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UNIVERSIDAD CIENTÍFICA DEL SUR

FACULTAD DE CIENCIAS AMBIENTALES

CARRERA PROFESIONAL DE BIOLOGÍA MARINA

"EVALUACIÓN DE AMINOÁCIDOS TIPO MICOSPORINA (MAAs) CON FUNCIÓN FOTOPROTECTORA FRENTE A LA RADIACIÓN UV EN DOS MACROALGAS ROJAS DE IMPORTANCIA ECONÓMICA DEL PERÚ"

Tesis para optar el título profesional de:

BIÓLOGA MARINA

Presentado por:

Fernanda Etsuko Miyagi Pita (0009-0007-9534-9756)

Asesor:

Juan Carlos Francioa Quiroz (0000-0001-5095-6623)

Lima – Perú

2026

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ACTA DE SUSTENTACIÓN DE TESIS

Lima, 20 de marzo de 2026

Los integrantes del Jurado de tesis:

Presidente: Mg. Leonardo Humberto Mendoza Carbajal
Miembro 1: Mg. Brisaida Maria Alejandra Castañeda Farro
Miembro 2: Blgo. Paul Martín Baltazar Guerrero

Se reúnen para evaluar la tesis titulada: "Evaluación de aminoácidos tipo micosporina (MAAs) con función fotoprotectora frente a la radiación UV en dos macroalgas rojas de importancia económica del Perú" con código de proyecto: N° PRE-5-2023-00455.

Presentada por la tesista:

- Fernanda Etsuko Miyagi Pita

Para optar el Título Profesional de Bióloga Marina.

Asesorada por: Mg. Juan Carlos Francia Quiroz

Luego de haber evaluado el informe final de tesis y evaluado el desempeño del estudiante de la carrera de Biología Marina en la sustentación, se concluye de manera unánime (X) por mayoría simple ( ) calificar a:

Tesista: Fernanda Etsuko Miyagi Pita			Nota (en letras): dieciocho
Aprobado ( )	Aprobado - Muy buena (X)	Aprobado - Sobresaliente ( )	Desaprobado ( )

Los miembros del jurado firman en señal de conformidad.

Mg. Leonardo Humberto Mendoza Carbajal
Presidente del Jurado

Mg. Juan Carlos Francia Quiroz
Asesor

Mg. Brisaida Maria Alejandra Castañeda Farro
Miembro 1

Blgo. Paul Martín Baltazar Guerrero
Miembro 2

ANEXO 21: DECLARACIÓN DE ORIGINALIDAD DE INFORME FINAL DE TESIS

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DECLARACIÓN DE ORIGINALIDAD DE CONTENIDO DE INFORME FINAL DE TESIS / TRABAJO DE INVESTIGACIÓN

Lima, 11 de febrero del 2026

Señor,
Dr. Percy Mayta Tristan
Vicerrectorado de Investigación
Universidad Científica del Sur
Presente. –

De nuestra consideración,

Yo: Fernanda Etsuko Miyagi Pita; egresada de la carrera de Biología Marina de la Universidad Científica del Sur, en conjunto con el asesor Juan Carlos Francia Quiroz, declaramos que este informe final de tesis de investigación titulado: "Evaluación de aminoácidos tipo micosporinas (maas) en *Chondracanthus chamissoi* Kützing 1843 y *Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 procedentes de la actividad acuícola (Pisco-Perú)", sustentado para obtener el Título Profesional de Biólogo Marino es original.

Es decir, no contiene plagio parcial ni total, cuando se utilizó información de fuentes externas se reconoció la autoría mediante la adecuada citación y los resultados obtenidos son producto entero de mi investigación y no han sido falseados ni fabricados. Todo esto en cumplimiento del Código de ética en la investigación, Reglamento del Comité de Integridad Científica, Reglamento de Propiedad Intelectual, Normas y procedimientos de los trabajos de investigación para la obtención de títulos profesionales y grados académicos[14], que afirmamos conocer en su totalidad.

Por ello, en caso de identificarse alguna situación de plagio, falsificación o fabricación de resultados, nos allanamos al proceso de investigación que establezca la Universidad y las posibles sanciones que pudieran surgir.

Firmamos en conformidad con lo declarado,



Firma tesista
DNI: 76391804



Firma del asesor de tesis
DNI: 40059556

13 En conformidad con la Resolución del Consejo Directivo N° 084-2022-SUNEDU/CD
14 Los documentos están en: https://investigacion.cientifica.edu.pe/reglamentos/

INFORME DE REVISIÓN DE ORIGINALIDAD

Título del documento evaluado. Evaluación de aminoácidos tipo micosporina (MAAs) con función fotoprotectora frente a la radiación UV en dos macroalgas rojas de importancia económica del Perú

Autores. Fernanda Etsuko Miyagi Pita

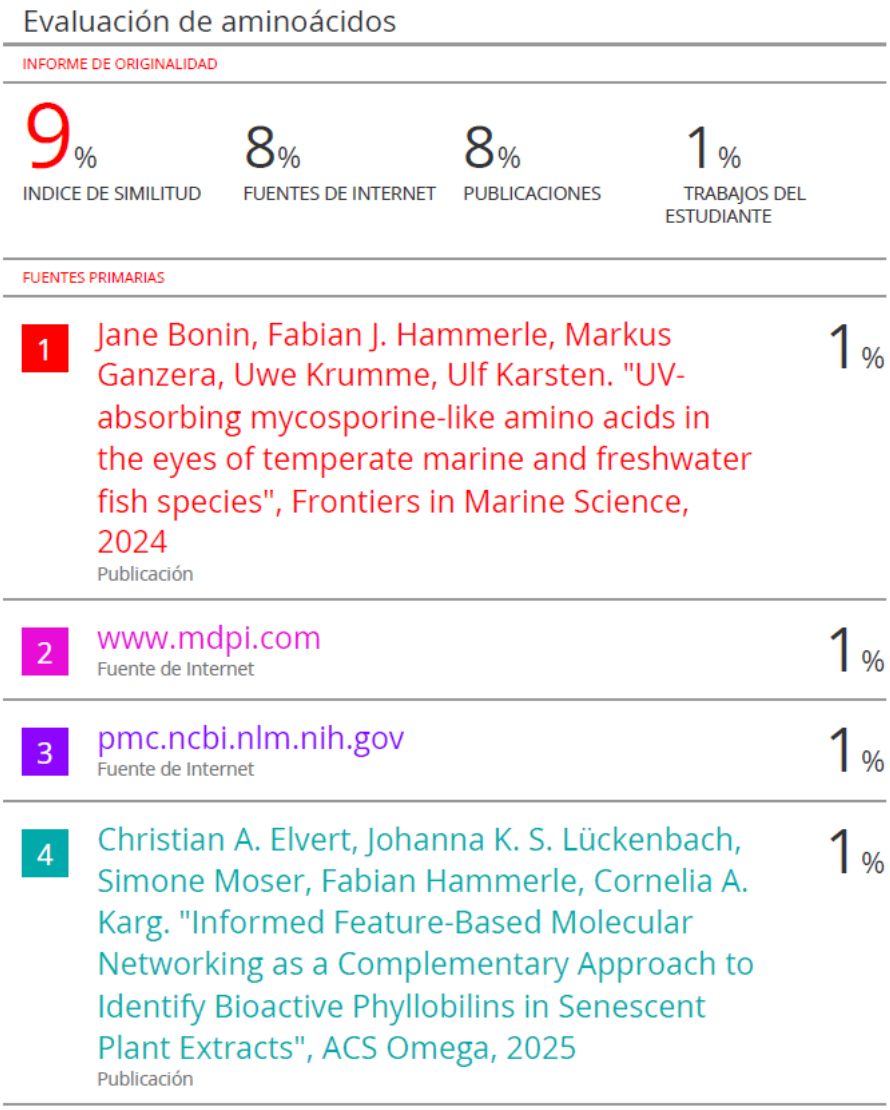
Mecanismo de revisión de originalidad. Evaluación con Turnitin (ID 2926095081)

Código de registro. PRE-5-2023-00455

Resultado de la evaluación. 9%

Revisado por. Magaly Kelly Guerrero Huaracallo.

Comentarios sobre la revisión. Filtros usados: excluir fuentes de menos de 12 palabras.



Evaluación de aminoácidos

INFORME DE ORIGINALIDAD

9%	8%	8%	1%
INDICE DE SIMILITUD	FUENTES DE INTERNET	PUBLICACIONES	TRABAJOS DEL ESTUDIANTE

FUENTES PRIMARIAS

1	Jane Bonin, Fabian J. Hammerle, Markus Ganzera, Uwe Krumme, Ulf Karsten. "UV-absorbing mycosporine-like amino acids in the eyes of temperate marine and freshwater fish species", Frontiers in Marine Science, 2024 Publicación	1%
2	www.mdpi.com Fuente de Internet	1%
3	pmc.ncbi.nlm.nih.gov Fuente de Internet	1%
4	Christian A. Elvert, Johanna K. S. Lückenbach, Simone Moser, Fabian Hammerle, Cornelia A. Karg. "Informed Feature-Based Molecular Networking as a Complementary Approach to Identify Bioactive Phyllobilins in Senescent Plant Extracts", ACS Omega, 2025 Publicación	1%

7. DEDICATORIA

Perseguir lo imposible, crear oportunidades donde parecía no haberlas y dar siempre lo mejor de mí: gracias por sembrar en mí la perseverancia y por enseñarme a no rendirme cuando el camino se vuelve incierto y desafiante.

Gracias, papás. Aunque nos separen miles de kilómetros, su presencia y su apoyo constante habita en cada decisión que tomo, en cada paso firme y también en cada duda superada.

Esta investigación representa, metafóricamente, el broche de oro de todo lo que me han enseñado a lo largo de mi vida. La persona que hoy hemos formado puede llamarse bióloga marina; pero más que un título, es una identidad construida con pasión, amor, esfuerzo y constancia.

8. AGRADECIMIENTOS

Quisiera expresar mi más sincero agradecimiento, en primer lugar, a mis supervisores Juan Carlos Francia-Quiroz, Ulf Karsten y Oscar Reátegui, quienes fueron el motor que impulso esta investigación hasta su culminación. Agradezco de manera especial a la Universidad de Rostock y al profesor Ulf Karsten por brindarme la oportunidad de desarrollar parte de este trabajo en su laboratorio, así como por su confianza, apertura académica y apoyo constante; sin su respaldo, la realización de esta investigación no habría sido posible.

Agradezco asimismo la valiosa colaboración de Fabian Hammerle y Markus Ganzera, cuyos aportes enriquecieron significativamente este estudio y contribuyeron a elevar la calidad de sus resultados. Del mismo modo, expreso mi gratitud a mi colega Barış Ballık por su compañerismo y apoyo constante durante el desarrollo del trabajo.

Extiendo mi agradecimiento a Paul Baltazar por facilitar el acceso al material biológico en coordinación con el LICMA y CONTRAPALMAR. Mi reconocimiento también a Arturo Mireyes, Max Castañeda y Antonio Calderón (LICMA) por su apoyo en el muestreo en campo, el procesamiento de muestras y la identificación de especies. Agradezco a Akemi Watanabe por su colaboración en el procesamiento de muestras en laboratorio, incluida la liofilización, y a Azam Omidi por su apoyo en la realización de los análisis estadísticos.

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Finalmente, expreso mi reconocimiento a los grupos de investigación que brindaron respaldo académico e institucional a este trabajo: Grupo CTS — Caracterización, Transformación y Sostenibilidad de los Recursos Naturales (Perú); LICMA — Grupo de Investigación en Acuicultura Sostenible (Perú); y el Grupo de Ecología Aplicada y Ficología, Instituto de Biociencias, Universidad de Rostock (Alemania).

9. RESUMEN

Los aminoácidos tipo micosporina (MAAs) son metabolitos multifuncionales que absorben radiación UV y presentan una creciente relevancia biotecnológica. Este estudio proporciona la primera caracterización integral de los perfiles de MAAs en dos macroalgas rojas peruanas de importancia comercial, *Chondracanthus chamissoi* y *Gracilariopsis lemaneiformis*, cultivadas en Pisco (Ica, Perú). Se analizaron diferencias estacionales (primavera vs. verano) y de estadio de vida (gametofito vs. esporofito) mediante HPLC–DAD para la cuantificación dirigida y UHPLC–HRMS/MS acoplada a redes moleculares basadas en características (FBMN) para el descubrimiento no dirigido de metabolitos. Ambas especies fueron confirmadas como altas productoras de MAAs (> 2 mg g^{-1} PS) y compartieron el mismo conjunto de 13 MAAs, aunque sus abundancias relativas variaron según la especie, la estación y el estadio de vida. *C. chamissoi* acumuló hasta 3,39 mg g^{-1} PS y presentó un perfil dominante en SH (shinorina (SH), palitina (PA) y asterina-330 (AS)), complementado por diez MAAs adicionales de baja abundancia detectados mediante FBMN. *G. lemaneiformis* alcanzó concentraciones totales más altas (hasta 3,69 mg g^{-1} PS) y mostró un repertorio más amplio de MAAs primarios, incluyendo porphyra-334 (PR), junto con nueve metabolitos secundarios detectados a niveles traza. El forzamiento estacional produjo máximos pronunciados en verano en la concentración total de MAAs en ambas especies, aunque la magnitud de la inducción fue sustancialmente mayor en *G. lemaneiformis* — consistente con una estrategia fotoprotectora inducible y dependiente del hábitat — mientras que *C. chamissoi* mostró una respuesta más constitutiva, alineada con la exposición persistente a radiación UV en el intermareal. También se evidenciaron efectos del estadio de vida: los esporofitos de ambas especies generalmente acumularon mayores niveles totales de MAAs y exhibieron perfiles más ricos en estos compuestos. El análisis mediante FBMN reveló varios MAAs previamente no reportados en ambos taxones, ampliando la diversidad química conocida de las Rhodophyta peruanas. En conjunto, estos hallazgos demuestran una fuerte modulación ambiental y ontogenética de la biosíntesis de MAAs y

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destacan a ambas especies — particularmente *G. lemaneiformis* — como fuentes prometedoras y sostenibles de compuestos naturales con protección UV para aplicaciones cosméticas, farmacéuticas y biomédicas.

