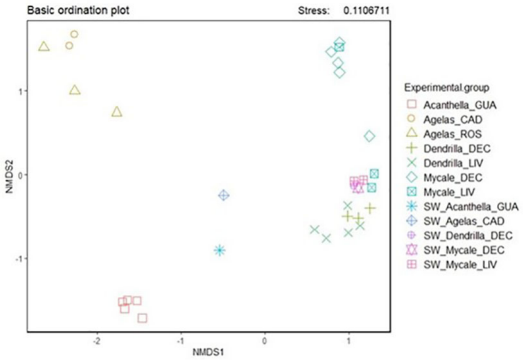


FIGURE 4
Non-linear multidimensional scaling representation of the different sponge species from the different geographic areas and sampling locations and their surrounding water. DEC, Deception Island; LIV, Livingston Island; GUA, Guam; ROS, Roses; CAD, Cadaqués; SW, seawater.

100% of the replicates of the sponge were affiliated to SAR11 Ia clade and *Polaribacter* (around 2% on average). Both were more abundant in seawater's community than in the sponges' communities: around ten times more abundant for the genus affiliated to SAR11 Ia clade, and around two times for *Polaribacter*. The other ASVs in *M. acerata* core community were less abundant than 1% on average.

The core bacterial community of *D. antarctica* from Deception Island included 36 ASVs, and that from Livingston Island, 19 ASVs. Considering all specimens from both locations, the core community of *D. antarctica* was composed of 9 ASVs. The five most abundant ASVs on average were affiliated to a unidentified genus of the SAR116 clade, *Polaribacter*, a unidentified genus of the NS4 marine group, a unidentified genus of the OM43 clade, and a unidentified genus of the Nitrincolaceae family. These unidentified ASVs showed the highest identity to genera *Thalassobaculum, Kordia, Rivicola*, and *Maribrevibacterium* from GenBank DB, respectively [Supplementary Table 3 (Table 1)]. SAR116 clade was not present in the seawater samples, the ASVs associated to a unidentified genus of the Nitrincolaceae family was equally abundant in the sponge specimens and seawater (~1% on average), and the other three were six to nine times more abundant in the sponge specimens compared to seawater.

The core bacterial community of the tropical sponge *A. cavernosa* included 65 ASVs. The five more abundant ASVs in the core bacterial community represented on average around 80% of the whole bacterial community. These ASVs affiliated to the genus *Cyanobium*, a unidentified genus of the Pirellulaceae family (showing the highest identity to genus *Botrimarina* from GenBank DB) [Supplementary Table 3 (Table 1)], *Ralstonia* genus and *Synechococcus* genus. All these five ASVs were present in the seawater community, with relative abundances under 0.5%, except for ASV34 (*Synechococcus* genus), which represented the 14% of the seawater community.

The core bacterial community of the temperate sponge *A. oroides* included 94 ASVs. Four out of the five more abundant ASVs on average (with abundances 4-7% of the community) belonged to bacteria in the Chloroflexi phylum. Specifically, these bacteria belonged either to class TK17, family A4b or class TK30 (showing the highest identity to genera *Litorilinea, Ornatilinea* and *Ammoniphilus*, respectively, from GenBank DB) [Supplementary Table 3 (Table 1)]. The third most abundant bacterial taxa in the core community of *A. oroides* was the KI89A clade (4.7% average relative abundance), belonging to the oligotrophic marine Gammaproteobacteria (OMG) group. These ASVs were either absent in the seawater bacterial community (class TK30, KI89A clade), or were 1000-2000 times more concentrated in the sponge specimens than in the seawater (class TK17, family A4b).

There were four ASVs (ASV 1, 2, 18, 112) that were shared among the four sponge species studied although not present in 100% of the replicates. The taxa assigned to them were a unidentified genus of the EC94 family, a unidentified genus of the SAR11 Ia clade, *Ralstonia*, and a unidentified genus of family Cryomorphaceae. These unidentified ASVs showed the highest identity to genera *Nitrosomonas*, *Pelagibacter* and *Phaeocystidibacter*, from GenBank DB) (Supplementary Table 1). The ASVs relative frequencies varied among the different sponge species being ASV 1 dominant in *M. acerata* (39%), with respect to the other sponge species (0.01-0.02%). The second most abundant shared ASV was ASV 18 in *A. cavernosa* (14%), being detected in the other sponges at lower relative abundances (with up to 4000 times lower relative abundance in *A. oroides*). The other left ASVs were present at lower frequencies (<2% in all of the sponge species). All except ASV1 were found ubiquitously in the surrounding seawaters, except ASV1, which was only found in the seawater sample from Deception Island at very low relative abundance (0.03%).

3.4 Effect of heat stress

The results of the effect of a heat wave on the bacterial communities of the sponge species are shown in Table 3 and Supplementary Table 2. In all four species, the reads from the adaptation control specimens mainly matched those of the natural habitat group (*M. acerata* 91.8%, *A. cavernosa* 61.6%, and *A. oroides* 82.8%), with lower percentage in *D. antarctica*, 46.6% (Table 3). At the end of the experiments, the percentage of sequences that were tracked to the natural habitat specimens had decreased in all cases. In the specimens exposed to extreme heat stress temperature, the source of the highest percentage of reads was the aquarium seawater in all cases. The experimental groups maintained at CT were those with a higher percentage of sequencing tracking the natural habitat specimens, although only *M. acerata* retained most sequences of the natural habitat specimens at CT condition. Taking into consideration the increasing temperatures, the percentages of shared sequences decreased as the temperature increased ($p<0.05$ in all comparisons of HST vs. CT, and EHST vs. CT, except for *D. antarctica* HST vs. CT).

In order to assess the effect of temperature in the communities' biodiversity, the richness of observed species and Shannon index were analyzed (Figure 5). A general decrease trend in the observed species richness and Shannon diversity index with the increasing temperature was observed for the tropical *A. cavernosa* and the temperate *A. oroides*. This trend was reversed for the Antarctic sponges, although in the case of *M. acerata* the diversity of the EHST experimental group was between HST and CT experimental groups. The diversity of the sponge specimens in their natural habitat was higher than the corresponding experimental group mimicking the natural conditions (CT) in the case of *D. antarctica* ($p<0.05$ for Shannon index difference) and lower in the case of *A. cavernosa* ($p<0.05$ for Shannon index difference). The diversity

TABLE 3 Average contribution of reads (%) of the possible sources to the microbiome of the different sponge species experimental groups detected in the aquaria exposed at different temperatures.

	Natural habitat		SW Natural habitat		SW Control group		SW CT		SW HST		SW EHST		Unknown	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
M. acerata Control	91.8	6.0	4.1	4.1	1.0	0.6	0.3	0.2	0.3	0.2	0.5	0.3	1.9	1.9
M. acerata CT	71.9	24.0	1.9	2.7	3.0	2.7	18.2	21.4	2.4	2.1	1.9	0.9	0.7	0.5
M. acerata HST	12.6	10.1	0.2	0.2	2.8	1.9	22.6	15.6	50.8	20.7	3.3	1.4	7.7	1.8
M. acerata EHST	15.3	6.3	0.2	0.4	27.3	5.4	5.2	2.7	1.1	0.4	49.6	7.7	1.2	0.4
D. antarctica Control	46.6	28.1	8.7	5.3	3.4	6.4	0.7	0.9	0.4	0.5	17.6	21.5	22.7	24.1
D. antarctica CT	25.3	8.9	0.9	0.2	0.8	0.3	38.6	16.3	0.4	0.4	19.4	6.1	14.5	25.5
D. antarctica HST	19.4	6.2	3.0	2.3	1.1	0.9	0.8	0.5	4.4	4.4	55.6	7.6	15.7	5.9
D. antarctica EHST	9.3	5.7	2.5	1.3	0.6	0.8	6.9	14.1	1.5	2.4	61.6	10.0	17.6	8.5
A. oroides Control	82.8	7.5	6.1	4.9	6.1	7.4	0.2	0.1	0.3	0.1	0.3	0.1	4.3	2.3
A. oroides CT	6.9	3.0	3.3	2.2	4.1	3.7	9.6	5.6	15.9	13.0	6.9	5.2	53.3	20.6
A. oroides HST	2.2	1.1	2.1	1.3	3.3	1.8	1.7	0.8	25.2	8.8	35.5	10.6	30.0	8.1
A. oroides EHST	1.4	1.3	0.7	0.8	0.4	0.3	0.2	0.1	1.2	0.6	68.1	2.9	27.9	3.5
A. cavernosa Control	61.6	29.1	0.1	0.1	0.4	0.3	1.5	1.9	5.6	10.7	4.5	6.6	26.3	18.8
A. cavernosa CT	12.7	7.5	0.1	0.0	0.8	0.2	1.5	0.8	1.6	0.9	4.3	3.3	79.0	6.6
A. cavernosa HST	1.1	0.7	0.1	0.0	0.4	0.2	1.9	0.6	48.2	6.8	11.8	3.1	36.6	3.4
A. cavernosa EHST	0.6	0.5	0.2	0.0	0.8	0.4	5.3	1.4	18.7	5.2	38.3	2.3	36.1	2.3

SW, seawater; CT, control temperature; HST, heat stress temperature; EHST, extreme heat stress temperature; SD, Standard deviation.

in natural habitat and CT sponge specimens was comparable in *M. acerata* ($p>0.05$ for Shannon index difference) and the same was observed in *A. oroides* ($p>0.05$ for Shannon index difference).

The diversity changes observed in the different experimental conditions were also assessed taxonomically (Figure 6). Changes were observed in the most abundant phyla among the different experimental groups. The phylum Proteobacteria decreased in *M. acerata* exposed to low heat stress (HST) compared to the other experimental groups. Instead, Bacteroidota increased in *M. acerata* exposed to HST (28±9%) compared to the natural habitat group, and slightly decreased at the extreme heat stress (EHST). Additionally, an enrichment in Campylobacterales was observed (14±2%), compared to the other experimental groups, where it was practically absent. Cyanobacteria decreased in *M. acerata* exposed to experimental temperatures and was almost absent in the EHST group.

In *D. antarctica* exposed to both heat stresses the abundance of Proteobacteria increased compared to the natural habitat group. Additionally, the proportion of Gamma- *versus* Alphaproteobacteria was inversed in all experimental groups compared to the natural habitat group. Bacteroidota was less abundant in the three experimental groups than in the natural habitat group. Firmicutes represented 25±17% of the bacterial community on average in CT sponge specimens but was practically absent in the other groups' communities.

In *A. oroides*, Proteobacteria phylum was less abundant in the sponge specimens of the heat stress experiment than in the natural habitat group. Moreover, while in the natural habitat communities' class Gammaproteobacteria was more abundant than class Alphaproteobacteria, this relationship was reversed in the communities of the three experimental groups. The three experimental groups were enriched in Bacteroidota compared to the natural habitat group, but HST and EHST sponge specimens were less enriched than CT. The phylum Chloroflexi represented 37±9% of the bacterial community of the natural habitat group in *A. oroides* but was practically absent in the communities of the experimental groups. Firmicutes was more abundant in the CT group than in the natural habitat group. HST group was more enriched in bacteria from this phylum than CT, and in EHST group, the abundance of Firmicutes was even higher than in HST.

In *A. cavernosa*, the communities from the groups exposed to the three experimental temperatures were enriched in Proteobacteria compared to the natural habitat sponge specimens' communities. Also, while in the natural habitat communities, class Gammaproteobacteria was more abundant than class Alphaproteobacteria, this relationship was reversed in

10.3389/fmars.2022.1072696

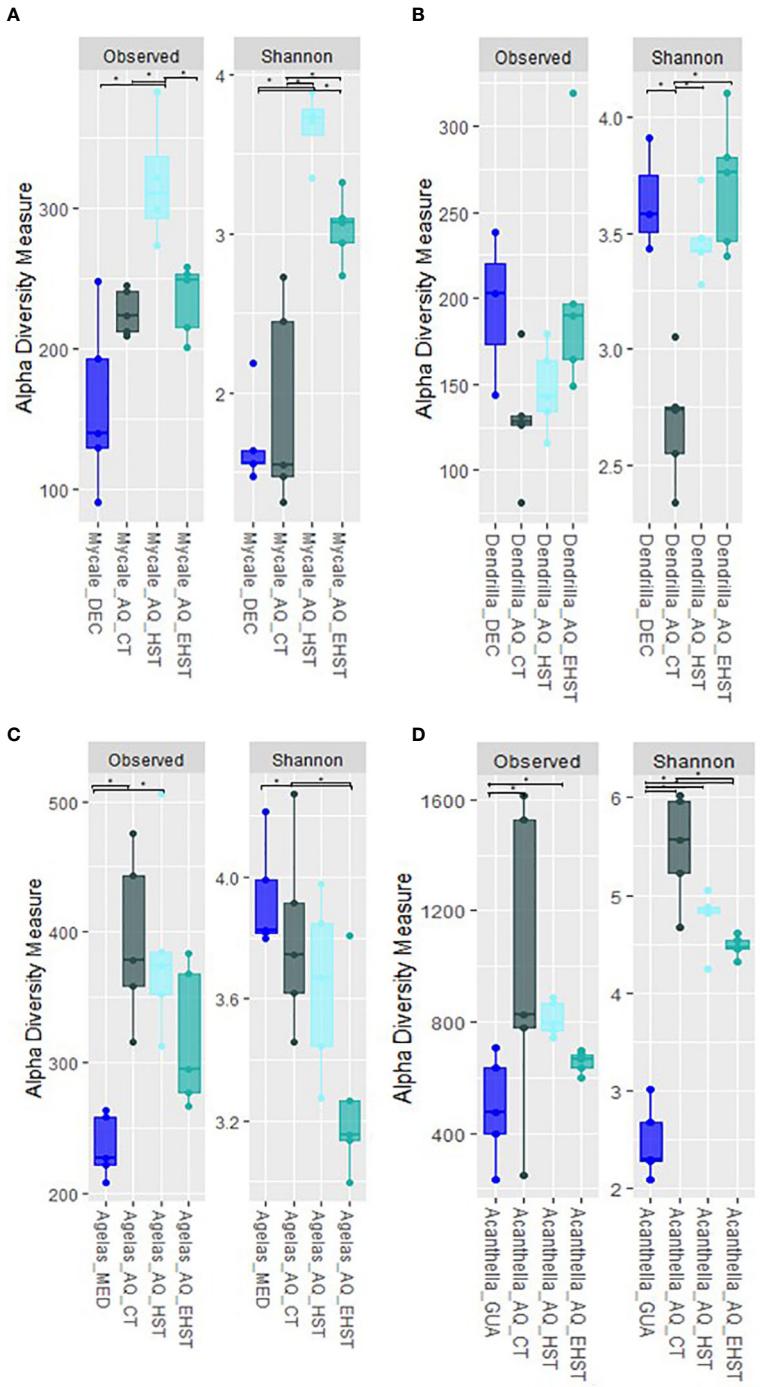


FIGURE 5
Alpha-diversity measurements of the bacterial communities of sponge specimens in aquaria experiments at the different experimental temperatures (CT, control temperature, HST, heat stress temperature, EHST, extreme heat stress temperature) compared to the natural habitat specimens: **(A)** *M. acerata* from Deception Island; **(B)** *D. antarctica* from Deception Island; **(C)** *A. oroides* from Cadaqués and Roses; **(D)** *A. cavernosa* from Guam. AQ, aquarium experiments. * p-value <0.05.

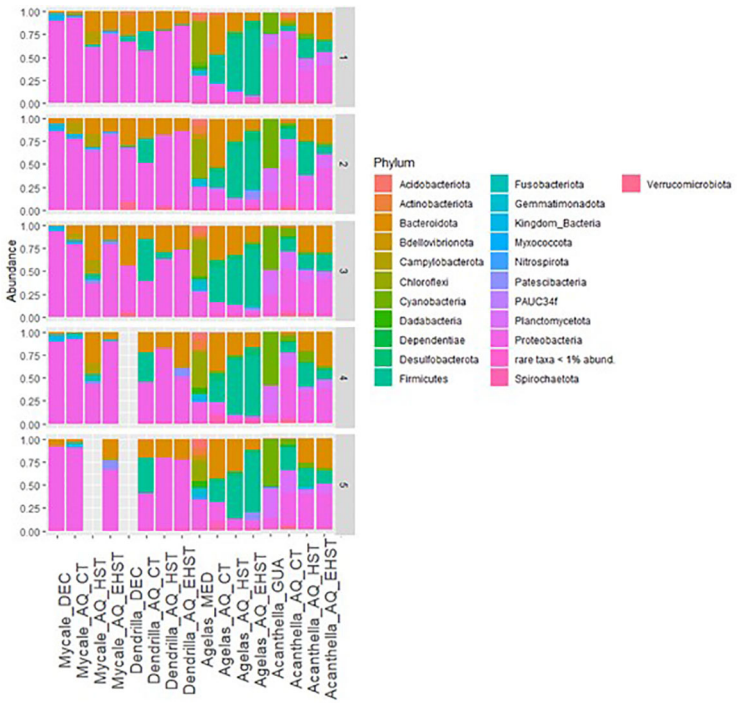


FIGURE 6
Taxonomic affiliation of ASVs at Phylum level in sponge species in their natural habitat and in the aquaria exposed at different temperatures (CT, control temperature, HST, heat stress temperature, EHST, extreme heat stress temperature). DEC, Deception Island; MED, Roses and Cadaqués origin; GUA, Guam; SW, seawater. Numbers on the right axis indicate sample replicates. AQ, aquarium experiments.

the communities of the three experimental groups in *A. cavernosa*. Bacteroidota showed and increase trend with the heat stress from lower than 1% in CT and natural habitat up to 38% in the EHST experimental group. Cyanobacteria was markedly less abundant in *A. cavernosa* in aquaria experiments compared to the natural habitat, being almost absent at *A. cavernosa* EHST group. The bacteria belonging to Planctomycetota were less abundant in *A. cavernosa* HST experimental group compared to the other groups. Firmicutes was more abundant in the CT group than in the natural habitat group and even more in the HST group. Nevertheless, in EHST group, the presence of Firmicutes was similar to CT.

Other phyla that were present in the natural habitat group but were not in the aquaria experimental groups were Acidobacteriota, Gemmatimonadota, and Actinobacteriota. Conversely, Desulfobacterota was more abundant in the communities of CT than natural habitat group, and its presence was higher in HST and even higher in EHST experimental groups. At genus level changes were also observed between the different experimental groups. If we focus on the core communities of the sponge species, the most abundant core taxa were maintained in the different experimental groups in the Antarctic sponges although with changing relative proportions (Figure 7). Nevertheless, some genera were practically lost from the core communities in all the experimental conditions such as *Oceanicoccus*, SAR92 clade, *Sulfitobacter*, *Rubritalea*, *in D. antarctica*, or *Arenicella*, *Rubidimonas*, *Ralstonia*, *Lutibacter* and *Winogradskyella* in *M. acerata*, among others.

The dominant taxa in the microbial communities of *M. acerata* natural habitat group were also hosted by the sponge specimens exposed to CT, HST and EHST (Figure 7A). The mean relative abundances in the 5 CT replicates were as follows: unidentified genus of family EC94 (67±22%), genus *Endozoicomonas* (2±5%). The unidentified genus of Family EC94 and genus *Endozoicomonas* were less abundant in CT sponge specimens with respect to natural habitat sponge specimens. In HST experimental group, family EC94 represented 12±9% of the bacterial community, while genus *Endozoicomonas* was 2±1%. Regarding EHST experimental group, unidentified genus of family EC94 represented 16±7% of the bacterial community, and *Endozoicomonas*, 0.1±0.1%.

Concerning the dominant taxa in *M. acerata* exposed to HST the dominant taxa were, after family EC94, genus *Pseudarcobacter* (8±4%), genus *Colwellia* (8±3%), genus *Crocinitomix* (7±4%), family Marinifilaceae (6±6%), and genus

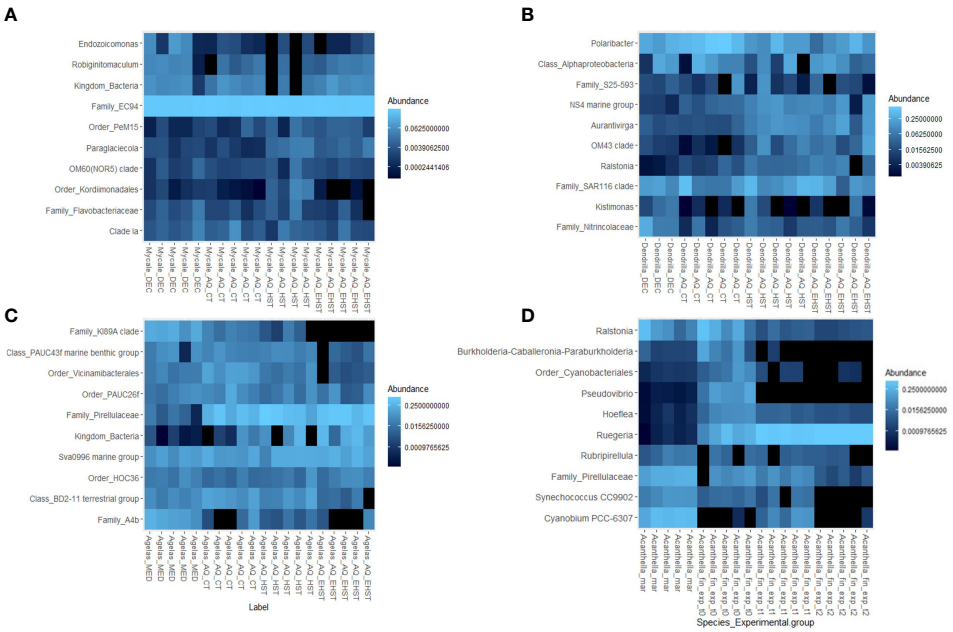


FIGURE 7
Heatmap representation of the abundance of the 10 most abundant genera from the core sponge communities of each sponge species in the different aquaria experimental groups exposed to different temperatures (CT, control temperature, HST, heat stress temperature, EHST, extreme heat stress temperature) and natural habitat: **(A)** *M. acerata* from Deception Island; **(B)** *D. antarctica* from Deception Island; **(C)** *A. oroides* from Cadaqués and Roses; **(D)** *A. cavernosa* from Guam. AQ, aquarium experiments.

Psychrobium (5±5%). All these taxa were present in the aquarium water, being 2-3 times less abundant in the water, except for genus *Crocinitomix*, which was almost equally abundant. In the EHST group bacterial communities, the most abundant taxa were genus *Colwellia* (27±3%), which was also the dominant taxa in the EHST aquarium water, family Rhodobacteraceae (15±7%), genus *Pseudoalteromonas* (5±2%), and genus *Polaribacter* (5±2%). All these taxa were practically absent in the natural habitat group sponges.

Concerning the most abundant taxa, the range of abundances in *D. antarctica* in the 5 CT replicates were as follows: genus *Polaribacter* (14±2%), unidentified genus of class Alphaproteobacteria (7±7%), clade SAR116 (6±8%), and unidentified genus of family Nitrincolaceae (1±1%) (Figure 7B). Genus *Polaribacter* was less abundant in CT sponge specimens with respect to natural habitat sponge specimens. Class Alphaproteobacteria and SAR116 clade had half relative abundance in CT sponge specimens than in the natural habitat sponge specimens. Family Nitrincolaceae was more than 10 times less abundant in CT sponge specimens than in natural habitat sponge specimens. Similarly, these taxa were present in the HST sponge specimens' communities, with similar abundances to CT sponge specimens, with the exception of *Polaribacter* (6±2%). The abundances of these taxa in the microbial communities of the sponge specimens exposed to EHST were lower than in HST sponge specimens, except for family Nitrincolaceae, which was more abundant in EHST sponge specimens.

The dominant taxon in *D. antarctica* HST group communities was group *Burkholderia-Caballeronia-Paraburkholderia* (34±5%), almost absent in the aquarium water. In the EHST group bacterial communities, the most abundant taxon was also group *Burkholderia-Caballeronia-Paraburkholderia* (29±17%). The relative abundance of *Burkholderia-Caballeronia-Paraburkholderia* was similar in the aquarium water communities and in EHST sponges. Genus *Burkholderia-Caballeronia-Paraburkholderia* were practically absent in the natural habitat group sponges.

In *A. oroides*, some genera were found in all experimental groups such as the class TK17 and Sva0996 marine group (Figure 7D). HST sponge specimens kept core taxa such as family A4b, class TK30, or clades KI89A and SAR202 in most of the specimens, which were lost in EHST sponge specimens. The mean abundances in the 5 CT replicates were as follows: family A4b (0.2±0.2%), KI89A clade (0.2-0.1%), class TK17 (0.04 ±0.03%), and class TK30 (0.1±0.041%). These had lower abundances in the microbial communities of the sponge specimens exposed to HST, and even lower in EHST.

The dominant taxa in *A. oroides* HST group communities were genus *Fusibacter* (23±6%), family Clostridiaceae (15±5%),

family Marinifilaceae (13±7%), order Peptostreptococcales-Tissierellales (5±2%), and genus *Halodesulfovibrio* (4±1%). In the EHST group bacterial communities, the most abundant taxa were family Clostridiaceae (40±7%), genus *Halodesulfovibrio* (7 ±3%), order Peptostreptococcales-Tissierellales (6±2%), and group Clostridia vadinBB60 (5±0.6%). Only two of the dominant taxa of the bacterial communities in HST and EHST experimental groups were present in the natural habitat group: family Clostridiaceae and genus *Halodesulfovibrio*, with abundances <0.01% on average.

In the tropical sponge *A. cavernosa*, most taxa were still present in CT sponge specimens although at lower abundance, but lost in HST and EHST groups such as the unidentified genus of Family EC94, *Filomicrobium* or *Blastopirellula*, or lost mostly at the highest temperatures (in EHST group), such as *Synecococcus* and *Cyanobium*. Still, the dominant taxa of the sponge species' core microbiota were present in HST sponge specimens (Figure 7C). The range of abundances in the 5 CT replicates were as follows: genus *Cyanobium*, 1±1%, family Pirellulaceae, 2±1%, genus *Ralstonia*, 5±5%, and genus *Synechococcus*, 0.3±0.1%. These had lower abundances in the microbial communities of the sponge specimens exposed to HST, and even lower in EHST.

The dominant taxa in *A. cavernosa* HST group communities were family Clostridiaceae (14±6%), family Flavobacteriaceae (7 ±2%), genus *Ruegeria* (6±2%), genus *Winogradskyella* (6±1%) and family Rhodobacteraceae (5±0.6%). In the EHST group bacterial communities, the most abundant taxa were family Flavobacteriaceae (17±3%), genus *Ruegeria* (15±2%), family Clostridiaceae (8±3%) and genus *Muricauda* (3±2%). All the dominant taxa of the bacterial communities in HST and EHST experimental groups were present in the natural habitat group, except for genera *Winogradskyella* and *Muricauda*. The abundances in the natural habitat group of the experimental groups dominant taxa were <0.7%.

The number of ASVs shared between the sponge specimens from natural habitat and those exposed to CT, HST and EHST for each sponge species are shown in Table 4. As shown, the number of ASVs shared between natural habitat specimens and those exposed to HST and EHST decreased notably with the increasing temperature specially for the temperate *A. oroides* and the tropical *A. cavernosa* sponge species.

4 Discussion

Sponges, which are important components of benthic environments both in biomass and in abundance, host species-specific microbial communities that can also be very abundant and diverse. In this study, we have described the microbiome of four sponge species from three different environments (polar, tropical and temperate) and examined the heat stress effect on the abundances and diversities of their bacterial communities. The effects of warming on the microbiome of sponges have been previously reported to be different for different sponge species (*e.g.* Fan et al., 2013; Lesser et al., 2016; Ramsby et al., 2018).

The microbiome of the four sponge species studied here has been analyzed also in previous works, although using different methodologies. The diversity and composition of the microbiota of the four studied species are comparable to those previously reported in the literature (Webster et al., 2004; Gerçe et al., 2011; Ribes et al., 2012; Björk et al., 2013; Blanquer et al., 2013; Ribes et al., 2015; Erwin et al., 2015; Ribes et al., 2016; Steinert et al., 2016; Cárdenas et al., 2018; Coelho et al., 2018; Cárdenas et al., 2019; Cleary et al., 2019; Díez-Vives et al., 2020; Papale et al., 2020; Sacristán-Soriano et al., 2020; Ruocco et al., 2021; Happel et al., 2022). Nevertheless, there are some remarkable differences that must be noted. Similar Shannon and Inverse of Simpson indices have been reported for *M. acerata* from other Antarctic Peninsula spots (Cárdenas et al., 2019; Sacristán-Soriano et al., 2020), while a higher Shannon index was reported for the microbiome of *M. acerata* from the distant Terra Nova Bay (Ross Sea, Antarctica) (Papale et al., 2020). However, Papale and colleagues amplified V1-V2 hypervariable regions from 16S rRNA gene, while our study and the other works cited amplified V4 or V4-V5 regions. Recent work showed high variability in the Shannon diversity index of *M. acerata*'s microbiome along the Western Antarctic Peninsula (0.65-3.79; Happel et al., 2022). The diversity found here in *D. antarctica* is comparable to that found previously (Díez-Vives et al., 2020), both regarding observed ASVs and Shannon index in Deception Island, but they found variability in diversity, with higher values of Shannon index (up to 5) in specimens from the neighbor King George Island (South Shetland Islands, Antarctica). In our study we have not detected significant changes in *M. acerata* or *D. antarctica* collected from either Livingston Island or Deception

TABLE 4 Number of ASVs shared between natural habitat sponge specimens and those from aquaria exposed to control temperature (CT), heat stress temperature (HST) and extreme heat stress temperature (EHST) in the different sponge species.

	Natural Habitat-AQ_CT	Natural Habitat-AQ_HST	Natural_AQ_EHST
M. acerata	212	178	184
D. antarctica	126	147	171
A. oroides	183	117	107
A. cavernosa	456	215	182

Island. The microbiome of *A. oroides* from our study harbored less bacterial richness (ASVs) than reported before from a nearby location (243±28 vs. 521±275), but similar Shannon indices (3.9±0.2 vs. 4.3±0.4) (Ribes et al., 2015). Concerning *A. cavernosa*, the bacterial community in our specimens was richer compared to previously reported results of the same species from Taiwan (Cleary et al., 2019), although our specimens had a similar Shannon index (2.2-3.2 vs. 2.9-4.3). Slight variations may be caused by the amplification of different 16S hypervariable regions. While we amplified the V4 hypervariable region, they amplified V3-V4 region.

The composition of the microbial communities observed in our specimens agrees with their previous HMA-LMA classification. The notably higher diversity indices (particularly the Inverse Simpson index) in *A. oroides* with respect to the other sponge species, agrees with the classification of this species as HMA. Moreover, according to (Giles et al., 2013; Simister et al., 2013), LMA sponges host microbiomes dominated by Proteobacteria or Cyanobacteria, while HMA sponges host Chloroflexi or other phyla as dominant (Moitinho-Silva et al., 2017b). In our work, among the LMA sponges, *M. acerata*, and *D. antarctica* were dominated by Proteobacteria, *A. cavernosa* by Cyanobacteria, while the HMA *A. oroides* was dominated by Chloroflexi. The presence of bacteria affiliated to phylum Poribacteria only in *A. oroides* microbiome and its absence in the other sponge species or the seawater samples adds evidence to the assignment of HMA status to this sponge species (Moitinho-Silva et al., 2017b). An unidentified genus affiliated to the gammaproteobacterial family EC94 was the dominant taxon of *M. acerata*'s bacterial microbiome, confirming what was observed in previous studies (Cárdenas et al., 2019; Sacristán-Soriano et al., 2020; Ruocco et al., 2021; Happel et al., 2022). This bacterial group has been reported to be dominant in the microbiome of other LMA sponges (Jackson et al., 2013; Jeong et al., 2015; Cárdenas et al., 2019; Happel et al., 2022) and of some corals (Yang et al., 2013; Gonzalez-Zapata et al., 2018). Recent research of the EC94 group suggested that these bacteria became sponge symbionts quite early in the evolutionary history and re-classified them as a new order, "*Candidatus* Tethybacterales" (Taylor et al., 2020). These EC94 bacteria are heterotrophs and can use various carbon, nitrogen, and sulfur sources (Taylor et al., 2020).

In a previous study in which the composition of the microbiota of four Antarctic sponges (including *D. antarctica*) was analyzed, a larger species intra-variability was found in the microbiome composition compared to the other sponge species studied (Sacristán-Soriano et al., 2020). Our results agree with this large inter-individual variability. The presence of the Phyla Proteobacteria and Bacteroidetes, accounting for most of the bacterial community in this species, has been reported before (Díez-Vives et al., 2020; Sacristán-Soriano et al., 2020). The most abundant ASV on average were an unidentified genus and one affiliated to family SAR116 clade, both affiliated to class Alphaproteobacteria. The role of the SAR116 clade in sponges is still unknown with the small number of cultured representatives but a significant role in the sulfur cycle has been reported (Roda-Garcia et al., 2021).

The microbiome of the temperate sponge *A. oroides* has been described as dominated by Chloroflexi phylum (Ribes et al., 2012; Björk et al., 2013; Blanquer et al., 2013; Ribes et al., 2016), except for one study that found Proteobacteria as the main phylum (Ribes et al., 2015). Bacteria belonging to the dominant Chloroflexi clades in *A. oroides* microbiome (Anaerolineae, Caldilineales, and SAR202) are involved in dissolved organic matter degradation. Specifically, clades Anaerolineae and Caldilineales have a wide gene repertoire in carbohydrates degradation, and SAR202 in amino acid degradation. Bacteria from SAR202 are also capable of cofactor production and therefore this HMA sponges characteristic phylum is thought to provide nutrients to the host, and also participate in nutrient cycling in the benthic ecosystem (Bayer et al., 2018; Campana et al., 2021). It is worth noting that while *A. oroides* studied in "The sponge microbiome project" had SAR202 as the most abundant Chloroflexi clade (Moitinho-Silva et al., 2017a), bacteria from class Anaerolineae were the dominant Chloroflexi in our *A. oroides* specimens.

The microbiome of *A. cavernosa* was dominated by one Cyanobacteria genus (*Cyanobium*), except for one specimen, enriched in a Gammaproteobacteria genus (*Ralstonia*). The microbiome of this tropical sponge has been reported before to be dominated by Proteobacteria or Acidobacteriota (Steinert et al., 2016; Coelho et al., 2018; Cleary et al., 2019). Differences in irradiance due to seasonal change or depth have been suggested to influence sponge-associated microbial communities, with some phototrophic symbionts being less abundant with depth (Morrow et al., 2016) or being absent in winter (Alex et al., 2012). Cyanobacteria from the *Cyanobium* genus have been reported from other marine sponges (Regueiras et al., 2017; Pagliara et al., 2021), but not from previously studied *A. cavernosa* specimens. However, bacteria from the same order, *Synechococcales*, were reported (Steinert et al., 2016; Coelho et al., 2018). Chemical extracts from *Cyanobium* strains have been reported to display cytotoxicity (Costa et al., 2015; Pagliara et al., 2021). Bacteria from genus *Ralstonia* have been found to be autotrophic ammonia oxidizers (Calvó et al., 2004). This genus has been previously reported in other sponges and macroalgae too (Florez et al., 2019).

Gradual ocean warming and more frequent heat wave episodes, along with other phenomena associated to anthropogenic climate change, have detrimental effects on benthic communities (Smale et al., 2019; Cooley et al., 2022). Environmental stress may cause imbalance of the microbial communities of sponges and other benthic components, disrupting or changing the holobiont's functions, and ultimately affecting the whole ecosystem (Pita et al., 2018; Ramsby et al., 2018; Rondon et al., 2020; Rubio-Portillo et al.,

2021; Vargas et al., 2021). Since responses to heat stress seem to be related to the sponge host species, we assessed the effect of heat stress and extreme heat stress in aquarium experiments on the four sponge species analyzed previously, which are important components of the benthos at different latitudes, and therefore, adapted to different climate conditions. According to our results, the microbiome of the sponges was quite stable despite the manipulation of the sponge specimens and after 24 h in the aquaria. The exception was *D. antarctica*, with its microbiome being the most altered at control adaptation aquarium group specimens. An "aquarium effect", described as the changes in the microbiome of the Control Temperature (CT) experimental group compared to the natural habitat sponge specimens, was observed for all sponge species with varying degrees, being the microbiome of *M. acerata* the most conserved, with more than 70% of the sequences of the natural habitat control group, followed by *D. antarctica* (25%).

Differences were also found in the response of each sponge species' microbiome to the heat stress experiments, being again the microbiomes of the Antarctic sponges, *M. acerata* and *D. antarctica*, those maintaining a higher percentage of reads when exposed to increasing temperatures (up to 20% for *D. antarctica*) compared to the tropical species. The microbiome of *D. antarctica* was highly altered at control specimens, but a fifth of the microbiome exposed to heat stress seemed to be maintained through the experiment, including the most abundant core taxa from the natural habitat sponge specimens. Both for the tropical *A. cavernosa* and the Mediterranean, *A. oroides* (up to 2% for *A. oroides*), the SourceTracker results suggest there was a shift in the microbiomes by the end of control temperature exposure in the aquarium experiment, with most sequences coming from unknown sources. The microbiomes exposed to heat stress also experienced a shift, modifying the proportions of rare or less abundant bacteria, but mostly becoming more similar to the microbiome found in the water in *A. oroides*. Concerning bacterial diversity, the microbiomes of the tropical and temperate sponge species lost diversity when exposed to a heat stress. However, while in *A. oroides* the highest diversity is found in the natural habitat specimens, in *A. cavernosa*, the diversity in the natural habitat group is lower than in the experimental groups (Figure 5). Contrastingly, the bacterial community associated with *M. acerata* became more diverse after heat stress exposure. We suggest warming boosted the activity of previously scarce or inactive bacteria that could not be detected previously due to the detection limit of the technique. Extreme heat stress seemed to dysregulate the microbiome leading to dysbiosis, and diversity was lost. The diversity in *D. antarctica* microbiome was similar in the sponges from natural habitat, HST and EHST, but was significantly lower in CT sponge specimens ($p<0.05$).

The changes in diversity were also reflected in changes in taxonomy. A shift in *A. oroides* microbiome occurred at sponge specimens exposed to CT even at phylum level (Figure 6). Changes at phylum level could also be observed in *D. antarctica* and *A. cavernosa* exposed to CT ("aquarium effect"), but not in *M. acerata* (Figure 6). Phyla Bacteroidota and Firmicutes were more abundant in the heat stress specimens in all sponge species, except for *D. antarctica*. Firmicutes was reported to also increased in diseased deep water *Geodia barretti* (Luter et al., 2017) and Bacteroidota abundance increased in heat stressed Antarctic sponge *Isodictya kerguelenensis* (Rondon et al., 2020) and diseased *Lubomirskia baicalensis* (Kulakova et al., 2018). Proteobacteria was more abundant after heat stress in *D. antarctica* and *A. cavernosa*, and less abundant after heat stress in *M. acerata* and *A. oroides*. Campylobacterota were enriched in HST *M. acerata*. Cyanobacteria was less abundant in *A. cavernosa*. Planctomycetota was less abundant after HST in *A. cavernosa*. Chloroflexi was practically absent in *A. oroides* exposed to HST.

The dominant taxa of the core microbiota were still present in the sponge specimens exposed to heat stress in all the four studied species. Nevertheless, different responses were observed among them. Both Antarctic sponges (*M. acerata* and *D. antarctica*) seem to host a microbiome that is more resistant to heat stress. After exposure to heat and extreme heat stress, not only the dominant core taxa were still present, but their relative abundance was notable. In *A. cavernosa* and *A.* oroides, the dominant taxa were present at very low abundances in heat stress exposed sponge specimens and were virtually absent in extreme heat stress sponge specimens. The exposure to heat stress caused important changes in the microbiomes at phylum level in many studies although not in all of them. Examples of sponge holobionts that have reported to be resistant to heat stress are found among tropical sponges (e.g. Simister et al., 2012a; Lesser et al., 2016) but there are also evidences of resistance in a polar deep sea sponge (Strand et al., 2017).

The most abundant taxon in the microbiome of the Antarctic sponge *M. acerata*, the EC94 group, was still present at the specimens at the end of both the heat stress and extreme heat stress experiments. We can presumably argue that the functions that this group develops within the holobiont may also be maintained even when exposed to stressful conditions. The microbiome of sponge specimens exposed to heat or extreme heat stress experienced a shift in composition, that was more or less prominent depending on the sponge species. There were some taxa, presumably opportunistic, that seemed to benefit from the heat stress-associated dysregulation in the sponge by occupying new niches in the holobiont, and other taxa already present at natural habitat sponge specimens that increased in abundance with temperature. In general, the most abundant taxa in the microbiome of heat stress sponge specimens were different for each sponge species. However, some taxa were shared among stressed species, such as bacteria from the family Marinifilaceae, more abundant in heat stressed *M. acerata* and *A. oroides*, or the family

Clostridiaceae, more abundant in the microbiome of *A. oroides* and *A. cavernosa*. Genus *Marinifilum* (Bacteroidota, family Marinifilaceae), and also genus *Fusibacter* (Firmicutes), which was also more abundant in heat stressed *A. oroides* specimens, were among the dominant taxa in the microbiome of necrotic *Rhopaloeides odorabile* (Fan et al., 2013). An ASV affiliated to family Marinifilaceae increased in abundance in the sponge *Leucetta chagosensis* exposed to pH 7.8, 30°C in comparison to those specimens exposed to present day conditions (Posadas et al., 2021). In our experiment with *M. acerata*, the specimens exposed to heat stress had higher abundances of genera *Colwellia*, *Crocinitomix*, *Pseudarcobacter*, and *Psychrobium*. Genus *Colwellia* (Gammaproteobacteria) was found to be much more abundant in injured specimens than it was in healthy specimens (Rondon et al., 2020). This genus was present in healthy *M. acerata* in previous studies (Ruocco et al., 2021). It is a denitrifying bacterium and produces natural compounds (Ruocco et al., 2021). Members from families Colwelliaceae and Flavobacteriaceae (Bacteroidota) were more abundant in injured specimens (Rondon et al., 2020), and were reported to be disease-related in necrotic and stressed sponge specimens (Simister et al., 2012a; Fan et al., 2013). They were associated with disease and stress in corals (Gajigan et al., 2017). Genera *Crocinitomix* (Flavobacteriales, Bacteroidota) and *Colwellia* were reported from other Antarctic benthic invertebrates such as sea anemones or soft corals (Webster and Bourne, 2007; Murray et al., 2016). Genus *Psychrobium* increased in abundance in the microbiota of marine bivalves after a viral infection (de Lorgeril et al., 2018) and after exposure to nanoplastics (Auguste et al., 2020). One bacterial genus that was more abundant in the *M. acerata* exposed to extreme heat stress than in the other groups in our study was genus *Pseudoalteromonas*. Strains from this genus have been found in other species of *Mycale* (Lau et al., 2005), and have been reported to have a great biotechnological potential, specially regarding cold-active enzymes (Borchert et al., 2017). On the other hand, a strain from *Pseudoalteromonas agarivorans* has been reported to be a pathogen of the tropical sponge *R. odorabile* (Choudhury et al., 2015).

In heat stress exposed *D. antarctica*, we observed an increase in the group *Burkholderia-Caballeronia-Paraburkholderia* (Gammaproteobacteria). ASVs affiliated to this group were among the most abundant in the microbiome of the coral *Acropora hyacinthus* (Kriefall et al., 2022). Contrastingly, in heat stressed *A. cavernosa*, we observed an increase in members of several bacterial families (Clostridiaceae, Flavobacteriaceae, Rhodobacteraceae) and two genera (*Ruegeria* and *Winogradskyella*). Genus *Clostridium* (family Clostridiaceae, Firmicutes) can be found in healthy *Xestospongia muta* specimens (Leal et al., 2022), and order Clostridiales in *Leucetta chagosensis* after experimental exposition to "present day" seawater temperature and pH conditions (Posadas et al., 2021). Moreover, class Clostridia was more abundant in diseased than in healthy corals (reviewed in Mouchka et al., 2010). Phyla Firmicutes and Bacteroidota were reported to be more abundant in diseased sponge specimens (Luter et al., 2017). Orders Flavobacteriales (Bacteroidota) and Rhodobacterales (Proteobacteria) were reported to be more abundant in various coral species exposed to stressors (reviewed in McDevitt-Irwin et al., 2017), as well as in sponge species such as *Isodictya kerguelenensis* (Rondon et al., 2020) and *Lubomirskia baicalensis* (Kulakova et al., 2018). Previous literature has found an important contribution of Operational Taxonomic Units (OTUs) from order Flavobacteriales to the pool of OTUs associated to coral diseases and stress (Gignoux-Wolfsohn and Vollmer, 2015). Bacteria from order Rhodobacterales (Alphaproteobacteria) have been reported to be fast growing and opportunistic (Teeling et al., 2012), have been found in both healthy and stressed corals (Meron et al., 2010; Sharp and Ritchie, 2012) and have the potential to bloom when new niches are available under stress periods (Welsh et al., 2017). Order Rhodobacterales was more abundant in the microbiome of the sponge *Leucetta chagosensis* experimentally exposed to warming and acidification scenarios (Posadas et al., 2021). Photoheterotrophic members of the Rhodobacteraceae family were more abundant in *Neopetrosia compacta* exposed to acidification and warming scenario. Those were suggested to provide heat tolerance to the sponge (Posadas et al., 2021). It also was more abundant in *Isodictya kerguelenensis* exposed to heat stress (Rondon et al., 2020). Genus *Rhodobacter* (family Rhodobacteraceae) was more abundant in diseased than in healthy corals (reviewed in (Mouchka et al., 2010)). Similarly, the Rhodobacteraceae family increased fourfold in coral white syndrome lesions compared to healthy tissues (Pollock et al., 2017; Rosales et al., 2020) and are implicated in Stony Coral Tissue Loss Disease that is currently affecting Caribbean reefs. Bacteria affiliated to genus *Winogradskyella* has been described from various sponge species. Species of this genus have been reported to produce antibiofilm compounds (Pinnaka et al., 2013; Schellenberg et al., 2017; Rizzo et al., 2021). *Ruegeria* (family Rhodobacteraceae) species were found to increase in abundance in diseased tissues of several Mediterranean corals collected during a marine heat wave (Rubio-Portillo et al., 2021). *Ruegeria* sp. was found to increase in corals exposed to stress conditions and was proposed to be a microbial bio-indicator of marine invertebrate diseases. This bacterium may be able to protect corals from pathogenic *Vibrio* spp. by growth inhibition (Miura et al., 2019; Rosado et al., 2019). Apart from genus *Fusibacter* and family Marinifilaceae, the microbiome of heat stressed *A. oroides* was enriched in family Clostridiaceae, order Peptostreptococcales-Tissierellales, and genera *Halodesulfovibio*. Genus *Halodesulfovibrio* has been found in corals and species from this genus seem to have antimicrobial properties (Shivani et al., 2017; Chen et al., 2021).

Despite all the differences observed between studies it must be noted that observed differences may be influenced by the use

of different methodologies, *e.g.* DNA extraction method, amplification primers targeting different 16S rRNA regions, sequencing platform, or bioinformatic pipeline. Sampling and experimental design should also be considered at comparisons.

Overall, we can say that the core microbiome is maintained in most sponge species after a heat stress, although whether they would recover to the normal conditions previous to the stress remains yet to be further investigated. Although the core bacteria may or may not change, it is clear that there is a change in transient bacteria appearing new species from unknown origin, some of which may be opportunistic pathogens finding this opportunity to grow after a dysbiosis occurs. According to our results, sponge species from Antarctic waters could be more resilient than tropical and temperate sponge species, and perhaps their recovery after the heat stress would be possible. Changes produced by an extreme heat stress however do not seem to be reversible for the sponge microbiomes in any of the sponge species tested. Remarkably, both the microbiome composition and the changes produced by the heat stress seem to be quite host species-specific, and thus, depend on the sponge species, being always different from the seawater. Therefore, we may conclude that although there are some general trends, heat stress will affect differently the different sponge species, as well as the different bacterium species, and thus we may see winners and losers when temperature increases high enough in the near future. Global change is impacting significantly in marine environments and the effects are particularly evident for sessile invertebrate species, which often exhibit narrow ranges of thermal tolerance. For all these reasons, tropical and temperate sponge species will probably be those suffering the most the heat stress. Consequently, the effects of global change may be dramatic for benthic ecosystems since sponges are a fundamental part of them.

Data availability statement

The data presented in the study are deposited in the Mendely Data public repository (DOI: 10.17632/9c6y62nv9z.1, DOI: 10.17632/d67pjpc47g.1, DOI: 10.17632/fnjhzsybxj.1). Further queries should be directed to the corresponding author.

Author contributions

Idea and conceptualization, PC-F, CA, EB, CG-A. Methodology, PC-F, CA, EB, CG-A. Investigation, PC-F, CA, EB, CG-A, JB, CA-P. Resources, CA, CG-A, EB. Sample collection: PC-F, CA, CA-P, JB. Data curation, PC-F, CA, EB, CG-A. Writing—original draft preparation, PC-F, CG-A. Writing—review and editing, PC-F, CA, EB, CG-A, CA-P. Supervision and project administration, CA, CG-A, EB. Funding acquisition, CA, CG-A, EB. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the BLUEBIO (CTM2016-78901/ANT) and CHALLENGE (PID2019-107979RB-I00/ANT) research grants to C.A. by the Spanish Government and the SGR (Research Quality Group) funding of the Generalitat de Catalunya (2014SGR336; 2017SGR1120). P.C.F. acknowledges financial support from an FPU Grant (2016-02877) of the MEFP (Spain) and the Water Research Institute (IdRA).

Acknowledgments

Thanks are due to all the researchers of the BLUEBIO and CHALLENGE projects for their support during sample collection and aquaria experiments in the Antarctic and Mediterranean sites, and A. Townsend (University of Guam Marine Laboratory) in Guam. Thanks are also due to the technicians from the Unitat de Tecnologia Marina (UTM-CSIC) for their support in the Antarctic sampling seasons. This work is a contribution to the Integrated Science to Inform Antarctic and Southern Ocean Conservation (Ant-ICON) Scientific Research Programme of the Scientific Committee on Antarctic Research (SCAR). We acknowledge the QGIS Development Team and the Norwegian Polar Institute's Quantarctica package.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1072696/full#supplementary-material

References

Alex, A., Vasconcelos, V., Tamagnini, P., Santos, A., and Antunes, A. (2012). Unusual symbiotic cyanobacteria association in the genetically diverse intertidal marine sponge hymeniacidon perlevis (Demospongiae, halichondrida). *PloS One* 7 (12), e51834. doi: 10.1371/journal.pone.0051834

Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75 (2), 129–137. doi: 10.3354/ame01753

Astudillo-García, C., Bell, J. J., Webster, N. S., Glasl, B., Jompa, J., Montoya, J. M., et al. (2017). Evaluating the core microbiota in complex communities: a systematic investigation. *Environ. Microbiol.* 19 (4), 1450–1462. doi: 10.1111/1462-2920.13647

Auguste, M., Lasa, A., Balbi, T., Pallavicini, A., Vezzulli, L., and Canesi, L. (2020). Impact of nanoplastics on hemolymph immune parameters and microbiota composition in mytilus galloprovincialis. *Mar. Environ. Res.* 159, 105017. doi: 10.1016/J.MARENVRES.2020.105017

Bayer, K., Jahn, M. T., Slaby, B. M., Moitinho-Silva, L., and Hentschel, U. (2018). Marine sponges as chloroflexi hot spots: Genomic insights and high-resolution visualization of an abundant and diverse symbiotic clade. *mSystems* 3 (6), e00150–e00118. doi: 10.1128/msystems.00150-18

Bell, J. J. (2008). The functional roles of marine sponges. *Estuar. Coast. Shelf Sci.* 79 (3), 341–353. doi: 10.1016/j.ecss.2008.05.002

Bennett, H. M., Altenrath, C., Woods, L., Davy, S. K., Webster, N. S., and Bell, J. J. (2017). Interactive effects of temperature and pCO2 on sponges: from the cradle to the grave. *Glob. Chang. Biol.* 23 (5), 2031–2046. doi: 10.1111/GCB.13474

Bengtson, S. (1998). Animal embryos in deep time. *Nature* 391 (6667), 529–530. doi: 10.1038/35245

Björk, J. R., Díez-Vives, C., Coma, R., Ribes, M., and Montoya, J. M. (2013). Specificity and temporal dynamics of complex bacteria-sponge symbiotic interactions. *Ecology* 94 (12), 2781–2791. doi: 10.1890/13-0557.1

Blanquer, A., Uriz, M. J., and Galand, P. E. (2013). Removing environmental sources of variation to gain insight on symbionts vs. transient microbes in high and low microbial abundance sponges. *Environ. Microbiol.* 15 (11), 3008–3019. doi: 10.1111/1462-2920.12261

Blanquer, A., Uriz, M. J., Cebrian, E., and Galand, P. E. (2016). Snapshot of a bacterial microbiome shift during the early symptoms of a massive sponge die-offin the western Mediterranean. *Front. Microbiol.* 7, 752. doi: 10.3389/FMICB.2016.00752/BIBTEX

Borchert, E., Knobloch, S., Dwyer, E., Flynn, S., Jackson, S. A., Jóhannsson, R., et al. (2017). Biotechnological potential of cold adapted pseudoalteromonas spp. isolated from 'deep sea' sponges. *Mar. Drugs* 15 (6), 184. doi: 10.3390/MD15060184

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13 (7), 518–583. doi: 10.1038/nmeth.3869

Calvó, L., Vila, X., Abella, C. A., and Garcia-Gil, L. J. (2004). Use of the ammonia-oxidizing bacterial-specific phylogenetic probe Nso1225 as a primer for fingerprint analysis of ammonia-oxidizer communities. *Appl. Microbiol. Biotechnol.* 63 (6), 715–721. doi: 10.1007/s00253-003-1433-1

Campana, S., Busch, K., Hentschel, U., Muyzer, G., and de Goeij, J. M. (2021). DNA-Stable isotope probing (DNA-SIP) identifies marine sponge-associated bacteria actively utilizing dissolved organic matter (DOM). *Environ. Microbiol.* 23 (8), 4489–4504. doi: 10.1111/1462-2920.15642

Cárdenas, C. A., Font, A., Steinert, G., Rondon, R., and González-Aravena, M. (2019). Temporal stability of bacterial communities in Antarctic sponges. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.02699

Cárdenas, C. A., González-Aravena, M., Font, A., Hestetun, J. T., Hajdu, E., Trefault, N., et al. (2018). High similarity in the microbiota of cold- water sponges of the genus mycale from two different geographical areas. *PeerJ* 6, e4935. doi: 10.7717/peerj.4935

Chen, Y. H., Yang, S. H., Tandon, K., Lu, C. Y., Chen, H. J., Shih, C. J., et al. (2021). Potential syntrophic relationship between coral-associated prosthecochloris and its companion sulfate-reducing bacterium unveiled by genomic analysis. *Microb. Genom.* 7 (5), 574. doi: 10.1099/mgen.0.000574

Choudhury, J. D., Pramanik, A., Webster, N. S., Llewellyn, L. E., Gachhui, R., and Mukherjee, J. (2015). The pathogen of the great barrier reef sponge rhopaloeides odorabile is a new strain of pseudoalteromonas agarivorans containing abundant and diverse virulence-related genes. *Mar. Biotechnol.* 17 (4), 463–478. doi: 10.1007/S10126-015-9627-Y

Clarke, A., Meredith, M. P., Wallace, M. I., Brandon, M. A., and Thomas, D. N. (2008). Seasonal and interannual variability in temperature, chlorophyll and macronutrients in northern Marguerite bay, Antarctica. *Deep-Sea Res. Part II-Top. Stud. Oceanogr.* 55 (18-19), 1988–2006. doi: 10.1016/j.dsr2.2008.04.035

Cleary, D. F. R., Becking, L. E., de Voogd, N. J., Pires, A. C. C., Polónia, A. R. M., Egas, C., et al. (2013). Habitat- and host-related variation in sponge bacterial symbiont communities in Indonesian waters. *FEMS Microbiol. Ecol.* 85 (3), 465–482. doi: 10.1111/1574-6941.12135

Cleary, D. F. R., Polónia, A. R. M., Huang, Y. M., Putchakarn, S., Gomes, N. C. M., and de Voogd, N. J. (2019). A comparison of prokaryote communities inhabiting sponges, bacterial mats, sediment and seawater in southeast Asian coral reefs. *FEMS Microbiol. Ecol.* 95 (12), fiz169. doi: 10.1093/femsec/fiz169

Coelho, F. J. R. C., Cleary, D. F. R., Gomes, N. C. M., Pólonia, A. R. M., Huang, Y. M., Liu, L. L., et al. (2018). Sponge prokaryote communities in Taiwanese coral reef and shallow hydrothermal vent ecosystems. *Microb. Ecol.* 75 (1), 239–254. doi: 10.1007/s00248-017-1023-x

Collins, M., Sutherland, M., Bouwer, L., Cheong, S.-M., Frölicher, T., Jacot Des Combes, H., et al. (2019). "Extremes, abrupt changes and managing risks," in *IPCC special report on the ocean and cryosphere in a changing climate*. Eds. H.-O. Pörtner, D. C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, et al (Cambridge, UK and New York: Cambridge University Press), 589–655. doi: 10.1017/9781009157964.008

Cooley, S., Schoeman, D., Bopp, L., Boyd, P., Donner, S., Ghebrehiwet, D. Y., et al. (2022). "Oceans and coastal ecosystems and their services," in *Climate change 2022: Impacts, adaptation and vulnerability*. Eds. H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, et al (Cambridge, UK and New York: Cambridge University Press), 379–550. doi: 10.1017/9781009325844.005

Costa, M. S., Costa, M., Ramos, V., Leão, P. N., Barreiro, A., Vasconcelos, V., et al. (2015). Picocyanobacteria from a clade of marine cyanobium revealed bioactive potential against microalgae, bacteria, and marine invertebrates. *J. Toxicol. Env. Health Part A* 78 (7), 432–442. doi: 10.1080/15287394.2014.991466

Cristi, A., Parada-Pozo, G., Morales-Vicencio, F., Cárdenas, C. A., and Trefault, N. (2022). Variability in host specificity and functional potential of Antarctic sponge-associated bacterial communities. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.771589

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., and Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 6 (1), 1–14. doi: 10.1186/s40168-018-0605-2

de Goeij, J. M., van Oevelen, D., Vermeij, M. J. A., Osinga, R., Middelburg, J. J., de Goeij, A. F. P. M., et al. (2013). Surviving in a marine desert: The sponge loop retains resources within coral reefs. *Science* 342 (6154), 108–110. doi: 10.1126/science.1241981

de Lorgeril, J., Lucasson, A., Petton, B., Toulza, E., Montagnani, C., Clerissi, C., et al. (2018). Immune-suppression by OsHV-1 viral infection causes fatal bacteraemia in pacific oysters. *Nat. Commun.* 9, 4215. doi: 10.1038/s41467-018-06659-3

Díez-Vives, C., Taboada, S., Leiva, C., Busch, K., Hentschel, U., and Riesgo, A. (2020). On the way to specificity-microbiome reflects sponge genetic cluster primarily in highly structured populations. *Mol. Ecol.* 29 (22), 4412–4427. doi: 10.1111/mec.15635

Dittami, S. M., Arboleda, E., Auguet, J. C., Bigalke, A., Briand, E., Cárdenas, P., et al. (2021). A community perspective on the concept of marine holobionts: Current status, challenges, and future directions. *PeerJ* 9, e10911. doi: 10.7717/peerj.10911

Edgar, R. C., and Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 31 (21), 3476–3482. doi: 10.1093/BIOINFORMATICS/BTV401

Ereskovsky, A., Ozerov, D. A., Pantyulin, A. N., and Tzetlin, A. B. (2019). Mass mortality event of white Sea sponges as the result of high temperature in summer 2018. *Polar Biol.* 42 (12), 2313–2318. doi: 10.1007/S00300-019-02606-0

Erwin, P. M., Coma, R., López-Sendino, P., Serrano, E., and Ribes, M. (2015). Stable symbionts across the HMA-LMA dichotomy: Low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts. *FEMS Microbiol. Ecol.* 91 (10), fiv115. doi: 10.1093/femsec/fiv115

Erwin, P. M., Pita, L., López-Legentil, S., and Turon, X. (2012). Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Appl. Environ. Microbiol.* 78 (20), 7358–7368. doi: 10.1128/aem.02035-12

Fan, L., Liu, M., Simister, R., Webster, N. S., and Thomas, T. (2013). Marine microbial symbiosis heats up: The phylogenetic and functional response of a sponge holobiont to thermal stress. *ISME J.* 7 (5), 991–1002. doi: 10.1038/ismej.2012.165

Fan, L., Reynolds, D., Liu, M., Stark, M., Kjelleberg, S., Webster, N. S., et al. (2012). Functional equivalence and evolutionary convergence in complex

communities of microbial sponge symbionts. *Proc. Natl. Acad. Sci. U.S.A.* 109 (27), E1878–E1887. doi: 10.1073/pnas.1203287109

Feuda, R., Dohrmann, M., Pett, W., Philippe, H., Rota-Stabelli, O., Lartillot, N., et al. (2017). Improved modeling of compositional heterogeneity supports sponges as sister to all other animals. *Curr. Biol.* 27 (24), 3864–3870.e4. doi: 10.1016/j.cub.2017.11.008

Flores, J. Z., Camus, C., Hengst, M. B., Marchant, F., and Bushmann, A. H. (2019). Structure of the epiphytic bacterial communities of Macrocystis pyrifera in localities with contrasting nitrogen concentrations and temperature. *Algal Res.* 44, 101706. doi: 10.1016/J.ALGAL.2019.101706

Freeman, C. J., Easson, C. G., Matterson, K. O., Thacker, R. W., Baker, D. M., and Paul, V. J. (2020). Microbial symbionts and ecological divergence of Caribbean sponges: A new perspective on an ancient association. *ISME J.* 14 (6), 1571–1583. doi: 10.1038/s41396-020-0625-3

Gajigan, A. P., Diaz, L. A., and Conaco, C. (2017). Resilience of the prokaryotic microbial community of acropora digitifera to elevated temperature. *MicrobiologyOpen* 6 (4), e00478. doi: 10.1002/mbo3.478

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., et al. (2009). Mass mortality in northwestern Mediterranean rocky benthic communities: Effects of the 2003 heat wave. *Glob. Change Biol.* 15 (5), 1090–1103. doi: 10.1111/j.1365-2486.2008.01823.x

Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., et al. (2022). Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Glob. Change Biol.* 28 (19), 5708–5725. doi: 10.1111/gcb.16301

Gerçe, B., Schwartz, T., Syldatk, C., and Hausmann, R. (2011). Differences between bacterial communities associated with the surface or tissue of Mediterranean sponge species. *Microb. Ecol.* 61 (4), 769–782. doi: 10.1007/s00248-011-9802-2

Gignoux-Wolfsohn, S. A., and Vollmer, S. V. (2015). Identification of candidate coral pathogens on white band disease-infected staghorn coral. *PloS One* 10 (8), e0134416. doi: 10.1371/journal.pone.0134416

Giles, E. C., Kamke, J., Moitinho-Silva, L., Taylor, M. W., Hentschel, U., Ravasi, T., et al. (2013). Bacterial community profiles in low microbial abundance sponges. *FEMS Microbiol. Ecol.* 83 (1), 232–241. doi: 10.1111/j.1574-6941.2012.01467.x

Gloeckner, V., Wehrl, M., Moitinho-Silva, L., Gernert, C., Hentschel, U., Schupp, P., et al. (2014). The HMA-LMA dichotomy revisited: An electron microscopical survey of 56 sponge species. *Biol. Bull.* 227 (1), 78–88. doi: 10.1086/BBLv227n1p78

Gonzalez-Acosta, B., Bashan, Y., Hernandez-Saavedra, N. Y., Ascencio, F., and de la Cruz-Agüero, G. (2006). Seasonal seawater temperature as the major determinant for populations of culturable bacteria in the sediments of an intact mangrove in an arid region. *FEMS Microbiol. Ecol.* 55 (2), 311–321. doi: 10.1111/j.1574-6941.2005.00019.x

Gonzalez-Zapata, F. L., Bongaerts, P., Ramírez-Portilla, C., Adu-Oppong, B., Walljasper, G., Reyes, A., et al. (2018). Holobiont diversity in a reef-building coral over its entire depth range in the mesophotic zone. *Front. Mar. Sci.* 5. doi: 10.3389/fmars.2018.00029

González-Aravena, M., Kenny, N. J., Osorio, M., Font, A., Riesgo, A. H., and Cárdenas, C. A. (2019). Warm temperatures, cool sponges: The effect of increased temperatures on the Antarctic sponge Isodictya sp. *PeerJ* 7, e8088. doi: 10.7717/peerj.8088

Happel, L., Rondon, R., Font, A., González-Aravena, M., and Cárdenas, C. A. (2022). Stability of the microbiome of the sponge mycale (Oxymycale) acerata in the Western Antarctic peninsula. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.827863

Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., et al. (2003). "Microbial diversity of marine sponges," in *Progress in molecular and subcellular biology*, vol. 37 . Ed. W. E. G. Müller (Berlin, Heidelberg: Springer), 59–88. doi: 10.1007/978-3-642-55519-0_3

Hentschel, U., Piel, J., Degnan, S. M., and Taylor, M. W. (2012). Genomic insights into the marine sponge microbiome. *Nat. Rev. Microbiol.* 10 (9), 641–654. doi: 10.1038/nrmicro2839

Hentschel, U., Usher, K. M., and Taylor, M. W. (2006). Marine sponges as microbial fermenters. *FEMS Microbiol. Ecol.* 55 (2), 167–177. doi: 10.1111/j.1574-6941.2005.00046.x

Hereu, B., and Kersting, D. K. (2016). Diseases of coralline algae in the Mediterranean Sea. *Coral Reefs* 35 (2), 713. doi: 10.1007/s00338-016-1428-x

Hooper, J. N. A., and van Soest, R. W. M. (2002). "Systema Porifera: A guide to the classification of sponges," in *Systema Porifera*. Eds. J. N. A. Hooper, R. W. M. Van Soest and P. Willenz (Boston, MA: Springer), 1–7. doi: 10.1007/978-1-4615-0747-5_1

Horn, H., Slaby, B. M., Jahn, M. T., Bayer, K., Moitinho-Silva, L., Förster, F., et al. (2016). An enrichment of CRISPR and other defense-related features in marine sponge-associated microbial metagenomes. *Front. Microbiol.* 7. doi: 10.3389/fmicb.2016.01751

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., et al. (2017). Global warming and recurrent mass bleaching of corals. *Nature* 543 (7645), 373–377. doi: 10.1038/nature21707

Jackson, S. A., Flemer, B., McCann, A., Kennedy, J., Morrissey, J. P., O'Gara, F., et al. (2013). Archaea appear to dominate the microbiome of inflatella pellicula deep sea sponges. *PloS One* 8 (12), e84438. doi: 10.1371/journal.pone.0084438

Jackson, S. A., Kennedy, J., Morrissey, J. P., O'Gara, F., and Dobson, A. D. W. (2012). Pyrosequencing reveals diverse and distinct sponge-specific microbial communities in sponges from a single geographical location in Irish waters. *Microb. Ecol.* 64 (1), 105–116. doi: 10.1007/s00248-011-0002-x

Janussen, D., and Downey, R. V. (2014). "Chapter 5.5. Porifera," in *Biogeographic atlas of the southern ocean*. Eds. C. de Broyer, P. Koubbi, H. J. Griffiths, B. Raymond, C. d'Udekem d'Acoz, A. Van de Putte, et al (Cambridge: Scientific Committee on Antarctic Research), 94–102.