10. ABSTRACT

Mycosporine-like amino acids (MAAs) are UV-absorbing, multifunctional metabolites with increasing biotechnological relevance. This study provides the first comprehensive characterization of MAAs profiles in two commercially important Peruvian red macroalgae, *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*, cultivated in Pisco (Ica, Peru). Seasonal (spring vs. summer) and life-stage (gametophyte vs. sporophyte) differences were analysed using HPLC–DAD for targeted quantification and UHPLC–HRMS/MS coupled with feature-based molecular networking (FBMN) for untargeted metabolite discovery. Both species were confirmed as high MAAs producers (> 2 mg g^{-1} DW) and shared the same set of 13 MAAs, although their relative abundances varied by species, season, and life stage. *C. chamissoi* accumulated up to 3.39 mg g^{-1} DW and exhibited a SH-dominant profile (shinorine (SH), palythine (PA) and asterina-330 (AS)), complemented by ten additional low-abundance MAAs detected by FBMN. *G. lemaneiformis* reached higher total concentrations (up to 3.69 mg g^{-1} DW) and displayed a broader primary-MAAs repertoire, including porphyra-334 (PR), together with nine secondary metabolites detected at trace levels. Seasonal forcing produced pronounced summer maxima MAAs total concentration in both species, although the magnitude of induction was substantially greater in *G. lemaneiformis* - consistent with an inducible, habitat-dependent photoprotective strategy - whereas *C. chamissoi* showed a more constitutive response aligned with persistent intertidal UV exposure. Life-stage effects were also evident, sporophytes of both species generally accumulated higher total MAAs levels and exhibited richer MAAs profiles. FBMN revealed several previously unreported MAAs in both taxa, expanding the known chemical diversity of Peruvian Rhodophyta. Collectively, these findings demonstrate strong environmental and ontogenetic modulation of MAAs biosynthesis and highlight both species - particularly *G. lemaneiformis* - as promising, sustainable sources of natural UV-protective compounds for cosmetic, pharmaceutical, and biomedical application

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11. VERSIÓN APROBADA PARA PUBLICAR

Evaluation of UV-sunscreen mycosporine-like amino acids (MAAs) in two economically important red macroalgae from Peru

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Abstract

Mycosporine-like amino acids (MAAs) are UV-absorbing, multifunctional metabolites with increasing biotechnological relevance. This study provides the first comprehensive characterization of MAAs profiles in two commercially important Peruvian red macroalgae, *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*, cultivated in Pisco (Ica, Peru). Seasonal (spring vs. summer) and life-stage (gametophyte vs. tetrasporophyte) differences were analysed using HPLC–DAD for targeted quantification and UHPLC–HRMS/MS coupled with feature-based molecular networking (FBMN) for untargeted metabolite discovery. Both species were confirmed as high MAAs producers (> 2 mg g^{-1} DW) and shared the same set of 13 MAAs, although their relative abundances varied by species, season, and life stage. *C. chamissoi* accumulated up to 3.39 mg g^{-1} DW and exhibited a SH-dominant profile (shinorine (SH), palythine (PA) and asterina-330 (AS)), complemented by ten additional low-abundance MAAs detected by FBMN. *G. lemaneiformis* reached higher total concentrations (up to 3.69 mg g^{-1} DW) and displayed a broader primary-MAAs repertoire, including porphyra-334 (PR), together with nine secondary metabolites detected at trace levels. Seasonal forcing produced pronounced summer maxima MAAs total concentration in both species, although the magnitude of induction was substantially greater in *G. lemaneiformis* - consistent with an inducible, habitat-dependent photoprotective strategy - whereas *C. chamissoi* showed a more constitutive response aligned with persistent intertidal UV exposure. Life-stage effects were also evident, tetrasporophytes of both species generally accumulated higher total MAAs levels and exhibited richer MAAs profiles. FBMN revealed several previously unreported MAAs in both taxa, expanding the known chemical diversity of Peruvian Rhodophyta. Collectively, these findings demonstrate strong environmental and ontogenetic modulation of MAAs biosynthesis and highlight both species - particularly *G. lemaneiformis* - as promising, sustainable sources of natural UV-protective compounds for cosmetic, pharmaceutical, and biomedical applications.

Keywords: *Chondracanthus chamissoi*, *Gracilariopsis lemaneiformis*, Mycosporine-like amino acids, FBMN, Seasonal variation, Life stages, Photoprotection, Peruvian Rhodophyta

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Introduction

Rising levels of ultraviolet radiation (UVR), driven by climate change and stratospheric ozone depletion, pose increasing risks to aquatic ecosystems (Neale et al. 2023). Subtropical regions of South America, including Peru, consistently record some of the highest global UV Index values, exacerbating the vulnerability of marine organisms to harmful radiation (Robinson and Erickson 2015).

Peru lies within a global biodiversity hotspot shaped by the Humboldt Current eastern boundary upwelling system, one of the most productive marine ecosystems worldwide (Kämpf and Chapman 2016). Despite this high productivity, elevated UVR due to the low latitudes imposes substantial physiological and ecological stress. Marine organisms, unlike many terrestrial taxa, lack protective structures or shaded refuges, leaving them directly exposed to UVR and photosynthetically active radiation (PAR). In primary producers such as macroalgae and phytoplankton, UVR can inhibit photosynthesis, degrade pigments, and impair electron transport in Photosystem II (Vass et al. 2005), leading to cascading impacts on productivity, trophic interactions, and ecosystem stability (Häder et al. 2007; Williamson et al. 2019).

To mitigate these stressors, many marine primary producers synthesize mycosporine-like amino acids (MAAs), a group of small (<400 Da), water-soluble secondary metabolites that act as natural sunscreens by absorbing and dissipating UVR as heat (Carreto and Carignan 2011; Muthumani et al. 2015; Geraldes and Pinto, 2021). MAAs are generated mainly via the shikimate and pentose phosphate pathways (Spence et al. 2012) and feature cyclohexenone or cyclohexenimine cores with nitrogenous substituents, enabling strong absorption across 268–362 nm with exceptionally high molar extinction coefficients (Balskus and Walsh 2010). Beyond photoprotection, MAAs exhibit antioxidant and thermoprotective properties, positioning them as multifunctional anti-stress compounds in diverse marine environments (Flaim et al. 2014; Torres et al. 2018). Their versatility has attracted growing interest in industry, particularly in cosmetics, pharmaceuticals, and biomedical applications (Rosic et al. 2023). As eco-friendly alternatives to synthetic UV filters, several MAAs also exhibit immunomodulatory, anti-inflammatory, and wound-healing activities (Vega et al. 2021; Görünmek et al. 2024).

Red macroalgae (Rhodophyta) are among the richest natural MAAs producers, with concentrations reaching up to 20 mg g^{-1} dry weight (DW), and some species exceeding this threshold (Lamare et al. 2004; Sun et al. 2020). Over 70 MAAs have been identified across

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marine taxa, with at least 22 reported from Rhodophyta, including ubiquitous compounds such as shinorine (SH), porphyra-334 (PR), palythine (PA), asterina-330 (AS), and palythinol (PAO) (Sun et al. 2020; Geraldes and Pinto 2021). However, the concentration and composition of MAAs in macroalgae are highly variable and shaped by a complex interplay of environmental and intrinsic factors (Lalegerie et al. 2020). Key drivers include UVR intensity, PAR, seasonal cycles, salinity, temperature, pH, and nutrient availability - particularly nitrogen and phosphate (Briani et al. 2018; Jofre et al. 2020; Geraldes and Pinto 2021). Intrinsic traits such as species identity, habitat depth, tissue type, life-stage differences, and genetic variability further modulate MAAs biosynthesis (Hoyer et al. 2001; Marshall and Newman, 2002; Boedeker and Karsten 2005; Carreto and Carignan, 2011).

Recent scientific advances have markedly expanded the known structural diversity of MAAs and improved analytical capabilities for their detection, particularly regarding their structural variability, molecular connectivity, and stereochemistry (Orfanoudaki et al. 2019, 2020a, 2023). These advances have not only expanded the number of known natural sunscreen compounds but have also driven the development of specialized analytical methods, ranging from validated CE–DAD protocols (Hartmann et al. 2017) to (U)HPLC–DAD workflows optimized for quantification (Orfanoudaki et al. 2020b; Zwerger and Ganzera 2022), now enabling the reliable profiling of both abundant and trace MAAs. More recently, untargeted metabolomic approaches, especially UHPLC–HRMS/MS combined with feature-based molecular networking (FBMN; Nothias et al. 2020), have become powerful tools in natural products chemistry for capturing full metabolic fingerprints and identifying related compound families (Hammerle et al. 2023; Pellissier et al. 2023; Quiros-Guerrero et al. 2024). Coupled with public MS^2 repositories such as GNPS (https://gnps.ucsd.edu/) and structural annotation tools like SIRIUS 4 (Dührkop et al. 2019), these strategies allow rapid, high-confidence annotation of known and novel MAAs. Their application in phycology is expanding, including recent analyses of macroalgae with this combined UHPLC–HRMS/MS–FBMN approach (Zwerger et al. 2023) and even MAAs-rich fish tissues (Hammerle et al. 2025).

Despite extensive research on MAAs in temperate and polar macroalgae, subtropical regions such as the Peruvian coast remain critically understudied (Hoyer et al., 2001; Briani et al., 2018; Sun et al., 2020). Peru hosts a rich Rhodophyta flora (Ávila-Peltroche & Padilla-Vallejos, 2020), yet no studies have characterized the MAAs profiles of its dominant commercial species. This gap is notable given the region's unique oceanographic context: shallow embayments and rocky platforms transitioning sharply into deeper subtidal zones influenced by the Humboldt

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Current's intense upwelling (Tarazona & Arntz, 2001; Avila-Peltroche & Padilla-Vallejos, 2020). These conditions generate steep gradients in light, temperature, nutrient supply, and turbidity over short spatial scales (Gutiérrez et al., 2011), creating environments that differ markedly from well-studied temperate coasts. As a result, Peruvian macroalgae are continually exposed to extreme UV radiation, nutrient-rich cold upwelled waters, and pronounced seasonal hydrographic shifts - conditions that likely shape their MAAs biosynthetic strategies in distinctive ways. Together, these features make the region an ecologically compelling and distinct natural laboratory for studying MAAs biosynthesis.

Currently, the commercially most important red macroalgae in Peru are *Chondracanthus chamissoi* ("yuyo") and *Gracilariopsis lemaneiformis* ("pelillo") (Rodríguez et al. 2018). *C. chamissoi* is harvested for human consumption and carrageenan extraction (Almeyda and Armas 2018; Arbaiza et al. 2019), while *G. lemaneiformis* is cultivated for agar production (Noriega 2016; Rodríguez et al. 2018). However, the Peruvian macroalgae industry remains focused on exporting raw biomass - primarily to China - with limited investment in value-added bioproducts. Between 2005 and 2014, macroalgae exports surged by 521%, from 5,585 to 29,089 metric tons dry weight (MTDW), peaking at 33,948 MTDW in 2019 before slightly declining to 29,642 MTDW in 2020 (Montero and Arbaiza, 2016; Díaz Ruíz et al. 2021; Avila-Peltroche and Villena-Sarmiento, 2022).

Despite the sector's economic growth, no studies have yet characterized the MAAs profiles of these key species or examined the environmental factors regulating their biosynthesis. This knowledge gap limits the potential for developing high-value, sustainable biotechnological applications based on Peruvian macroalgae. Therefore, this study aims to characterize for the first time the detailed MAAs profiles of *C. chamissoi* and *G. lemaneiformis* cultivated in Ica, one of Peru's regions with the highest UV radiation levels (SENAMHI 2024). By analyzing seasonal variation (spring vs. summer) and life stage differences (gametophyte vs. tetrasporophyte), this research seeks to elucidate the environmental and biological drivers shaping MAAs production in these species. Ultimately, this work provides a foundation for the development of sustainable, high-value photoprotective compounds derived from Peruvian macroalgae and contributes to a broader understanding of MAAs biosynthesis in subtropical coastal ecosystems.

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Materials and methods

Macroalgae Harvesting

Samples of *Chondracanthus chamissoi* (C.Agardh) Kützing and *Gracilariopsis lemaneiformis* (Bory) E.Y.Dawson, Acleto and Foldvik were collected from a marine bottom cultivation system at La Puntilla Beach, Paracas, Ica, Peru (13°48′25.57″S, 76°15′06.64″W). The site is managed by the Cooperativa de Trabajadores Pesqueros Artesanales Algas Marinas (COTRAPALMAR) in collaboration with the Laboratorio de Investigación en Cultivos Marinos (LICMA) (Suppplementary Fig. S1).

Sampling was conducted on two dates: 6th of November 2023 (spring) and 5th of March 2024 (summer). For each species, up to 500 g of fresh biomass was collected from the intertidal zone (0–50 cm depth), placed in sterile plastic bags, and transported in cooled containers to the laboratory at Universidad Científica del Sur.

To minimize climatic variability, samples were collected on sunny days and within a restricted time window (10:00-12:00 h), following recommendations of Velasco-Charpentier et al. (2016) and Navarro et al. (2014). In situ measurements of surface seawater temperature, dissolved oxygen, salinity, and pH were taken using a YSI PLUS multiparameter probe. Historical and seasonal oceanographic data (2023-2024) were obtained from the Laboratorio Costero de Pisco, Instituto del Mar del Perú (IMARPE), as part of its marine environmental monitoring program (https://www.imarpe.pe/imarpe/accesoinformacion/). Light-related parameters, including all-sky surface photosynthetically active radiation (PAR) and UV index, were retrieved all from the NASA POWER Data Access Viewer (https://power.larc.nasa.gov/data-access-viewer/). All physicochemical parameters are summarized in Table 1.

Species were taxonomically identified based on morphological characteristics. Identifications were verified by specialists from LICMA and formally confirmed at the Natural History Museum of the Universidad Ricardo Palma. Isomorphic life stages were distinguished based on reproductive structures, with fertilized female gametophytes identified by the presence of cystocarps and tetrasporophytes by the presence of tetrasporangia (Supplementary Fig. S2). All gametophytes analyzed in this study corresponded exclusively to fertilized female gametophytes (cystocarpic plants).

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MAAs extraction

Fresh material was rinsed with distilled water to remove epiphytes, salt, and debris, then lyophilized (-108 °C, 0.013 mbar, 24 h). Dried samples were ground, sieved (< 250 µm), and stored in amber jars at -20 °C. MAAs were extracted following Karsten et al. (2009), with modifications. Ten milligrams of freeze-dried algal powder (three replicates per treatment combination: species, season and life stage) were mixed with 1 mL of 25% aqueous methanol, vortexed, and incubated in a water bath at 45 °C for 2 h in tightly sealed screw-cap vials to allow solvent extraction. After incubation, samples were centrifuged at 13,000 × *g* for 5 min, and 800 µL of the clear supernatant were transferred to new vials and evaporated to dryness under vacuum. The dried residues were reconstituted in 800 µL of distilled water, vortexed, centrifuged again to remove particulates, and finally filtered through 0.22 µm membrane filters before transfer to HPLC vials (Suppplementary Fig. S3).

Quantification and identification of MAAs

The MAAs content of extracts was determined by high-performance liquid chromatography with diode array detection (HPLC-DAD), following Orfanoudaki et al. (2020b). Analyses were carried out on a Merck–Hitachi Elite La Chrom instrument (Tokyo, Japan) equipped with an organizer (SYSTEM O/B), a quaternary pump (L-2100), a degasser (Degasys DG-2410; Uniflows Co.,Ltd., Tokyo, Japan), an autosampler (L-2200), a column oven (L-2300), and a diode-array detector (L-2455). As stationary phase, a YMC-Pack ODS column (250 mm × 4.60 mm, 5 µm; YMC Europe, Dinslaken, Germany) in combination with an appropriate guard column was used. The mobile phase consisted of 20 mM ammonium formate and 0.25% (v/v) formic acid in water (A) and methanol (B). The applied gradient was as follows: 100% A from 0 to 20 min, 80% A at 30 min, 2% A at 35 min, and held at this composition for 5 min (total runtime of 40 min); followed by 15 min of re-equilibration under the initial conditions. Flow rate, temperature, and injection volume were adjusted to 0.65 mL min^{-1}, 9 °C and 5.0 µL, respectively. Detection wavelengths were set to 310, 330, and 350 nm. The area under the curve of target peaks was determined with EZChrom Elite software using the chromatograms recorded at 330 nm.

As described previously (Bonin et al. 2024; Hammerle et al. 2025), calibration was performed using stock solutions of shinorine (SH), palythine (PA), asterina-330 (AS), and porphyra-334 (PR), originally isolated and characterized in earlier work (Orfanoudaki et al. 2020b). These

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standards, along with additional reference compounds including mycosporine-glycine (MG), mycosporine-glycine-alanine (MGA), aplysiapalythine A (APA), aplysiapalythine B (APB), mycosporine-methylamine-threonine (MMT), usujirene (US), and palythene (PAE), were stored at −20 °C and protected from light to minimize degradation. Stock solutions were periodically refreshed by re-isolation, and the stability and concentration of calibrator solutions were routinely verified prior to analysis. MAAs are known to be chemically stable under these storage conditions, as documented in previous studies (Orfanoudaki et al. 2019).

UHPLC–VWD–HR-MS² Analysis and Feature-based Molecular Networking (FBMN)

UHPLC–HRMS/MS analyses were carried out in collaboration with the University of Innsbruck. Extracts, blanks (ultra-pure water), and reference standards (Orfanoudaki et al. 2020b) were analyzed following the workflows of Zwerger et al. (2023) and Hammerle et al. (2025), with minor modifications.

Chromatographic separations were conducted on a Vanquish UHPLC system (Thermo Scientific, USA), consisting of a binary pump (VC-P10-A), an autosampler (VC-A12-A), a column oven (VC-C10-A), and a variable wavelength detector (VC-D40-A) connected to a Thermo Scientific Exploris 120 Orbitrap HRMS unit. A Luna Omega C18 100 Å column (100 mm × 2.1 mm; particle size 1.6 µm; Phenomenex, Torrance, USA), protected by a SecurityGuard ULTRA guard C18 pre-column, served as stationary phase. The mobile phase comprised water with 0.25% formic acid and 20 mM ammonium formate (A) and acetonitrile (B). The gradient program was: 0–10 min, 0–5% B; 10–11 min, 5–90% B; 11–13 min, 90% B; followed by 20 min re-equilibration at initial conditions. The flow rate, column oven temperature, auto-sampler temperature, and injection volume were adjusted to 0.3 mL min^{-1}, 17 °C, 20 °C, and 1 µL, respectively. UV detection was performed at 330 and 350 nm, the data collection rate to 2.0 Hz, the response time to 2.0 s, and the peak width to 0.2 min.

The system was controlled by Thermo Scientific Xcalibur 4.4 software. Calibration of the mass analyzer was done via the Thermo Scientific proprietary calibration mix and the respective automatic calibration function. The mass spectrometric parameters were as follows: heated-ESI ionization source, static spray voltage (positive: 3500 V), sheath gas (N2): 30 arbitrary units, auxiliary gas (N2): 17 arbitrary units, sweep gas (N2): 0 arbitrary units. Temperature of the ion transfer tube and vaporizer was adjusted to 370 and 420 °C, respectively. MS data (range 70-1000 *m/z*) were recorded from 0 to 20 min with a resolution of 60,000 FWHM for MS^1. The

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RF lens parameter was set to 70%. Data-dependent experiments were conducted with stepped collision energy mode and normalized collision energy type using HCD collision energies of 15, 30, and 45% at a resolution of 15,000 FWHM. The number of dependent scans was set to 3. The following selection of filters was employed: intensity threshold filter (1.0E5), dynamic exclusion (auto), isotope exclusion (assigned), charge state (perform dependent scans on single charged precursors only), and apex filter (desired apex window: 75%). In addition, a specific exclusion list was created for the measurement using HPLC-grade water as a background extract with an IODA Mass Spec notebook (Zuo et al. 2021).

Raw data were converted using **MSConvert** (Chambers et al. 2012) and processed in **MZmine 4.3.0** (Schmid et al. 2023). Feature tables and MS^2 spectra were then submitted to the **FBMN workflow on GNPS** (Nothias et al. 2020) to generate molecular networks, which were visualized in **Cytoscape** (Shannon et al. 2003). Metabolite annotation was performed using **SIRIUS 6.1.1** (Dührkop et al. 2015, 2019, 2021), and compound-class predictions from **CANOPUS** (Kim et al. 2021) were used to further characterize feature groups. Detailed processing and annotation parameters are provided in the Supplementary Material.

To identify clusters potentially containing MAAs, we followed the workflow described by Zwerger et al. (2023), applying a multi-criteria annotation strategy. Experimental MS^2 spectra were first compared against an in-house MAAs reference database during data preprocessing in MZmine, using accurate $[M+H]^+$ masses within a defined mass error tolerance. In parallel, MS^2 spectra were searched against the GNPS community spectral library, enabling spectral similarity-based matching and analog searches. Molecular formula predictions were further refined using SIRIUS, and compound-class annotations were assigned with CANOPUS to visualize NPC chemotaxonomic categories (Kim et al. 2021) within the molecular network, highlighting features classified as “mycosporines and mycosporine-like amino acids.”

In addition, a Cytoscape-compatible UV330 filter was applied to the network to identify MS features co-eluting with chromatographic peaks exhibiting strong absorbance at 330 nm, a diagnostic wavelength for MAAs. Only features supported by multiple, independent lines of evidence—including accurate mass, MS/MS spectral similarity, UV absorption behavior, retention time, and chemotaxonomic consistency—were retained as putative MAAs.

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Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics v30 (IBM Corp., Armonk, NY, USA). For each species (*C. chamissoi* and *G. lemaneiformis*), the effects of season (spring vs. summer) and life stage (gametophyte vs. tetrasporophyte) on (i) total MAAs concentration and (ii) the concentrations of individual major MAAs (SH, PA, AS, and PR) were evaluated.

Samples corresponded to independent biological replicates, defined as different thalli randomly collected from the cultivation system until approximately 500 g of fresh biomass was obtained for each treatment combination. After lyophilization, dried material from each treatment was used to prepare the MAAs extract, which was made in three independent replicates (n = 3).

Data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When assumptions were met, a two-way analysis of variance (ANOVA) was applied with season and life stage as fixed factors. When significant effects were detected, Tukey's honestly significant difference (HSD) test was used for post hoc pairwise comparisons.

For selected pairwise comparisons, independent-samples *t*-tests were additionally applied to further explore differences between specific groups. Statistical significance was set at $p < 0.05$, and exact *p*-values are reported whenever possible.

Results are expressed as mean ± standard deviation (SD), where *n* refers to the number of independent biological replicates ($n = 3$). Given the small sample size, statistical outcomes were interpreted cautiously and supported by consistent trends across seasons and life stages. For *G. lemaneiformis*, tetrasporophytes were absent from the cultivation system during spring; therefore, seasonal comparisons for this life stage were not performed.

All graphs were generated using Microsoft Excel 2021 and display mean values with error bars representing ± SD. Different letters above the bars indicate statistically significant differences among groups based on Tukey's HSD post hoc tests ($p < 0.05$), whereas bars sharing the same letter do not differ significantly.

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Results

MAAs Profiles

Chromatographic analyses revealed both shared and species-specific patterns of MAA accumulation in *C. chamissoi* and *G. lemaneiformis* (Fig. 1). HPLC–DAD quantified the primary MAAs - defined here as the major contributors to total MAAS content, whereas UHPLC–HRMS/MS combined with FBMN expanded the detectable chemical diversity by resolving low-abundance compounds, herein referred to as secondary MAAs. Across both species, 13 MAAs were putatively annotated: *C. chamissoi* contained three primary and ten secondary MAAs, while *G. lemaneiformis* contained four primary and nine secondary MAAs (Fig. 2; Table 2).

HPLC–DAD Quantification

Analysis using four calibrators and seven reference standards showed that *C. chamissoi* was dominated by SH, PA, and AS, whereas *G. lemaneiformis* exhibited a broader primary profile that also included PR (Fig. 2). Total MAA concentrations differed between species, with *G. lemaneiformis* showing a wider range of values and reaching higher maximum concentrations (1.73–3.69 mg g^{-1} DW) compared with *C. chamissoi* (2.37–3.39 mg g^{-1} DW), indicating species-specific differences in biosynthetic capacity.

UHPLC–VWD–HR-MS² and FBMN

UHPLC–VWD–HRMS/MS analysis of algal extracts and blanks, followed by data processing and feature-based molecular networking in the GNPS environment, generated a molecular network comprising 881 nodes and 1,367 edges, organized into 45 clusters containing three or more nodes. To identify features potentially corresponding to MAAs, the workflow established by Zwerger et al. (2023) was applied, including UV absorbance filtering at 330 nm and MS/MS spectral similarity. Approximately 40% of all nodes exhibited UV-absorption characteristics consistent with MAAs. Based on these criteria, three clusters and three individual nodes were identified as putative MAA-related features (Fig. 3A).

Beyond the MAAs quantified by HPLC–DAD (SH, PA, AS, and PR), LC–MS/MS analysis revealed a broader chemical diversity, with 13 putatively identified MAAs detected across both

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species, including several compounds present only at trace levels (Table 2). Comparison with in-house reference spectra obtained from multiple previous studies enabled high-confidence putative annotation of APC, MG, APA, MGA, APB, MMT, mycosporine-lysine (ML), US, and PAE (Fig. 3B). Additional nodes corresponding to less common analogues, such as palythine-serine, palythine-threonine, and shinorine methyl ester, were also observed (Fig. 4).

Cluster 1 contained 42 nodes, eight of which were classified as MAAs at the compound-class level by SIRIUS, including well-known compounds such as SH, PA, AS, and several aplysiapalythines. Structurally related MAAs, including the cis–trans pair US and PAE, as well as the group comprising SH, AS, and APA, formed distinct subregions within this cluster. Cluster 2 comprised 11 features, including PR and MMT, which share close structural similarity, with MMT representing the decarboxylated analogue of PR. Cluster 3 consisted of MG together with closely related low-mass ions (m/z 246 and 188), indicative of structurally related derivatives.

Detailed annotation results are provided in the Supplementary Material (Table S1). Annotation confidence was assessed using a multi-criteria strategy that combined accurate mass and isotope patterns, MS/MS spectral similarity within the GNPS molecular network, diagnostic UV absorption at 330 nm, comparison with in-house reference data, and compound-class predictions generated by SIRIUS/CANOPUS. Discrepancies between GNPS and in-house annotations reflect differences in the underlying data sources (MS^2-based versus MS^1-based matching) and were resolved through integrated evaluation of all available orthogonal information, in accordance with current best practices for untargeted metabolomics reporting (Alseekh et al. 2021).

Based on this analysis, clear species-specific secondary MAA profiles were identified. In *C. chamissoi*, the secondary profile was dominated by ML and aplysiapalythines, particularly APC and APB, which generally occurred at medium abundance (10^6–10^7 mAU). In contrast, *G. lemaneiformis* exhibited a broader secondary profile, characterized by the consistent high abundance of APA ($>1.0 \times 10^7$ mAU), while APB was detected at medium levels across all samples.

Seasonal variations in MAAs production

Total MAAs concentrations differed significantly between seasons in both species, with consistently higher values recorded in summer (Fig. 2). In *C. chamissoi*, gametophytes

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increased from 2.37 ± 0.06 mg g^{-1} DW in spring to 3.01 ± 0.05 mg g^{-1} DW in summer (two-way ANOVA, Tukey's HSD, $p = 0.0009$), while tetrasporophytes rose from 2.68 ± 0.06 to 3.39 ± 0.22 mg g^{-1} DW over the same period ($p = 0.0004$).

G. lemaneiformis exhibited a pronounced seasonal increase in total MAAs. Gametophyte total MAAs concentrations more than doubled from 1.73 ± 0.02 mg g^{-1} DW in spring to 3.53 ± 0.17 mg g^{-1} DW in summer (two-way ANOVA, Tukey's HSD, $p = 4 \times 10^{-5}$). Summer tetrasporophytes reached 3.69 ± 0.11 mg g^{-1} DW; however, tetrasporophytes were absent from the cultivation system during spring, precluding seasonal comparisons for this life stage.

Taken together, these results demonstrate that summer conditions strongly promote MAA accumulation in both species. In *C. chamissoi*, seasonal increases ranged from 26–27% across life stages, whereas *G. lemaneiformis* gametophytes exhibited a markedly larger relative increase (104%). These proportional relative differences indicate a stronger seasonal response in *G. lemaneiformis.*

Seasonal changes were also evident in MAA composition. In *C. chamissoi*, SH remained the dominant primary compound and increased significantly during summer (two-way ANOVA, $p < 0.01$), whereas PA and AS showed no significant seasonal variation (Fig. 2). Several secondary MAAs, including PR, US (restricted to gametophytes), and PAE, were comparatively enriched in summer samples, while MGA was more abundant in spring. APC decreased specifically in summer tetrasporophytes, indicating stage-specific regulation (Table 2).

In *G. lemaneiformis*, all four primary MAAs (SH, PA, AS, and PR) increased more than twofold from spring to summer (two-way ANOVA, Tukey's HSD, $p < 0.00001$), with SH and PR becoming the major constituents in summer (Fig. 2). Seasonal shifts were also evident among secondary MAAs in gametophytes, where MG, US, and PAE were more abundant in spring (Table 2).

Collectively, both species exhibited higher MAA abundance during summer, reflecting enhanced accumulation of primary compounds accompanied by species-specific patterns in secondary MAA composition under high-irradiance conditions.

Life Stage Influences on MAAs Biosynthesis

Life stage significantly influenced MAAs accumulation in *C. chamissoi*. Tetrasporophytes consistently contained higher total MAAs concentrations than gametophytes in both seasons (two-way ANOVA, $p < 0.05$; Fig. 2), indicating a stage-dependent capacity for photoprotective metabolite production. In contrast, *G. lemaneiformis* showed no significant differences in total

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MAAs concentration between life stages during summer. Seasonal comparisons for spring were not possible due to the absence of tetrasporophytes from the cultivation system during this period.