Jeong, J. B., Kim, K. H., and Park, J. S. (2015). Sponge-specific unknown bacterial groups detected in marine sponges collected from Korea through barcoded pyrosequencing. *J. Microbiol. Biotechnol.* 25 (1), 1–10. doi: 10.4014/jmb.1406.06041

Kennedy, J., Flemer, B., Jackson, S. A., Morrissey, J. P., O'Gara, F., and Dobson, A. D. W. (2014). Evidence of a putative deep sea specific microbiome in marine sponges. *PloS One* 9 (3), e91092. doi: 10.1371/journal.pone.0091092

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., et al. (2011). Bayesian Community-wide culture-independent microbial source tracking. *Nat. Methods* 8 (9), 761–763. doi: 10.1038/nmeth.1650

Koutsouveli, V., Taboada, S., Moles, J., Cristobo, J., Rı, P., Bertran, A., et al. (2018). Insights into the reproduction of some Antarctic dendroceratid, poecilosclerid, and haplosclerid demosponges. *PloS One* 13 (2), e0192267. doi: 10.1371/journal.pone.0192267

Kriefall, N. G., Kanke, M. R., Aglyamova, G. V., and Davies, S. W. (2022). Reef environments shape microbial partners in a highly connected coral population. *Proc. R. Soc B-Biol. Sci.* 289 (1967), 20212459. doi: 10.1098/rspb.2021.2459

Kulakova, N. V., Sakirko, M. V., Adelshin, R. V., Khanaev, I. V., Nebesnykh, I. A., and Pérez, T. (2018). Brown rot syndrome and changes in the bacterial community of the baikal sponge lubomirskia baicalensis. *Microb. Ecol.* 75 (4), 1024–1034. doi: 10.1007/S00248-017-1097-5

Lau, S. C. K., Tsoi, M. M. Y., Li, X., Dobretsov, S., Plakhotnikova, Y., Wong, P. K., et al. (2005). Pseudoalteromonas spongiae sp. nov., a novel member of the γ-proteobacteria isolated from the sponge mycale adhaerens in Hong Kong waters. *Int. J. Syst. Evol. Microbiol.* 55 (4), 1593–1596. doi: 10.1099/IJS.0.63638-0

Leal, C. V., Avelino-Alves, D., Salazar, V., Omachi, C., Thompson, C., Berlinck, R. G. S., et al. (2022). Sponges present a core prokaryotic community stable across tropical Western Atlantic. *Sci. Total Environ.* 835, 155145. doi: 10.1016/j.scitotenv.2022.155145

Lee, O. O., Wang, Y., Yang, J., Lafi, F. F., Al-Suwailem, A., and Qian, P. Y. (2011). Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the red Sea. *ISME J.* 5 (4), 650–664. doi: 10.1038/ismej.2010.165

Leray, M., Wilkins, L. G. E., Apprill, A., Bik, H. M., Clever, F., Connolly, S. R., et al. (2021). Natural experiments and long-term monitoring are critical to understand and predict marine host-microbe ecology and evolution. *PloS Biol.* 19 (8), e3001322. doi: 10.1371/journal.pbio.3001322

Lesser, M. P., Fiore, C., Slattery, M., and Zaneveld, J. (2016). Climate change stressors destabilize the microbiome of the Caribbean barrel sponge. *Exp. Mar. Biol. Ecol.* 475, 11–18. doi: 10.1016/j.jembe.2015.11.004

Lie, H. J., and Lee, S. (2010). Reconstruction of regular time series from bi-monthly temperature data in the yellow Sea and the northwestern East China Sea. *Ocean Sci. J.* 45 (2), 139–149. doi: 10.1007/S12601-010-0012-5

Lo Giudice, A., Azzaro, M., and Schiaparelli, S. (2019). "Microbial symbionts of Antarctic marine benthic invertebrates," in *The ecological role of micro-organisms in the Antarctic environment*. Ed. S. Castro-Sowinski (Cham: Springer), 277–296. doi: 10.1007/978-3-030-02786-5_13

Luter, H. M., Bannister, R. J., Whalan, S., Kutti, T., Pineda, M. C., and Webster, N. S. (2017). Microbiome analysis of a disease affecting the deep-sea sponge geodia barretti. *FEMS Microbiol. Ecol.* 93 (6), fix074. doi: 10.1093/femsec/fix074

Maldonado, M., Aguilar, R., Bannister, R. J., Bell, J. J., Conway, K. W., Dayton, P. K., et al. (2015). "Sponge grounds as key marine habitats: a synthetic review of types, structure, functional roles, and conservation concerns," in *Marine animal forests*. Eds. S. Rossi, L. Bramanti, A. Gori, C. Orejas and C. Saco del Valle (Cham: Springer), 1–39. doi: 10.1007/978-3-319-17001-5_24-1

McDevitt-Irwin, J. M., Baum, J. K., Garren, M., and Vega Thurber, R. L. (2017). Responses of coral-associated bacterial communities to local and global stressors. *Front. Mar. Sci.* 4. doi: 10.3389/fmars.2017.00262

McMurdie, P. J., and Holmes, S. (2013). Phyloseq: An r package for reproducible interactive analysis and graphics of microbiome census data. *PloS One* 8 (4), e61217. doi: 10.1371/journal.pone.0061217

Meron, D., Atias, E., Iasur Kruh, L., Elifantz, H., Minz, D., Fine, M., et al (2010). The impact of reduced pH on the microbial community of the coral Acropora eurystoma. *ISME J.* 5 (1), 51–60. doi: 10.1038/ismej.2010.102

Miura, N., Motone, K., Takagi, T., Aburaya, S., Watanabe, S., Aoki, W., et al. (2019). Ruegeria sp. strains isolated from the reef-building coral galaxea fascicularis inhibit growth of the temperature-dependent pathogen vibrio coralliilyticus. *Mar. Biotechnol.* 21 (1), 1–8. doi: 10.1007/S10126-018-9853-1

Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G. L., Cerrano, C., et al. (2017a). The sponge microbiome project. *GigaScience* 6 (10), gix077. doi: 10.1093/gigascience/gix077

Moitinho-Silva, L., Steinert, G., Nielsen, S., Hardoim, C. C. P., Wu, Y. C., McCormack, G. P., et al. (2017b). Predicting the HMA-LMA status in marine sponges by machine learning. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00752

Moreno-Pino, M., Cristi, A., Gillooly, J. F., and Trefault, N. (2020). Characterizing the microbiomes of Antarctic sponges: A functional metagenomic approach. *Sci. Rep.* 10 (1), 1–12. doi: 10.1038/s41598-020-57464-2

Morrow, K. M., Fiore, C. L., and Lesser, M. P. (2016). Environmental drivers of microbial community shifts in the giant barrel sponge, xestospongia muta, over a shallow to mesophotic depth gradient. *Environ. Microbiol.* 18 (6), 2025–2038. doi: 10.1111/1462-2920.13226

Mouchka, M. E., Hewson, I., and Harvell, C. D. (2010). Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. *Integr. Comp. Biol.* 50 (4), 662–674. doi: 10.1093/icb/icq061

Murray, A. E., Rack, F. R., Zook, R., Williams, M. J. M., Higham, M. L., Broe, M., et al. (2016). Microbiome composition and diversity of the ice-dwelling sea anemone, edwardsiella andrillae. *Integr. Comp. Biol.* 56 (4), 542–555. doi: 10.1093/icb/icw095

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., et al. (2022). "Vegan: Community ecology package," in *R package version 2.6-2*. Available at: https://CRAN.R-project.org/package=vegan.

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L., et al. (2018). Longer and more frequent marine heatwaves over the past century. *Nat. Commun.* 9 (1), 1–12. doi: 10.1038/s41467-018-03732-9

Pagliara, P., de Benedetto, G. E., Francavilla, M., Barca, A., and Caroppo, C. (2021). Bioactive potential of two marine picocyanobacteria belonging to cyanobium and synechococcus genera. *Microorganisms* 9 (10), 2048. doi: 10.3390/microorganisms9102048

Papale, M., Rizzo, C., Fani, R., Bertolino, M., Costa, G., Payturí-Gallart, A., et al. (2020). Exploring the diversity and metabolic profiles of bacterial communities associated with Antarctic sponges (Terra Nova bay, Ross Sea). *Front. Ecol. Evol.* 8. doi: 10.3389/fevo.2020.00268

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18 (5), 1403–1414. doi: 10.1111/1462-2920.13023

Peck, L. S., and Conway, L. Z. (2000). The myth of metabolic cold adaptation: Oxygen consumption in stenothermal Antarctic bivalves. *Geol. Soc Spec. Publ.* 177 (1), 441–450. doi: 10.1144/GSL.SP.2000.177.01.29

Perkins, N. R., Monk, J., Soler, G., Gallagher, P., and Barrett, N. S. (2022). Bleaching in sponges on temperate mesophotic reefs observed following marine heatwave events. *Clim. Change Ecol.* 3, 100046. doi: 10.1016/J.ECOCHG.2021.100046

Pinnaka, A. K., Ara, S., Singh, A., and Shivaji, S. (2013). Draft genome sequence of winogradskyella psychrotolerans rs-3t, isolated from the marine transect of kongsfjorden, ny-Ålesund, Svalbard, Arctic ocean. *Genome Announc.* 1 (4), e00630–e00613. doi: 10.1128/genomea.00630-13

Pita, L., Rix, L., Slaby, B. M., Franke, A., and Hentschel, U. (2018). The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome* 6 (1), 46. doi: 10.1186/s40168-018-0428-1

Pollocks, J. F., Katz, S. M., van de Water, J. A. J. M., Davies, S. W., Hein, M., and Torda, G. (2017). Coral larvae for restoration and research: A large-scale method for rearing Acropora millepora larvae, inducing settlement, and establishing symbiosis. *PeerJ* 3, e3732. doi: 10.7717/PEERJ.3732

Posadas, N., Baquiran, J. I. P., Nada, M. A. L., Kelly, M., and Conaco, C. (2021). Microbiome diversity and host immune functions influence survivorship of sponge holobionts under future ocean conditions. *ISME J.* 16 (1), 58–67. doi: 10.1038/S41396-021-01050-5

Ramsby, B. D., Hoogenboom, M. O., Whalan, S., and Webster, N. S. (2018). Elevated seawater temperature disrupts the microbiome of an ecologically important bioeroding sponge. *Mol. Ecol.* 27 (8), 2124–2137. doi: 10.1111/mec.14544

Regueiras, A., Alex, A., Pereira, S., Costa, M. S., Antunes, A., and Vasconcelos, V. (2017). Cyanobacterial diversity in the marine sponge hymeniacidon perlevis from a temperate region (Portuguese coast, northeast Atlantic). *Aquat. Microb. Ecol.* 79 (3), 259–272. doi: 10.3354/ame01830

Reveillaud, J., Maignien, L., Eren, M. A., Huber, J. A., Apprill, A., Sogin, M. L., et al. (2014). Host-specificity among abundant and rare taxa in the sponge microbiome. *ISME J.* 8 (6), 1198–1209. doi: 10.1038/ismej.2013.227

Ribes, M., Calvo, E., Movilla, J., Logares, R., Coma, R., and Pelejero, C. (2016). Restructuring of the sponge microbiome favors tolerance to ocean acidification. *Environ. Microbiol. Rep.* 8 (4), 536–544. doi: 10.1111/1758-2229.12430

Ribes, M., Dziallas, C., Coma, R., and Riemann, L. (2015). Microbial diversity and putative diazotrophy in high- and low-Microbial-Abundance Mediterranean sponges. *Appl. Environ. Microbiol.* 81 (17), 5683–5693. doi: 10.1128/aem.01320-15

Ribes, M., Jiménez, E., Yahel, G., López-Sendino, P., Diez, B., Massana, R., et al. (2012). Functional convergence of microbes associated with temperate marine sponges. *Environ. Microbiol.* 14 (5), 1224–1239. doi: 10.1111/j.1462-2920.2012.02701.x

Riesgo, A., Farrar, N., Windsor, P. J., Giribet, G., and Leys, S. P. (2014). The analysis of eight transcriptomes from all poriferan classes reveals surprising genetic complexity in sponges. *Mol. Biol. Evol.* 31 (5), 1102–1120. doi: 10.1093/molbev/msu057

Rizzo, C., Zammuto, V., Lo Giudice, A., Rizzo, M. G., Spanò, A., Laganà, P., et al. (2021). Antibiofilm activity of Antarctic sponge-associated bacteria against pseudomonas aeruginosa and staphylococcus aureus. *J. Mar. Sci. Eng.* 9 (3), 243. doi: 10.3390/jmse9030243

Roda-Garcia, J. J., Haro-Moreno, J. M., Huschet, L. A., Rodriguez-Valera, F., and López-Pérez, M. (2021). Phylogenomics of SAR116 clade reveals two subclades with different evolutionary trajectories and an important role in the ocean sulfur cycle. *mSystems* 6 (5), e00944–e00921. doi: 10.1128/msystems.00944-21

Rodríguez-Marconi, S., de la Iglesia, R., Díez, B., Fonseca, C. A., Hajdu, E., Trefault, N., et al. (2015). Characterization of bacterial, archaeal and eukaryote symbionts from Antarctic sponges reveals a high diversity at a three-domain level and a particular signature for this ecosystem. *PloS One* 10 (9), e0138837. doi: 10.1371/journal.pone.0138837

Rondon, R., González-Aravena, M., Font, A., Osorio, M., and Cárdenas, C. A. (2020). Effects of climate change stressors on the prokaryotic communities of the Antarctic sponge isodictya kerguelenensis. *Front. Ecol. Evol.* 8. doi: 10.3389/fevo.2020.00262

Rosado, P. M., Leite, D. C. A., Duarte, G. A. S., Chaloub, R. M., Jospin, G., Nunes da Rocha, U., et al. (2019). Marine probiotics: Increasing coral resistance to bleaching through microbiome manipulation. *ISME J.* 13 (4), 921–936. doi: 10.1038/s41396-018-0323-6

Rosales, S. M., Clark, A. S., Huebner, L. K., Ruzicka, R. R., and Muller, E. M. (2017). Rhodobacterales and Rhizobiales are associated with stony coral tissue loss disease and its suspected sources of transmission. *Front. Microbiol.* 11, 681. doi: 10.3389/FMICB.2020.00681

Rubio-Portillo, E., Izquierdo-Muñoz, A., Gago, J. F., Rosselló-Mora, R., Antón, J., and Ramos-Esplá, A. A. (2016). Effects of the 2015 heat wave on benthic invertebrates in the tabarca marine protected area (southeast Spain). *Mar. Environ. Res.* 122, 135–142. doi: 10.1016/j.marenvres.2016.10.004

Rubio-Portillo, E., Ramos-Esplá, A. A., and Antón, J. (2021). Shifts in marine invertebrate bacterial assemblages associated with tissue necrosis during a heat wave. *Coral Reefs* 40 (2), 395–404. doi: 10.1007/s00338

Ruocco, N., Esposito, R., Bertolino, M., Zazo, G., Sonnessa, M., Andreani, F., et al. (2021). A metataxonomic approach reveals diversified bacterial communities in Antarctic sponges. *Mar. Drugs* 19 (3), 173. doi: 10.3390/md19030173

Sacristán-Soriano, O., Pérez Criado, N., and Avila, C. (2020). Host species determines symbiotic community composition in Antarctic sponges (Porifera: Demospongiae). *Front. Mar. Sci.* 7. doi: 10.3389/fmars.2020.00474

Schellenberg, J., Busse, H. J., Hardt, M., Schubert, P., Wilke, T., Kämpfer, P., et al. (2017). Winogradskyella haliclonae sp. nov., isolated from a marine sponge of the genus haliclona. *Int. J. Syst. Evol. Microbiol.* 67 (12), 4902–4910. doi: 10.1099/ijsem.0.002192

Schmitt, S., Hentschel, U., and Taylor, M. W. (2012a). Deep sequencing reveals diversity and community structure of complex microbiota in five Mediterranean sponges. *Hydrobiologia* 687, 341–351. doi: 10.1007/s10750-011-0799-9

Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., et al. (2012b). Assessing the complex sponge microbiota: Core, variable and species-specific bacterial communities in marine sponges. *ISME J.* 6 (3), 564–576. doi: 10.1038/ismej.2011.116

Sharp, K. H., and Ritchie, K. B. (2021). Multi-partner interactions in corals in the face of climate change. *Biol. Bull* 223 (1), 66–77. doi: 10.1086/BBLV223N1P66

Shivani, Y., Subhash, Y., Sasikala, C., and Ramana, C. H. V. (2017). Halodesulfovibrio spirochaetisodalis gen. nov. sp. nov. and reclassification of four desulfovibrio spp. *Int. J. Syst. Evol. Microbiol.* 67 (1), 87–93. doi: 10.1099/ijsem.0.001574

Simion, P., Philippe, H., Baurain, D., Jager, M., Richter, D. J., di Franco, A., et al. (2017). A large and consistent phylogenomic dataset supports sponges as the sister

group to all other animals. *Curr. Biol.* 27 (7), 958–967. doi: 10.1016/j.cub.2017.02.031

Simister, R., Taylor, M. W., Rogers, K. M., Schupp, P. J., and Deines, P. (2013). Temporal molecular and isotopic analysis of active bacterial communities in two new Zealand sponges. *FEMS Microbiol. Ecol.* 85 (1), 195–205. doi: 10.1111/1574-6941.12109

Simister, R., Taylor, M. W., Tsai, P., Fan, L., Bruxner, T. J., Crowe, M. L., et al. (2012a). Thermal stress responses in the bacterial biosphere of the great barrier reef sponge, rhopaloeides odorabile. *Environ. Microbiol.* 14 (12), 3232–3246. doi: 10.1111/1462-2920.12010

Simister, R., Taylor, M. W., Tsai, P., and Webster, N. (2012b). Sponge-microbe associations survive high nutrients and temperatures. *PloS One* 7 (12), e52220. doi: 10.1371/journal.pone.0052220

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., et al. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat. Clim. Change* 9 (4), 306–312. doi: 10.1038/s41558-019-0412-1

Southwell, M. W., Weisz, J. B., Martens, C. S., and Lindquist, N. (2008). *In situ* fluxes of dissolved inorganic nitrogen from the sponge community on conch reef, key largo, Florida. *Limnol. Oceanogr.* 53 (3), 986–996. doi: 10.4319/LO.2008.53.3.0986

Steinert, G., Taylor, M. W., Deines, P., Simister, R. L., de Voogd, N. J., Hoggard, M., et al. (2016). In four shallow and mesophotic tropical reef sponges from Guam the microbial community largely depends on host identity. *PeerJ* 4, e1936. doi: 10.7717/peerj.1936

Steinert, G., Wemheuer, B., Janussen, D., Erpenbeck, D., Daniel, R., Simon, M., et al. (2019). Prokaryotic diversity and community patterns in Antarctic continental shelf sponges. *Front. Mar. Sci.* 6. doi: 10.3389/fmars.2019.00297

Stevens, M. A., Riebesell, U., McDevitt-Irwin, J. M., Karlsson, R., Kanbar, J., Gifford, I., et al. (2017). A communal catalogue reveals earth's multiscale microbial diversity. *Nature* 551 (7681), 457–463. doi: 10.1038/nature24621

Strand, R., Whalan, S., Webster, N. S., Kutti, T., Fang, J. K. H., Luter, H. M., et al. (2017). The response of a boreal deep-sea sponge holobiont to acute thermal stress. *Sci. Rep.* 7 (1), 1–12. doi: 10.1038/s41598-017-01091-x

Taylor, M. W., Radax, R., Steger, D., and Wagner, M. (2007). Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol. Mol. Biol. Rev.* 71 (2), 295–347. doi: 10.1128/mmbr.00040-06

Taylor, M. W., Schupp, P. J., Dahllöf, I., Kjelleberg, S., and Steinberg, P. D. (2004). Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. *Environ. Microbiol.* 6 (2), 121–130. doi: 10.1046/J.1462-2920.2003.00545.x

Taylor, M. W., Tsai, P., Simister, R. L., Deines, P., Botte, E., Ericson, G., et al. (2013). 'Sponge-specific' bacteria are widespread (but rare) in diverse marine environments. *ISME J.* 7 (2), 438–443. doi: 10.1038/ismej.2012.111

Taylor, J. A., Palladino, G., Wemheuer, B., Steinert, G., Sipkema, D., and Williams, T. J. (2020). Phylogeny resolved, metabolism revealed: functional radiation within a widespread and divergent clade of sponge symbionts. *ISME J.* 15 (2), 503–519. doi: 10.1038/s41396-020-00791-z

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., et al. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336 (6081), 608–611. doi: 10.1126/science.1218344

Thébault, J., Chauvaud, L., Clavier, J., Guarini, J., Dunbar, R. B., Fichez, R., et al. (2007). Reconstruction of seasonal temperature variability in the tropical pacific ocean from the shell of the scallop, comptopallium radula. *Geochim. Cosmochim. Acta* 71 (4), 918–928. doi: 10.1016/j.gca.2006.10.017

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., et al. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. *Nat. Commun.* 7 (1), 1–12. doi: 10.1038/ncomms11870

Thomas, T., Rusch, D., DeMaere, M. Z., Yung, P. Y., Lewis, M., Halpern, A., et al. (2010). Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. *ISME J.* 4 (12), 1557–1567. doi: 10.1038/ismej.2010.74

Vacelet, J., and Donadey, C. (1977). Electron microscope study of the association between some sponges and bacteria. *J. Exp. Mar. Biol. Ecol.* 30 (3), 301–314. doi: 10.1016/0022-0981(77)90038-7

Vargas, S., Leiva, L., and Wörheide, G. (2021). Short-term exposure to high-temperature water causes a shift in the microbiome of the common aquarium sponge lendenfeldia chondrodes. *Microb. Ecol.* 81 (1), 213–222. doi: 10.1007/s00248-020-01556-z

Webster, N., and Bourne, D. (2007). Bacterial community structure associated with the Antarctic soft coral, alcyonium antarcticum. *FEMS Microbiol. Ecol.* 59 (1), 81–94. doi: 10.1111/j.1574-6941.2006.00195.x

Webster, N. S., Cobb, R. E., and Negri, A. P. (2008). Temperature thresholds for bacterial symbiosis with a sponge. *ISME J.* 2 (8), 830–842. doi: 10.1038/ismej.2008.42

Webster, N. S., Negri, A. P., Munro, M. M. H. G., and Battershill, C. N. (2004). Diverse microbial communities inhabit Antarctic sponges. *Environ. Microbiol.* 6 (3), 288–300. doi: 10.1111/j.1462-2920.2004.00570.x

Webster, N. S., and Taylor, M. W. (2012). Marine sponges and their microbial symbionts: love and other relationships. *Environ. Microbiol.* 14 (2), 335–346. doi: 10.1111/j.1462-2920.2011.02460.x

Webster, N. S., Taylor, M. W., Behnam, F., Lücker, S., Rattei, T., Whalan, S., et al. (2010). Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environ. Microbiol.* 12 (8), 2070–2082. doi: 10.1111/j.1462-2920.2009.02065.x

Webster, N. S., and Thomas, T. (2016). The sponge hologenome. *mBio* 7 (2), e00135–e00116. doi: 10.1128/mBio.00135-16

Webster, N. S., Webb, R. I., Ridd, M. J., Hill, R. T., and Negri, A. P. (2001). The effects of copper on the microbial community of a coral reef sponge. *Environ. Microbiol.* 3 (1), 19–31. doi: 10.1046/j.1462-2920.2001.00155.x

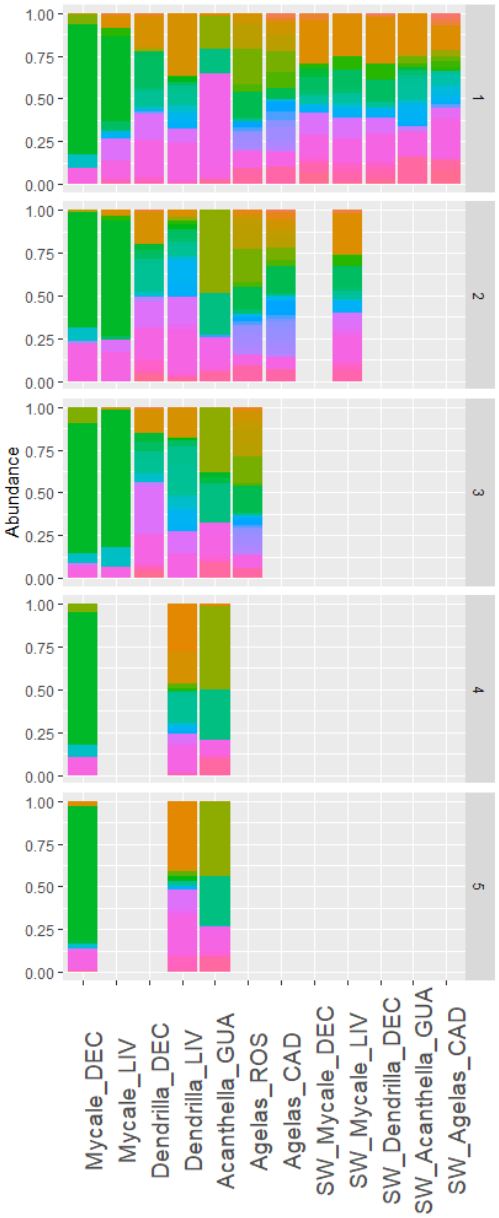
Welsh, R. M., Rosales, S. M., Zaneveld, J. R., Payet, J. P., McMinds, R., Hubbs, S. L., et al. (2017). Alien vs. predator: bacterial challenge alters coral microbiomes unless controlled by halobacteriovorax predators. *PeerJ* 5, e3315. doi: 10.7717/peerj.3315

Yang, S., Sun, W., Zhang, F., and Li, Z. (2013). Phylogenetically diverse denitrifying and ammonia-oxidizing bacteria in corals alcyonium gracillimum and tubastraea coccinea. *Mar. Biotechnol.* 15, 540–551. doi: 10.1007/S10126-013-9503-6

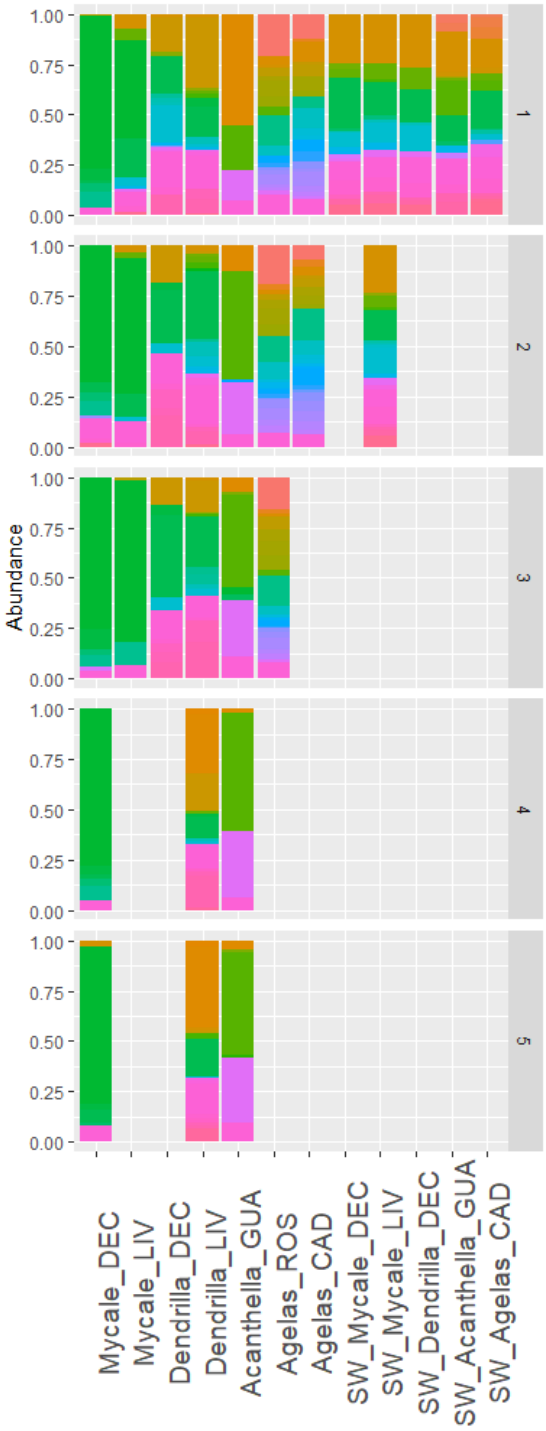
Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures

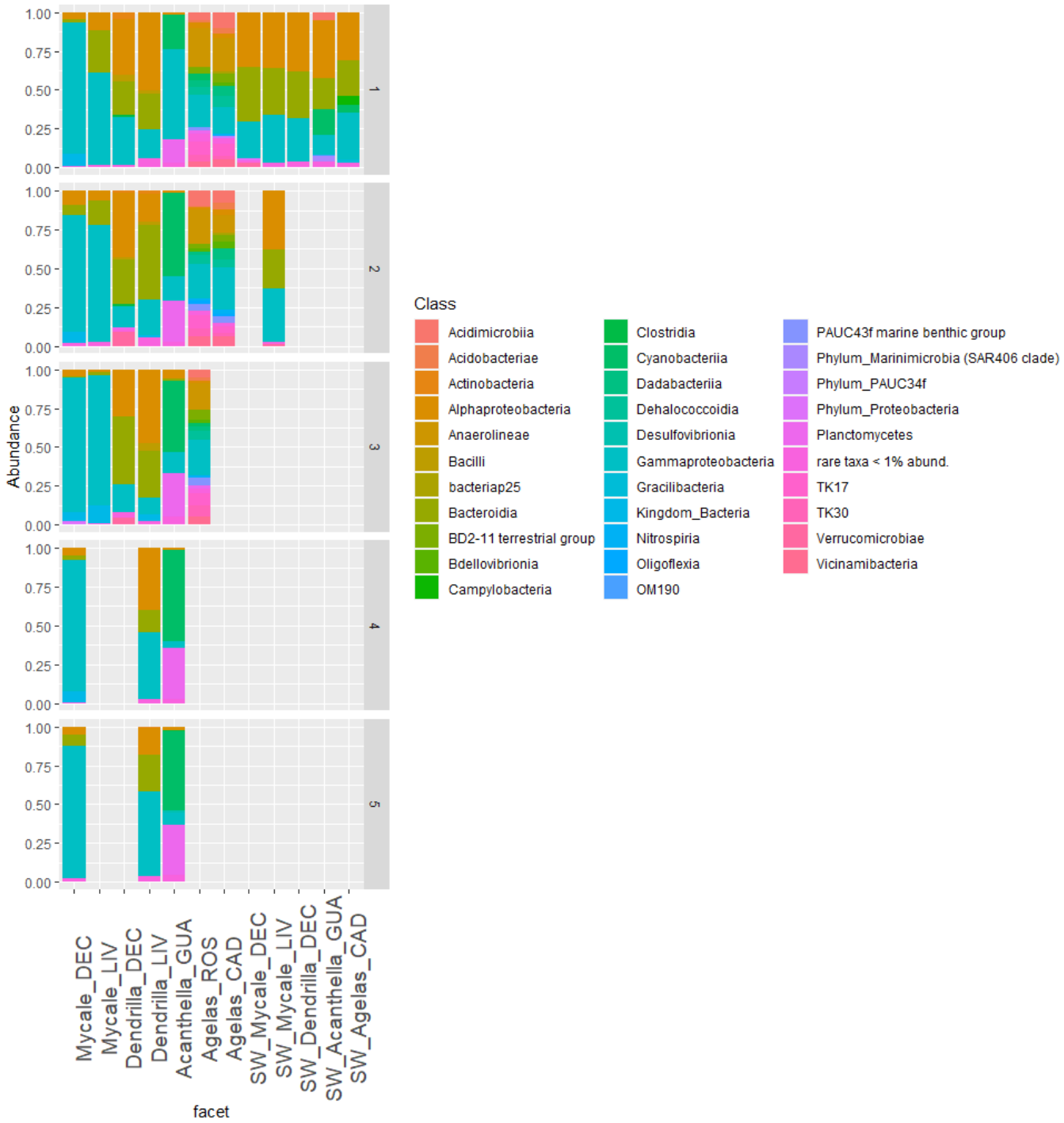


Supplementary Figure 1. Taxonomic affiliation of ASVs detected in sponges and sea water in their natural habitat at Genus level. DEC, Deception Island; LIV, Livingston Island; GUA, Guam; ROS, Roses; CAD, Cadaqués; SW, sea water. Numbers on the right axis indicate sample replicates.


Abundance
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
Mycale_DEC
Mycale_LIV
Dendrilla_DEC
Dendrilla_LIV
Acanthella_GUA
Agelas_ROS
Agelas_CAD
SW_Mycale_DEC
SW_Mycale_LIV
SW_Dendrilla_DEC
SW_Acanthella_GUA
SW_Agelas_CAD

Supplementary Figure 2. Taxonomic affiliation of ASVs detected in sponges and sea water in their natural habitat at Family level. DEC, Deception Island; LIV, Livingston Island; GUA, Guam; ROS, Roses; CAD, Cadaqués; SW, sea water. Numbers on the right axis indicate sample replicates.

Supplementary Figure 3. Taxonomic affiliation of ASVs detected in sponges and sea water in their natural habitat at Order level. DEC, Deception Island; LIV, Livingston Island; GUA, Guam; ROS, Roses; CAD, Cadaqués; SW, sea water. Numbers on the right axis indicate sample replicates.



Supplementary Figure 4. Taxonomic affiliation of ASVs detected in sponges and sea water in their natural habitat at Class level. DEC, Deception Island; LIV, Livingston Island; GUA, Guam; ROS, Roses; CAD, Cadaqués; SW, sea water. Numbers on the right axis indicate sample replicates.

1.2. Supplementary Tables

Supplementary Table 1. Taxonomic affiliation of the 15 most abundant ASVs from the different sponge species analyzed.

	ASV	Reads	SILVA DB	GenBank DB	Accession Number	% identity
M. acerata	1	160461	Family_EC94	*Nitrosomonas stercoris*	NR_146824.1	87.45
	2	8138	Clade Ia	*Pelagibacter ubique*	NR_074224	100.00
	3	104104	Family_EC94	*Nitrosomonas oligotropha*	NR_114770.1	90.55
	12	9081	*Polaribacter*			
	25	1961	Family_Flavobacteriaceae	*Altibacter lentus*	NR_126240.1	94.49
	42	2735	Family_Nitrincolaceae	*Maribrevibacterium harenarium*	NR_173646.1	94.07
	47	16213	Family_EC94	*Nitrosomonas oligotropha*	NR_114770.1	89.37
	53	1860	Family_Nitrincolaceae	*Maribrevibacterium harenarium*	NR_173646.1	94.47
	69	13510	Kingdom_Bacteria	*Sediminispirochaeta bajacaliforniensis*	NR_029033.1	83.72
	70	1862	*Aurantivirga*			
	77	1751	*Ulvibacter*			
	112	3611	Family_Cryomorphaceae	*Phaeocystidibacter marisrubri*	NR_136475.1	92.94
	177	7399	*Robiginitomaculum*			

	223	8722	*Endozoicomonas*			
	503	2915	Kingdom_Bacteria	*Chakrabartia godavariana*	NR_165009.1	80.31
D. antarctica	12	32418	*Polaribacter*			
	24	4295	*Sulfitobacter*			
	53	4473	Family_Nitrincolaceae	*Maribrevibacterium harenarium*	NR_173646.1	94.47
	62	10020	*Burkholderia-Caballeronia-Paraburkholderia*			
	77	5751	*Ulvibacter*			
	85	43275	Class_Alphaproteobacteria	*Pseudorhodobacter wandonensis*	NR_109461.1	84.31
	93	5015	*Polaribacter*			
	100	31825	Family_SAR116 clade	*Thalassobaculum salexigens*	NR_116122.1	89.80
	141	6651	NS4 marine group	*Kordia ulvae*	NR_149793.1	92.49
	169	4663	OM43 clade	*Rivicola pingtungensis*	NR_133846.1	96.05
	289	9210	Family_Nitrincolaceae	*Maribrevibacterium harenarium*	NR_173646.1	94.07
	336	7329	*Polaribacter*			
	358	9569	Class_Alphaproteobacteria	*Varunaivibrio sulfuroxidans*	NR_152005.1	88.67
	362	7493	Family_S25-593	*Parasphingorhabdus pacifica*	NR_134813.1	88.19
	363	8631	*Kistimonas*			
A. oroides	56	48359	Class_TK17	*Litorilinea aerophila*	NR_132330.1	83.4

	98	44992	Family_A4b	*Ornatilinea apprima*	NR_109544.1	83.4
	101	21238	Class_PAUC43f marine benthic group	*Longimicrobium terrae*	NR_149251.1	85.55
	109	32967	Family_KI89A clade	*Pseudomonas marincola*	NR_117186.1	93.25
	113	30684	Class_TK30	*Ammoniphilus oxalaticus*	NR_026432.1	84.98
	127	24174	Sva0996 marine group	*Rhabdothermincola sediminis*	NR_173681.1	87.06
	135	15012	Order_Subgroup 9	*Luteitalea pratensis*	NR_156918.1	85.83
	154	26209	Family_A4b	*Bellilinea caldifistulae*	NR_041354.1	82.61
	156	15401	Order_PAUC26f	*Paludibaculum fermentans*	NR_134120.1	84.25
	168	12584	Class_BD2-11 terrestrial group	*Longimicrobium terrae*	NR_149251.1	83.13
	184	16133	Sva0996 marine group	*Actinomarinicola tropica*	NR_171428.1	93.31
	192	12611	Order_Dadabacteriales	*Thioalkalivibrio thiocyanoxidans*	NR_025129.1	84.40
	194	20750	Family_A4b	*Quisquiliibacterium transsilvanicum*	NR_159181.1	80.31
	202	16631	Family_Caldilineaceae	*Litorilinea aerophila*	NR_132330.1	85.77
	205	13881	Family_KI89A clade	*Alcanivorax sediminis*	NR_174285.1	91.67
A. cavernosa	5	154922	Family_Pirellulaceae	*Botrimarina hoheduenensis*	NR_173585.1	90.87
	8	196687	*Cyanobium* PCC-6307			
	11	1966	*Halodesulfovibrio*			

18	87947	*Ralstonia*			
34	20932	*Synechococcus* CC9902			
57	60818	*Cyanobium* PCC-6307			
62	2146	*Burkholderia-Caballeronia-Paraburkholderia*			
200	13498	*Rubripirellula*			
217	14347	*Synechococcus* CC9902			
225	3375	*Ralstonia*			
227	4388	Family_Geminicoccaceae	*Arboricoccus pini*	NR_157671.1	89.72
349	7201	*Synechococcus* CC9902			
469	6031	Family_EC94	*Kushneria pakistanensis*	NR_136435.1	87.40
600	3259	*Synechococcus* CC9902			
687	2632	Order_PeM15	*Geodermatophilus africanus*	NR_108882.1	93.68

CAPÍTULO IV

A chemo-ecological investigation on *Dendrilla antarctica* Topsent, 1905: identification of deceptionin and effects of heat stress and predation pressure on its terpene profiles

Paula De Castro-Fernández[1,2,3,4], Carlos Angulo-Preckler[5], Cristina García-Aljaro[3], Conxita Avila[1,2], and Adele Cutignano[4]

[1]Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals (BEECA), Facultat de Biologia, Universitat de Barcelona (UB), Av. Diagonal 645, Barcelona 08028, Spain

[2]Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Barcelona, Spain

[3]Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona (UB), Av. Diagonal 645, Barcelona 08028, Spain

[4]Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica Biomolecolare (ICB), Pozzuoli, NA, Italy

[5]Red Sea Research Center and Computational Bioscience Research Center, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900 Saudi Arabia

Abstract

Marine sponges usually host a wide array of secondary metabolites that play crucial roles in their biological interactions, displaying deterrent, toxic or anti-fouling activities, among others. The factors that influence the intraspecific variability in the metabolic profile of organisms, their production or their ecological function remain unknown in most cases. Understanding this may help predict changes in biological relationships due to environmental variations as a consequence of ongoing climate change. The Antarctic sponge *Dendrilla antarctica* is common in shallow rocky bottoms of the Antarctic Peninsula, and is known to produce a series of diterpenic compounds that are supposed to have defensive roles in the sponge. Here, we used GC-MS to determine the major diterpenes in two populations of *D. antarctica* from two islands, Livingston and Deception Island (South Shetland Islands). In order to assess the potential effect of heat stress, we performed experiments in aquaria exposing the sponge to a control temperature (similar to local), a heat stress (five degrees higher) and an extreme heat stress (ten degrees higher). To test for defense induction by predation pressure, we exposed the sponges to the omnivorous seastar *Odontaster validus* and the amphipod *Cheirimedon femoratus*. Seven major diterpenes were isolated and identified from the samples. While six of them were already reported in the literature, we identified one new aplysulphurane derivative that was more abundant in the samples from Deception Island, so we

named it deceptionin (**7**). The samples separated in the PCA bi-plot space according to the Island of collection, with 9,11-dihydrogracilin A (**1**) being more abundant in the samples from Livingston Island, and deceptionin (**7**) in the samples from Deception Island. We found a slight effect of heat stress on the diterpene profiles of *D. antarctica*, with tetrahydroaplysulphurin-1 (**6**) being more abundant in the group exposed to heat stress. However, predation pressure did not seem to influence the metabolite production in the sponge. Further research on the bioactivity of *D. antarctica* secondary metabolites, and the responses to environmental changes will help better understand the functioning and fate of the Antarctic benthic ecosystems.

1. Introduction

Antarctic shallow rocky bottom communities are sponge-rich associations of organisms with a complex network of biological interactions (Dayton et al., 1970). In these communities, sponges provide habitat and food for many other species, being a fundamental element of these benthic ecosystems (Janussen and Downey, 2014; Cárdenas and Montiel, 2017; Costello and Chaudhary, 2017; Cárdenas et al., 2018). Many of the species living in these communities use chemical communication and therefore are rich in natural products (Lebar et al., 2007; Avila et al., 2008; Figuerola et al., 2013; Moles et al., 2015; Núñez-Pons and Avila, 2015; Soldatou and Baker, 2017; Angulo-Preckler et al., 2018b). As the rest of organisms, sponges produce biologically active natural compounds to avoid predation, competition, pathogenic infections, or fouling, among others (Puglisi et al., 2019; Carroll et al., 2022).

Dendrilla antarctica Topsent, 1905, is a common demosponge inhabiting shallow waters in coastal areas of Antarctica (Goodwin et al., 2019). Its distribution area comprises the Antarctic Peninsula but also Southern South America, the Falkland Islands, the subantarctic and other locations in Antarctica (including McMurdo Sound, Wilheim II Coast, Victoria Land, and Graham Coast) from depths 10 – 549 m (Koltun, 1976; Brueggeman, 1998). It usually forms large and massive yellow carpets covering rocks and other surfaces, sometimes reaching a few meters in size. Its surface is bright in color and presents conspicuous spiky conules. It usually adopts a lobular or an encrusting shape. *Dendrilla antarctica* forms three dimensional structures where other organisms may also live (von Salm et al., 2022). It may also grow on top of other organisms (macroalgae or other invertebrates).

Its chemical defenses, mostly diterpenoids, provide the sponge with protection to avoid predation (Molinski and Faulkner, 1987; Baker et al., 1995; Fontana et al., 1997; Ankisetty et al., 2004b; Salm et al., 2016; Angulo-Preckler et al., 2018b; Bory et al., 2020; Prieto et al., 2022). Potential predators for the species include sympatric generalist predators like the sea star *Odontaster validus*, omnivorous amphipods, or the common nudibranch *Doris kerguelenensis* (Barnes and Bullough, 1996; Amsler et al., 2009). A recent study has shown that abundances of amphipod communities living near the sponges are related to the metabolic profile of the sponge (von Salm et al., 2022).

The role of chemical ecology is pivotal in these communities (Avila, 2016), and the relevance for sponges has become clear over recent years (Peters et al., 2009, 2010; Prieto et al., 2022; von Salm et al., 2022). For *D. antarctica*, the defensive role of its metabolites represents an ecological advantage that may be affected by global change, since increasing water temperatures may affect, for example, the production of these chemicals and thus the sponge protection against potential predators. Similarly, changes in predation pressures may also produce changes in the natural products (Cronin and Hay, 1996). Some of the sponge compounds may also display relevant bioactivities in the laboratory (Molinski and Faulkner, 1987; Ankisetty et al., 2004a; von Salm et al., 2016; Bory et al., 2020; Shilling et al., 2020; Ottaviani et al., 2022). *Dendrilla antarctica* compounds have been described as having antibacterial, antifungal, or cytotoxic activity, among others. Both 9,11-dihydrogracilin A and membranolide showed activity against *B. subtilis* (Molinski and Faulkner, 1987), whereas membranolide and darwinolide inhibited methicillin resistant *S. aureus* biofilm (von Salm et al., 2016). The compounds 9,11-dihydrogracilin A and 9,11-dihydrogracillinone A were found to have antifouling activity (Prieto et al., 2022). Tetrahydroaplysulphurin-1 displayed low micromolar activity against the Leishmania parasite *L. donovani* (Shilling et al., 2020). Ciaglia and co-workers suggested that 9,11-dihydrogracilin A exerts anti-inflammatory effects and has anti-edema activity in vivo (Ciaglia et al., 2017).

Sponges have abundant and common associations with a wide range of microorganisms, forming the "holobiont" (Thomas et al., 2016; Pita et al., 2018; Posadas et al., 2021). The sponge microbiota may play a role in either the biological production or the compounds variability reported in Antarctic species (Cárdenas et al., 2018; lo Giudice et al., 2019; Murray et al., 2020; Sacristán-Soriano et al., 2020; Happel et al., 2022). Their species composition is affected by geographic, environmental, and host factors. This is relevant for the role they play within the sponge (Easson et al., 2020; Freeman

et al., 2021). The microbiota of *Dendrilla antarctica* has been previously studied (Sacristán-Soriano et al., 2020; De Castro-Fernández et al., 2023).

In this study we aimed at (1) analyzing the secondary metabolites profile in the populations of the sponge *Dendrilla antarctica* from Livingston and Deception Islands (South Shetland Island), and (2) assessing the effects of heat stress and predation pressure in the chemical profile of the sponge. The identification and structural characterization of a new terpene derivative from specimens collected in Deception Island is presented.

2. Materials and Methods

2.1. Sample collection

Healthy whole specimens of *D. antarctica* were collected by hand using scuba from different stations in Livingston (n=13) and Deception (n=30) Islands (South Shetland Islands, Antarctica) (**Figure 1**). Additionally, bulk *D. antarctica* specimens were collected for the extraction of chemical standards needed for quantification in study specimens. Collection sites were shallow (depths of 20-25 m) rocky bottoms. Collection took place during January 2018 and January 2019. Sponge specimens were kept in plastic containers and transported to the lab within less than 1 h. There, some specimens were directly frozen at -20°C and some others were used for the aquaria experiments.

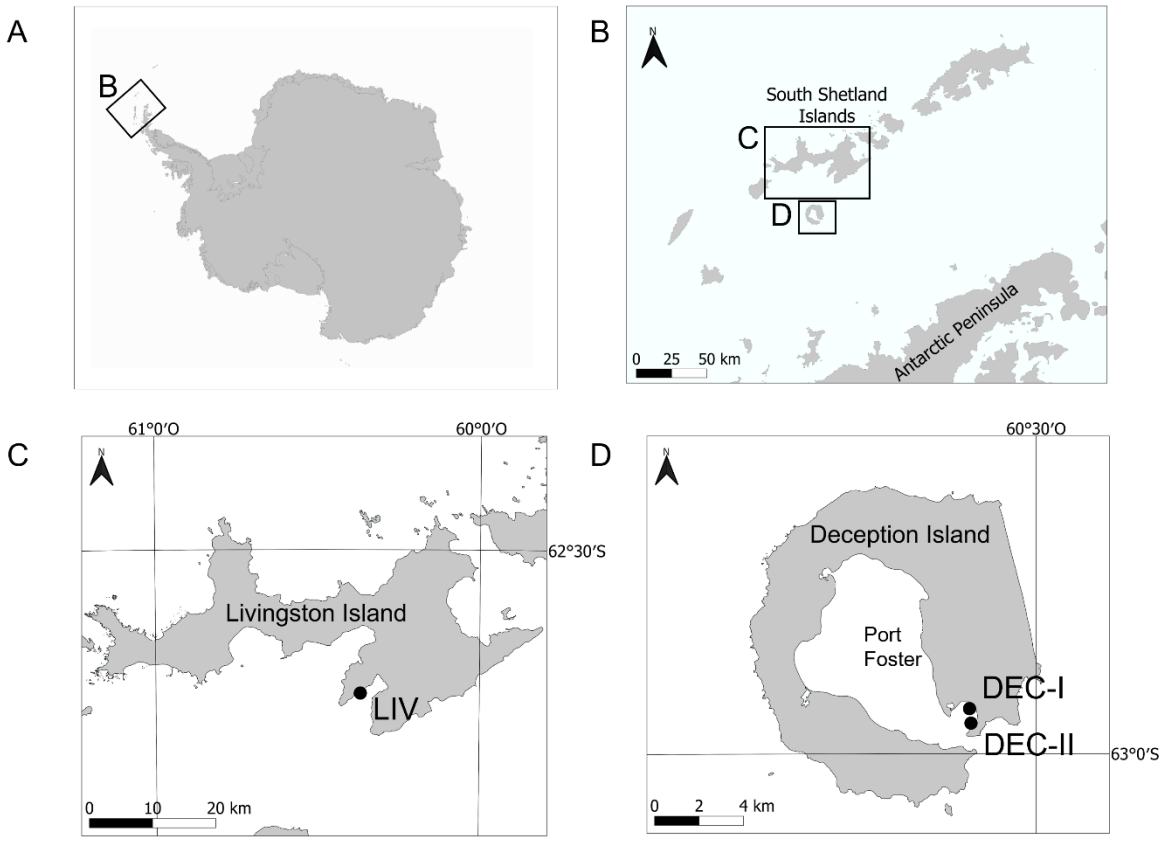


Figure 1. Location of the sampling sites. A. Antarctic continent. B. Tip of the Antarctic Peninsula and South Shetland Islands. C. Livingston Island (South Shetland Islands). D. Deception Island (South Shetland Islands). Sampling stations are marked with dots.

2.2. Heat stress and predation pressure experiments in aquaria

To measure the effect of the heat stress in the production of metabolites, 15 specimens of *D. antarctica* were placed in aquaria at three different temperatures, including local seawater temperature, to be used as control temperature (CT), 0.5±0.3 °C (mean±SD temperature along the experiment), and at two higher temperatures: heat stress temperature (HST), 5.4±0.4 °C, and extreme heat stress temperature (EHST), 9.7±0.8 °C. Temperatures were chosen according to the increase predicted by the IPCC and other reports, by duplicating the expected values and higher, and to the aquarium possibilities available for us to carry out the experiments. Water temperature

was measured and controlled using a digital controller (Aqua Medic T controller twin) connected to heating (Sera 50 W or 150 W) and/or cooling (Aqua Medic Titan 150) units.

To measure the effect of the predation pressure in the production of metabolites, five specimens of *D. antarctica* were placed in an aquarium at local seawater temperature with two specimens per sponge of the red seastar *Odontaster validus*, an omnivorous predator with circumpolar distribution. On a separate tank, five specimens of *D.* antarctica were placed along with ~200 specimens per sponge of the amphipod *Cheirimedon femoratus*, a spongivorous species very common in these shallow benthic assemblages (De Broyer and Jazdzewska, 2014).

For both experiments, the system was kept steady all along the experiment. Sponge specimens were kept in compartmented tanks (volumes ranging between 24-112.5 L according to the organism sizes), with seawater circulating through all the compartments, and were incubated for ca. three weeks.

2.3. General analysis

Optical rotation was measured on Jasco P-2000 digital polarimeter at 589 nm. FT-IR spectrum was recorded on a Jasco FT/IR 4100 spectrophotometer. UV spectrum was acquired on a Jasco V-650 Spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker AVANCE™ III HD-400, equipped with a CryoProbe™ Prodigy or on a Bruker DRX-600 equipped with TXI CryoProbe™ in $CDCl_3$ (δ_H values reported refer to $C\underline{H}Cl_3$ protons at 7.26; δ_C values refer to $\underline{C}DCl_3$ carbon at 77.0 ppm). High resolution mass spectra were acquired on a Q-Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Milan, Italy). GC-MS analyses were performed on an ion-trap MS instrument in EI mode (70eV) (Thermo, Polaris Q) connected with a GC system (Thermo, GCQ) by a 5% phenyl/methyl polysiloxane column (30 m x 0.25 mm x 0.25 µm, Agilent, VF-5ms) using Helium as gas carrier. HPLC analyses were performed on a Shimadzu high-performance liquid chromatography system (Shimadzu, Milan, Italy) equipped with binary LC-20AD pumps in line with a Diode Array Detector SPD-M20A. TLC plates (KieselGel 60 F254) and silica gel powder (Kieselgel 60 0.063-0.200 mm) were from Merck (Darmstadt, Germany). Chemicals were of analytical reagent grade and solvents of HPLC/LCMS grade (Sigma-Aldrich, Milan, Italy) and were used without any further purification.

2.4. Metabolomic analysis

Ca 100 mg of each freeze-dried study specimen were extracted with dichloromethane (DCM) (3 x 10 ml). Phytyl acetate (80 µg) was added as an internal standard before the first extraction. Combined extracts were concentrated under N_2 stream and redissolved in DCM (500 µl) for GC-MS analysis. The following temperature gradient was applied: initial 160°C holding for 3 min, then increase of 3°C min^{-1} up to 260°C followed by 30°C min^{-1} up to 310°C, holding for 3 min at 310°C; split flow 10 ml min^{-1}; Transfer line T= 280°C; Inlet T=290°C; Ion source T=250°C; full scan *m/z* 50 – 450. Injection of 2 µl. Analytical runs were processed by using Xcalibur software (vers. 2.2 SP1.48) (Thermo-Scientific). All samples were analyzed in duplicate.

2.5. Isolation and characterization of diterpenes from D. antarctica.

Three freeze-dried specimens (35.6 g) of *D. antarctica* collected in Livingston Island, were pooled together and extracted with methanol (MeOH) (6 x 400 ml); the raw extract (16.1 g) was redissolved in MeOH/H_2O 9:1 and partitioned with *n*-hexane (4 x 100 ml) following the Kupchan method (Kupchan et al., 1973). The *n*-hexane extract (1.9 g) was fractionated by silica chromatography on column eluted with a gradient of solvents from 100% petroleum ether (PE) to 100% diethyl ether (EE). Fraction PDC19l eluting with PE/EE 9:1 afforded pure 9,11-dihydrogracilin A (**1**) (10.1 mg).

Twelve freeze-dried specimens (30 g) collected in Deception Island were pooled together and extracted with DCM (1 x 200 ml, 6 x 300 ml); the raw extract (1.4 g) was fractionated by silica chromatography on column with gradient elution from 100% PE to 100% EE. The fractions eluted by PE/EE 9:1 were further fractionated by normal phase-high performance liquid chromatography (NP-HPLC) (Phenomenex Luna Silica column (250 x 4.6 mm, 5 µm), flow rate 1 ml min^{-1}) with PDA detector; for the elution *n*-hexane (A) and *n*-hexane:isopropanol (97:3) (B) were used applying an isocratic method: initial 60% A and 40% B holding for 30 min. Peak collected at Rt 8 min was analyzed by MS and NMR and identified as **3** (0.7 mg) by comparison with literature data.

The fractions eluted by PE/EE 1:1 were further fractionated by normal phase-high performance liquid chromatography (NP-HPLC) (Kromasil KR100-5-Sil column (250 x 10 mm, 5 µm), flow rate 3.5 ml min^{-1}) with PDA detector; for the elution *n*-hexane (A) and *n*-hexane:isopropanol (97:3) (B) were used applying the following gradient: initial 60% A and 40% B holding for 35 min, followed by increase to 100% B in 15 min, then returning to original conditions in one min; a re-equilibration

time of 9 min was included between runs. Peaks collected at Rt 25, 31 and 42 min were analyzed by MS and NMR and identified as **2** (0.6 mg), tetrahydroaplysulphurin-1 (**6**) (0.4 mg) and membranolide (**4**) (1.2 mg) by comparison with literature data.

The fractions eluted by PE/EE 8:2 were further purified by silica chromatography on column and then on NP-HPLC-PDA by using *n*-hexane (A) and *n*-hexane:isopropanol (97:3) (B) (Kromasil KR100-5-Sil column (250 x 10 mm, 5 µm), flow rate 3.5 ml min^{-1}) with PDA detector; for the elution *n*-hexane (A) and *n*-hexane:isopropanol (97:3) (B) were used applying the following gradient: initial 80% A and 20% B, then increase to 30% B in 35 min, followed by increase to 65% B in 7.5 min, then returning to original conditions in one min; a re-equilibration time of 6.5 min was included between runs. Peaks collected at Rt 15 and 20 min were analyzed by MS and NMR and identified as aplysulphurin (**5**) (4.1 mg) and deceptionin (**7**) (1.9 mg) by comparison with literature data.

2.6. Deceptionin (7): $[\alpha]_D$ -7.38° (c 0.05, MeOH); IR (film) ν_{max} = 2929, 2851, 1739, 1561, 1454, 1367, 1234, 1084, 1006, 933 cm^{-1}; UV λ_{max} (ε) =206 (3409); HRESIMS$^+$: m/z 415.24348 $[M+Na]^+$ $C_{23}H_{36}O_5Na^+$ (theor. 415.24550). NMR data see **Table 1**.

2.7. Data analysis

The diterpenes were quantified using the internal standard phytyl acetate. The µg of each metabolite was calculated from the peak area, and then normalized by the sponge sample dry weight. Transformation of the data using fourth root was applied to the natural habitat samples subset to achieve homoscedasticity. Untransformed data was used for the heat stress and predation pressure experimental samples. Each subset of samples was standardized. Euclidean matrix distance was generated after a fourth root transformation was applied to the natural habitat data set, and a dendrogram was plotted with samples clustered using the complete method. Principal Components Analysis and Permutational Analysis of Variance (PERMANOVA) were performed for the three subsets of samples: natural habitat samples, heat stress experiment samples, and predation pressure experiment samples. Analysis of variance (ANOVA) was performed for each compound on the three subsets of samples. Wilcoxon pair-wise tests were used for comparisons between the control group and the experimental group.

Statistical tests were performed using R (version 4.2.0).

2.8. Antimicrobial assays

The Kirby-Bauer disk diffusion susceptibility test was used to assess the potential antimicrobial activity of some of the isolated diterpenoids of *D. antarctica*. The metabolites 9,11-dihydrogracilin A (**1**), membranolide (**4**), aplysulphurin (**5**), tetrahydroaplysulphurin-1 (**6**), and deceptionin (**7**) were tested against the human pathogens *E. coli* O157:H7 (ATCC 43888) and *S. aureus* (ATCC 9144). Two hundred µg of each compound were loaded on 6-mm paper disks in a volume of 20 µl of chloroform, which saturated the disk. Pure cultures of the bacterial strains were grown on TSA plates for 24 h. Then, isolated colonies were suspended using a sterile cotton swab in a NaCl 0.85% solution until reaching the turbidity of a McFarland 0.5 standard. A spread culture in Mueller-Hinton agar plates was prepared from this solution using a sterile cotton swab. Disks were placed on the seeded plates and plates were incubated at 37°C. A chloramphenicol disk (10 ug) was used as a positive control. An unloaded disk and a disk loaded only with 20 µl of the solvent were used as negative controls. The test was performed in duplicate. After 24 h, activity was checked observing the halo of growth inhibition around the disks. When there was a halo, the diameter was measured. Growth inhibition activity was classified following Mahon and co-workers criteria (Mahon et al., 2003).