Life-stage effects on MAA composition differed between species (Fig. 2). In *C. chamissoi*, PA and AS concentrations were consistently higher in tetrasporophytes (two-way ANOVA, $p < 0.001$), whereas SH showed no significant variation between life stages. Among secondary MAAs, APC declined specifically in summer tetrasporophytes, while US was more abundant in spring tetrasporophytes than in gametophytes (Table 2).

In *G. lemaneiformis*, all four primary MAAs were detected in both life stages (Fig. 2), but only AS was significantly enriched in tetrasporophytes compare to gametophytes during summer (two-way ANOVA, $p < 0.01$). Life-stage differences among secondary MAAs were restricted to specific compounds (Table 2). MG, US, and PAE were more abundant in tetrasporophytes during summer, whereas the remaining secondary MAAs showed comparable abundances between life stages. In contrast, during spring, MG, US and PAE exhibited higher relative abundances in gametophytes.

Overall, these results indicate that life-stage effects on MAA biosynthesis and composition are species-specific. Such effects were clearly detectable in *C. chamissoi*, whereas in *G. lemaneiformis* they were either not statistically significant or restricted to specific compounds and dependent on seasonal context.

Discussion

The integrated analysis using HPLC–DAD and UHPLC–HRMS/MS coupled with FBMN revealed species-, seasonal-, and life-stage-dependent differences in MAA concentration and composition in *C. chamissoi* and *G. lemaneiformis* cultivated under identical conditions. A total of 13 MAAs were putatively annotated in both species (Fig. 2; Table 2); however, *C. chamissoi* exhibited a more restricted primary profile and a secondary profile dominated by aplysiapalythines and ML, whereas *G. lemaneiformis* displayed a broader primary profile that included PR and reached higher maximum total MAAs concentrations. MAA production was strongly modulated by seasonality, with significantly higher total concentrations recorded in summer in both species. In contrast, life-stage effects in total MAA accumulation was species-specific and evident only in *C. chamissoi*, where tetrasporophytes consistently accumulated higher total MAAs than gametophytes.

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Species Differences in MAAs Profiles

According to Sun et al. (2020), both *C. chamissoi* and *G. lemaneiformis* classify as high-MAA producers (>2 mg g-1 DW), yet they differ markedly in their MAAs capacities and chemical diversity. In *C. chamissoi*, total MAAs concentrations (2.37–3.39 mg g^{-1} DW) reach the upper range reported for Chilean natural populations of the same species (0.8–3.3 mg g^{-1} DW; Véliz et al. 2019). The higher values observed in this study likely reflect shallower cultivation (~1 m) and thus greater irradiance than the 3–4 m semi-protected sites sampled by Véliz et al. (2019). In *G. lemaneiformis*, total MAAs concentrations (1.73–3.69 mg g^{-1} DW) exceed those documented for congeners, including *G. longissima* (0.69–1.6 mg g^{-1} DW; Álvarez-Gómez et al. 2019; Chaves-Peña et al. 2019) and *G. tenuifrons* (<1.5 mg g^{-1} DW even under high PAR; Torres et al. 2016), demonstrating a strong intrinsic capacity for MAAs accumulation.

Primary MAAs composition also differed between species. *C. chamissoi* was dominated by SH, followed by PA and AS - a pattern typical of intertidal Rhodophyta (Sun et al. 2020) and in part consistent with Chilean populations of *C. chamissoi*, which feature SH and PA (Véliz et al. 2019). UHPLC–HRMS/MS extended the previously reported Chilean MAA profile for this species by revealing ten additional low-abundance MAAs (Table 2), underscoring the value of high-resolution workflows for resolving co-eluting or trace metabolites (Hartmann et al. 2015; Zwerger and Ganzera 2022). The differences observed between Peruvian and Chilean populations of the same species challenge the notion of strictly species-fixed MAAs profiles (Boedeker and Karsten 2005) and instead suggest that environmentally driven plasticity shapes population-specific chemical fingerprints. Comparative work along Peruvian–Chilean gradients would help disentangle genetic from environmental contributions.

In contrast, *G. lemaneiformis* displayed a richer and broader MAAs spectrum, comprising four primary MAAs (SH, PA, AS, PR) and nine secondary metabolites, providing the first detailed profile for this species. Earlier reports relied solely on absorbance at ~325 nm (Zheng and Gao 2009), precluding compound-level identification. The resolved profile aligns in part with general Gracilariaceae patterns (e.g., *G. longissima*: SH, PA, AS, PR, palythinol; Álvarez-Gómez et al. 2019; *G. tenuifrons*: AS, palythinol, US, PAE; Torres et al. 2016) and supports the existence of a conserved biosynthetic core modulated by species-specific tuning. Secondary MAAs identified via FBMN further emphasized interspecific differences. *C. chamissoi* was enriched in ML and aplysiapalythines (notably APC and APB), forming a classic cluster comprising glycine substituted cyclohexenimines (Cluster 1) characteristic of Rhodophyta (Zwerger et al. 2023). In contrast, *G. lemaneiformis* exhibited a more abundant secondary profile, including consistently high levels of APA, together with US, PAE and MG (Clusters 2-

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3), indicative of greater metabolic accumulation. Less common MAAs such as palythine-serine, palythine-threonine, and shinorine methyl ester were putatively identified in both species, highlighting the power of high-resolution MS/MS combined with FBMN to capture rare photoprotective metabolites that would otherwise remain undetected.

The combined consideration of ecological setting, morphological traits, and phylogenetic background provides a coherent framework to explain these interspecific contrasts under identical culture conditions. *G. lemaneiformis* typically inhabits turbid, soft-bottom, semi-protected environments down to approximately 6 m depth, where underwater light fields are spatially and spectrally heterogeneous due to turbidity and variable attenuation (Obrador and Pretus 2008; Carbajal et al. 2019). Such conditions are consistent with the expression of inducible and compositionally broader photoprotective systems, including profiles enriched in PR. In contrast, *C. chamissoi* predominantly colonizes wave-exposed rocky substrates in the lower intertidal and shallow subtidal zone (to ~8 m) (Carbajal et al. 2019), where organisms experience consistently high irradiance and UV exposure. These environments are associated with constitutively elevated but less compositionally variable MAA profiles, dominated by SH, reflecting a strategy of sustained photoprotection under stable high-light conditions.

Thallus architecture may further contribute to the observed interspecific differences. *G. lemaneiformis* forms cylindrical, fleshy, and highly branched thalli (> 1 m) that create internal light gradients where complementary UV-A/UV-B absorbers are advantageous (Hoyer et al. 2001; Karsten et al. 2005). In contrast, *C. chamissoi* develops membranous, flattened blades (0.3–2.5 cm wide, up to 50 cm long) with a high surface-to-volume ratio, exposing most cells directly to incident radiation. This architecture is consistent with reliance on a more limited set of MAAs, such as SH and related derivatives (Carbajal et al. 2019). However, direct links between thallus morphology, internal light fields, and MAA allocation remain poorly resolved and require dedicated experimental approaches to be conclusively established. Phylogenetically, Gracilariaceae (e.g., *G. lemaneiformis*) tend to exhibit greater inducibility of MAAs pathways than Gigartinaceae (e.g., *C. chamissoi*) (Sun et al. 2020). Together, these ecological, morphological, and phylogenetic factors help explain why *G. lemaneiformis* attained both higher concentrations and a broader MAAs spectrum than *C. chamissoi* under the same culture conditions.

Overall, both species emerge as promising MAAs sources. Notably, *G. lemaneiformis* combines high total MAAs content with a broad composition of primary and secondary MAAs, which

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may be particularly advantageous for biotechnological applications targeting different spectral windows.

Environmental and Seasonal Drivers for MAAs Production

At the subtropical latitude, seasonal SST contrasts are modest (ΔSST ≈ 2 °C), yet solar radiation remains intense year-round, with midsummer UV indices exceeding 12 (SENAMHI, 2024). This creates a background of chronic UV stress for intertidal and shallow subtidal macroalgae. The Pisco–San Juan upwelling system (Peru) operates year-round but reaches peak intensity during late winter and spring, introducing cold, nutrient-rich waters into the coastal zone and thereby strongly influencing nitrogen availability and water clarity (Gutiérrez et al. 2011). These pulses are critical because MAAs are nitrogen-rich metabolites, and their biosynthesis is sensitive to nitrogen supply. In addition, episodic ozone depletion in early spring further increases biologically effective UVR (Williamson et al. 2019; Barnes et al. 2022), adding an additional seasonal stressor that may stimulate photoprotective responses.

Our study period was contemporaneous with the 2023–2024 Coastal El Niño, which was superimposed on a major basin-scale El Niño event. The event began in early 2023, produced regional SST anomalies, and disrupted normal upwelling along the Peruvian coast. Eastern Pacific SST anomalies exceeded +2 °C during November–December 2023 before declining by April 2024 (Peng et al. 2024, 2025). Although significant warming was observed at the basin scale, local thermal anomalies in Pisco remained modest (Table 1), a condition likely mitigated by persistent coastal upwelling. This buffering action restricted extreme surface warming but consequently altered nutrient delivery, water column structure, and hydrographic stability. Such indirect effects can substantially influence algal metabolism, particularly nitrogen-dependent pathways like MAAs biosynthesis, where the interaction of light and nutrient availability serves to modulate accumulation.

Taken together, the seasonal increase in both MAA concentration and compositional complexity observed in *C. chamissoi* and *G. lemaneiformis* is best explained by the convergence of multiple environmental drivers (Briani et al. 2018). In the present study, these drivers may include peak summer irradiance, the partial recovery of nutrient availability following spring limitation, and episodic environmental anomalies, rather than irradiance alone, which is often emphasized in the literature (Sun et al. 2020).

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Seasonal effects on MAAs concentration

Both species reached their maximum total MAAs levels in summer (Fig. 2). Despite the relatively small seasonal variability in SST, dissolved oxygen, salinity, pH, PAR, and UV index recorded at the site (Table 1), MAAs accumulation increased markedly, indicating that no single measured parameter alone explains the observed pattern. We therefore propose that nutrient limitation during spring—associated with reduced or irregular upwelling at the onset of the 2023–2024 Coastal El Niño—constrained MAA biosynthesis in both species. By summer, nutrient availability partially recovered while UV and PAR levels remained high, creating conditions favorable for enhanced MAA production. This light–nutrient interaction is consistent with experimental evidence showing that MAA biosynthesis is not solely light-driven but also depends on nitrogen availability, as nitrogen-rich amino acid precursors are directly incorporated into MAA biosynthetic pathways (Korbee et al. 2005; Álvarez-Gómez et al. 2017).

C. chamissoi exhibited a modest, steady seasonal increase in total MAAs, consistent with its adaptation to chronically high UV and comparatively stable photoprotective requirements. This pattern complements Chilean observations where geographic and latitudinal gradients overshadowed seasonality (Véliz et al. 2019). In contrast, *G. lemaneiformis* showed a more drastic response: summer gametophytes more than doubled their MAAs content relative to spring, indicative of strong inducibility. This is consistent with the high plasticity documented in Gracilariaceae species, where combined UVR and nutrient enrichment significantly enhance MAAs accumulation (Álvarez-Gómez et al. 2017). Such inducibility likely reflects the species' ecology, inhabiting turbid and variable habitats in Peru, it benefits from a flexible metabolic strategy that rapidly boosts photoprotection during periods of high stress.

Together, these results indicate contrasting seasonal strategies of photoprotection. *C. chamissoi* displays a more conservative, constitutive response to seasonal forcing, whereas *G. lemaneiformis* relies on a more inducible strategy, showing greater sensitivity to seasonal and hydrographic variability. These complementary strategies highlight the ecological differentiation of MAAs regulation in red macroalgae and have implications for their resilience and biotechnological potential.

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Seasonal effects on MAAs composition

Compound-specific modulation mirrored changes in total MAAs content. In *C. chamissoi*, SH remained dominant across seasons and increased significantly in summer, whereas PA and AS remained comparatively stable (Fig. 2). This pattern suggests preferential up-regulation of SH, consistent with its strong UV absorption ($\lambda_{max} \approx 332–334$ nm), antioxidant capacity, and photostability (Karsten et al. 1998; Hartmann et al. 2015; Orfanoudaki et al. 2019; Geraldes and Pinto 2021; Rosic et al. 2023). Within the secondary MAAs set, PR, US (only for gametophytes) and PAE increased in summer, while MGA was more abundant in spring.

In *G. lemaneiformis,* summer profiles shifted toward SH and PR dominance, whereas spring was more evenly distributed among SH, PA, AS, and PR. This preferential allocation to broad-spectrum absorbers (SH and PR) under high-irradiance conditions resembles patterns reported for other Gracilariaceae (Pliego-Cortés et al., 2019) and for *Gracilaria vermiculophylla*, in which the timing and magnitude of seasonal MAA maxima vary among localities (Barceló-Villalobos et al., 2017). In contrast, several secondary MAAs (MG, US, and PAE) were more abundant in spring gametophytes than in summer. Because UV radiation and PAR were comparable between seasons, this pattern is unlikely to be driven by irradiance alone and more plausibly reflects seasonal differences in nutrient availability linked to intensified spring upwelling and early El Niño–related hydrographic variability. Such conditions may favor the synthesis of specific secondary MAAs, whereas summer profiles appear to prioritize broad-spectrum primary MAAs (SH and PR).

Collectively, these shifts indicate that seasonality modulates not only total MAA levels but also the relative balance among MAA types, thereby enhancing the photoprotective repertoire during periods of increased environmental stress.

Life stage influences on MAAs biosynthesis

Both *C. chamissoi* and *G. lemaneiformis* exhibit triphasic life cycles with isomorphic gametophytes and tetrasporophytes (Kain and Destombe 1995; Ávila et al. 2011). Despite their morphological similarity, life stages can differ physiologically as a consequence of ploidy level, reproductive status, and metabolic allocation, factors known to influence the production of secondary metabolites, including MAAs (Hoyer et al. 2001; Boedeker and Karsten 2005; Korbee et al. 2005; García-Jiménez and Robaina 2015; Vieira et al. 2021).

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In the present study, all gametophytes analyzed corresponded exclusively to fertilized female gametophytes. The high energetic and nutritional costs associated with cystocarp development are well documented in red algae and are known to reduce growth and pigment content (Guillemin et al. 2013). Such reproductive trade-offs may indirectly constrain MAA biosynthesis or modify allocation among compounds, as reported for other secondary metabolites (Kain and Destombe 1995).

In *C. chamissoi*, tetrasporophytes accumulated significantly higher total MAA concentrations than gametophytes across both seasons. This contrasts with observations from Chilean populations, where female gametophytes were reported to dominate MAA accumulation (Véliz et al. 2019), indicating that life-stage patterns are context-dependent and modulated by environmental conditions such as culture depth and UV exposure. Under shallow, consistently high irradiance, higher MAA levels in tetrasporophytes likely reflect ploidy-related physiological regulation and life-stage-specific metabolic allocation, whereas cystocarpic gametophytes may prioritize reproductive investment over secondary metabolite production, rather than morphological differences per se.

In *G. lemaneiformis*, no statistically significant differences in total MAA concentrations were detected between life stages during summer, and tetrasporophytes were absent from the cultivation system in spring, precluding seasonal comparisons. Consequently, life-stage effects on MAA accumulation could not be robustly evaluated for this species. The absence of tetrasporophytes during spring coincided with atypical conditions associated with the 2023–2024 Coastal El Niño, which may have influenced life-cycle expression, as seasonal and temperature-dependent shifts in ploidy ratios have been reported in Gracilariaceae (Feng et al. 2022).

Overall, these results indicate that life stage is a significant driver of MAA accumulation in *C. chamissoi* under the conditions studied, whereas comparable effects could not be confirmed for *G. lemaneiformis*, where MAA variability appears to be more influenced by seasonal forcing.

Life stage effects on MAAs composition

Ontogenetic differences also influenced MAAs composition in both species. In *C. chamissoi*, PA and AS were enriched in tetrasporophytes, whereas SH showed no clear life-stage-specific variation. Given the isomorphic morphology of life stages, this effect is more plausibly

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attributed to ploidy-dependent physiological regulation and metabolic allocation rather than structural differences (Kain and Destombe 1995). PA absorbs primarily in the UV-B range (λmax ≈ 320 nm) and exhibits antioxidant activity, while AS absorbs in the UV-A range (λmax ≈ 330 nm), together broadening spectral photoprotection and complementing the broad-spectrum role of SH (Karsten et al. 1998; de la Coba et al. 2019).

In *G. lemaneiformis*, all four primary MAAs (SH, PA, AS, and PR) were present in both life stages; however, AS was significantly more abundant in tetrasporophytes during summer. Because morphological differences between life stages are also minimal in this genus, this pattern likely reflects physiological regulation associated with ploidy and/or reproductive status rather than anatomical traits.

Secondary MAAs exhibited life-stage-dependent variation, although differences were restricted to specific compounds rather than indicating a generally more complex profile in tetrasporophytes. Collectively, these findings suggest that life-stage modulation of MAAs composition operates through selective, compound-level regulation driven by physiological and reproductive constraints, interacting with seasonal and species-specific factors to shape photoprotective metabolism.

Conclusion

This study provides the first comprehensive characterization of MAAs in two commercially important red macroalgae from Peru, *C. chamissoi* and *G. lemaneiformis,* integrating targeted HPLC–DAD quantification with UHPLC–HRMS/MS-based untargeted metabolomics and FBMN. Both species were confirmed as high-MAAs producers and shared the same set of 13 MAAs, yet they differed markedly in their biochemical and regulatory strategies.

Seasonality emerged as the primary driver of MAA accumulation and composition in both species, with consistently higher concentrations observed in summer. Life stage further modulated MAA biosynthesis in *C. chamissoi*, where tetrasporophytes accumulated higher total MAAs and exhibited distinct compositional patterns, indicating species-specific ontogenetic regulation. In contrast, comparable life-stage effects could not be confirmed for *G. lemaneiformis*, suggesting that MAA variability in this species is more constrained by seasonal and environmental forcing.

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The application of FBMN substantially expanded the known MAAs repertoire of both species, enabling the detection of low-abundance and rare palythine derivatives that would likely remain unresolved using targeted approaches alone. These results underscore the value of integrating untargeted metabolomics to reveal hidden chemodiversity with relevance for photoprotection and natural product discovery.

Overall, *C. chamissoi* and *G. lemaneiformis* represent promising and sustainable sources of natural UV-protective compounds. In particular, the strong seasonal inducibility observed in *G. lemaneiformis* highlights its potential for optimized aquaculture strategies aimed at enhancing MAAs yields and tailoring spectral coverage. Strengthening Peru's foundational knowledge of algal bioactive compounds, together with promoting local processing and valorization of seaweed biomass rather than its export as raw material, could support the development of a national bioeconomy centered on high-value photoprotective metabolites.

Future research integrating multi-site field comparisons, controlled cultivation experiments, molecular approaches, and analyses of thallus architecture, together with targeted evaluation of MAAs production across different life stages—particularly among gametophyte expressions (male, unfertilized female, and fertilized female)—will be essential to disentangle environmental, genetic, and reproductive drivers of MAAs biosynthesis and to fully realize the biotechnological potential of these key Peruvian Rhodophyta.

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Author contributions

F.M.: Investigation, methodology, visualization, writing—original draft. **J.F.:** Supervision, writing—review and editing. **F.H.:** Methodology, visualization, writing—original draft, writing—review and editing. **M.G.:** Funding acquisition, writing—review and editing. **B.B.:** Visualization, writing—original draft, writing—review and editing. **O.R.:** Supervision, methodology, writing—review and editing. **U.K.:** Supervision, writing—original draft, writing—review and editing, funding acquisition.

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Data availability

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

Competing interests

The authors declare that they have no competing interests.

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Figure legends

Fig. 1 Representative UHPLC–HRMS extracted ion chromatograms (EICs) monitored at 330 nm for annotated mycosporine-like amino acids (MAAs) in *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*. Chromatograms correspond to extracts obtained from spring gametophytes of each species. Major peaks are labeled as shinorine (SH), palythine (PA), asterina-330 (AS), porphyra-334 (PR), aplysiapalythine A (APA), aplysiapalythine B (APB),

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usujirene (US), and palythene (PAE). Additional MAAs detected at lower abundance are listed in Table 2.

Fig. 2 Concentration and composition of primary mycosporine-like amino acids (MAAs), and total MAA content (mg g^{-1} DW), determined by HPLC–DAD in gametophytes and tetrasporophytes of *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis* collected in spring 2023 and summer 2024. Values represent means ± standard deviation (SD) of three independent biological replicates (n = 3). Bars sharing the same letter are not significantly different; bars with different letters or without letters indicate statistically significant differences (Tukey's HSD, $p < 0.05$). Abbreviations: SH, shinorine; PA, palythine; AS, asterina-330; PR, porphyra-334. Tetrasporophytes of *G. lemaneiformis* were absent in spring.

Fig. 3 A | Feature-based molecular network generated from the extracts of *C. chamissoi* and *G. lemaneiformis*. Clusters containing mycosporine-like amino acids (MAAs) are highlighted with red boxes and corresponding labels. Nodes annotated as potential MAAs via SIRIUS are shown in red. Node size reflects UV absorption characteristics, with larger nodes indicating features coeluting with a peak at 330 nm (UV330 filter: 1). Node border thickness represents retention time, increasing with later elution. **B** | Detailed view of MAA-containing clusters. The relative abundance of each feature in the algal extracts is displayed as a pie chart on the respective node. Nodes putatively annotated as MAAs by SIRIUS are depicted as octagons. Annotation results are shown on the right, including molecular structures, observed m/z values, trivial names, and corresponding retention times. Node size again represents the UV filter variable, with larger nodes indicating absorption at 330 nm. The numbering of annotated compounds corresponds to the node labels in the network.

Fig. 4 Fragment spectra mirror plots of nodes putatively identified as palythine (ID 665), palythine-serine (ID 624), and palythine-threonine (ID 874). Matching fragment ions are highlighted in blue, while matching neutral losses are indicated in orange with dotted lines and arrows. The top section of each plot shows the modified cosine score and the number of matched signals.

Supplements legends

Fig. S1 Schematic illustration of the marine bottom cultivation system at San Andrés Beach, Pisco Province, Ica Region, Peru. The site is a shallow coastal habitat (0.5–1.0 m depth) with rocky substrates and interspersed sand. The system consists of horizontal mainlines anchored

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with moorings (nets filled with pebbles) and supporting 20 cultivation units each. Units are constructed from raffia to ensure neutral buoyancy, maintaining algae slightly above the seabed. Each was initially inoculated with ~40 g of juvenile red algal thalli and fixed at regular intervals. A surface buoy marks each line for retrieval. Illustration created with BioRender.com.

Fig. S2 Photographic overview of the cultivation site and macroscopic differentiation of fertilized female gametophytes and tetrasporophytes in *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*.

Fig. S3 Supplementary details of the extraction protocol used for the isolation of mycosporine-like amino acids (MAAs) from lyophilized macroalgal samples.

Fig. S4 Clusters containing MAAs, with nodes labeled according to their corresponding feature IDs. Relative peak areas across different algal extracts are visualized as pie charts.

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Table 1. Physicochemical parameters recorded during spring and summer 2023-2024 at the cultivation site in Pisco, Peru, compared with historical seasonal and annual data from the Laboratorio Costero de Pisco (IMARPE). PAR = photosynthetically active radiation; DO = dissolved oxygen; SSTa = sea surface temperature anomaly. All values are expressed in SI units.

Parameter	Spring (06 Nov 2023)	Summer (05 Mar 2024)	2023 (range or mean ± SD)	2024 (range or mean ± SD)	Spring (Sept–Dec)	Summer (Dec–Mar)
Coordinates	S 13°48′25.57″ / W 76°15′6.64″		S 13°47′17.0″ / W 76°14′30.0″			
Sea Surface Temperature (°C)	19.50	21.50	18.84 – 23.61	17.84 – 23.94	18.84 – 21.80	21.24 – 23.94
Anomalies (ATSM)	–	–	0.48 ± 0.75	0.03 ± 0.56	0.28 ± 0.38	–0.80 ± 0.83
Dissolved Oxygen (DO, mg L^{-1})	10.46	7.93	4.44 ± 1.35	4.69 ± 1.24	5.06 ± 0.73	5.53 ± 0.43
Salinity (PSU)	36.10	34.30	33.99 ± 2.99	34.92 ± 0.25	35.08 ± 0.12	35.12 ± 0.13
pH	8.20	7.40	7.77 ± 0.30	7.71 ± 0.25	7.90 ± 0.32	7.67 ± 0.13
All sky surface total PAR (MJ m^{-2} day^{-1})	11.83	11.54	8.98 ± 2.26	9.08 ± 2.22	10.15 ± 1.17	11.24 ± 0.36
All sky surface UV Index (W m^{-2} × 40)	2.78	2.99	2.14 ± 0.63	2.26 ± 0.65	2.41 ± 0.39	2.93 ± 0.15

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Table 2. Relative abundance of secondary MAAs putatively identified by UHPLC–VWD–HRMS/MS followed by feature-based molecular networking in gametophytes and tetrasporophytes of *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis* (spring 2023, summer 2024). Abundance categories: low (L) (< 1.0×10^6 mAU, green), medium (M) (1.0×10^6–1.0×10^7 mAU, yellow), and high (H) (> 1.0×10^7 mAU, red). *Porphyra-334 is classified as a primary MAAs only in *G. lemaneiformis* and is indicated in purple with the letter "P." m/z = mass-to-charge ratio; RT = retention time (min). Color coding is explained within the table for ease of interpretation.

m/z	RT (min)	MAA Type	*Chondracanthus chamissoi*				*Gracilariopsis lemaneiformis*		
			Spring		Summer		Spring	Summer	
			Gametophyte	Tetrasporophyte	Gametophyte	Tetrasporophyte	Gametophyte	Gametophyte	Tetrasporophyte
347.14	2.26	Porphyra-334	L	L	M	M	P	P	P
259.13	2.32	Aplysiapalythine C	M	M	M	L	L	L	L
246.10	2.82	Mycosporine-glycine	L	L	L	L	M	L	M
303.15	3.02	Aplysiapalythine A	L	L	L	L	H	H	H
317.13	3.33	Mycosporine-glycine-alanine	M	M	L	L	L	L	L
273.14	4.77	Aplysiapalythine B	M	M	M	M	M	M	M
303.16	5.03	Mycosporine-methylamine-threonine	L	L	L	L	L	L	L
317.17	6.27	Mycosporine-lysine	M	M	M	M	L	L	L
285.14	8.70	Usujirene	L	M	M	M	H	M	H

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285.14	8.95	Palythene	L		L		M		M	H	M	H

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12. VERSIÓN INICIAL ENVIADA A PUBLICAR

Evaluation of UV-sunscreen mycosporine-like amino acids (MAAs) in two economically important red macroalgae from Peru

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Abstract

Mycosporine-like amino acids (MAAs) are UV-absorbing, multifunctional metabolites with increasing biotechnological relevance. This study provides the first comprehensive characterization of MAAs profiles in two commercially important Peruvian red macroalgae, *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*, cultivated in Pisco (Ica, Peru). Seasonal (spring vs. summer) and life-stage (gametophyte vs. sporophyte) differences were analysed using HPLC–DAD for targeted quantification and UHPLC–HRMS/MS coupled with feature-based molecular networking (FBMN) for untargeted metabolite discovery. Both species were confirmed as high MAAs producers (> 2 mg g^{-1} DW) and shared the same set of 13 MAAs, although their relative abundances varied by species, season, and life stage. *C. chamissoi* accumulated up to 3.39 mg g^{-1} DW and exhibited a SH-dominant profile (shinorine (SH), palythine (PA) and asterina-330 (AS)), complemented by ten additional low-abundance MAAs detected by FBMN. *G. lemaneiformis* reached higher total concentrations (up to 3.69 mg g^{-1} DW) and displayed a broader primary-MAAs repertoire, including porphyra-334 (PR), together with nine secondary metabolites detected at trace levels. Seasonal forcing produced pronounced summer maxima MAAs total concentration in both species, although the magnitude of induction was substantially greater in *G. lemaneiformis* - consistent with an inducible, habitat-dependent photoprotective strategy - whereas *C. chamissoi* showed a more constitutive response aligned with persistent intertidal UV exposure. Life-stage effects were also evident, sporophytes of both species generally accumulated higher total MAAs levels and exhibited richer MAAs profiles. FBMN revealed several previously unreported MAAs in both taxa, expanding the known chemical diversity of Peruvian Rhodophyta. Collectively, these findings demonstrate strong environmental and ontogenetic modulation of MAAs biosynthesis and highlight both species - particularly *G. lemaneiformis* - as promising, sustainable sources of natural UV-protective compounds for cosmetic, pharmaceutical, and biomedical applications.

Keywords: *Chondracanthus chamissoi*, *Gracilariopsis lemaneiformis*, Mycosporine-like amino acids, FBMN, Seasonal variation, Life stages, Photoprotection, Peruvian Rhodophyta

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Introduction

Rising levels of ultraviolet radiation (UVR), driven by climate change and stratospheric ozone depletion, pose increasing risks to aquatic ecosystems (Neale et al. 2023). Subtropical regions of South America, including Peru, consistently record some of the highest global UV Index values, exacerbating the vulnerability of marine organisms to harmful radiation (Robinson and Erickson 2015).