3. Results

3.1. Major diterpenes in *Dendrilla antarctica* populations from two different localities

We analyzed the chemical profile of *Dendrilla antarctica* specimens collected from Deception (n=5) and Livingston (n=13) Islands, in the South Shetland Archipelago, Antarctica. Data from Gas Chromatography-Mass Spectrometry (GC-MS) revealed seven most prominent peaks in the extracts, putatively identified as terpenes. The identity of six of them was confirmed after their purification from the raw extract as 9,11-dihydrogracilin A (**1**) (Molinski & Faulkner 1987) (Rt=25.86) and the gracilane norditerpene **2** (Díaz-Marrero et al. 2004) (Rt=24.21), the glaciolane norditerpene **3** (Mayol et al. 1988) (Rt=27.89), and three aplysulphurane derivatives, membranolide (**4**) (Molinski & Faulkner 1987) (Rt=29.14), aplysulphurin (**5**) (Karuso et al. 1984) (Rt=35.34) and tetrahydroaplysulphurin-1 (**6**) (Karuso et al. 1986) (Rt=35.51) (**Figure 2**). The spectroscopic data of the remaining compound did not match with any of the known terpenes and was characterized as

a new aplysulphurane derivative especially abundant in the specimens from Deception Island, which therefore we named deceptionin (Rt=20.2) (**7**, **Figure 3**).

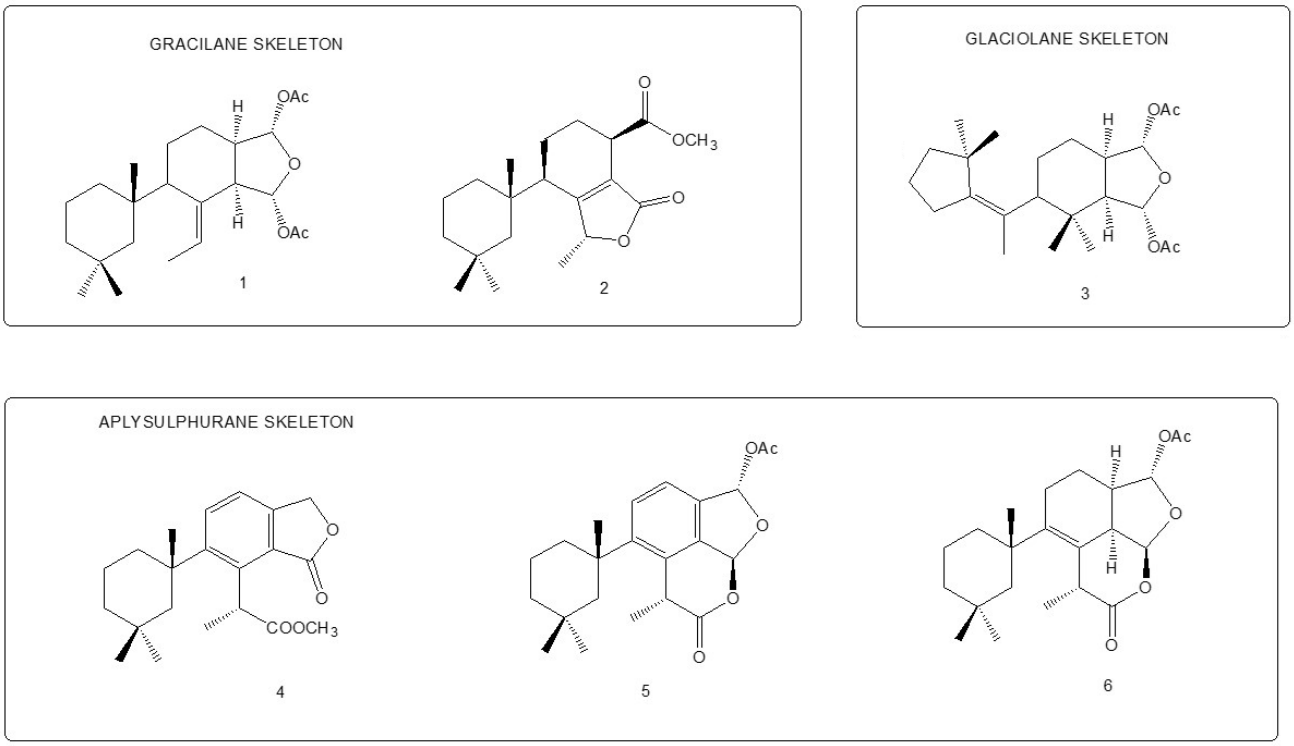


Figure 2. Diterpene metabolites identified in *Dendrilla antarctica* in this study.

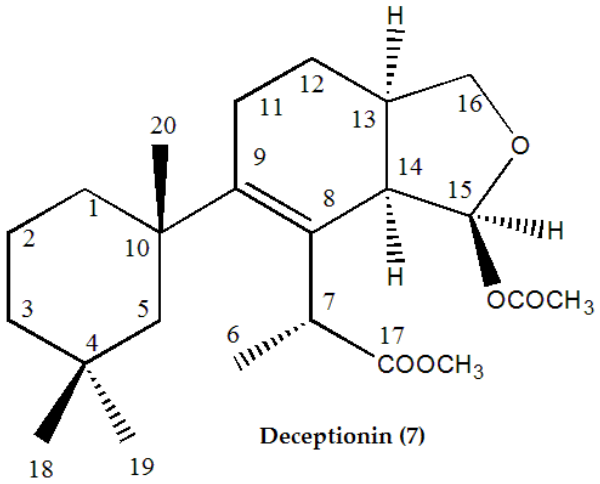


Figure 3. Chemical structure of deceptionin (**7**).

3.2. Structural characterization of deceptionin (7).

Deceptionin (**7**) gave a molecular ion adduct $[M+Na]^+$ in HRESIMS at *m/z* 415.24348 which was consistent with the molecular formula $C_{23}H_{36}O_5Na^+$ (theor. 415.24550) requiring six degrees of unsaturation. The ^{13}C NMR spectrum showed four resonances attributable to sp^2 carbons. In particular, quaternary carbon signals at d 128.8 and 146.0 ppm accounted for a tetra-substituted non conjugated double bond (C-8/C-9) while two downfield shifted signals at d 174.9 and 170.6 ppm were assigned to carbonyl ester groups, in agreement with the stretching band observed in the FT-IR spectrum at 1739.48 cm^{-1}. In particular, the loss of 60 (AcOH) observed in ESIMS/MS spectra of the molecular ion suggested the presence of an acetyl group while a deep interpretation of the GCMS spectrum allowed to infer the occurrence of a methoxycarbonyl function due to consecutive losses of MeOH and CO from the M-Me-AcOH fragment at *m/z* 317.12 (Supporting material). Hence, the three remaining formal unsaturations had to be assigned to cycles. A diagnostic methine carbon at 103.5 ppm (C-15), bearing a proton resonating as doublet at d 5.87 (*J*=2.4 Hz) in the ^1H NMR spectrum, was indicative of an acetal function which fulfilled the number of oxygens required by the molecular formula. Starting from this latter proton, COSY and TOCSY correlations allowed to easily depict the spin system of the bicyclic substructure including a cyclohexene ring fused with a cyclic acetal (Figure xxx). In fact, the acetal proton (H-15) was coupled to an allylic proton at 2.91 (dd, J= 8.1,2.4 Hz, H-14) in turn coupled with a methine at 2.41 (H-13). This latter signal showed cross-peaks in the COSY spectrum with one of the two protons of the oxygenated methylene C16 at d 4.09 (dd, J= 8.6, 5.6 Hz) and the proton signal at d 1.26 of the methylene at C-12. The methylene H_2-12 was coupled with the allylic methylene at C-11; thus, with the support of HSQC and HMBC data the planar structure of the bicyclic system was unambiguously assigned. The linkage with the remaining cycle was secured by the HMBC correlation of the singlet methyl resonance at d 1.08 with the olefinic carbon at 146.0 through the quaternary carbon at 40.5 ppm. The cyclohexane ring bearing two geminal methyl groups at d 0.88 and 0.89 on C-4 was easily assigned by combining homo and heteronuclear correlation data. Finally, a bis-allylic methine quartet at d 4.22 (*J*=6.9 Hz) coupled to a methyl group at d 1.24, connected the carboxymethyl function to the olefinic carbon at 128.8 ppm thus completing the elucidation of the aplysulphuran skeleton of the new diterpene molecule. Spectroscopic signals of this metabolite resulted highly overlapping to the methyl ester derivative obtained from the Pourewic acid A (Keyzers, EurJOC 2004) that are listed in Table 2 for comparative purposes from which it differed having an acetoxy instead of a methoxy acetal system. Also relative stereochemistry, determined by measuring vicinal coupling constants and inspecting nuclear

Overhauser effects (nOe) in NOESY experiments, was suggested to be the same assigned for this derivative and the tetrahydroaplysulphurin-1 (Karuso, 1986; Buckleton 1987); in particular, diagnostic nOe correlations were observed between the acetal proton H-15 (d 5.88) and the methine H-14 and between this latter with H-13 thus indicating that they lay from the same face of the molecular plane; on the other hand, a clear cross-peak was observed between the bis-allylic proton H-7 and the methyl moiety at d 1.07 (H_3-20) as well as between the methyl group at d 1.24 (H_3-6) and the proton at d 2.91 (H-14), thus securing the relative stereochemistry as depicted in **Figure 3** for deceptionin (**7**).

Table 1. ^{1}H and ^{13}C NMR data of deceptionin (**7**). (400 MHz, $CDCl_3$). Spectroscopic data of methyl pourewate A are reported as a comparison from reference (Keyzers 2004).

Position	**Deceptionin**		**Methyl Pourewate A**	
	^{1}H, d, mult, *J* (Hz)	^{13}C, ppm	^{1}H, d, mult, *J* (Hz)	^{13}C, ppm
1	1.19, m; 2.09, m	39.1	1.26, m; 2.18, m	39.2
2	1.49, m; 1.80, m	20.1	1.49, m; 1.94, m	20.0

3		1.21, m; 1.33, m	40.0		1.18, m; 1.38, m	40.1	
4		-	31.6		-	31.6	
5		1.06, m; 1.77, d, 13.5	50.7		0.97, m; 1.84, m	50.9	
6		1.24, d, 6.9	15.9		1.20, d, 6.8	16.1	
7		4.22, q, 6.9	41.9		4.18, q, 4.4	42.0	
8		-	128.8		-	128.2	
9		-	146.0		-	143.9	
10		-	41.5		-	41.6	
11		1.94 (ax), ddd, 16.0, 14.0, 4.2; 2.23 (eq), ddd, 16.0, 4.2, 4.2	27.4		1.86, m; 2.20, m	27.7	

12		1.26 (ax), m; 1.64 (eq), dq, 13.0, 4.2	30.2		1.24, m; 1.65, m	31.2	
13		2.41, m	37.8		2.34, q, 7.6	38.0	
14		2.91, dd, 8.1, 2.5	47.1		2.65, dd, 8.1, 2.4	49.0	
15		5.87, d, 2.5	103.5		4.62, d, 2.4	110.8	
16		3.85, d, 8.6; 4.09, dd, 8.6, 5.6	76.0		3.76, dd, 8.8, 3.4; 4.02, dd, 8.5, 6.4		
17		-	175.0		-	174.7	
18		0.89, s	27.2		0.92	26.6	
19		0.88, s	32.6		0.88	33.3	
20		1.08, s	30.4		1.02	30.9	
COCH_3		-	170.6				

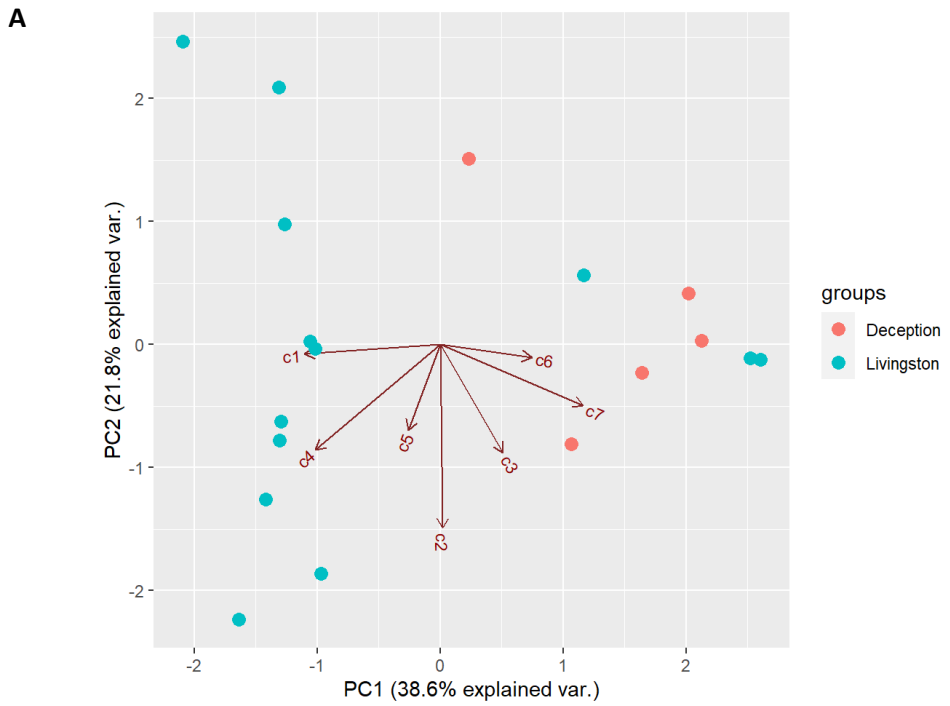
CO*CH₃*		2.04, s	21.5				
OCH_3		3.59, s	50.8		3.67	51.7	

3.3. Terpene profile of sponges at their natural habitat

The results indicate that there is a high variability in the chemical profiles of the *Dendrilla antarctica* specimens analyzed, as observed in standard deviation values for natural habitat samples (**Table 2**). PCA extracted three components with an eigenvalue >1, which explained 80.2% of the overall variability in the metabolites profiles. The first component (PC1) explained 38.6% of the overall variability in the metabolites profiles and mostly opposed the abundance of **7** (positive value) to that of **1** and **4** (negative values). The second component (PC2) explained 21.8% of the overall variability in the metabolites profiles and was influenced by the abundance of **2** (negative value). The third component (PC3) explained 19.8% of the overall variability in the metabolites profiles and mostly opposed the abundance of **5** (positive value) to that of **3** (negative value). PCA revealed that the sponge specimens separated in the biplot space according to the island of origin. In general, sponge specimens from Deception Island were enriched in **7** whereas those from Livingston Island were enriched in **1** and **4** (**Figure 4**). There were three specimens from Livingston Island that grouped with Deception Island sponges, and one specimen from Deception Island that was enriched in **5** and depleted in 2 and **3**. PERMANOVA revealed the chemical profiles of the two groups of sponges were significantly different ($p<0.05$) between islands. Univariate tests revealed that the concentrations of **1** and **7** were significantly different between the two groups of sponges (Mann-Whitney-Wilcoxon test; $p<0.05$). Also, total terpenes concentration was significantly higher on average in samples from Livingston (6.18±3.61 mg/g dw) than in samples from Deception Island (2.48±0.75 mg/g dw) ($p<0.05$).

Table 2. Concentration values of the main terpene derivatives **1-7** in *D. antarctica* specimens. Values are represented as mean±sd (mg/g dw). CT=control temperature, HST= heat stress temperature, EHST= extreme heat stress temperature.

	1	2	3	4	5	6	7
Natural habitat							
Deception Island	0.00±0.01	0.13±0.10	0.14±0.12	0.92±0.98	0.10±0.18	0.01±0.03	1.16±0.30
Livingston Island	2.39±2.00	0.29±0.23	0.12±0.10	2.99±2.76	0.03±0.07	0.07±0.16	0.30±0.42
Heat stress experiment							
CT	0.02±0.03	0.05±0.06	0.20±0.11	1.44±0.85	0.55±0.38	0.15±0.14	1.04±0.53
HST	0.01±0.01	0.17±0.19	0.20±0.09	1.29±1.53	0.67±0.92	0.35±0.24	1.57±0.49
EHST	0.00±0.00	0.07±0.09	0.12±0.10	0.45±0.39	0.01±0.02	0.41±0.54	0.79±0.62
Predation experiment							
Macropredation	0.04±0.04	0.14±0.11	0.19±0.06	1.34±0.51	0.35±0.40	0.12±0.18	1.32±0.15
Micropredation	0.02±0.03	0.06±0.08	0.21±0.04	0.81±0.84	0.21±0.34	0.22±0.28	1.28±0.43



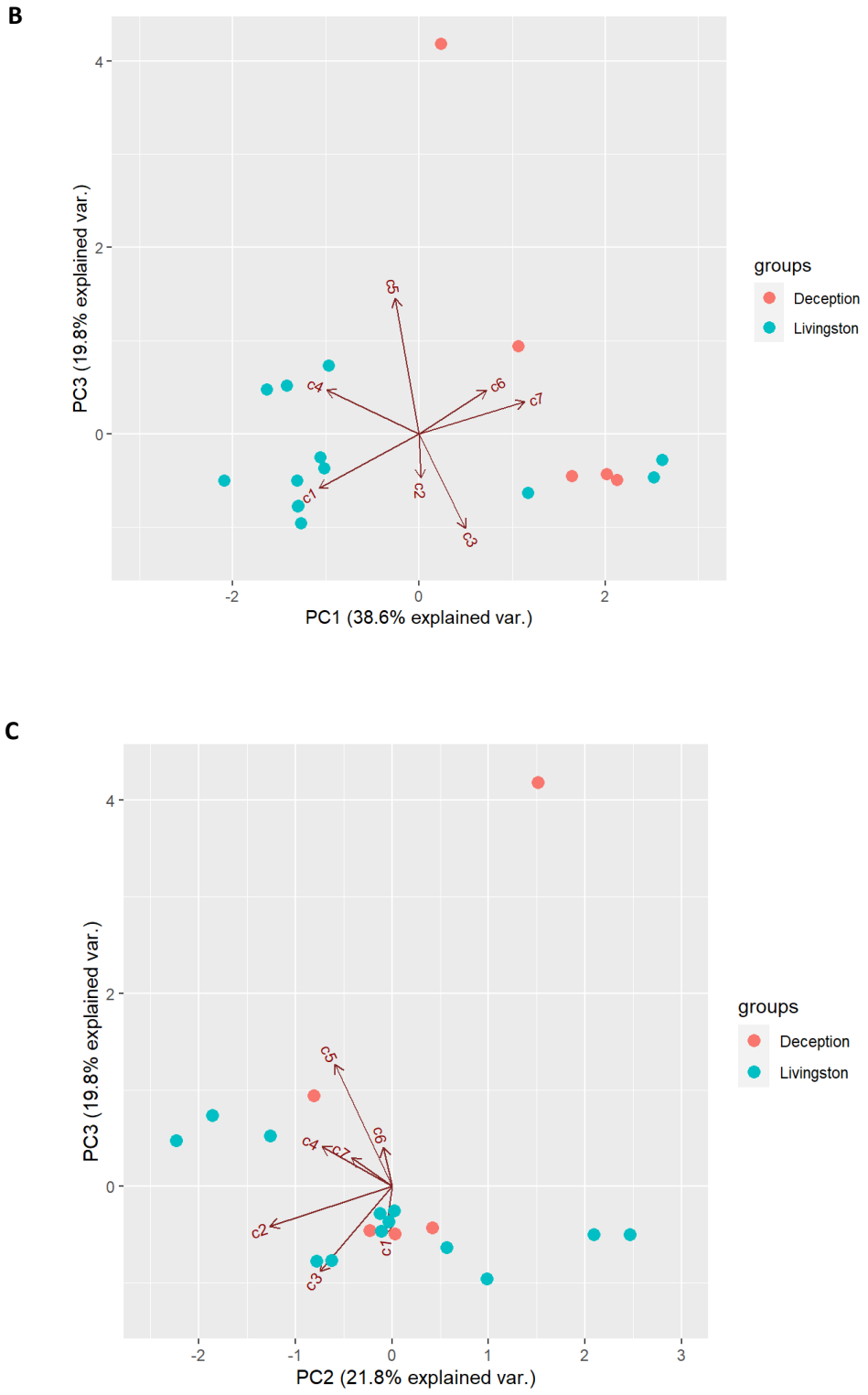


Figure 4. Biplots of the first three Principal Components extracted in the PCA of the samples from the natural habitat. **A.** PC1 vs. PC2. **B.** PC1 vs. PC3. **C.** PC2 vs. PC3. Deception=samples from Deception Island, Livingston=samples from Livingston Island. c(**1-7**)=compounds analyzed.

3.4. Chemotyping

The relative abundance and dominance of the 7 major metabolites within individual samples indicates that there are chemically distinct phenotypes within the *D. antarctica* populations of Deception and Livingston Islands. The samples were clustered according to the abundance of the metabolites and six chemotypes were identified in the natural habitat sponges (**Figure 5**). All Livingston Island samples but three were clustered into three different chemotypes (chemotypes 4 to 6). Chemotype 4 was characterized by dominance of **1** over all other metabolites (**Figure 6**). Chemotypes 5 and 6 were characterized by dominance of **4** and, to a lesser extent, **1**, but chemotype 6 presented metabolites **5** and **6**, virtually absent in chemotype 5. The other three Livingston samples clustered with Deception Island samples in chemotypes 2 and 3. Chemotypes 1, 2, and 3 were characterized by higher abundance of **7**, almost absent in chemotypes 4-6. One natural habitat sample from Deception conformed chemotype 1, since it had only **4**, **5**, and **7** and in different proportions than the other samples. The main difference between chemotypes 2 and 3 is the presence of **6** in chemotype 2, absent in chemotype 3 (**Figure 7**).

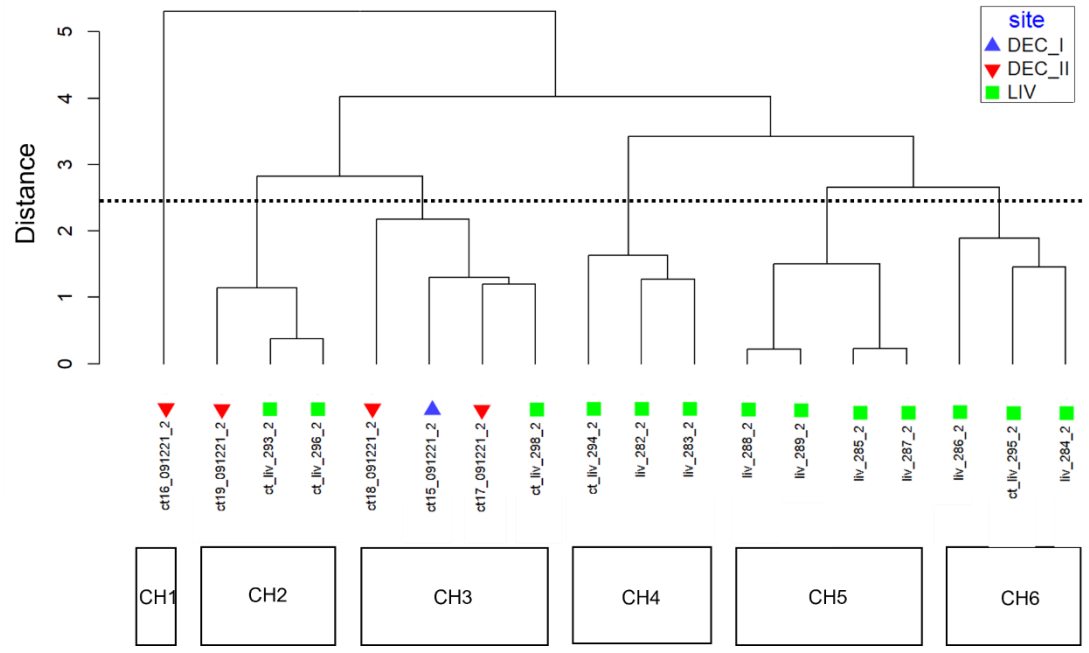


Figure 5. Chemotype clustering of the natural habitat samples. CH=Chemotype. DEC-I, DEC-II=Sampling sites in Deception Island. LIV=Sampling site in Livingston Island.

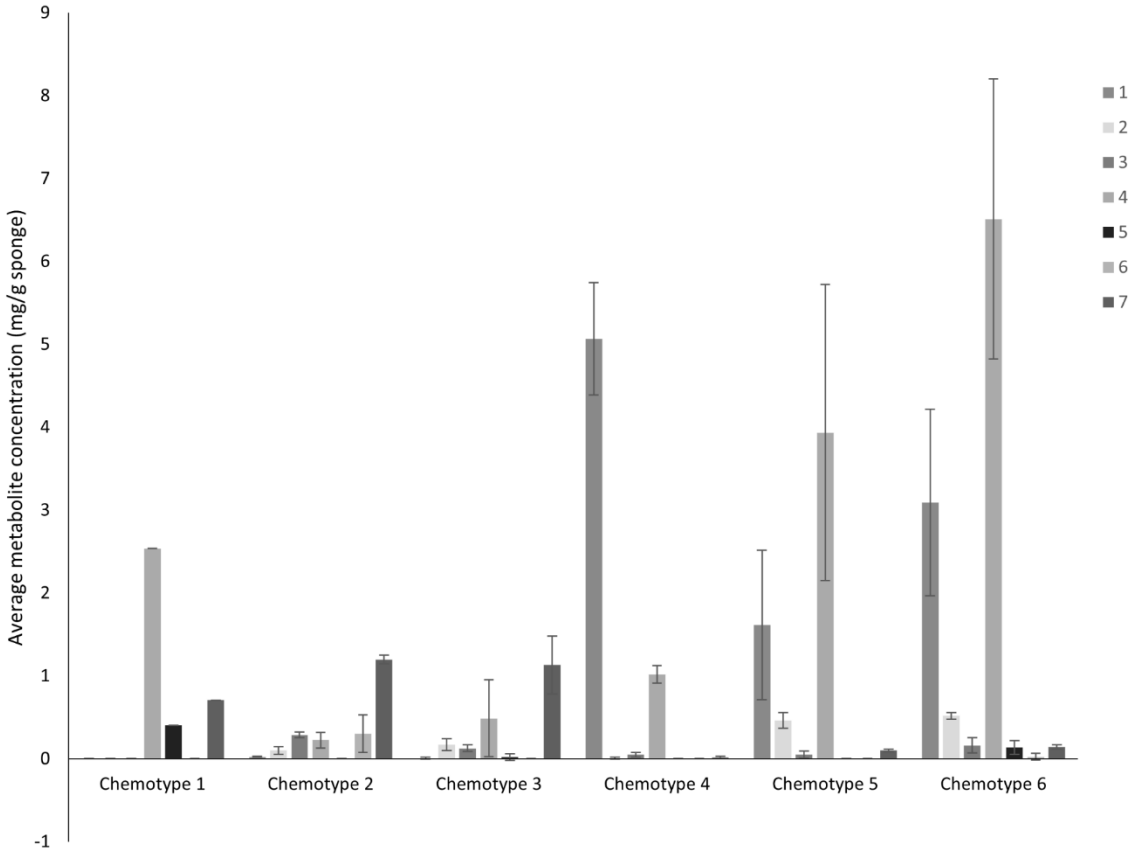


Figure 6. Average concentration of the seven most abundant diterpenes **1-7** from the natural habitat *D. antarctica* for each chemotype. IS= phytyl acetate.

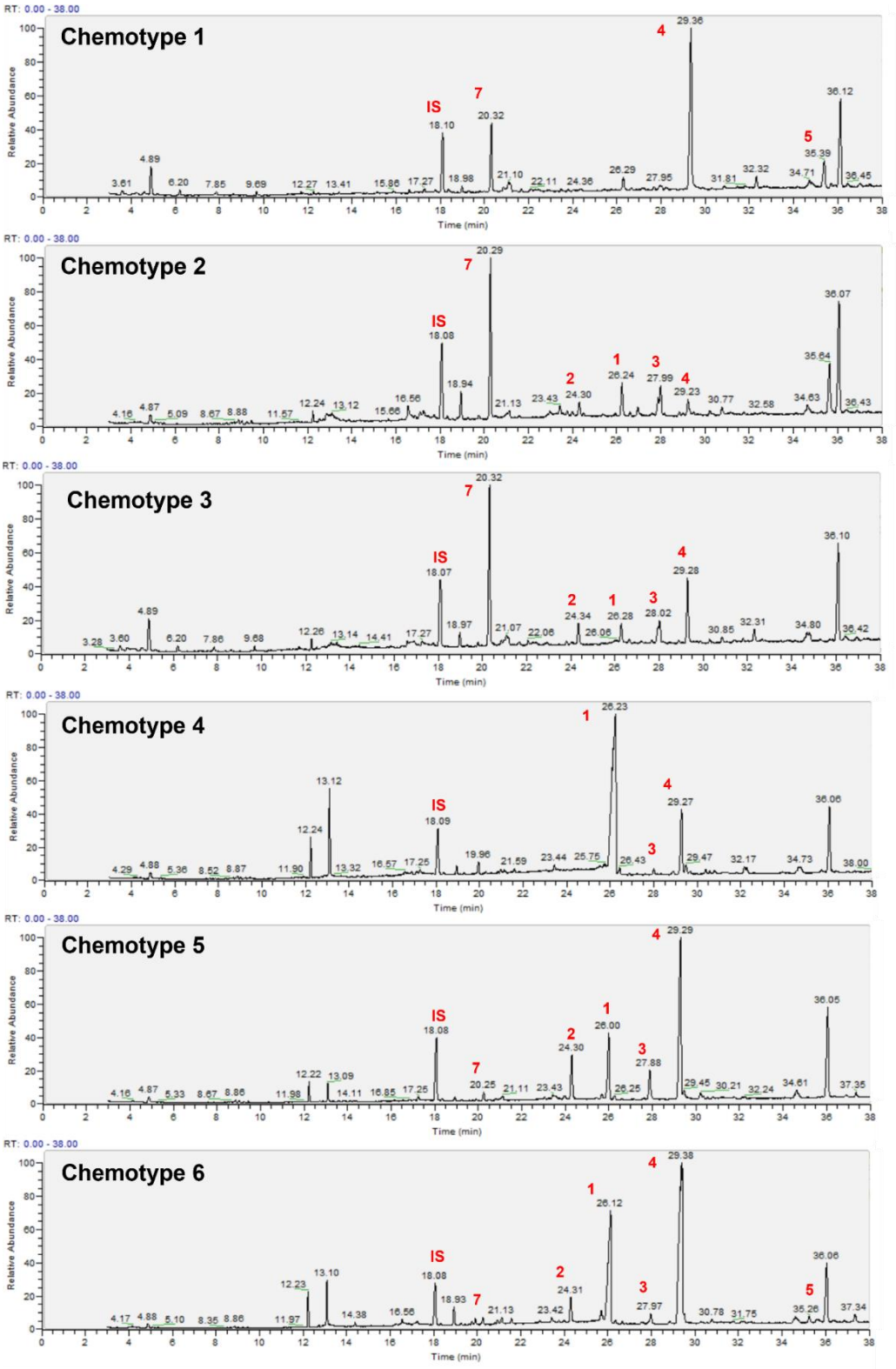


Figure 7. Representative chemical profiles of each chemotype for the natural habitat samples of *D. antarctica*. Main terpene derivatives **1-7** are reported. IS=Internal Standard.