Peru lies within a global biodiversity hotspot shaped by the Humboldt Current eastern boundary upwelling system, one of the most productive marine ecosystems worldwide (Kämpf and Chapman 2016). Despite this high productivity, elevated UVR due to the low latitudes imposes substantial physiological and ecological stress. Marine organisms, unlike many terrestrial taxa, lack protective structures or shaded refuges, leaving them directly exposed to UVR and photosynthetically active radiation (PAR). In primary producers such as macroalgae and phytoplankton, UVR can inhibit photosynthesis, degrade pigments, and impair electron transport in Photosystem II (Vass et al. 2005), leading to cascading impacts on productivity, trophic interactions, and ecosystem stability (Häder et al. 2007; Williamson et al. 2019).

To mitigate these stressors, many marine primary producers synthesize mycosporine-like amino acids (MAAs), a group of small (<400 Da), water-soluble secondary metabolites that act as natural sunscreens by absorbing and dissipating UVR as heat (Carreto and Carignan 2011; Geraldes and Pinto, 2021; Muthumani et al. 2015). MAAs are generated mainly via the shikimate and pentose phosphate pathways (Spence et al. 2012) and feature cyclohexenone or cyclohexenimine cores with nitrogenous substituents, enabling strong absorption across 268–362 nm with exceptionally high molar extinction coefficients (Balskus and Walsh 2010). Beyond photoprotection, MAAs exhibit antioxidant and thermoprotective properties, positioning them as multifunctional anti-stress compounds in diverse marine environments (Torres et al. 2018; Flaim et al. 2014). Their versatility has attracted growing interest in industry, particularly in cosmetics, pharmaceuticals, and biomedical applications (Rosic et al. 2023). As eco-friendly alternatives to synthetic UV filters, several MAAs also exhibit immunomodulatory, anti-inflammatory, and wound-healing activities (Vega et al. 2021; Görünmek et al. 2024).

Red macroalgae (Rhodophyta) are among the richest natural MAAs producers, with concentrations reaching up to 20 mg g^{-1} dry weight (DW), and some species exceeding this threshold (Lamare et al. 2004; Sun et al. 2020). Over 70 MAAs have been identified across

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marine taxa, with at least 22 reported from Rhodophyta, including ubiquitous compounds such as shinorine (SH), porphyra-334 (PR), palythine (PA), asterina-330 (AS), and palythinol (PAO) (Geraldes and Pinto 2021; Sun et al. 2020). However, the concentration and composition of MAAs in macroalgae are highly variable and shaped by a complex interplay of environmental and intrinsic factors (Lalegerie et al. 2020). Key drivers include UVR intensity, PAR, seasonal cycles, salinity, temperature, pH, and nutrient availability - particularly nitrogen and phosphate (Geraldes and Pinto 2021; Jofre et al. 2020; Briani et al. 2018). Intrinsic traits such as species identity, habitat depth, tissue type, life-stage differences, and genetic variability further modulate MAAs biosynthesis (Boedeker and Karsten 2005; Hoyer et al. 2001; Carreto and Carignan, 2011; Marshall and Newman, 2002).

Recent scientific advances have markedly expanded the known structural diversity of MAAs and improved analytical capabilities for their detection, particularly regarding their structural variability, molecular connectivity, and stereochemistry (Orfanoudaki et al. 2019, 2020a, 2023). These advances have not only expanded the number of known natural sunscreen compounds but have also driven the development of specialized analytical methods, ranging from validated CE–DAD protocols (Hartmann et al. 2017) to (U)HPLC–DAD workflows optimized for quantification (Orfanoudaki et al. 2020b; Zwerger and Ganzera 2022), now enabling the reliable profiling of both abundant and trace MAAs. More recently, untargeted metabolomic approaches, especially UHPLC–HRMS/MS combined with feature-based molecular networking (FBMN; Nothias et al. 2020), have become powerful tools in natural products chemistry for capturing full metabolic fingerprints and identifying related compound families (Pellissier et al. 2023; Quiros-Guerrero et al. 2024; Hammerle et al. 2023). Coupled with public MS^2 repositories such as GNPS (https://gnps.ucsd.edu/) and structural annotation tools like SIRIUS 4 (Dührkop et al. 2019), these strategies allow rapid, high-confidence annotation of known and novel MAAs. Their application in phycology is expanding, including recent analyses of macroalgae with this combined UHPLC–HRMS/MS–FBMN approach (Zwerger et al. 2023) and even MAAs-rich fish tissues (Hammerle et al. 2025).

Despite extensive research on MAAs in temperate and polar macroalgae, subtropical regions such as the Peruvian coast remain critically understudied (Briani et al., 2018; Sun et al., 2020; Hoyer et al., 2001). Peru hosts a rich Rhodophyta flora (Ávila-Peltroche & Padilla-Vallejos, 2020), yet no studies have characterized the MAAs profiles of its dominant commercial species. This gap is notable given the region's unique oceanographic context: shallow embayments and rocky platforms transitioning sharply into deeper subtidal zones influenced by the Humboldt

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Current's intense upwelling (Avila-Peltroche & Padilla-Vallejos, 2020; Tarazona & Arntz, 2001). These conditions generate steep gradients in light, temperature, nutrient supply, and turbidity over short spatial scales (Gutiérrez et al., 2011), creating environments that differ markedly from well-studied temperate coasts. As a result, Peruvian macroalgae are continually exposed to extreme UV radiation, nutrient-rich cold upwelled waters, and pronounced seasonal hydrographic shifts—conditions that likely shape their MAAs biosynthetic strategies in distinctive ways. Together, these features make the region an ecologically compelling and distinct natural laboratory for studying MAAs biosynthesis.

Currently, the commercially most important red macroalgae in Peru are *Chondracanthus chamissoi* ("yuyo") and *Gracilariopsis lemaneiformis* ("pelillo") (Rodríguez et al. 2018). *C. chamissoi* is harvested for human consumption and carrageenan extraction (Almeyda and Armas 2018; Arbaiza et al. 2019), while *G. lemaneiformis* is cultivated for agar production (Noriega 2016; Rodríguez et al. 2018). However, the Peruvian macroalgae industry remains focused on exporting raw biomass - primarily to China - with limited investment in value-added bioproducts. Between 2005 and 2014, macroalgae exports surged by 521%, from 5,585 to 29,089 metric tons dry weight (MTDW), peaking at 33,948 MTDW in 2019 before slightly declining to 29,642 MTDW in 2020 (Montero and Arbaiza, 2016; Díaz Ruíz et al. 2021; Avila-Peltroche and Villena-Sarmiento, 2022).

Despite the sector's economic growth, no studies have yet characterized the MAAs profiles of these key species or examined the environmental factors regulating their biosynthesis. This knowledge gap limits the potential for developing high-value, sustainable biotechnological applications based on Peruvian macroalgae. Therefore, this study aims to characterize for the first time the detailed MAAs profiles of *C. chamissoi* and *G. lemaneiformis* cultivated in Ica, one of Peru's regions with the highest UV radiation levels (SENAMHI 2024). By analyzing seasonal variation (spring vs. summer) and life stage differences (gametophyte vs. sporophyte), this research seeks to elucidate the environmental and biological drivers shaping MAAs production in these species. Ultimately, this work provides a foundation for the development of sustainable, high-value photoprotective compounds derived from Peruvian macroalgae and contributes to a broader understanding of MAAs biosynthesis in subtropical coastal ecosystems.

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Materials and methods

Macroalgae Harvesting

Samples of *Chondracanthus chamissoi* (C.Agardh) Kützing and *Gracilariopsis lemaneiformis* (Bory) E.Y.Dawson, Acleto and Foldvik were collected from a marine bottom cultivation system at La Puntilla Beach, Paracas, Ica, Peru (13°48′25.57″S, 76°15′06.64″W; Fig. 1). The site is managed by the Cooperativa de Trabajadores Pesqueros Artesanales Algas Marinas (COTRAPALMAR) in collaboration with the Laboratorio de Investigación en Cultivos Marinos (LICMA) (Annex 1).

Sampling was conducted on two dates: 6th of November 2023 (spring) and 5th of March 2024 (summer). For each species, up to 500 g of fresh biomass was collected from the intertidal zone (0–50 cm depth), placed in sterile plastic bags, and transported in cooled containers to the laboratory at Universidad Científica del Sur.

To minimize climatic variability, samples were collected on sunny days and within a restricted time window (10:00-12:00 h), following recommendations of Velasco-Charpentier et al. (2016) and Navarro et al. (2014). In situ measurements of surface seawater temperature, dissolved oxygen, salinity, and pH were taken using a YSI PLUS multiparameter probe. Historical and seasonal oceanographic data (2023-2024) were obtained from the Laboratorio Costero de Pisco, Instituto del Mar del Perú (IMARPE), as part of its marine environmental monitoring program (https://www.imarpe.pe/imarpe/accesoinformacion/). Light-related parameters, including all-sky surface photosynthetically active radiation (PAR) and UV index, were retrieved all from the NASA POWER Data Access Viewer (https://power.larc.nasa.gov/data-access-viewer/). All physicochemical parameters are summarized in Table 1.

Species were taxonomically identified based on morphological characteristics, and life stages (gametophyte or sporophyte) were determined from reproductive structures. Identifications were verified by specialists from LICMA (Annex 2) and formally confirmed at the Natural History Museum of Universidad Ricardo Palma.

MAAs extraction

Fresh material was rinsed with distilled water to remove epiphytes, salt, and debris, then lyophilized (-108 °C, 0.013 mbar, 24 h). Dried samples were ground, sieved (< 250 µm), and

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stored in amber jars at -20 °C. MAAs were extracted following Karsten et al. (2009), with modifications. Ten milligrams of freeze-dried algal powder (three replicates per species and season) were mixed with 1 mL of 25% aqueous methanol, vortexed, and incubated in a water bath at 45 °C for 2 h in tightly sealed screw-cap vials to allow solvent extraction. After incubation, samples were centrifuged at 13,000 × *g* for 5 min, and 800 µL of the clear supernatant were transferred to new vials and evaporated to dryness under vacuum. The dried residues were reconstituted in 800 µL of distilled water, vortexed, centrifuged again to remove particulates, and finally filtered through 0.22 µm membrane filters before transfer to HPLC vials.

Quantification and identification of MAAs

The MAAs content of extracts was determined by high-performance liquid chromatography with diode array detection (HPLC-DAD), following Orfanoudaki et al. (2020b). Analyses were carried out on a Merck–Hitachi Elite La Chrom instrument (Tokyo, Japan) equipped with an organizer (SYSTEM O/B), a quaternary pump (L-2100), a degasser (Degasys DG-2410; Uniflows Co.,Ltd., Tokyo, Japan), an autosampler (L-2200), a column oven (L-2300), and a diode-array detector (L-2455). As stationary phase, a YMC-Pack ODS column (250 mm × 4.60 mm, 5 µm; YMC Europe, Dinslaken, Germany) in combination with an appropriate guard column was used. The mobile phase consisted of 20 mM ammonium formate and 0.25% (v/v) formic acid in water (A) and methanol (B). The applied gradient was as follows: 100% A from 0 to 20 min, 80% A at 30 min, 2% A at 35 min, and held at this composition for 5 min (total runtime of 40 min); followed by 15 min of re-equilibration under the initial conditions. Flow rate, temperature, and injection volume were adjusted to 0.65 mL min^{-1}, 9 °C and 5.0 µL, respectively. Detection wavelengths were set to 310, 330, and 350 nm. The area under the curve of target peaks was determined with EZChrom Elite software using the chromatograms recorded at 330 nm.

As described before (Bonin et al. 2024; Hammerle et al. 2025), calibration was performed using stock solutions of shinorine (SH), palythine (PA), asterina-330 (AS), and porphyra-334 (PR), which came from a previous study (Orfanoudaki et al. 2020b). Additionally, standards for mycosporine-glycine (MG), mycosporine-glycine-alanine (MGA), aplysiapalythine A (APA), aplysiapalythine B (APB), mycosporine-methylamine-threonine (MMT), usujirene (US), and palythene (PAE) were included.

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UHPLC–VWD–HR-MS² Analysis and Feature-based Molecular Networking (FBMN)

UHPLC–HRMS/MS analyses were carried out in collaboration with the University of Innsbruck. Extracts, blanks (ultra-pure water), and reference standards (Orfanoudaki et al. 2020b) were analyzed following the workflows of Zwerger et al. (2023) and Hammerle et al. (2025), with minor modifications.

Chromatographic separations were conducted on a Vanquish UHPLC system (Thermo Scientific, USA), consisting of a binary pump (VC-P10-A), an autosampler (VC-A12-A), a column oven (VC-C10-A), and a variable wavelength detector (VC-D40-A) connected to a Thermo Scientific Exploris 120 Orbitrap HRMS unit. A Luna Omega C18 100 Å column (100 mm × 2.1 mm; particle size 1.6 µm; Phenomenex, Torrance, USA), protected by a SecurityGuard ULTRA guard C18 pre-column, served as stationary phase. The mobile phase comprised water with 0.25% formic acid and 20 mM ammonium formate (A) and acetonitrile (B). The gradient program was: 0–10 min, 0–5% B; 10–11 min, 5–90% B; 11–13 min, 90% B; followed by 20 min re-equilibration at initial conditions. The flow rate, column oven temperature, auto-sampler temperature, and injection volume were adjusted to 0.3 mL min^{-1}, 17 °C, 20 °C, and 1 µL, respectively. UV detection was performed at 330 and 350 nm, the data collection rate to 2.0 Hz, the response time to 2.0 s, and the peak width to 0.2 min.

The system was controlled by Thermo Scientific Xcalibur 4.4 software. Calibration of the mass analyzer was done via the Thermo Scientific proprietary calibration mix and the respective automatic calibration function. The mass spectrometric parameters were as follows: heated-ESI ionization source, static spray voltage (positive: 3500 V), sheath gas (N2): 30 arbitrary units, auxiliary gas (N2): 17 arbitrary units, sweep gas (N2): 0 arbitrary units. Temperature of the ion transfer tube and vaporizer was adjusted to 370 and 420 °C, respectively. MS data (range 70-1000 *m/z*) were recorded from 0 to 20 min with a resolution of 60 000 FWHM for MS^1. The RF lens parameter was set to 70%. Data-dependent experiments were conducted with stepped collision energy mode and normalized collision energy type using HCD collision energies of 15, 30, and 45% at a resolution of 15 000 FWHM. The number of dependent scans was set to 3. The following selection of filters was employed: intensity threshold filter (1.0E5), dynamic exclusion (auto), isotope exclusion (assigned), charge state (perform dependent scans on single charged precursors only), and apex filter (desired apex window: 75%). In addition, a specific

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exclusion list was created for the measurement using HPLC-grade water as a background extract with an IODA Mass Spec notebook (Zuo et al. 2021).