3.5. Effect of experimental heat stress and predation pressure on the secondary metabolites of *Dendrilla antarctica*

There was an effect of the temperature in the natural products of *D. antarctica.* PCA extracted two components with an eigenvalue > 1, which explained 66% of the overall variability in the metabolites profiles (**Figure 8**). The first component (PC1) explained 35.7% of the overall variability in the metabolites profiles and mostly opposed the abundance of **4** and **5** (negative values) to that of **2** and **7** (positive values). The second component (PC2) explained 30.3% of the overall variability in the metabolites profiles and was influenced by the abundance of **1** and **3** (negative values). The samples did not seem to follow any pattern in the biplot space, and PERMANOVA revealed that the chemical profile was not significantly different between the experimental groups ($p>0.05$). Univariate ANOVAs revealed no significant difference in concentration of any of the seven compounds between the experimental groups ($p>0.05$). Pair-wise tests of each compound revealed a significant difference in the concentration of **6** between the control and the HST group ($p<0.05$). Also, total terpenes concentration was higher on average in the HST group (4.25±2.03 mg/g dw) than in the natural habitat group (2.48±0.75 mg/g dw), although the difference was not statistically significant ($p>0.05$).

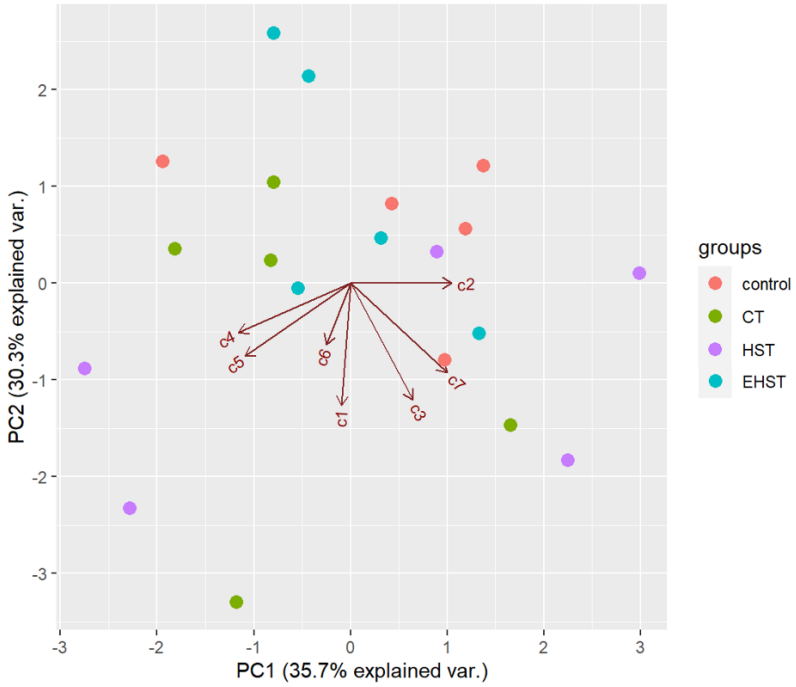
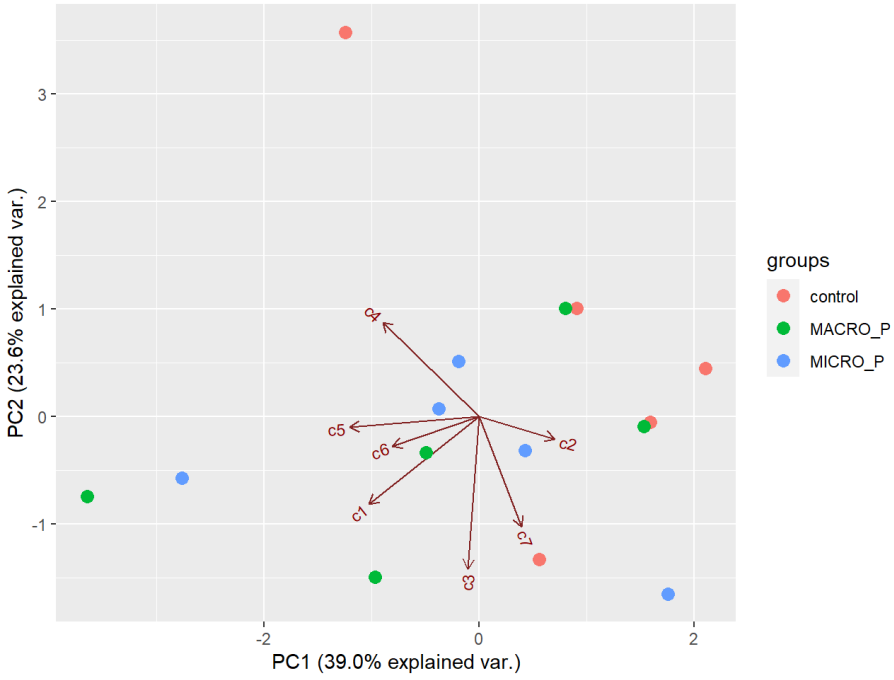


Figure 8. Biplot of the PCA of the samples from the heat stress experiment. control=natural habitat samples (Deception Island), CT=control temperature, HST= heat stress temperature, EHST= extreme heat stress temperature. c(**1-7**)=compounds analyzed.

There was no effect of predation in the natural products of *D. antarctica.* PCA extracted three components with an eigenvalue > 1, which explained 76.9% of the overall variability in the metabolites profiles (**Figure 9**). The first component (PC1) explained 39% of the overall variability in the metabolites profiles and was influenced by the abundance of **1** and **5** (negative values). The second component (PC2) explained 23.6% of the overall variability in the metabolites profiles and was influenced by **3** and **7** (negative values). The third component (PC3) explained 14.3% of the overall variability in the metabolites profiles and mostly opposed the abundance of **6** (positive value) to that of **2** (negative value). The samples did not seem to follow any pattern in the biplot space, and PERMANOVA revealed that the chemical profile was not significantly different between the experimental groups ($p>0.05$). Univariate ANOVAs revealed no significant differences in concentration of any of the seven compounds between the experimental groups ($p>0.05$). Pair-wise tests of each compound neither revealed significant difference in the concentration of the individual compounds between the control and the macro-predation group, nor between the control and the micro-predation group ($p>0.05$). However, concentration of metabolite **1** was more abundant in the macropredation samples than in the natural habitat samples, although the difference was not statistically significant ($p=0.075$). Also, total terpenes concentration was higher on average in the macropredation samples (3.52±0.91 mg/g dw) than in the natural habitat group (2.48±0.75 mg/g dw), although the difference was not statistically significant ($p=0.085$).

A



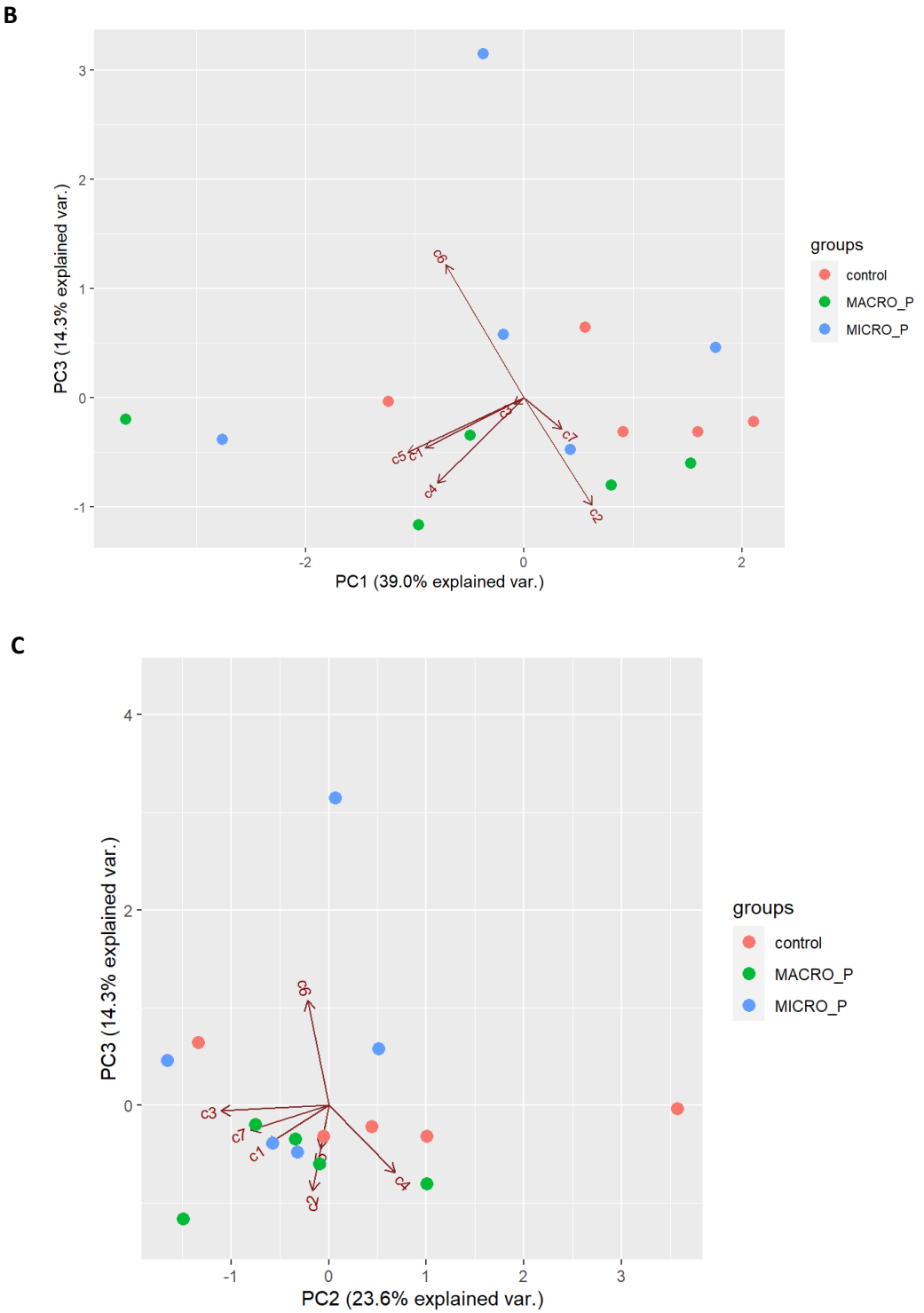


Figure 9. Biplot space of the PCA of the samples from the predation pressure experiment. **A.** PC1 vs. PC2. **B.** PC1 vs. PC3. **C.** PC2 vs. PC3. control=natural habitat samples (Deception Island), MACRO_P=samples exposed to macropredation, MICRO-P=samples exposed to micropredation. c(**1-7**)=compounds analyzed.

3.6. Antimicrobial assays

Membranolide (**4**) showed antimicrobial activity against *E. coli* O157:H7 at 200 µg, presenting a zone of inhibition of 10 mm in the two replicates performed. Following Mahon and co-workers criteria, this could be considered as "strong" (+++) growth inhibition (Mahon et al., 2003). The other compounds tested did not show any activity against the bacterial strains tested.

4. Discussion

Benthic invertebrates in general, and sponges in particular, are a rich source of bioactive molecules that could serve as base for developing new drugs or have an industrial application. The samples of *Dendrilla antarctica* contained seven major diterpenes, one of which was characterized as a new aplysulphurane derivative, deceptionin (**7**). Further antimicrobial assays using different pathogenic strains, as well as cytotoxicity and other bioactivity tests should be done to search for a potential bioactivity of the compound.

The diterpenes profile was highly variable among the samples, even in the samples collected from the same sampling site. Not only all seven metabolites were not present at all the samples, but also the concentrations and proportions among compounds varied. This variability in the chemical profile between individuals of the same species is not uncommon (Puyana et al., 2003; Cutignano et al., 2011; Rohde et al., 2012), and was already reported for *D. antarctica* populations around Palmer Station (Anvers Island) (von Salm et al., 2022). Von Salm and co-workers also found high variability in the terpenes concentration even between replicates from the same sampling site (von Salm et al., 2022). Despite the high interindividual variability, in general the samples grouped according to the island of origin. There were two main differences in the diterpenes profile between the sponges from Livingston and Deception Island. One of them was the considerably higher concentration of total terpenes in the sponges from Livingston Island than in the sponges from Deception Island. The second one was the concentration of two of the major diterpenes: 9,11-dihydrogracilin A (**1**), more abundant in Livingston Island samples, and deceptionin (**7**), more abundant in Deception Island samples, while almost absent in Livingston Island samples.

Although both collection sites are shallow rocky bottoms harboring relatively similar benthic communities, there are environmental factors that may be playing a role in *D. antarctica* chemical profile: waters of Port Foster (the inner bay and caldera of the volcanic Deception Island) have

higher water temperature (Ortiz et al., 1992), presence of suspended volcaniclastic particles (Smith et al., 2003) and chemicals from local geothermal activity (Elderfield, 1972; Deheyn et al., 2005). We could expect that sponges exposed to higher environmental pressures, presumably those from Deception Island, would have higher concentration of defensive compounds (Puglisi et al., 2019). However, regarding total terpenes concentration, we observed significantly higher concentration of compounds in Livingston than in Deception Island. This could perhaps be related to a higher predation pressure in Livingston Island, which should be further analyzed. The higher presence of deceptionin (**7**) in *D. antarctica* from Deception Island could be a response to a particular stress characteristic of the environment of Deception Island coasts.

The populations of *D. antarctica* analyzed so far are not chemically homogenous around the continent. In McMurdo Sound, *D. antarctica* was reported to yield 9,11-dihydrogracilin A (**1**) and membranolide (**4**) (Molinski and Faulkner 1987), and also dendrillin in a later study (Baker et al., 1995). The ecological role of 9,11-dihydrogracilin A (**1**) has not been reported yet. It has been suggested to play a role against predators found in areas not dominated by macroalgae and thus with less abundance of amphipods (Shilling, 2019) Specimens from Terra Nova Bay, instead, presented only 9,11-dihydrogracilin (**1**) and dendrinolide (Fontana et al., 1997). Populations from around Palmer Station (Western Antarctic Peninsula) have been studied several times, with reports including aplysulphurin (**5**), tetrahydroaplysulphurin-1 (**6**), and membranolide (**4**) as the major diterpenoids (Ankisetty et al., 2004a; von Salm et al., 2022), another study including also darwinolide (von Salm et al., 2016), and a more recent analysis identifying up to eleven diterpene derivatives, the major constituents being 9,11-dihydrogracilin A (**1**), membranolide (**4**), aplysulphurin (**5**), and tetrahydroaplysulphurin-1 (**6**), and the minor constituents glaciolide, the norditerpene **3**, cadlinolide C, and dendrillins A-D (Bory et al., 2020). To our knowledge, just one study considered the effect of location on the chemical diversity of *D. antarctica*, but they found no significant effect. Sampling locations were all within a small area around Palmer Station (von Salm et al., 2022). From the evidence so far, we could suggest that there is not a single diterpenoid derivative found in every studied population of the sponge. However, it seems that some compounds are found in the two main regions studied (the Ross Sea and the Western Antarctic Peninsula), like 9,11-dihydrogracilin A (**1**) and membranolide (**4**), while aplysulphurin (**5**), tetrahydroaplysulphurin-1 (**6**) and darwinolide are only found in *D. antarctica* from the Antarctic Peninsula, and dendrinolide was only reported in McMurdo Sound (Ross Sea). However, further studies on the influence of location on the chemical profile of the sponge should be done. Also, the same populations collected around Palmer Station

(Anvers Island, Western Antarctic Peninsula) showed differential diterpenoids profile depending on the year of collection (Shilling et al., 2020).

Metabolic and genetic studies of some Antarctic benthic species, such as the rhodophyte *Plocamium cartilagineum* (Young et al., 2013; Shilling et al., 2021) and the nudibranch *Doris kerguelenensis* (Wilson et al., 2013) have shown significant metabolic variations between specimens mostly corresponding to different phylogroups. Cycles of glaciation have isolated regions, allowing independent divergence among individuals of the same species, which could lead to speciation, as shown for *Doris kerguelenensis* (Maroni et al., 2022). High genetic connectivity and subsequent homogeneity was found for *D. antarctica* populations from different locations off the South Shetland Islands and Northern Antarctic Peninsula (Leiva et al., 2019). However, signs of local adaptation were found for the samples of Deception Island, showing activation of genes mostly involved in immune and stress responses. This was associated to the physicochemical particularities of the island as an active volcano, with higher temperatures and distinct environmental conditions than the surrounding islands. Specimens from Livingston Island were not analyzed in this genetic study.

Significant differences between *D. antarctica* collections or populations led Von Salm and co-workers to hypothesize that selective predation pressures may be driving its chemical diversity (von Salm et al., 2022). These authors found different diterpene profiles in *D. antarctica* in the different habitats where they lived: shallower zones dominated by canopy-forming macroalgae and with abundance in omnivorous amphipods harbored *D. antarctica* that had more concentration of tetrahydroaplysulphurin-1 (**6**) than those collected from deeper, more exposed, habitats depleted in amphipods. In our study, the collection sites in both Islands are similar shallow rocky bottoms dominated by similar species. However, Deception Island is a very particular place with peculiar shallow water communities (Angulo-Preckler et al., 2018a). The bottoms of this island are covered by volcanic pyroclasts, with a huge abundance of echinoderms, and with some rocky areas fully covered by filter-feeding organisms (Angulo-Preckler et al., 2017). This particular environment may have driven the selection and evolution of the natural metabolites production abilities present there.

Methanolic extracts of *D. antarctica* showed chemotactic food response in the spongivorous seastar *Perknaster fuscus* (Mcclintock, 1994; Baker et al., 1995) and lipophilic extracts showed deterrence on the omnivorous amphipod *Gondogeneia antarctica* in feeding deterrence assays (Amsler et al.,

2009). However, which molecules are responsible for this activity is yet to be discovered. Membranolide (**4**) deterred the amphipod *G. antarctica* (Maschek, 2011), but was not found in higher concentrations in the sponges from the high amphipod abundance habitat (von Salm et al., 2022). In our study, we did not find any significant pattern of the effect of micropredation on the diterpenoids profile of *D. antarctica*. Similarly, we did not find an effect of macropredation. Exposure to predator species that may not elicit an antipredatory activity may have hindered the effect of predation pressures. Maybe the exposure to predator species that are known to be deterred by compounds produced by the sponge may elicit the chemical response of the sponge. Also, using exclusively individuals with the same chemotype would help better ascertain the potential effect of the factors tested on the chemistry of the sponge.

Although we did not find any significant changes in the chemical profile of sponges exposed to predation pressure of macro- (*Odontaster validus*) and micropredator (*Cheirimedon femoratus*) species, tetrahydroaplysulphurin-1 (**6**) was the only compound that was significantly more abundant in the samples exposed to a heat stress of around 5°C above the local seawater temperature than in the natural habitat samples from Deception Island.

Terpenes and terpenoids are a large class of natural products commonly regarded as of fungal and plant origin whose biosynthesis by bacteria is attracting increasing research interest (Yamada et al., 2015). Considering the broad distribution of terpene/terpenoid synthase genes across bacterial genomes (Yamada et al., 2015), it is tempting to argue that terpenoid biosynthesis in marine sponges could be mediated by bacterial symbionts, emerging as a further mechanism possibly conferring host defense against natural enemies or mediating microbe–microbe interactions within the sponge host.

Further characterisation of the chemical profile in *Dendrilla antarctica* from different sites should be done to keep gaining insight into the factors affecting or regulating the chemical ecology in benthic organisms. Further experiments with a larger number of samples and/or testing specimens with the same chemotype or clones would allow a better observation of the effect of these stressors, that clearly pose a risk to Antarctic communities in the frame of the current global change.

5. References

Amsler, C. D., Iken, K., Mcclintock, B., and Baker, B. J. (2009). Defenses of polar macroalgae against herbivores and biofoulers. 52, 535–545. doi: 10.1515/BOT.2009.070.

Angulo-Preckler, C., Figuerola, B., Núñez-Pons, L., Moles, J., Martín-Martín, R., Rull-Lluch, J., et al. (2018a). Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica. *Cont Shelf Res* 157, 20–31. doi: 10.1016/J.CSR.2018.02.005.

Angulo-Preckler, C., San Miguel, O., García-Aljaro, C., and Avila, C. (2018b). Antibacterial defenses and palatability of shallow-water Antarctic sponges. *Hydrobiologia* 806, 123–138. doi: 10.1007/s10750-017-3346-5.

Angulo-Preckler, C., Tuya, F., and Avila, C. (2017). Abundance and size patterns of echinoderms in coastal soft-bottoms at Deception Island (South Shetland Islands, Antarctica). *Cont Shelf Res* 134, 1–11. doi: 10.1016/j.csr.2016.12.010.

Ankisetty, S., Amsler, C. D., McClintock, J. B., and Baker, B. J. (2004a). Further membranolide diterpenes from the Antarctic sponge *Dendrilla membranosa*. *J Nat Prod* 67, 1172–1174. doi: 10.1021/np0340551.

Ankisetty, S., Nandiraju, S., Win, H., Park, Y. C., Amsler, C. D., Mcclintock, J. B., et al. (2004b). Chemical Investigation of Predator-Deterred Macroalgae from the Antarctic. *J Nat Prod* 67, 1295–1302. doi: 10.1021/np049965c.

Avila, C. (2016). Biological and chemical diversity in Antarctica: from new species to new natural products. *Biodiversity* 17, 5-11. doi: 10.1080/14888386.2016.1176957.

Avila, C., Taboada, S., and Nu, L. (2008). Antarctic marine chemical ecology: what is next? 29, 1–71. doi: 10.1111/j.1439-0485.2007.00215.x.

Baker, B. J., Yoshida, W. Y., McClintock, J. B., and Kopitzke, R. W. (1995). Chemical and ecological studies of the Antarctic sponge *Dendrilla membranosa*. *J Nat Prod* 58, 1459–1462. doi: 10.1021/np50123a020.

Barnes, D. K. A., and Bullough, L. W. (1996). Some observations on the diet and distribution of nudibranchs at Signy Island, Antarctica. *J Mollus Stud* 62, 281-287. doi: 10.1093/mollus/62.3.281

Bory, A., Shilling, A. J., Allen, J., Azhari, A., Roth, A., Shaw, L. N., et al. (2020). Bioactivity of spongian diterpenoid scaffolds from the Antarctic sponge *Dendrilla antarctica*. *Mar Drugs* 18. doi: 10.3390/md18060327.

Brueggeman, P. (1998). Echinodermata-Asteroidea: seastars. Underwater field guide to Ross Island & McMurdo Sound, Antarctica. Available at: www.norbertwu.com.

Cárdenas, C. A., González-Aravena, M., Font, A., Hestetun, J. T., Hajdu, E., Trefault, N., et al. (2018). High similarity in the microbiota of cold-water sponges of the Genus *Mycale* from two different geographical areas. *PeerJ* 2018. doi: 10.7717/peerj.4935.

Cárdenas, C. A., and Montiel, A. (2017). Coexistence in Cold Waters: Animal Forests in Seaweed-Dominated Habitats in Southern High Latitudes. *Marine animal forests: the ecology of benthic biodiversity hotspots*, 257–276. doi: 10.1007/978-3-319-17001-5.

Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2022). Marine natural products. *Nat Prod Rep* 39, 1122–1171. doi: 10.1039/D1NP00076D.

Ciaglia, E., Malfitano, A. M., Laezza, C., Fontana, A., Id, G. N., Id, A. C., et al. (2017). Immuno-Modulatory and Anti-Inflammatory Effects of Dihydrogracilin A, a Terpene Derived from the Marine Sponge *Dendrilla membranosa*. doi: 10.3390/ijms18081643.

Costello, M. J., and Chaudhary, C. (2017). Marine Biodiversity, Biogeography, Deep-Sea Gradients, and Conservation. *Current Biology* 27, R511–R527. doi: 10.1016/J.CUB.2017.04.060.

Cronin, G., and Hay, M. E. (1996). Induction of Seaweed Chemical Defenses by Amphipod Grazing. *Ecology* 77, 2287–2301. doi: 10.2307/2265731.

Cutignano, A., Zhang, W., Avila, C., Cimino, G., and Fontana, A. (2011). Intrapopulation Variability in the Terpene Metabolism of the Antarctic Opisthobranch Mollusc *Austrodoris kerguelenensis*. *European J Org Chem* 2011, 5383–5389. doi: 10.1002/ejoc.201100552.

Dayton, P., Robilliard, G. A., and Paine, R. T. (1970). Benthic Faunal Zonation as a Result of Anchor Ice at McMurdo Sound, Antarctica. In M. W. Holdgate (Ed.), *Antarctic ecology* (Vol. 1, pp. 244–258). Academic Press.

De Broyer, C., and Jazdzewska, A. (2014). Biogeographic patterns of Southern Ocean benthic Amphipods. In: De Broyer C., Koubbi P., Griffiths H.J., Raymond B., Udekem d'Acoz C. d' et al. (eds)

Biogeographic Atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, pp. 155–165. doi: 10.13140/RG.2.1.1716.3289

De Castro-Fernández, P., Ballesté, E., Angulo-Preckler, C., Biggs, J., Avila, C., and García-Aljaro, C. (2023). How does heat stress affect sponge microbiomes? Structure and resilience of microbial communities of marine sponges from different habitats. *Front Mar Sci* 9:1072696. doi: 10.3389/fmars.2022.1072696.

Deheyn, D. D., Gendreau, P., Baldwin, R. J., and Latz, M. I. (2005). Evidence for enhanced bioavailability of trace elements in the marine ecosystem of Deception Island, a volcano in Antarctica. *Mar Environ Res* 60, 1–33. doi: 10.1016/J.MARENVRES.2004.08.001.

Easson, C. G., Chaves-Fonnegra, A., Thacker, R. W., and Lopez, J. v. (2020). Host population genetics and biogeography structure the microbiome of the sponge *Cliona delitrix*. *Ecol Evol* 10, 2007–2020. doi: 10.1002/ECE3.6033.

Elderfield, H. (1972). Effects of volcanism on water chemistry, Deception Island, Antarctica. *Mar Geol* 13, M1–M6. doi: 10.1016/0025-3227(72)90066-7.

Figuerola, B., Núñez-Pons, L., Moles, J. and Avila, C. (2013). Feeding repellence in Antarctic bryozoans. doi: 10.1007/s00114-013-1112-8.

Fontana, A., Scognamiglio, G., and Cimino, G. (1997). Dendrinolide, a New Degraded Diterpenoid from the Antarctic Sponge *Dendrilla membranosa*. 3864, 475–477. doi: 10.1021/np960712w.

Freeman, C. J., Easson, C. G., Fiore, C. L., and Thacker, R. W. (2021). Sponge–Microbe Interactions on Coral Reefs: Multiple Evolutionary Solutions to a Complex Environment. *Front Mar Sci* 8, 917. doi: 10.3389/FMARS.2021.705053/BIBTEX.

Goodwin, C. E., Berman, J., and Hendry, K. R. (2019). Demosponges from the sublittoral and shallow-circalittoral (<24m depth) Antarctic Peninsula with a description of four new species and notes on in situ identification characteristics. *Zootaxa* 4658, zootaxa.4658.3.3-zootaxa.4658.3.3. doi: 10.11646/ZOOTAXA.4658.3.3.

Happel, L., Rondon, R., Font, A., González-Aravena, M., and Cárdenas, C. A. (2022). Stability of the Microbiome of the Sponge *Mycale (Oxymycale) acerata* in the Western Antarctic Peninsula. *Front Microbiol* 13. doi: 10.3389/fmicb.2022.827863.

Janussen, D., and Downey, R. V. (2014). Porifera. In: De Broyer C., Koubbi P., Griffiths H.J., Raymond B., Udekem d'Acoz C. d', et al. (eds.) Biogeographic Atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, pp. 94-102.

Koltun, V. M. (1976). Porifera-Part 1: Antarctic Sponges. Report B.A.N.Z. Antarctic Research Expedition 1929-1931, (B, Zoology and Botany), 5, 153-198.

Kupchan, S. M., Tsou, G., and Sigel, C. W. (1973). Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from *Datisca glomerata*. *J Org Chem* 38, 1420–1421. doi: 10.1021/JO00947A041.

Lebar, M. D., Heimbegner, J. L., Baker, B. J., and Baker, B. (2007). Cold-water marine natural products. 774–797. doi: 10.1039/b516240h.

Leiva, C., Taboada, S., Kenny, N. J., Combosch, D., Giribet, G., Jombart, T., et al. (2019). Population substructure and signals of divergent adaptive selection despite admixture in the sponge *Dendrilla antarctica* from shallow waters surrounding the Antarctic Peninsula. *Mol Ecol* 28, 3151–3170. doi: 10.1111/mec.15135.

lo Giudice, A., Azzaro, M., and Schiaparelli, S. (2019). "Microbial Symbionts of Antarctic Marine Benthic Invertebrates," in, 277–296. doi: 10.1007/978-3-030-02786-5_13.

Mahon, A. R., Amsler, C. D., McClintock, J. B., Amsler, M. O., and Baker, B. J. (2003). Tissue-specific palatability and chemical defenses against macropredators and pathogens in the common articulate brachiopod *Liothyrella uva* from the Antarctic Peninsula. *J Exp Mar Biol Ecol* 290, 197–210. doi: 10.1016/S0022-0981(03)00075-3.

Maroni, P. J., Baker, B. J., Moran, A. L., Woods, H. A., Avila, C., Johnstone, G. J., et al. (2022). One Antarctic slug to confuse them all: the underestimated diversity of *Doris kerguelenensis*. *Invertebr Syst*, 1–17. doi: 10.1071/IS21073.

Maschek, J. A. (2011). Chemical Investigation of the Antarctic Marine Invertebrates *Austrodoris kerguelenensis* & *Dendrilla membranosa* and the Antarctic Red Alga *Gigartina skottsbergii* [Doctoral dissertation, University of South Florida]. Graduate Theses and Dissertations. Available at: https://scholarcommons.usf.edu/etd/3236.

Mcclintock, J. B. (1994). Trophic biology of antarctic shallow-water echinoderms. *Mar Ecol Prog Ser* 111, 191–202. doi: 10.3354/meps111191.

Moles, J., Núñez, L., Taboada, S., and Figuerola, B. (2015). Anti-predatory chemical defences in Antarctic benthic fauna. *Mar Biol* 162, 1813–1821. doi: 10.1007/s00227-015-2714-9.

Molinski, T. F., and Faulkner, D. J. (1987). Metabolites of the Antarctic Sponge *Dendrilla membranosa*. *Journal of Organic Chemistry* 52, 296–298. doi: 10.1021/jo00378a031.

Murray, A. E., Avalon, N. E., Bishop, L., Davenport, K. W., Delage, E., Dichosa, A. E. K., et al. (2020). Uncovering the Core Microbiome and Distribution of Palmerolide in *Synoicum adareanum* Across the Anvers Island Archipelago, Antarctica. *Marine Drugs 2020, Vol. 18, Page 298* 18, 298. doi: 10.3390/MD18060298.

Núñez-Pons, L., and Avila, C. (2015). Natural products mediating ecological interactions in Antarctic benthic communities: a mini-review of the known molecules. *Nat Prod Rep* 32, 1114–1130. doi: 10.1039/c4np00150h.

Ortiz, R., Vila, J., García, A., Camacho, A. G., Diez, J. L., Aparicio, A., et al. (1992). Geophysical features of Deception Island. *Recent progress in Antarctic earth science*, 443–448.

Ottaviani, A., Welsch, J., Agama, K., Pommier, Y., Desideri, A., Baker, B. J., et al. (2022). From Antarctica to cancer research: a novel human DNA topoisomerase 1B inhibitor from Antarctic sponge *Dendrilla antarctica*. *https://doi.org/10.1080/14756366.2022.2078320* 37, 1404–1410. doi: 10.1080/14756366.2022.2078320.