Raw data were converted using **MSConvert** (Chambers et al. 2012) and processed in **MZmine 4.3.0** (Schmid et al. 2023). Feature tables and MS^2 spectra were then submitted to the **FBMN workflow on GNPS** (Nothias et al. 2020) to generate molecular networks, which were visualized in **Cytoscape** (Shannon et al. 2003). Metabolite annotation was performed using **SIRIUS 6.1.1** (Dührkop et al. 2015, 2019, 2021), and compound-class predictions from **CANOPUS** (Kim et al. 2021) were used to further characterize feature groups. Detailed processing and annotation parameters are provided in the Supplementary Material.

To identify clusters potentially containing MAAs, we followed the workflow of Zwerger et al. (2023). Briefly, experimental MS^2 spectra were matched against an in-house MAAs reference library (exact $[M+H]^+$ masses), compared against the GNPS spectral library, and refined through SIRIUS-based formula predictions. CANOPUS classifications were used to visualize NPC chemotaxonomic categories, highlighting nodes annotated as "mycosporines and mycosporine-like amino acids." In addition, a Cytoscape-compatible UV330 filter was applied to flag features coeluting with peaks exhibiting strong absorption at **330 nm**, a characteristic wavelength for many MAAs.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics v30 (IBM Corp., Armonk, NY, USA). For each species (*C. chamissoi* and *G. lemaneiformis*), the effects of season (spring vs. summer) and life stage (gametophyte vs. sporophyte) on (i) total MAAs concentration and (ii) the concentrations of individual major MAAs (SH, PA, AS, and PR) were evaluated.

Samples corresponded to independent biological replicates, defined as different thalli randomly collected from the cultivation system until approximately 500 g of fresh biomass was obtained for each treatment combination. After lyophilization, dried material from each treatment was used to prepare the MAAs extract, which was made in three analytical (technical) replicates. Only technical replication was considered for statistical analyses (n = 3).

Data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When assumptions were met, a two-way analysis of variance (ANOVA)

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was applied with season and life stage as fixed factors. When significant effects were detected, Tukey's honestly significant difference (HSD) test was used for post hoc pairwise comparisons.

For selected pairwise comparisons, independent-samples *t*-tests were additionally applied to further explore differences between specific groups. Statistical significance was set at $p < 0.05$, and exact *p*-values are reported whenever possible.

Results are expressed as mean ± standard deviation (SD), where *n* refers to the number of independent biological replicates ($n = 3$). Given the small sample size, statistical outcomes were interpreted cautiously and supported by consistent trends across seasons and life stages. For *G. lemaneiformis*, sporophytes were absent from the cultivation system during spring; therefore, seasonal comparisons for this life stage were not performed.

All graphs were generated using Microsoft Excel 2021 and display mean values with error bars representing ± SD. Different letters above the bars indicate statistically significant differences among groups based on Tukey's HSD post hoc tests ($p < 0.05$), whereas bars sharing the same letter do not differ significantly.

Results

MAAs Profiles

Chromatographic analyses revealed both shared and species-specific patterns of MAAs accumulation in *C. chamissoi* and *G. lemaneiformis* (Fig. 2). HPLC–DAD quantified the primary MAAs - defined here as the major contributors to total MAAS content - while UHPLC-HRMS/MS combined with FBMN expanded the detectable chemical diversity. Across both species, 13 MAAs were putatively annotated: *C. chamissoi* contained three primary MAAs and ten secondary MAAs (low-abundance metabolites), while *G. lemaneiformis* contained four primary MAAs and nine secondary metabolites (Table 2).

HPLC–DAD Quantification

Analysis using four calibrators and seven reference standards showed that *C. chamissoi* was dominated by SH, PA, and AS, whereas *G. lemaneiformis* exhibited a broader primary profile also including PR (Fig. 3). Total MAAS concentrations were consistently higher in *G.*

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lemaneiformis (1.73–3.69 mg g^{-1} DW) than in *C. chamissoi* (2.37–3.39 mg g^{-1} DW), indicating species-specific differences in biosynthetic capacity.

UHPLC–VWD–HR-MS² and FBMN

UHPLC-VWD-HRMS/MS analysis of the algal extracts and the corresponding blank, followed by data processing and feature-based molecular networking in the GNPS environment, yielded a network comprising 881 nodes and 1367 edges, organized into 45 clusters containing three or more nodes. To identify clusters potentially containing MAAs, the workflow established by Zwerger *et al.* (2023) was applied. In brief, experimental MS² spectra were searched against an in-house MAAS library (containing exact $[M+H]^+$ masses) using **mzmine**, compared with entries in the GNPS community library, and further annotated with **SIRIUS**. Compound class predictions from **CANOPUS** were used to visualize the NPC chemotaxonomy (Kim et al. 2021) within the network, highlighting nodes classified as "Mycosporine and Mycosporine-like amino acids". Additionally, a Cytoscape-compatible filter variable was generated to identify MS features coeluting with peaks in the 330 nm chromatogram, a wavelength at which MAAs typically exhibit strong UV absorption. Approximately 40% of all nodes showed such absorption characteristics. Based on these criteria, three clusters and three individual nodes were identified as potentially representing MAAs in the analyzed dataset (Fig. 4A).

Beyond the MAAs that could be quantified (i.e., SH, PA, AS, and PR), LC-MS/MS analysis revealed a broader chemical diversity, detecting a total of 13 putatively identified MAAs across both species, including several ones present only at trace levels (Table 2). Based on comparison with in-house reference data collected over several studies, the compounds APC, MG, APA, MGA, APB, MMT, mycosporine-lysine (ML), US, and PAE were putatively annotated with high confidence (Fig. 4B). In addition, nodes potentially corresponding to less common compounds such as palythine-serine, palythine-threonine, and shinorine methyl ester were also observed (Fig. 5).

In cluster 1, eight out of 42 nodes were classified as potential MAAs by SIRIUS at the NPC class level. This cluster contained many well-known MAAs, including SH, PA, AS, and several aplysiapalythines. Closely related structures, such as the cis–trans pair US and PAE, as well as the structurally similar group comprising SH, AS, and APA, appeared in distinct subregions of the cluster. Features corresponding to less common analogues, including palythine-serine and palythinol, were also observed. Cluster 2 comprised 11 features, three of which were annotated

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as MAAs on the compound class level. Two nodes corresponded to PR and MMT, which show close structural similarity, with MMT representing the decarboxylated analogue of PR. Cluster 3 consisted of MG together with closely related low-mass ions at m/z 246 and 188.

Further annotation results are provided in the Supplementary Material (Table S5), following current best practices for metabolomic data reporting (Alseekh et al. 2021).

In *C. chamissoi*, ML and Aplysiapalythines (notably APC and APB) dominated the secondary profile and generally occurred at medium abundance (10^6–10^7 mAU). Seasonal modulation was evident: US and PAE increased from low to medium levels in summer, whereas MGA was most abundant in spring. APC decreased specifically in summer sporophytes, indicating stage-specific regulation.

In *G. lemaneiformis*, the secondary profile was broader and more variable. APA was consistently abundant at high levels (>1.0 × 10^7 mAU) throughout the year. US and PAE displayed strong seasonal and stage-dependent enrichment, peaking in spring gametophytes and summer sporophytes in high levels. MG remained at medium abundance across all samples, with highest levels in spring gametophytes and summer sporophytes. APB occurred consistently at medium levels across all categories.

Overall, secondary MAAs diversity increased during summer in both species, with sporophytes generally exhibiting richer profiles than gametophytes (Table 2).

Seasonal variations in MAAs production

Total MAAs concentrations differed significantly between seasons in both species, with consistently higher values recorded in summer (Fig. 3). In *C. chamissoi*, gametophytes increased from 2.37 ± 0.06 mg g^{-1} DW in spring to 3.01 ± 0.05 mg g^{-1} DW in summer (two-way ANOVA, Tukey's HSD, $p = 0.0009$), while sporophytes rose from 2.68 ± 0.06 to 3.39 ± 0.22 mg g^{-1} DW over the same period ($p = 0.0004$).

G. lemaneiformis exhibited an even stronger seasonal response. Gametophyte total MAAs concentrations more than doubled from 1.73 ± 0.02 mg g^{-1} DW in spring to 3.53 ± 0.17 mg g^{-1} DW in summer (two-way ANOVA, Tukey's HSD, $p = 4 \times 10^{-5}$). Summer sporophytes reached 3.69 ± 0.11 mg g^{-1} DW; however, sporophytes were absent from the cultivation system during spring, precluding seasonal comparisons for this life stage.

Taken together, these results indicate that summer conditions strongly promote MAAs accumulation in both species, although the magnitude of the response is more pronounced in *G. lemaneiformis*.

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Seasonal changes were also reflected in MAAs composition in both species. In *C. chamissoi*, SH remained the dominant compound and increased significantly in summer (two-way ANOVA, $p < 0.01$), whereas PA and AS showed no marked seasonal differences. Several secondary MAAs, including PR, US, and PAE, were comparatively enriched in summer samples, while MGA was more abundant in spring (Table 2).

In *G. lemaneiformis*, all four primary MAAs (SH, PA, AS, and PR) increased more than twofold from spring to summer, with SH and PR becoming the major constituents in summer. Seasonal shifts were also evident among secondary MAAs in gametophytes, where MG, US, and PAE were more abundant in spring, whereas APA remained consistently abundant throughout the year ($> 1 \times 10^7$ mAU), and APB maintained medium-level abundance across all samples.

Collectively, MAA profiles were more abundant in summer in both species, reflecting enhanced accumulation of both primary and secondary compounds under high-irradiance conditions.

Life Stage Influences on MAAs Biosynthesis

Life stage significantly influenced MAAs accumulation in *C. chamissoi*. Sporophytes consistently contained higher total MAAs concentrations than gametophytes in both seasons (two-way ANOVA, $p < 0.05$; Fig. 3), indicating a stage-dependent capacity for photoprotective metabolite production.

In contrast, *G. lemaneiformis* showed no significant differences in total MAAs concentration between life stages during summer. Seasonal comparisons for spring were not possible due to the absence of sporophytes from the cultivation system during this period.

Life-stage effects on MAA composition differed between species (Fig. 3, Table 2). In *C. chamissoi*, PA and AS concentrations were consistently higher in sporophytes, whereas SH showed no significant variation between life stages. Among secondary MAAs, APC declined specifically in summer sporophytes, while US was more abundant in spring sporophytes than in gametophytes.

In *G. lemaneiformis*, all four primary MAAs were detected in both life stages, but only AS was significantly enriched in sporophytes compare to gametophytes during summer (two-way ANOVA, $p < 0.01$). The secondary MAA profile was generally richer in sporophytes than gamethophytes during summer, whereas US and PAE showed higher relative abundances in spring gametophytes and summer sporophytes. APA remained consistently abundant across life stages.

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Taken together, life-stage-dependent differences were more pronounced in *C. chamissoi,* whereas seasonal forcing exerted a stronger influence on MAAs composition in *G. lemaneiformis.*

Discussion

Species Differences in MAAs Profiles

According to Sun et al. (2020), both *C. chamissoi* and *G. lemaneiformis* classify as high-MAA producers (>2 mg g^{-1} DW), yet they differ markedly in their MAAs capacities and chemical diversity. In *C. chamissoi*, total MAAs concentrations (2.37–3.39 mg g^{-1} DW) reach the upper range reported for Chilean natural populations of the same species (0.8–3.3 mg g^{-1} DW; Véliz et al. 2019). The higher values observed in this study likely reflect shallower cultivation (~1 m) and thus greater irradiance than the 3–4 m semi-protected sites sampled by Véliz et al. (2019). In *G. lemaneiformis*, total MAAs concentrations (1.73–3.69 mg g^{-1} DW) exceed those documented for congeners, including *G. longissima* (0.69–1.6 mg g^{-1} DW; Chaves-Peña et al. 2019; Álvarez-Gómez et al. 2019) and *G. tenuifrons* (<1.5 mg g^{-1} DW even under high PAR; Torres et al. 2016), demonstrating a strong intrinsic capacity for MAAs accumulation.

Primary MAAs composition also differed between species. *C. chamissoi* was dominated by SH, followed by PA and AS - a pattern typical of intertidal Rhodophyta (Sun et al. 2020) and in part consistent with Chilean populations of *C. chamissoi*, which feature SH and PA (Véliz et al. 2019). UHPLC–HRMS/MS extended this profile, revealing ten additional low-abundance MAAs (Table 2), underscoring the value of high-resolution workflows for resolving co-eluting or trace metabolites (Hartmann et al. 2015; Zwerger and Ganzera 2022). The differences observed between Peruvian and Chilean populations of the same species challenge the notion of strictly species-fixed MAAs profiles (Boedeker and Karsten 2005) and instead suggest that environmentally driven plasticity shapes population-specific chemical fingerprints. Comparative work along Peruvian–Chilean gradients would help disentangle genetic from environmental contributions.

In contrast, *G. lemaneiformis* displayed a richer and broader MAAs spectrum, comprising four primary MAAs (SH, PA, AS, PR) and nine secondary metabolites, providing the first detailed profile for this species. Earlier reports relied solely on absorbance at ~325 nm (Zheng and Gao 2009), precluding compound-level identification. The resolved profile aligns in part with general Gracilariaceae patterns (e.g., *G. longissima*: SH, AS, PR, PA, palythinol; Álvarez-

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Gómez et al. 2019; *G. tenuifrons*: AS, palythinol, US, PAE; Torres et al. 2016) and supports the existence of a conserved biosynthetic core modulated by species-specific tuning.

Secondary MAAs identified via FBMN further emphasized interspecific differences. *C. chamissoi* was enriched in ML and aplysiapalythines (notably APC and APB), forming a classic cluster comprising glycine substituted cyclohexenimines (Cluster 1) characteristic of Rhodophyta (Zwerger et al. 2023). In contrast, *G. lemaneiformis* exhibited a more abundant secondary profile, including consistently high levels of APA, together with US, PAE and MG (Clusters 2-3), indicative of greater metabolic accumulation. Less common MAAs such as palythine-serine, palythine-threonine, and shinorine methyl ester were putatively identified in both species, highlighting the power of high-resolution MS/MS combined with FBMN to capture rare photoprotective metabolites that would otherwise remain undetected.

The combined consideration of ecology, morphology, and phylogeny helps to explain these interspecific contrasts under identical culture conditions. *G. lemaneiformis* typically inhabits turbid, soft-bottom, semi-protected habitats down to ~6 m, where light intensity and spectral quality are highly variable (Carbajal et al. 2019; Obrador and Pretus 2008). Such settings favor inducible and compositionally broader photoprotective systems, including PR-rich profiles. *C. chamissoi*, by contrast, colonizes wave-exposed rocky substrates in the lower intertidal and shallow subtidal (to ~8 m) (Carbajal et al. 2019), where persistently high UV exposure selects for constitutively elevated but less variable MAAs profiles centered on SH.