Peters, K. J., Amsler, C. D., Mcclintock, J. B., and Baker, B. J. (2010). Potential chemical defenses of Antarctic sponges against sympatric microorganisms. 649–658. doi: 10.1007/s00300-009-0741-z.

Peters, K. J., Amsler, C. D., Mcclintock, J. B., Soest, R. W. M. van, and Baker, B. J. (2009). Palatability and chemical defenses of sponges from the western Antarctic Peninsula. 385, 77–85. doi: 10.3354/meps08026.

Pita, L., Rix, L., Slaby, B. M., Franke, A., and Hentschel, U. (2018). The sponge holobiont in a changing ocean: from microbes to ecosystems. *Microbiome* 6, 46. doi: 10.1186/s40168-018-0428-1.

Posadas, N., Baquiran, J. I. P., Nada, M. A. L., Kelly, M., and Conaco, C. (2021). Microbiome diversity and host immune functions influence survivorship of sponge holobionts under future ocean conditions. *The ISME Journal 2021 16:1* 16, 58–67. doi: 10.1038/S41396-021-01050-5.

Prieto, I. M., Paola, A., Pérez, M., García, M., Blustein, G., Schejter, L., et al. (2022). Antifouling Diterpenoids from the Sponge *Dendrilla antarctica*. *Chem Biodivers* 19. doi: 10.1002/cbdv.202100618.

Puglisi, M. P., Sneed, J. M., Ritson-Williams, R., and Young, R. (2019). Marine chemical ecology in benthic environments. *Nat Prod Rep* 36, 410–429. doi: 10.1039/C8NP00061A.

Puyana, M., Fenical, W., and Pawlik, J. R. (2003). Are there activated chemical defenses in sponges of the genus *Aplysina* from the Caribbean? *Mar Ecol Prog Ser* 246, 127–135. doi: 10.3354/MEPS246127.

Rohde, S., Gochfeld, D. J., Ankisetty, S., Avula, B., Schupp, P. J., and Slattery, M. (2012). Spatial Variability in Secondary Metabolites of the Indo-Pacific Sponge *Stylissa massa*. *J Chem Ecol* 38, 463–475. doi: 10.1007/S10886-012-0124-8/TABLES/3.

Sacristán-Soriano, O., Pérez Criado, N., and Avila, C. (2020). Host Species Determines Symbiotic Community Composition in Antarctic Sponges (Porifera: Demospongiae). *Front Mar Sci* 7. doi: 10.3389/fmars.2020.00474.

Shilling, A. J. (2019). The Chemical Ecology and Drug Discovery Potential of the The Chemical Ecology and Drug Discovery Potential of the Antarctic Red Alga Plocamium cartilagineum and the Antarctic Sponge *Dendrilla membranosa*. [Doctoral dissertation, University of South Florida]. Graduate Theses and Dissertations. Available at: https://scholarcommons.usf.edu/etd.

Shilling, A. J., Heiser, S., Amsler, C. D., McClintock, J. B., and Baker, B. J. (2021). Hidden diversity in an antarctic algal forest: Metabolomic profiling linked to patterns of genetic diversification in the Antarctic red alga *Plocamium* sp. *Mar Drugs* 19. doi: 10.3390/md19110607.

Shilling, A. J., Witowski, C. G., Maschek, J. A., Azhari, A., Vesely, B. A., Kyle, D. E., et al. (2020). Spongian Diterpenoids Derived from the Antarctic Sponge Dendrilla antarctica Are Potent Inhibitors of the Leishmania Parasite. *J Nat Prod* 83, 1553–1562. doi: 10.1021/acs.jnatprod.0c00025.

Smith, K. L., Baldwin, R. J., Kaufmann, R. S., and Sturz, A. (2003). Ecosystem studies at Deception Island, Antarctica: an overview. *Deep Sea Research Part II: Topical Studies in Oceanography* 50, 1595–1609. doi: 10.1016/S0967-0645(03)00081-X.

Soldatou, S., and Baker, B. J. (2017). Cold-water marine natural products, 2006 to 2016. *Nat Prod Rep* 34, 585–626. doi: 10.1039/c6np00127k.

Ternon, E., Perino, E., Manconi, R., Pronzato, R., and Thomas, O. P. (2017). How Environmental Factors Affect the Production of Guanidine Alkaloids by the Mediterranean Sponge *Crambe crambe*. *Marine Drugs 2017, Vol. 15, Page 181* 15, 181. doi: 10.3390/MD15060181.

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., et al. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun* 7. doi: 10.1038/ncomms11870.

von Salm, J. L., Witowski, C. G., Amsler, M. O., Amsler, C. D., McClintock, J. B., and Baker, B. J. (2022). Amphipod diversity and metabolomics of the Antarctic sponge *Dendrilla antarctica*. *Antarct Sci* 34, 349–360. doi: 10.1017/s0954102022000268.

von Salm, J. L., Witowski, C. G., Fleeman, R. M., McClintock, J. B., Amsler, C. D., Shaw, L. N., et al. (2016). Darwinolide, a New Diterpene Scaffold That Inhibits Methicillin-Resistant Staphylococcus aureus Biofilm from the Antarctic Sponge *Dendrilla membranosa*. *Org Lett* 18, 2596–2599. doi: 10.1021/acs.orglett.6b00979.

Wilson, N. G., Maschek, J. A., and Baker, B. J. (2013). A Species Flock Driven by Predation? Secondary Metabolites Support Diversification of Slugs in Antarctica. *PLoS One* 8, e80277. doi: 10.1371/JOURNAL.PONE.0080277.

Young, R. M., von Salm, J. L., Amsler, M. O., Lopez-Bautista, J., Amsler, C. D., McClintock, J. B., et al. (2013). Site-Specific Variability in the Chemical Diversity of the Antarctic Red Alga *Plocamium cartilagineum*. *Marine Drugs 2013, Vol. 11, Pages 2126-2139* 11, 2126–2139. doi: 10.3390/MD11062126.

DISCUSIÓN GENERAL

El objetivo principal de esta tesis ha sido profundizar en el conocimiento de las interacciones ecológicas del bentos antártico y evaluar el impacto antropogénico desde dos perspectivas diferentes, el impacto de la contaminación en las redes tróficas y el aumento de temperatura asociado al calentamiento global. El aislamiento geográfico, junto con las condiciones ambientales extremas debido a su latitud, hacen de las comunidades biológicas antárticas focos de biodiversidad, con alto grado de endemismos, y adaptaciones únicas a un ambiente de bajas temperaturas, fuerte estacionalidad, o presencia de hielo marino. Las interacciones biológicas juegan un papel clave en la estructuración de estas comunidades. En las comunidades bentónicas marinas, las interacciones más relevantes en esta estructuración serían las relaciones tróficas, las interacciones mediadas por compuestos químicos, y las que existen entre el huésped y su comunidad microbiana simbionte. Entre los factores más importantes que pueden alterar estos ecosistemas se encuentra la presencia del ser humano, impulsor del cambio global al que nos estamos enfrentando, caracterizado por el calentamiento y acidificación de los océanos, o el aumento de la frecuencia de fenómenos extremos como las olas de calor, entre muchos otros. La Península Antártica es una de las áreas que ha sufrido una de las mayores tasas de calentamiento en las últimas décadas, además de un aumento de la intensidad de las olas de calor, consecuencia también del cambio climático (González-Herrero et al., 2022). El bentos antártico, por las características mencionadas anteriormente, constituye uno de los hábitats más atractivos para explorar estas interacciones biológicas y cómo el impacto antropogénico puede alterarlas y desequilibrar así el ecosistema, y en consecuencia alterar el planeta de manera global.

Para poder observar y predecir los efectos y consecuencias de los impactos antropogénicos y el cambio global sobre los ecosistemas, es necesario conocer la estructura y el funcionamiento de las comunidades biológicas presentes en el ecosistema. Como hemos visto, las interacciones biológicas, además de las condiciones ambientales, juegan un papel clave en la estructuración de las comunidades. Las relaciones tróficas son una de las interacciones entre organismos que nos aporta mucha información sobre la estructura de la comunidad. Algunos depredadores de esponjas antárticas de aguas poco profundas son asteroideos como las especies espongívoras *Perknaster fuscus*, depredador principal de *Mycale acerata* (McClintock et al., 2005), o *Acodontaster conspicuous*. Otros depredadores como *Odontaster validus* o algunas especies de anfípodos son omnívoros que incluyen esponjas en sus dietas (Gillies et al., 2012; Mcclintock, 1994). *Doris kerguelenensis* es un molusco heterobranquio con distribución circumpolar, que se encuentra frecuentemente sobre diversas especies de esponjas y cuyas presas principales se consideran en

general las esponjas hexactinélidas de las especies *Rossella nuda* y *R. racovitzae* y *Scolymastra (Anoxycalyx) joubini*. En los fondos someros rocosos de Bahía Balleneros en Isla Decepción (Archipiélago de las Shetland del Sur, al norte de la Península Antártica) no se encuentran esponjas de la clase Hexactinellida a poca profundidad, y se ha observado al molusco *D. kerguelenensis* sobre diversas especies de demoesponjas. El análisis de isótopos estables de C y N, y el perfil de ácidos grasos (capítulo I) nos han permitido determinar parcialmente la dieta de este molusco. Los resultados sugieren que de las siete especies de presas potenciales analizadas (*Axinella crinita*, *Dendrilla antarctica*, *Mycale acerata*, *Sphaerotylus antarcticus*, *Hemigellius pilosus*, *Kirkpatrickia variolosa*, y *Haliclona* sp.), las cuales dominan la comunidad macroscópicamente, el molusco estaría obteniendo el C de *D. antarctica*, *M. acerata*, *A. crinita* y *Haliclona* sp. Sin embargo, las esponjas no supondrían la única fuente de carbono de *D. kerguelenensis*, ya que la señal isotópica de carbono indica que existe otra presa más empobrecida en C. Esta fuente de carbono desconocida podría consistir en las bacterias simbiontes de dichas esponjas, o bien otra presa alternativa baja en C, como por ejemplo una ascidia. Esto último sería bastante atípico y requiere de muchos más estudios detallados. Los nudibranquios se consideran en general carnívoros, aunque se han descrito ejemplos de estrategias alimentarias que contrastan con la norma general, como el caso de *Polycerella emertoni* (Camps-Castella et al., 2020). El uso de un factor de discriminación isotópica entre los tejidos y la dieta (DTDF por sus siglas en inglés) general para invertebrados y no específico para *D. kerguelenensis* puede tener influencia en los resultados, y pone énfasis en la importancia de utilizar DTDFs específicos siempre que sea posible.

Las relaciones tróficas pueden también estudiarse a partir del análisis de elementos que pueden biomagnificarse lo largo de las cadenas tróficas. En nuestro estudio (capítulo II), en el que se incluyen algunos de los organismos más relevantes de la comunidad bentónica de fondos rocosos someros en isla Decepción (Islas Shetland del Sur), el mercurio (Hg) y el plomo (Pb) mostraron cierta biomagnificación entre las estrellas de mar depredadoras *Odontaster validus* y *Diplasterias brucei,* y la lapa herbívora *Nacella concinna*. El hecho de que los productores primarios analizados (materia orgánica particulada y las especies de macroalgas dominantes) tuvieran concentraciones de los elementos traza mucho mayores que las de los animales nos permitió rechazarlas como posible fuente de carbono para los consumidores. El uso de marcadores tróficos nos ha permitido no sólo conocer las relaciones tróficas entre ellos, sino también conocer el grado de contaminación de estos. El análisis de la concentración de determinados elementos traza en distintos puntos a lo largo de gran parte de la latitud de la península Antártica reflejó una concentración similar a la que existe en

otras partes del planeta. Por ello, no se puede considerar que sigue siendo una zona prístina, ya que el impacto humano es muy relevante.

Las esponjas han desarrollado a lo largo de millones de años de evolución una serie de defensas químicas para evitar el *fouling* o crecimiento de otros organismos en su superficie, evitar ser depredadas, o infectadas por microorganismos patógenos (Peters et al., 2010; Thakur & Singh, 2016). Las esponjas de las que presumiblemente se alimenta *D. kerguelenensis* en Isla Decepción están protegidas químicamente. La población de la esponja *D. antarctica* en esta isla posee siete diterpenos derivados del *espongiano,* que utiliza para defenderse de la depredación. Sin embargo, parece que estos compuestos no serían efectivos para disuadir a *D. kerguelenensis*. A diferencia de otros depredadores de esponjas u otros invertebrados, se ha visto que la tasa de alimentación del molusco es muy baja, causando un impacto ínfimo en esponja, no comprometiendo su integridad ni supervivencia (Barnes & Bullough, 1996; Dayton et al., 1974). Quizás esto explicaría que las defensas de *D. antactica* no sean efectivas contra *D. kerguelenensis*.

D. kerguelenensis posee también un arsenal de metabolitos secundarios, entre los cuales se encuentran diterpenos (Diyabalanage, 2006). Sin embargo, se ha sugerido que estos no derivan de la dieta, como ocurre en muchos nudibranquios, si no que *D. kerguelenensis* sería capaz de biosintetizarlos, ya que en las zonas en las que sus supuestas únicas fuentes de carbono son esponjas hexactinélidas como *Rossella ruda*, *R. racovitzae*, y *Anoxycalyx (Scolymastra) joubini* o demoesponjas distintas de *Dendrilla antarctica*, que no poseen compuestos diterpénicos de este tipo, *D. kerguelenensis* sigue produciendo los compuestos diterpénicos (Iken et al., 2002). Los diterpenos son metabolitos secundarios de estructura isoprenoide con 20C, que no tienen función conocida en el metabolismo primario de los organismos que los producen, formando uno de los grupos más importantes de productos naturales. Son producidos tanto por plantas como por hongos, además de algunos animales. Sin embargo, las sintasas implicadas en su producción se han estudiado sobre todo en plantas, hongos, y bacterias (Yamada et al., 2015).

Se cree que los simbiontes bacterianos, que juegan un papel importante en la fisiología de la esponja, podrían estar en el origen de muchos de los compuestos químicos que se han detectado en esponjas marinas (Rust et al., 2020). Así pues, el estudio de estos simbiontes y sus metabolitos ha atraído el interés de los investigadores en los últimos años, ya que representan una fuente para la búsqueda de nuevos compuestos bioactivos. Por ejemplo, se ha demostrado que las proteobacterias y las actinobacterias producen metabolitos secundarios que complementan las

defensas inmunitarias del huésped (Hentschel et al., 2001). En esta tesis se han estudiado las comunidades bacterianas de especies de esponjas de diferentes latitudes no sólo para conocer el microbioma de estas especies sino para evaluar también el impacto del aumento de temperatura sobre estas.

Experimentos de laboratorio en los que se han simulado los efectos del calentamiento y acidificación del mar han mostrado que en general estos organismos son más sensibles al calentamiento y no se ven tan afectados por la acidificación (revisado en (Bell et al., 2018)). Sin embargo, se observó que no todas las especies de esponjas tienen la misma respuesta a estos estresores, por lo que es posible que la composición de las comunidades de esponjas cambiase, desapareciendo aquellas especies más vulnerables, y aumentando sus poblaciones aquellas más tolerantes. Comparando las esponjas de zonas tropicales con otros organismos que forman parte de las mismas comunidades, concretamente con corales calcáreos en los arrecifes, se sugirió que las esponjas serían "ganadoras" en un escenario de cambio climático del futuro próximo, ya que los corales son más sensibles a la acidificación y calentamiento del océano, y al disminuir sus poblaciones, disminuiría la competencia por el espacio y las esponjas podrían aumentar sus poblaciones (Bell et al., 2018). Sin embargo, otros estudios sugieren que habría otros factores que afectarían a las esponjas y limitarían su crecimiento. (Lesser & Slattery, 2020) sugieren que una disminución de la concentración de oxígeno en el océano limitaría la productividad primaria, y por tanto la obtención de carbono de las esponjas. Un aumento de la abundancia absoluta de esponjas requeriría de un aumento de la productividad. A diferencia de los arrecifes tropicales, las consecuencias del cambio climático para las esponjas de zonas templadas y polares parecen más difíciles de predecir (Bell et al., 2018). A pesar de que en general se considera que los organismos de zonas templadas podrían ser más tolerantes a cambios ambientales ya que están adaptados a mayores variaciones de las condiciones ambientales que aquellos de zonas tropicales o polares, se observó que esponjas de zonas templadas expuestas a olas de calor eran vulnerables a estas (Bell et al., 2023). En dichos estudios se evaluaba la supervivencia de los individuos, pero no se comprobó qué cambios en las esponjas llevaban a la pérdida de funciones y eventual muerte de los organismos.

Según la información disponible, el cambio climático ya podría estar afectando muchos organismos antárticos de diferentes maneras (Ashton et al., 2017; Constable et al., 2014; Griffiths et al., 2017; Poloczanska et al., 2016; Turner et al., 2014). Por ejemplo, los pingüinos se ven afectados por muchos más parásitos (Diaz et al., 2017), mientras que algunas especies bentónicas pueden

experimentar cambios en su distribución (Barnes et al., 2009; Fillinger et al., 2013; Gutt et al., 2011; Pasotti et al., 2015), o incluso reducir algunas de sus relaciones interespecíficas (Barnes et al., 2014). Se ha estudiado la capacidad de responder fisiológicamente al estrés térmico en diferentes taxones antárticos durante varios años (revisado por Peck, 2018). Los resultados mostraron escasas capacidades de supervivencia, pero parece haber diferencias a nivel de especie (Ashton et al., 2017). En cuanto a los productos naturales, no se conoce bien todavía si los cambios de temperatura, pH, calcificación y otros, pueden afectar la producción y/o el uso de productos químicos por organismos bentónicos marinos en la Antártida. Metabolitos relacionados con una palatabilidad desagradable, como monoterpenos halogenados (como anverene y epi-plocamene) que definen las relaciones entre las macroalgas y herbívoros simpátricos, puede variar según las condiciones ambientales, y por lo tanto, las relaciones tróficas en los ecosistemas antárticos podrían estar fuertemente afectadas por el cambio climático (IPCC, 2022). La macroalga *Desmarestia menziesii*, por ejemplo, aumenta la producción de florotaninos cuando se expone a la acidificación (Schoenrock et al., 2015). Otro ejemplo podría ser la inducción de defensas químicas (austrodoral y ácido austrodórico) como respuesta al estrés potencial en el nudibranquio *Doris kerguelenensis* (Gavagnin et al., 2003), que forman parte del diverso arsenal químico de este molusco (Avila et al., 2018), aunque es necesaria más investigación en este sentido. También se ha citado alguna variación en los productos químicos de la macroalga *Plocamium cartilagineum* de diferentes localidades (Shilling et al., 2021; Young et al., 2013), así como en *D. kerguelenensis* (Wilson et al., 2013).

En nuestro estudio, el aumento de temperatura no sólo afectó a las comunidades bacterianas de las esponjas, sino que también, como se ha mostrado en el capítulo IV, afectó a la producción de uno de los diterpenos que produce la esponja *D. antarctica*: la concentración de tetrahidroaplisulfurina-1 fue mayor en el grupo expuesto a un estrés térmico. Este incremento de la producción no va asociado a una mayor concentración de diterpenos totales en las esponjas, por lo que parecería que los recursos se concentran en producir más cantidad de este compuesto quizás en detrimento de otros. Los principales metabolitos se siguen produciendo en los especímenes de *D. antarctica* tras la exposición a un estrés térmico, y no varía significativamente la cantidad de terpenos totales que se producen. A priori, se podría pensar que, por ejemplo, el aumento de la temperatura del agua sería un factor estresante que podría inducir a los organismos a dejar de producir metabolitos secundarios, ya que esta es una estrategia costosa. Por el contrario, para algunas especies, una temperatura más alta podría conducir a un aumento de las defensas químicas como reacción para protegerse contra el propio estrés. Nuestros experimentos sugieren que la esponja *D. antarctica*

estaría utilizando esta segunda estrategia, produciendo en mayor cantidad el diterpeno tetrahidroaplishulfurina-1 al estar expuesta a un estrés térmico. Factores como la estrategia de producción de las defensas químicas deben tenerse en cuenta a la hora de analizar los efectos de los estresores. Por ejemplo, es diferente estudiar un organismo que biosintetiza directamente los compuestos, como podría ser el caso del molusco *D. kerguelenensis*, que aquellos en los que el microbioma del organismo está quizás implicado en la producción, como podría ser el caso de las esponjas.

Hay que tener en cuenta también que las variaciones de temperatura que pueden soportar las comunidades microbianas pueden ser mayores que las de sus huéspedes. Así pues, aunque el estrés térmico produjo cambios en la comunidad de bacterias simbiontes de *D. antarctica* y *M. acerata*, las bacterias que formaban parte de la comunidad *core* se mantuvieron presentes tras la exposición al estrés. No hemos realizado un estudio de la supervivencia o los efectos sobre la fisiología de las esponjas de las tres regiones estudiadas, por tanto no podemos asociar una mayor resistencia al aumento de temperatura de la comunidad *core* de las esponjas antárticas con una mayor resistencia de las propias esponjas. A pesar de que las esponjas antárticas estudiadas (*M. acerata* y *D. antarctica*) parece que mantienen mejor las comunidades bacterianas frente a un aumento de temperatura respecto a especies tropicales o templadas, no podemos olvidar como la supervivencia de los especímenes puede verse comprometida ante este estresor, y quizás no sea necesario un gran cambio en la composición de esas comunidades bacterianas, sino que seguramente, pequeños cambios, o de determinados grupos, tienen una gran influencia en la *fitness* o eficacia biológica de la especie.

La producción de metabolitos secundarios en *D. antarctica* también mostró variabilidad en cuanto a la producción de los principales diterpenos que produce esta esponja en función de su procedencia geográfica, isla Livingston e isla Decepción (Islas Shetland del Sur). El hecho de que el nuevo compuesto descrito, decepcionina, esté más presente en la población de *D. antarctica* de una zona de alto estrés ambiental como es isla Decepción podría implicar que posee una bioactividad beneficiosa para la esponja (y tal vez con interés médico o industrial). También se observaron variaciones en el perfil químico de otros diterpenos mayoritarios. Estas variaciones podrían estar relacionadas con la genética, la presión de depredación, o el microbioma de las especies, pero también con las condiciones ambientales de cada zona. Los análisis del perfil de compuestos

diterpénicos realizados hasta el momento en esta especie sugieren que algunos de estos compuestos solo se producen en ciertas regiones del área de distribución de la especie (Bory et al., 2020), pero ningún estudio hasta la fecha había comparado diferentes localidades. Si esto podría estar relacionado con la adaptación al cambio ambiental requiere de más investigación. Las diversas aproximaciones a la biología y ecología de *D. antarctica* muestran que se trata de una especie con alta variabilidad intraespecífica química, genética, y microbiológica, aunque seguramente no se deben a poblaciones genéticamente aisladas (Leiva et al., 2019); esta tesis). Futuros estudios en los que se compare la composición química y microbiológica nos permitirán conocer las relaciones entre estos dos aspectos vitales de la esponja.

Conocer en profundidad la estructura y función de las comunidades bentónicas antárticas nos permitirá establecer estrategias para promover la conservación de una biota diversa, en gran parte propia de esta región, y analizar el impacto de los cambios ambientales sobre estas. Los resultados obtenidos en esta tesis contribuyen a ampliar el conocimiento en diversos aspectos de la ecología bentónica antártica, haciendo énfasis en la importancia de analizar los diferentes factores que estructuran las comunidades biológicas y los cambios que está provocando el fenómeno del cambio global.

CONCLUSIONES

Las conclusiones que se derivan de los diferentes estudios realizados en esta tesis doctoral son:

1. El análisis de isótopos estables de C y N y el análisis del perfil de ácidos grasos ha permitido determinar la dieta del molusco *Doris kerguelenensis* en la Isla de Decepción. De acuerdo a los perfiles de ácidos grasos obtenidos *D. kerguelenensis* se alimenta de las esponjas *Axinella crinita*, *Dendrilla antarctica*, *Mycale acerata* y *Haliclona* sp. aunque el análisis indica también que estas esponjas no sus únicas fuentes de alimento.

2. Las discrepancias entre la relación de isótopos estables en *D. kerguelenensis* y sus potenciales fuentes de alimento son principalmente en los valores de $\delta^{13}C$, ya que el DTDF descrito en la literatura para gasterópodos carnívoros proporciona una buena predicción para los valores de $\delta^{15}N$. Esto sugiere que las diferencias se deben a la ausencia de una presa principal en el modelo. La idea de que *D. kerguelenensis* es un espongívoro especializado parece pues que se debería cuestionar, ya que nuestros resultados indican que presenta una dieta más diversa.

3. La comunidad bentónica antártica de la península antártica y las islas Shetland del Sur está afectada por contaminación antropogénica del mismo modo que otras partes del planeta ya que se detectaron concentraciones de elementos traza (Cr, Hg y Pb) similares a las que se detectan en otras regiones. Esta contaminación no sigue ningún gradiente latitudinal, si bien posiblemente esto pueda estar enmascarado por factores locales relacionados con los puntos de muestreo, zonas costeras protegidas en vez de lugares abiertos.

4. El análisis de Cr, Hg y Pb en la red trófica del bentos antártico en la que se incluyen tres macroalgas, *Palmaria decipiens*, *Desmarestia anceps* y *Desmarestia menziesii,* como productores primarios, el molusco gasterópodo *Nacella concinna* y dos estrellas de mar, *Odontaster validus* y *Diplasterias brucei* como consumidores, y la materia orgánica particulada ha mostrado diferencias en la concentración de éstos entre especies y en los diferentes puntos de muestreo pero sin seguir un determinado patrón.

5. Los productores primarios analizados tienen una mayor concentración de elementos traza que los macroinvertebrados situados en los niveles superiores de la red trófica lo cual sugiere que estos productores primarios no constituyen su fuente de carbono principal.

6. Se ha demostrado bioacumulación de Hg y Pb (biomagnificación) obteniéndose mayores concentraciones de estos elementos traza en las estrellas de mar, que son los que se encuentran en el nivel superior de la red trófica, que en el molusco gasterópodo.

7. La tecnología de secuenciación masiva del amplicon 16S rRNA ha permitido caracterizar la comunidad bacteriana asociada a las esponjas de tres zonas climáticas diferentes: *Mycale acerata* y *Dendrilla antarctica* (antárticas), *Acanthella cavernosa* (tropical) y *Agelas oroides* (templada). Estas esponjas presentan una comunidad bacteriana propia, dependiendo de la especie, que se diferencia de la del agua de mar en la que habitan.

8. El estrés térmico ligero, ha provocado una pérdida general de especies microbianas respecto a las esponjas mantenidas a la temperatura de su hábitat, aunque mayoritariamente se mantiene la microbiota *core* de las esponjas estudiadas.

9. Respecto a los índices de biodiversidad bacteriana, el estrés térmico ligero ha provocado una disminución significativa de la biodiversidad en las esponjas de clima tropical (*A. cavernosa*) y de clima templado (*A. oroides*). Sin embargo, las comunidades bacterianas asociadas a esponjas antárticas han mostrado una mayor resiliencia al aumento de temperatura, aunque la metodología empleada no permite diferenciar el estado metabólico de las bacterias detectadas ni de las esponjas.

10. El sometimiento a un estrés térmico extremo provoca la pérdida general de la microbiota específica de las diferentes esponjas, la cual acaba asemejándose a la detectada en el agua de los acuarios. Esto afecta también a la supervivencia de las esponjas.

11. El perfil químico obtenido en *Dendrilla antarctica* ha mostrado siete compuestos de tipo diterpeno presentes en estas esponjas en las islas de Decepción y Livingston, detectándose perfiles diferentes en función del lugar de procedencia de las esponjas, aunque también se ha detectado cierta variabilidad a nivel de individuo. Los siete compuestos mayoritarios han permitido agrupar los diferentes individuos en seis quimiotipos diferentes en función de la distribución y abundancia de estos compuestos.

12. Se ha aislado un nuevo compuesto químico especialmente abundante en individuos de *Dendrilla antarctica* procedentes de la isla de Decepción, la decepcionina. Es un diterpeno con un esqueleto aplisulfurano, cuya potencial bioactividad es aún desconocida.

13. El estrés térmico provoca cambios en la abundancia y en el perfil químico de los terpenos mayoritarios de *Dendrilla antarctica* particularmente en el compuesto tetrahidroaplisulfurina-1, cuya concentración es mayor en los individuos expuestos al estrés térmico en experimentos de laboratorio.

14. La exposición de *Dendrilla antarctica* a dos de sus depredadores, el macrodepredador *Odontaster validus* y el microdepredador *Cheirimedon femoratus,* provoca un leve aumento en la concentración de terpenos, en experimentos de laboratorio.

15. El estudio de los ecosistemas bentónicos antárticos ha permitido disponer de una imagen global de su estado actual que permitirá valorar en los próximos años el avance del impacto antropogénico y del cambio global al que nos enfrentamos. Los niveles de contaminación detectados son superiores a los que serían esperables en un lugar prístino como la península antártica, por lo que es necesario una monitorización de estas regiones, así como también continuar explorando los efectos del cambio global en las especies que habitan en estos ecosistemas.

BIBLIOGRAFÍA

Angot, H., Magand, O., Helmig, D., Ricaud, P., Quennehen, B., Gallée, H., et al. (2016). New insights into the atmospheric mercury cycling in central Antarctica and implications on a continental scale. *Atmos Chem Phys* 16, 8249–8264. doi: 10.5194/ACP-16-8249-2016.

Angulo-Preckler, C., Castro-Fernandez, P. de, Martín-Martín, R., Figuerola, B., and Avila, C. (2020). Chemical ecology in the Southern Ocean. *Life in Extreme Environments*, 251–278. doi: 10.1017/9781108683319.015.

Angulo-Preckler, C., Cid, C., Oliva, F., and Avila, C. (2015). Antifouling activity in some benthic Antarctic invertebrates by “in situ” experiments at Deception Island, Antarctica. *Mar Environ Res* 105, 30–38. doi: 10.1016/j.marenvres.2015.02.001.

Angulo-Preckler, C., Figuerola, B., Núñez-Pons, L., Moles, J., Martín-Martín, R., Rull-Lluch, J., et al. (2018a). Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica. *Cont Shelf Res* 157, 20–31. doi: 10.1016/J.CSR.2018.02.005.

Angulo-Preckler, C., San Miguel, O., García-Aljaro, C., and Avila, C. (2018b). Antibacterial defenses and palatability of shallow-water Antarctic sponges. *Hydrobiologia* 806, 123–138. doi: 10.1007/s10750-017-3346-5.

Ashton, G. v., Morley, S. A., Barnes, D. K. A., Clark, M. S., and Peck, L. S. (2017). Warming by 1°C Drives Species and Assemblage Level Responses in Antarctica’s Marine Shallows. *Current Biology* 27, 2698-2705.e3. doi: 10.1016/J.CUB.2017.07.048.

Avila, C. (2016). Ecological and Pharmacological Activities of Antarctic Marine Natural Products. *Planta Med* 82, 767–774. doi: 10.1055/s-0042-105652.

Avila, C., and Angulo-Preckler, C. (2021). A Minireview on Biodiscovery in Antarctic Marine Benthic Invertebrates. *Front Mar Sci* 8, 976. doi: 10.3389/FMARS.2021.686477/TEXT.

Avila, C., Angulo-Preckler, C., Martín-Martín, R. P., Figuerola, B., Griffiths, H. J., and Waller, C. L. (2020). Invasive marine species discovered on non–native kelp rafts in the warmest Antarctic island. *Scientific Reports 2020 10:1* 10, 1–9. doi: 10.1038/s41598-020-58561-y.

Avila, C., Núñez-Pons, L., and Moles, J. (2018). From the Tropics to the Poles : Chemical Defense Strategies in Sea Slugs (Mollusca: Heterobranchia). *Chemical Ecology*, 71–163. doi: 10.1201/9780429453465-3.

Avila, C., Taboada, S., and Nu, L. (2008). Antarctic marine chemical ecology: what is next? 29, 1–71. doi: 10.1111/j.1439-0485.2007.00215.x.

Balzarini, J., Haller-Meier, F., de Clercq, E., and Meier, C. (2016). Antiviral activity of cyclosaligenyl prodrugs of acyclovir, carbovir and abacavir. *Antiviral Chemistry and Chemotherapy* 12, 301–306. doi: 10.1177/095632020101200505.

Barbraud, C., and Welmerskirch, H. (2001). Emperor penguins and climate change. *Nature* 411, 183–186. doi: 10.1038/35075554.

Bargagli, R. (2008). Environmental contamination in Antarctic ecosystems. *Science of The Total Environment* 400, 212–226. doi: 10.1016/J.SCITOTENV.2008.06.062.

Barker, P. F., and Burrell, J. (1977). The opening of Drake Passage. *Mar Geol* 25, 15–34. doi: 10.1016/0025-3227(77)90045-7.

Barker, P. F., Filippelli, G. M., Florindo, F., Martin, E. E., and Scher, H. D. (2007). Onset and role of the Antarctic Circumpolar Current. *Deep Sea Research Part II: Topical Studies in Oceanography* 54, 2388–2398. doi: 10.1016/J.DSR2.2007.07.028.

Barnes, D. K. A., and Bullough, L. W. (1996). Some observations on the diet and distribution of nudibranchs at Signy Island, Antarctica. *J Mollus Stud* 62, 281-287. doi: 10.1093/mollus/62.3.281

Barnes, D. K. A., and Clarke, A. (1995). Seasonality of feeding activity in Antarctic suspension feeders. *Polar Biol* 15, 335–340 (1995). doi: 10.1007/BF00238483.

Barnes, D. K. A., Conlan, K. E., Barnes, D. K. A., and Conlan, K. E. (2007). Disturbance, colonization and development of Antarctic benthic communities. *Philos Trans R Soc B-Biol Sci* 362, 11-38. doi: 10.1098/rstb.2006.1951.

Barnes, D. K. A., Fenton, M., and Cordingley, A. (2014). Climate-linked iceberg activity massively reduces spatial competition in Antarctic shallow waters. *Curr Biol* 24, R553–R554. doi: 10.1016/J.CUB.2014.04.040.

Barnes, D. K. A., Griffiths, H. J., and Kaiser, S. (2009). Geographic range shift responses to climate change by Antarctic benthos: where we should look. *Mar Ecol Prog Ser* 393, 13–26. doi: 10.3354/MEPS08246.

Bell, J. J., Bennett, H. M., Rovellini, A., and Webster, N. S. (2018). Sponges to be winners under near-future climate scenarios. *Bioscience* 68, 955–968. doi: 10.1093/biosci/biy142.

Bell, J. J., Davy, S. K., Jones, T., Taylor, M. W., and Webster, N. S. (2013). Could some coral reefs become sponge reefs as our climate changes? *Glob Chang Biol* 19, 2613–2624. doi: 10.1111/gcb.12212.

Bell, J. J., Smith, R. O., Micaroni, V., Strano, F., Balemi, C. A., Caiger, P. E., et al. (2023). Marine heat waves drive bleaching and necrosis of temperate sponges. *Curr Biol* 33, 158-163.e2. doi: 10.1016/J.CUB.2022.11.013.

Bengtson Nash, S., Xiao, H., Nash, B., Schlabach, M., King, C., Stark, J. S., et al. (2011). Contaminant profiles of air and soil around Casey station, Antarctica; Discerning local and distant contaminant sources. *Society for Environmental Toxicology and Chemistry (SETAC), Europe Proceedings* 21.

Bory, A., Shilling, A. J., Allen, J., Azhari, A., Roth, A., Shaw, L. N., et al. (2020). Bioactivity of spongian diterpenoid scaffolds from the Antarctic sponge *Dendrilla antarctica*. *Mar Drugs* 18. doi: 10.3390/md18060327.

Callewaert, C., Quinn, R. A., Debelius, J., Thompson, L. R., Kosciolek, T., Aksenov, A., et al. (2018). Best practices for analysing microbiomes. *Nat Rev Microbiol* 16, 410–422. doi: 10.1038/s41579-018-0029-9.

Camps-Castellà, J., Ballesteros, M., Trobajo, R., Pontes, M., and Prado, P. (2020). Not all nudibranchs are carnivorous: trophic ecology of *Polycerella emertoni* in the Ebro Delta. *Mar Ecol Prog Ser* 645, 67–82. doi: 10.3354/MEPS13379.

Cárdenas, C. A., González-Aravena, M., Font, A., Hestetun, J. T., Hajdu, E., Trefault, N., et al. (2018). High similarity in the microbiota of cold- water sponges of the Genus *Mycale* from two different geographical areas. *PeerJ* 2018. doi: 10.7717/peerj.4935.

Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2022). Marine natural products. *Nat Prod Rep* 39, 1122–1171. doi: 10.1039/D1NP00076D.

Chen, C. Y., and Mao, Y. B. (2020). Research advances in plant–insect molecular interaction. *F1000Res* 9. doi: 10.12688/F1000RESEARCH.21502.1.

Clark, G. F., Stark, J. S., Palmer, A. S., Riddle, M. J., and Johnston, E. L. (2017). The Roles of Sea-Ice, Light and Sedimentation in Structuring Shallow Antarctic Benthic Communities. *PLoS One* 12, e0168391. doi: 10.1371/JOURNAL.PONE.0168391.

Constable, A. J., Melbourne-Thomas, J., Corney, S. P., Arrigo, K. R., Barbraud, C., Barnes, D. K. A., et al. (2014). Climate change and Southern Ocean ecosystems I: how changes in physical habitats directly affect marine biota. *Glob Chang Biol* 20, 3004–3025. doi: 10.1111/GCB.12623.

Cossa, D., Heimbürger, L. E., Lannuzel, D., Rintoul, S. R., Butler, E. C. V., Bowie, A. R., et al. (2011). Mercury in the Southern Ocean. *Geochim Cosmochim Acta* 75, 4037–4052. doi: 10.1016/J.GCA.2011.05.001.

da Silva, J. R. M. C., Bergami, E., Gomes, V., and Corsi, I. (2023). Occurrence and distribution of legacy and emerging pollutants including plastic debris in Antarctica: Sources, distribution and impact on marine biodiversity. *Mar Pollut Bull* 186, 114353. doi: 10.1016/J.MARPOLBUL.2022.114353.

Dayton, P. K., Robilliard, G. A., Paine, R. T., and Dayton, L. B. (1974). Biological Accommodation in the Benthic Community at McMurdo Sound, Antarctica. *Ecol Monogr* 44, 105–128. doi: 10.2307/1942321.

Diaz, J. I., Fusaro, B., Vidal, V., González-Acuña, D., Costa, E. S., Dewar, M., et al. (2017). Macroparasites in Antarctic Penguins. *Parasitology Research Monographs* 9, 183–204. doi: 10.1007/978-3-319-46343-8_9.

Diyabalanage, T. (2006). Chemical Investigation of two Antarctic Invertebrates, *Synoicum adareanum* (Chordata: Ascidiaceae; Enterogona; Polyclinidae) and *Austrodoris kergulenensis* (Molusca; Gastropoda; Nudibranchia; Doridiidae). [Doctoral dissertation, University of South Florida]. Graduate Theses and Dissertations. Available at: https://digitalcommons.usf.edu/etd/2505.

Dommergue, A., Sprovieri, F., Pirrone, N., Ebinghaus, R., Brooks, S., Courteaud, J., et al. (2010). Overview of mercury measurements in the Antarctic troposphere. *Atmos Chem Phys* 10, 3309–3319. doi: 10.5194/ACP-10-3309-2010.

Downey, R. v, Griffiths, H. J., Linse, K., and Janussen, D. (2012). Diversity and Distribution Patterns in High Southern Latitude Sponges. 7. doi: 10.1371/journal.pone.0041672.

Echeverría, C. A., Paiva, P. C., and Alves, V. C. (2005). Composition and biomass of shallow benthic megafauna during an annual cycle in Admiralty Bay , King George Island , Antarctica. 17, 312–318. doi: 10.1017/S0954102005002762.

Ferraccioli, F., Jones, P. C., Vaughan, A. P. M., and Leat, P. T. (2006). New aerogeophysical view of the Antarctic Peninsula: More pieces, less puzzle. *Geophys Res Lett* 33. doi: 10.1029/2005GL024636.

Figuerola, B., Núñez-Pons, L., Moles, J., and Avila, C. (2013). Feeding repellence in Antarctic bryozoans. 100, 1069–1081. doi: 10.1007/s00114-013-1112-8.

Fillinger, L., Janussen, D., Lundälv, T., and Richter, C. (2013). Rapid Glass Sponge Expansion after Climate-Induced Antarctic Ice Shelf Collapse. *Current Biology* 23, 1330–1334. doi: 10.1016/J.CUB.2013.05.051.

Francis, J. E., Marenssi, S., Levy, R., Hambrey, M., Thorn, V. C., Mohr, B., et al. (2008). Chapter 8 From Greenhouse to Icehouse – The Eocene/Oligocene in Antarctica. *Developments in Earth and Environmental Sciences* 8, 309–368. doi: 10.1016/S1571-9197(08)00008-6.

Fuerst, J. A. (2014). Diversity and biotechnological potential of microorganisms associated with marine sponges. *Appl Microbiol Biotechnol* 98, 7331–7347. doi: 10.1007/S00253-014-5861-X/FIGURES/2.

Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., et al. (2022). Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Glob Chang Biol* 28, 5708–5725. doi: 10.1111/GCB.16301.

Gavagnin, M., Carbone, M., Mollo, E., and Cimino, G. (2003). Austrodoral and austrodoric acid: nor-sesquiterpenes with a new carbon skeleton from the Antarctic nudibranch *Austrodoris kerguelenensis*. *Tetrahedron Lett* 44, 1495–1498. doi: 10.1016/S0040-4039(02)02849-6.

Gillies, C. L., Stark, J. S., Johnstone, G. J., and Smith, S. D. A. (2012). Carbon flow and trophic structure of an Antarctic coastal benthic community as determined by δ13C and δ15N. *Estuar Coast Shelf Sci* 97, 44–57. doi: 10.1016/J.ECSS.2011.11.003.

Gillies, C. L., Stark, J. S., Johnstone, G. J., and Smith, S. D. A. (2013). Establishing a food web model for coastal Antarctic benthic communities: A case study from the Vestfold Hills. *Mar Ecol Prog Ser* 478, 27–41. doi: 10.3354/meps10214.

González-Herrero, S., Barriopedro, D., Trigo, R. M., López-Bustins, J. A., and Oliva, M. (2022). Climate warming amplified the 2020 record-breaking heatwave in the Antarctic Peninsula. *Communications Earth & Environment* 3, 1–9. doi: 10.1038/s43247-022-00450-5.

Gordon, B. R., and Leggat, W. (2010). *Symbiodinium*—Invertebrate Symbioses and the Role of Metabolomics. *Mar Drugs* 8, 2546–2568. doi: 10.3390/MD8102546.

Griffiths, H. J., Meijers, A. J. S., and Bracegirdle, T. J. (2017). More losers than winners in a century of future Southern Ocean seafloor warming. *Nature Climate Change 2017 7:10* 7, 749–754. doi: 10.1038/nclimate3377.

Griffiths, H. U. W. J., Linse, K., and Barnes, D. K. A. (2008). Distribution of macrobenthic taxa across the Scotia Arc, Southern Ocean. 20, 213–226. doi: 10.1017/S0954102008001168.

Gutt, J., Barratt, I., Domack, E., d'Udekem d'Acoz, C., Dimmler, W., Grémare, A., et al. (2011). Biodiversity change after climate-induced ice-shelf collapse in the Antarctic. *Deep-Sea Res Part II-Top Stud Oceanogr* 58, 74–83. doi: 10.1016/J.DSR2.2010.05.024.

Gutt, J., Griffiths, H. J., and Jones, C. D. (2013). Circumpolar overview and spatial heterogeneity of Antarctic macrobenthic communities. *Mar Biodivers* 43, 481–487. doi: 10.1007/S12526-013-0152-9.

Gutt, J., Isla, E., Xavier, J. C., Adams, B. J., Ahn, I. Y., Cheng, C. H. C., et al. (2021). Antarctic ecosystems in transition – life between stresses and opportunities. *Biological Reviews* 96, 798–821. doi: 10.1111/BRV.12679.

Häder, D. P., Banaszak, A. T., Villafañe, V. E., Narvarte, M. A., González, R. A., and Helbling, E. W. (2020). Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. *Sci Total Environ* 713, 136586. doi: 10.1016/J.SCITOTENV.2020.136586.

Happel, L., Rondon, R., Font, A., González-Aravena, M., and Cárdenas, C. A. (2022). Stability of the microbiome of the sponge *Mycale* (*Oxymycale*) *acerata* in the Western Antarctic Peninsula. *Front Microbiol* 13. doi: 10.3389/fmicb.2022.827863.

Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., et al. (2003). Microbial diversity of marine sponges. *Prog Mol Subcell Biol* 37, 59–88. doi: 10.1007/978-3-642-55519-0_3.

Hentschel, U., Schmid, M., Wagner, M., Fieseler, L., Gernert, C., and Rg Hacker, J. (2001). Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. *FEMS Microbiol Ecol* 35, 305–312. doi: 10.1111/J.1574-6941.2001.TB00816.X.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., et al. (2017). Global warming and recurrent mass bleaching of corals. *Nature* 543, 373–377. doi: 10.1038/nature21707.

Iken, K., Avila, C., Fontana, A., and Gavagnin, M. (2002). Chemical ecology and origin of defensive compounds in the Antarctic nudibranch *Austrodoris kerguelenensis* (Opisthobranchia: Gastropoda). *Mar Biol* 141, 101–109. doi: 10.1007/s00227-002-0816-7.

IPCC (2022). *Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, et al. (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.

Jackson, S. A., Kennedy, J., Morrissey, J. P., O'Gara, F., and Dobson, A. D. W. (2012). Pyrosequencing Reveals Diverse and Distinct Sponge-Specific Microbial Communities in Sponges from a Single Geographical Location in Irish Waters. *Microb Ecol* 64, 105–116. doi: 10.1007/s00248-011-0002-x.

Khalifa, S. A. M., Elias, N., Farag, M. A., Chen, L., Saeed, A., Hegazy, M. E. F., et al. (2019). Marine Natural Products: A Source of Novel Anticancer Drugs. *Mar Drugs* 17, 491. doi: 10.3390/MD17090491.

Kim, S., and Thurber, A. (2007). Comparison of seastar (Asteroidea) fauna across island groups of the Scotia Arc. *Polar Biol* 30, 415–425. doi: 10.1007/S00300-006-0198-2.

Krymarys, M. (2011). Distribution patterns in the biomass of macrozoobenthic communities in Admiralty Bay (King George Island, South Shetlands, Antarctic). 489–500. doi: 10.1007/s00300-010-0903-z.

Leiva, C., Taboada, S., Kenny, N. J., Combosch, D., Giribet, G., Jombart, T., et al. (2019). Population substructure and signals of divergent adaptive selection despite admixture in the sponge *Dendrilla antarctica* from shallow waters surrounding the Antarctic Peninsula. *Mol Ecol* 28, 3151–3170. doi: 10.1111/mec.15135.

Lesser, M. P., and Slattery, M. (2020). Will coral reef sponges be winners in the Anthropocene? *Glob Chang Biol* 26, 3202–3211. doi: 10.1111/GCB.15039.

Lin, X., Zhai, X., Wang, Z., and Munday, D. R. (2018). Mean, Variability, and Trend of Southern Ocean Wind Stress: Role of Wind Fluctuations. *J Clim* 31, 3557–3573. doi: 10.1175/JCLI-D-17-0481.1.

Linse, K., Cope, T., Lörz, A. N., and Sands, C. (2007). Is the Scotia Sea a centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae). *Polar Biol* 30, 1059–1068. doi: 10.1007/S00300-007-0265-3.

Livermore, R., Eagles, G., Morris, P., and Maldonado, A. (2004). Shackleton Fracture Zone: No barrier to early circumpolar ocean circulation. *Geology* 32, 797–800. doi: 10.1130/G20537.1.

Lyle, M., Gibbs, S., Moore, T. C., and Rea, D. K. (2007). Late Oligocene initiation of the Antarctic Circumpolar Current: Evidence from the South Pacific. *Geology* 35, 691–694. doi: 10.1130/G23806A.1.

Massom, R. A., and Stammerjohn, S. E. (2010). Antarctic sea ice change and variability – Physical and ecological implications. *Polar Sci* 4, 149–186. doi: 10.1016/J.POLAR.2010.05.001.

Matulja, D., Wittine, K., Malatesti, N., Laclef, S., Turks, M., Markovic, M. K., et al. (2020). Marine Natural Products with High Anticancer Activities. *Curr Med Chem* 27, 1243–1307. doi: 10.2174/0929867327666200113154115.

Mcclintock, J. B. (1994). Trophic biology of Antarctic shallow-water echinoderms. *Mar Ecol Prog Ser* 111, 191–202. doi: 10.3354/meps111191

McClintock, J. B., Amsler, C. D., Baker, B. J., and van Soest, R. W. M. (2005). Ecology of Antarctic Marine Sponges: An Overview. *Integr Comp Biol* 45, 359–368. doi: 10.1093/ICB/45.2.359.

Mincks, S. L., Smith, C. R., and DeMaster, D. J. (2005). Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment 'food bank.' *Mar Ecol Prog Ser* 300, 3–19. doi: 10.3354/MEPS300003.

Moles, J., Núñez, L., Taboada, S., and Figuerola, B. (2015). Anti-predatory chemical defences in Antarctic benthic fauna. *Mar Biol* 162, 1813–1821. doi: 10.1007/s00227-015-2714-9.

Molina, A. N., Pulgar, J. M., Rezende, E. L., and Carter, M. J. (2022). Heat tolerance of marine ectotherms in a warming Antarctica. *Glob Chang Biol*. doi: 10.1111/gcb.16402.

Morley, S. A., Chu, J. W. F., Peck, L. S., and Bates, A. E. (2022a). Temperatures leading to heat escape responses in Antarctic marine ectotherms match acute thermal limits. *Front Physiol* 13. doi: 10.3389/fphys.2022.1077376.

Morley, S. A., Souster, T. A., Vause, B. J., Gerrish, L., Peck, L. S., and Barnes, D. K. A. (2022b). Benthic Biodiversity, Carbon Storage and the Potential for Increasing Negative Feedbacks on Climate Change in Shallow Waters of the Antarctic Peninsula. *Biology (Basel)* 11. doi: 10.3390/biology11020320.

Newman, D. J., and Cragg, G. M. (2016). Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod* 79, 629–661. doi: 10.1021/ACS.JNATPROD.5B01055.

Norkko, A., Thrush, S. F., Cummings, V. J., Gibbs, M. M., Andrew, N. L., Norkko, J., et al. (2007). Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. *Ecology* 88, 2810–2820. doi: 10.1890/06-1396.1.

Núñez-Pons, L., and Avila, C. (2015). Natural products mediating ecological interactions in Antarctic benthic communities: a mini-review of the known molecules. *Nat Prod Rep* 32, 1114–1130. doi: 10.1039/c4np00150h.

Núñez-Pons, L., Rodríguez-Arias, M., Gómez-Garreta, A., Ribera-Siguán, A., and Avila, C. (2012). Feeding deterrency in Antarctic marine organisms: bioassays with the omnivore amphipod Cheirimedon femoratus. 462, 163–174. doi: 10.3354/meps09840.

Oliver, T. A., and Palumbi, S. R. (2011). Many corals host thermally resistant symbionts in high-temperature habitat. *Coral Reefs* 30, 241–250. doi: 10.1007/s00338-010-0696-0.

Orejas, C., Gili, J. M. 1953-, Arntz, W. E., Ros, J. 1946-, López, P. J., Teixidó Ullod, N., et al. (2000). Benthic suspension feeders, key players in Antarctic marine ecosystems? *CONTRIBUTIONS to SCIENCE* 1, 299–311. ISSN: 1575-6343

Pasotti, F., Saravia, L. A., de Troch, M., Tarantelli, M. S., Sahade, R., and Vanreusel, A. (2015). Benthic Trophic Interactions in an Antarctic Shallow Water Ecosystem Affected by Recent Glacier Retreat. *PLoS One* 10, e0141742. doi: 10.1371/JOURNAL.PONE.0141742.

Paul, V. J., Arthur, K. E., Ritson-Williams, R., Ross, C., Paul, J., Arthur, E., et al. (2007). Chemical Defenses: From Compounds to Communities. *Biol Bull* 213, 226–251.

Peck, L. S. (2005). Prospects for surviving climate change in Antarctic aquatic species. *Front Zool* 8, 1–8. doi: 10.1186/1742-9994-2-9.

Peck, L. S. (2018). Antarctic marine biodiversity: adaptations, environments and responses to change. In: S.J. Hawkins, A.J. Evans, A.C. Dale, L.B. Firth & I.P. Smith (eds.) Oceanography and Marine Biology: An Annual Review 56, 2-133. CRC Press. ISBN 978-0-429-45445-5 (ebk).

Peck, L. S., and Conway, L. Z. (2000). The myth of metabolic cold adaptation: Oxygen consumption in stenothermal Antarctic bivalves. *Geol Soc Spec Publ* 177, 441–450. doi: 10.1144/GSL.SP.2000.177.01.29.

Pereira, R. V., Filgueiras, C. C., Dória, J., Peñaflor, M. F. G. V., and Willett, D. S. (2021). The Effects of Biostimulants on Induced Plant Defense. *Frontiers in Agronomy* 3, 45. doi: 10.3389/FAGRO.2021.630596.

Pertierra, L. R., Hughes, K. A., Vega, G. C., and Olalla-Tárraga, M. Á. (2017). High resolution spatial mapping of human footprint across Antarctica and its implications for the strategic conservation of avifauna. *PloS one* 12, e0168280. doi: doi.org/10.1371/journal.pone.0168280.

Pertierra, L. R., Santos-Martin, F., Hughes, K. A., Avila, C., Caceres, J. O., de Filippo, D., et al. (2021). Ecosystem services in Antarctica: Global assessment of the current state, future challenges and managing opportunities. *Ecosyst Serv* 49, 101299. doi: 10.1016/j.ecoser.2021.101299.

Peters, K. J., Amsler, C. D., Mcclintock, J. B., and Baker, B. J. (2010). Potential chemical defenses of Antarctic sponges against sympatric microorganisms. 649–658. doi: 10.1007/s00300-009-0741-z.

Pfuhl, H. A., and McCave, I. N. (2005). Evidence for late Oligocene establishment of the Antarctic Circumpolar Current. *Earth Planet Sci Lett* 235, 715–728. doi: 10.1016/J.EPSL.2005.04.025.

Piepenburg, D., Schmid, K., and Gerdes, D. (2002). The benthos off King George Island (South Shetland Islands, Antarctica): further evidence for a lack of a latitudinal biomass cline in the Southern Ocean. 146–158. doi: 10.1007/s003000100322.

Pollock, J., Laura, G., Trong, W., and Mick, W. (2018). The madness of microbiome: attempting to find consensus “best practice” for 16S microbiome studies. *Appl Environ Microbiol* 84, e02627-17. doi: 10.1128/AEM.02627-17.

Poloczanska, E. S., Burrows, M. T., Brown, C. J., Molinos, J. G., Halpern, B. S., Hoegh-Guldberg, O., et al. (2016). Responses of marine organisms to climate change across oceans. *Front Mar Sci* 3, 62. doi: 10.3389/FMARS.2016.00062/BIBTEX.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703-718. doi: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.

Ramsby, B. D., Hoogenboom, M. O., Whalan, S., and Webster, N. S. (2018). Elevated seawater temperature disrupts the microbiome of an ecologically important bioeroding sponge. *Mol Ecol* 27, 2124–2137. doi: 10.1111/mec.14544.

Ranjan, R., Rani, A., Metwally, A., McGee, H. S., and Perkins, D. L. (2016). Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem Biophys Res Commun* 469, 967–977. doi: 10.1016/J.BBRC.2015.12.083.

Ren, X., Xie, X., Chen, B., Liu, L., Jiang, C., and Qian, Q. (2021). Marine Natural Products: A Potential Source of Anti-hepatocellular Carcinoma Drugs. *J Med Chem*. doi: 10.1021/ACS.JMEDCHEM.0C02026.

Ruocco, N., Esposito, R., Bertolino, M., Zazo, G., Sonnessa, M., Andreani, F., et al. (2021). A metataxonomic approach reveals diversified bacterial communities in antarctic sponges. *Mar Drugs* 19. doi: 10.3390/md19030173.

Rust, M., Helfrich, E. J. N., Freeman, M. F., Nanudorn, P., Field, C. M., Rückert, C., et al. (2020). A multiproducer microbiome generates chemical diversity in the marine sponge Mycale hentscheli. *Proc Natl Acad Sci U S A* 117, 9508–9518. doi: 10.1073/PNAS.1919245117.

Sacristán-Soriano, O., Pérez Criado, N., and Avila, C. (2020). Host Species Determines Symbiotic Community Composition in Antarctic Sponges (Porifera: Demospongiae). *Front Mar Sci* 7. doi: 10.3389/fmars.2020.00474.

Sangrà, P., Gordo, C., Hernández-Arencibia, M., Marrero-Díaz, A., Rodríguez-Santana, A., Stegner, A., et al. (2011). The Bransfield current system. *Deep Sea Research Part I: Oceanographic Research Papers* 58, 390–402. doi: 10.1016/J.DSR.2011.01.011.

Schoenrock, K. M., Schram, J. B., Amsler, C. D., McClintock, J. B., and Angus, R. A. (2015). Climate change impacts on overstory *Desmarestia* spp. from the western Antarctic Peninsula. *Mar Biol* 162, 377–389. doi: 10.1007/S00227-014-2582-8.

Shilling, A. J., Heiser, S., Amsler, C. D., McClintock, J. B., and Baker, B. J. (2021). Hidden diversity in an antarctic algal forest: Metabolomic profiling linked to patterns of genetic diversification in the antarctic red alga *Plocamium* sp. *Mar Drugs* 19. doi: 10.3390/md19110607.

Shinde, P., Banerjee, P., and Mandhare, A. (2019). Marine natural products as source of new drugs: a patent review (2015–2018). *Expert opinion on therapeutic patents* 29, 283–309. doi: 10.1080/13543776.2019.1598972.

Smale, D. A. (2007). Ice disturbance intensity structures benthic communities in nearshore Antarctic waters. 349, 89–102. doi: 10.3354/meps07104.

Sotomayor, E. M., Piantadosi, S., Miller, C. B., Karp, J. E., Jones, R. J., Rowley, S. D., et al. (2002). Long-term follow-up of intensive ara-C-based chemotherapy followed by bone marrow transplantation for adult acute lymphoblastic leukemia: impact of induction Ara-C dose and post-remission therapy. *Leuk Res* 26, 461–471. doi: 10.1016/S0145-2126(01)00175-8.

Staley, J. T., and Konopka, A. (1985). Measurement of in situ activities for non-photosynthetic microorganisms in aquatic and terrestrial habitats. *Ann Rev Microbiol*, 321–346.

Sun, W., Dai, S., Jiang, S., Wang, G., Liu, G., Wu, H., et al. (2010). Culture-dependent and culture-independent diversity of Actinobacteria associated with the marine sponge *Hymeniacidon perleve* from the South China Sea. *Antonie van Leeuwenhoek* 98, 65–75. doi: 10.1007/S10482-010-9430-8.

Taboada, C., Millán, R., and Míguez, I. (2010). Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. *J Sci Food Agric* 90, 445–449. doi: 10.1002/JSFA.3836.

Takahashi, T., Sweeney, C., Hales, B., Chipman, D. W., Newberger, T., Goddard, J. G., et al. (2012). The changing carbon cycle in the Southern Ocean. *Oceanography* 25, 26–37.

Taylor, M. W., Radax, R., Steger, D., and Wagner, M. (2007). Sponge-Associated Microorganisms: Evolution, Ecology, and Biotechnological Potential. *Microbiology and Molecular Biology Reviews* 71, 295–347. doi: 10.1128/mmbr.00040-06.

Teixidó, N., Garrabou, J., and Arntz, W. E. (2002). Spatial pattern quantification of Antarctic benthic communities using landscape indices. *Mar Ecol Prog Ser* 242, 1–14. doi: 10.3354/MEPS242001.

Thakur, N. L., and Singh, A. (2016). Chemical Ecology of Marine Sponges. In: Pallela, R., Ehrlich, H. (eds) Marine Sponges: Chemicobiological and Biomedical Applications. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2794-6_3

Tian, L., Yin, Y., Jin, H., Bing, W., Jin, E., Zhao, J., et al. (2020). Novel marine antifouling coatings inspired by corals. *Mater Today Chem* 17, 100294. doi: 10.1016/J.MTCHEM.2020.100294.

Turner, D., Lucieer, A., Malenovský, Z., King, D. H., and Robinson, S. A. (2014a). Spatial co-registration of ultra-high resolution visible, multispectral and thermal images acquired with a micro-UAV over Antarctic moss beds. *Remote Sensing* 6, 4003–4024. doi: 10.3390/RS6054003.

Turner, J., Barrand, N. E., Bracegirdle, T. J., Convey, P., Hodgson, D. A., Jarvis, M., et al. (2014b). Antarctic climate change and the environment: an update. *Polar Record* 50, 237–259. doi: 10.1017/S0032247413000296.

Ueoka, R., Uria, A. R., Reiter, S., Mori, T., Karbaum, P., Peters, E. E., et al. (2015). Metabolic and evolutionary origin of actin-binding polyketides from diverse organisms. *Nature Chemical Biology 2015 11:9* 11, 705–712. doi: 10.1038/nchembio.1870.

Vaughan, D. G., Marshall, G. J., Connolley, W. M., Parkinson, C., Mulvaney, R., Hodgson, D. A., et al. (2003). Recent rapid regional climate warming on the Antarctic Peninsula. *Clim Change* 60, 243–274. doi: 10.1023/A:1026021217991/METRICS.

Webster, N. S., Negri, A. P., Munro, M. M. H. G., and Battershill, C. N. (2004). Diverse microbial communities inhabit Antarctic sponges. *Environ Microbiol* 6, 288–300. doi: 10.1111/J.1462-2920.2004.00570.X.

Wilkinson, C. R. (1978). Microbial associations in sponges. I. Ecology, physiology and microbial populations of coral reef sponges. *Mar Biol* 49, 161–167. doi: 10.1007/BF00387115/METRICS.

Wilson, N. G., Maschek, J. A., and Baker, B. J. (2013). A Species Flock Driven by Predation? Secondary Metabolites Support Diversification of Slugs in Antarctica. *PLoS One* 8, e80277. doi: 10.1371/JOURNAL.PONE.0080277.

Yamada, Y., Kuzuyama, T., Komatsu, M., Shin-ya, K., Omura, S., Cane, D. E., et al. (2015). Terpene synthases are widely distributed in bacteria. *Proc Natl Acad Sci U S A* 112, 857–862. doi: 10.1073/PNAS.1422108112/SUPPL_FILE/PNAS.201422108SI.PDF.

Young, R. M., von Salm, J. L., Amsler, M. O., Lopez-Bautista, J., Amsler, C. D., McClintock, J. B., et al. (2013). Site-Specific Variability in the Chemical Diversity of the Antarctic Red Alga Plocamium cartilagineum. *Marine Drugs 2013, Vol. 11, Pages 2126-2139* 11, 2126–2139. doi: 10.3390/MD11062126.

Diseño de portada y contraportada: Mar de Castro Fernández
Foto de portada: Paula de Castro Fernández
Foto de contraportada: Marc Sentís Hernández

IRBio

UNIVERSITAT DE BARCELONA