Tissue architecture likely reinforces these trends. *G. lemaneiformis* forms cylindrical, fleshy, branched thalli (> 1 m) that create internal light gradients where complementary UV-A/UV-B absorbers are advantageous (Hoyer et al. 2001; Karsten et al. 2005). *C. chamissoi* develops membranous, flattened blades (0.3–2.5 cm wide, up to 50 cm long) with high surface-to-volume ratios, favoring reliance on SH and related derivatives (Carbajal et al. 2019). Phylogenetically, Gracilariaceae (e.g., *G. lemaneiformis*) tend to exhibit greater inducibility of MAAs pathways than Gigartinaceae (e.g., *C. chamissoi*) (Sun et al. 2020). Together, these ecological, morphological, and phylogenetic factors help explain why *G. lemaneiformis* attained both higher concentrations and a broader MAAs spectrum than *C. chamissoi* under the same culture conditions.

Overall, both species emerge as promising MAAs sources. Notably, *G. lemaneiformis* combines high total MAAs content with a broad composition of primary and secondary MAAs, which may be particularly advantageous for biotechnological applications targeting different spectral windows.

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Environmental and Seasonal Drivers for MAAs Production

At the subtropical latitude, seasonal SST contrasts are modest (ΔSST ≈ 2 °C), yet solar radiation remains intense year-round, with midsummer UV indices exceeding 12 (SENAMHI, 2024). This creates a background of chronic UV stress for intertidal and shallow subtidal macroalgae. The Pisco–San Juan upwelling system (Peru) operates year-round but reaches peak intensity during late winter and spring, introducing cold, nutrient-rich waters into the coastal zone and thereby strongly influencing nitrogen availability and water clarity (Gutiérrez et al. 2011). These pulses are critical because MAAs are nitrogen-rich metabolites, and their biosynthesis is sensitive to nitrogen supply. In addition, episodic ozone depletion in early spring further increases biologically effective UVR (Williamson et al. 2019; Barnes et al. 2022), adding an additional seasonal stressor that may stimulate photoprotective responses.

Our study period was contemporaneous with the 2023–2024 Coastal El Niño, which was superimposed on a major basin-scale El Niño event. The event began in early 2023, produced regional SST anomalies, and disrupted normal upwelling along the Peruvian coast. Eastern Pacific SST anomalies exceeded +2 °C during November–December 2023 before declining by April 2024 (Peng et al. 2024, 2025). Although significant warming was observed at the basin scale, local thermal anomalies in Pisco remained modest (Table 1), a condition likely mitigated by persistent coastal upwelling. This buffering action restricted extreme surface warming but consequently altered nutrient delivery, water column structure, and hydrographic stability. Such indirect effects can substantially influence algal metabolism, particularly nitrogen-dependent pathways like MAAs biosynthesis, where the interaction of light and nutrient availability serves to modulate accumulation.

Taken together, the seasonal rise in MAAs concentration and composition in both *C. chamissoi* and *G. lemaneiformis* is best explained by the convergence of multiple environmental drivers (Briani et al. 2018) - in this case high summer irradiance, partial recovery of nutrient availability after spring limitation, and episodic anomalies - rather than by irradiance alone, which is often highlighted in the literature (Sun et al. 2020). This interpretation agrees with experimental work demonstrating that MAAs induction depends on the combined availability of light and nitrogen precursors (Korbee et al. 2005; Álvarez-Gómez et al. 2017).

Seasonal effects on MAAs concentration

Both species reached their maximum total MAAs levels in summer (Fig. 3). Despite the relatively small seasonal variability in SST, dissolved oxygen, salinity, pH, PAR, and UV index

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recorded at the site (Table 1), MAAs accumulation increased markedly, indicating that no single measured parameter alone explains the observed pattern.

We propose that nutrient limitation in spring, associated with reduced or irregular upwelling at the onset of the 2023–2024 Coastal El Niño, constrained MAAs biosynthesis in both species. By summer, nutrient availability has partially recovered, while UV and PAR remained high, providing conditions favorable for enhanced MAAs production. This nutrient-light interaction aligns with previous studies showing that MAAs synthesis is not solely light-driven but also nitrogen-dependent, as nitrogen-rich amino acid precursors feed directly into MAAs biosynthetic pathways (Korbee et al. 2005; Álvarez-Gómez et al. 2017).

C. chamissoi exhibited a modest, steady seasonal increase in total MAAs, consistent with its adaptation to chronically high UV and comparatively stable photoprotective requirements. This pattern complements Chilean observations where geographic and latitudinal gradients overshadowed seasonality (Véliz et al. 2019). In contrast, *G. lemaneiformis* showed a more drastic response: summer gametophytes more than doubled their MAAs content relative to spring, indicative of strong inducibility. This is consistent with the high plasticity documented in Gracilariaceae species, where combined UVR and nutrient enrichment significantly enhance MAAs accumulation (Álvarez-Gómez et al. 2017). Such inducibility likely reflects the species' ecology, inhabiting turbid and variable habitats in Peru, it benefits from a flexible metabolic strategy that rapidly boosts photoprotection during periods of high stress.

Altogether, these findings demonstrate that while *C. chamissoi* maintains a conservative, steady increase in response to seasonal forcing, *G. lemaneiformis* exhibits a more pronounced, inducible response. This contrast illustrates the coexistence of constitutive versus inducible photoprotective strategies in red macroalgae, both effective under seasonal stress but differing in ecological foundations and biotechnological potential.

Seasonal effects on MAAs composition

Compound-specific modulation mirrored changes in total MAAs content. In *C. chamissoi*, SH remained dominant across seasons and increased significantly in summer, whereas PA and AS remained comparatively stable (Fig. 3). This pattern suggests preferential up-regulation of SH, consistent with its strong UV absorption ($\lambda_{max} \approx 332–334$ nm), antioxidant capacity, and photostability (Karsten et al. 1998; Hartmann et al. 2015; Geraldes and Pinto 2021; Orfanoudaki

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et al. 2019; Rosic et al. 2023). Within the secondary MAAs set, PR, US and PAE increased in summer, while MGA was more abundant in spring.

In *G. lemaneiformis,* summer profiles shifted toward SH and PR dominance, whereas spring was more evenly distributed among SH, PA, AS, and PR. This preferential allocation to broad-spectrum absorbers (SH and PR) under high-irradiance conditions resembles patterns reported for other Gracilariaceae (Pliego-Cortés et al., 2019) and for *G. vermiculophylla*, in which the timing and magnitude of seasonal MAA maxima vary among localities (Barceló-Villalobos et al., 2017). In contrast, several secondary MAAs (MG, US, and PAE) were more abundant in spring gametophytes than in summer. Because UV radiation and PAR were comparable between seasons, this pattern is unlikely to be driven by irradiance alone and more plausibly reflects seasonal differences in nutrient availability linked to intensified spring upwelling and early El Niño–related hydrographic variability. Such conditions may favor the synthesis of specific secondary MAAs, whereas summer profiles appear to prioritize broad-spectrum primary MAAs (SH and PR).

Collectively, these shifts indicate that seasonality modulates not only total MAA levels but also the relative balance among MAA types, thereby enhancing the photoprotective repertoire during periods of increased environmental stress.

Life stage influences on MAAs biosynthesis

Both *C. chamissoi* and *G. lemaneiformis* exhibit isomorphic triphasic life cycles (Ávila et al. 2011; Kain and Destombe 1995), with gametophytes and sporophytes that are morphologically similar but can differ physiologically (Hoyer et al. 2001; Boedeker and Karsten 2005; Korbee et al. 2005; García-Jiménez and Robaina 2015). Such differences are often linked to ploidy level, tissue architecture, and metabolic allocation (Vieira et al. 2021) and can influence the production of secondary metabolites, including MAAs.

In *C. chamissoi*, sporophytes consistently accumulated higher total MAA concentrations than gametophytes regardless of the season. This contrasts with Chilean populations, where gametophytes have been reported to dominate (Véliz et al. 2019), suggesting that environmental context - particularly shallower culture depth and stronger UV exposure - can invert life-stage

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patterns. Under the conditions studied here, sporophytes, being diploid and structurally more complex, may require enhanced protection to ensure reproductive success under intense UV.

In *G. lemaneiformis*, sporophytes tended to have higher MAAs levels than gametophytes in summer, but differences were not statistically significant, and sporophytes were absent in spring. This pattern suggests a seasonally restricted development of the sporophytic phase, potentially linked to environmental drivers, as ploidy ratios in wild populations of Gracilariaceae have been shown to vary with temperature and seasonal conditions (Feng et al. 2022). The absence of sporophytes under El Niño–related anomalies in spring supports the idea that environmental stress can constrain reproductive development and thereby indirectly affect MAAs dynamics. More broadly, *C. chamissoi* behaves as a euryoecious species with constitutive photoprotection, while *G. lemaneiformis* appears more stenoecious, relying on inducible defenses and displaying greater sensitivity to seasonal and hydrographic variability.

Life stage effects on MAAs composition

Ontogenetic differences also affected MAAs composition. In *C. chamissoi*, PA and AS were enriched in sporophytes, whereas SH showed no clear stage-specific variation. This pattern suggests a strategy of broadening the spectral coverage of photoprotection in the diploid phase: PA absorbs mainly in the UV-B range ($\lambda_{max} \approx 320$ nm) and has antioxidant properties, whereas AS absorbs in the UV-A range ($\lambda_{max} \approx 330$ nm) and mitigates longer-wavelength stress (Karsten et al. 1998; de la Coba et al. 2019). Their enrichment in sporophytes may therefore enhance resilience of diploid tissues to fluctuating irradiance, complementing SH's broad-spectrum role.

In *G. lemaneiformis*, all four primary MAAs (SH, PA, AS, PR) were present in both stages, but AS was significantly more abundant in sporophytes during summer. This enrichment is consistent with the thicker and structurally more complex morphology of sporophytes (Wiencke et al. 2000), in which deeper tissue layers are exposed to attenuated yet biologically effective UV-A radiation and therefore require enhanced photoprotection (Neale et al. 2023). Comparable ontogenetic shifts in MAAs allocation have been reported in other macroalgae, where developmental stage and tissue organization interact with the internal light field to regulate photoprotective compound distribution (Hoyer et al. 2001; Ávila et al. 2011; Álvarez-Gómez et al. 2017).

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Secondary MAAs were generally more abundant in sporophytes of both species, further supporting the notion that life-stage-specific morphology modifies internal optical environments and promotes more complex MAAs profiles. This life-stage-dependent modulation complements the seasonal and interspecific patterns observed in this study and highlights the dynamic, multi-scale regulation of MAAs biosynthesis in red macroalgae.

Conclusion

This study provides the first comprehensive characterization of MAAs in two commercially important red macroalgae from Peru, *C. chamissoi* and *G. lemaneiformis,* integrating targeted HPLC–DAD quantification with UHPLC–HRMS/MS-based untargeted metabolomics and FBMN. Both species were confirmed as high-MAAs producers and shared the same set of 13 MAAs, yet they differed markedly in their biochemical strategies.

Seasonality was of great importance for the MAAs profile in both species. Life stage further modulated these responses, with sporophytes generally accumulating higher total MAAs and exhibiting richer secondary-MAAs profiles, signalling ontogenetic control of photoprotection.

Beyond ecological insights, this work significantly expands the known MAAs repertoire for both species, including rare palythine derivatives—through FBMN-guided annotation. These findings highlight the importance of integrating untargeted metabolomics to reveal hidden chemodiversity relevant for natural product discovery.

Overall, *C. chamissoi* and *G. lemaneiformis* represent promising, sustainable resources for the production of natural UV-protective compounds. The strong inducibility of *G. lemaneiformis* in particular highlight its potential for optimized aquaculture aimed at enhancing MAAs yields and tailoring spectral coverage. Strengthening Peru's foundational knowledge of algal bioactive compounds and promoting the processing and valorization of seaweed biomass - rather than its export primarily as raw material - could foster a national bioeconomy centered on high-value photoprotective metabolites. Continued research integrating multi-site comparisons, controlled cultivation experiments, and molecular approaches will be essential to disentangle environmental and genetic drivers of MAAs biosynthesis and fully unlock the biotechnological potential of these key Peruvian Rhodophyta.

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Author contributions

F.M.: Investigation, methodology, visualization, writing—original draft. **J.F.:** Supervision, writing—review and editing. **F.H.:** Methodology, visualization, writing—original draft, writing—review and editing. **M.G.:** Funding acquisition, writing—review and editing. **B.B.:** Visualization, writing—original draft, writing—review and editing. **O.R.:** Supervision, methodology, writing—review and editing. **U.K.:** Supervision, writing—original draft, writing—review and editing, funding acquisition.

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Data availability

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

Competing interests

The authors declare that they have no competing interests.

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Figure legends

Fig. 1 Map of the Ica region, Peru, showing the location of the marine bottom cultivation site (COTRAPALMAR, Pisco province) where *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis* were sampled.

Fig. 2 Representative UHPLC–HRMS extracted ion chromatograms (EICs) at 330 nm for annotated mycosporine-like amino acids (MAAs) in *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*. Major peaks are labeled: shinorine (SH), palythine (PA), asterina-330 (AS), porphyra-334 (PR), aplysiapalythine A (APA), aplysiapalythine B (APB), usujirene (US), and palythene (PAE). Additional low-abundance compounds are listed in Table 2.

Fig. 3 Concentration and composition of primary mycosporine-like amino acids (MAAs), and total MAAs content (mg g^{-1} DW), determined by HPLC–DAD in gametophytes and sporophytes of *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis* collected in spring 2023 and summer 2024. Values represent means ± standard deviation (SD) of three replicates (n = 3). Bars sharing the same letter are not significantly different (Tukey's HSD, $p < 0.05$): Abbreviations: SH, shinorine; PA, palythine; AS, asterina-330; PR, porphyra-334. Sporophytes of *G. lemaneiformis* were absent in spring.

Fig. 4 A | Feature-based molecular network generated from the extracts of *C. chamissoi* and *G. lemaneiformis*. Clusters containing mycosporine-like amino acids (MAAs) are highlighted with red boxes and corresponding labels. Nodes annotated as potential MAAs via SIRIUS are shown in red. Node size reflects UV absorption characteristics, with larger nodes indicating features coeluting with a peak at 330 nm (UV330 filter: 1). Node border thickness represents retention time, increasing with later elution. **B** | Detailed view of MAA-containing clusters. The relative abundance of each feature in the algal extracts is displayed as a pie chart on the respective node. Nodes putatively annotated as MAAs by SIRIUS are depicted as octagons. Annotation results are shown on the right, including molecular structures, observed m/z values, trivial names, and

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corresponding retention times. Node size again represents the UV filter variable, with larger nodes indicating absorption at 330 nm. The numbering of annotated compounds corresponds to the node labels in the network.

Fig. 5 Fragment spectra mirror plots of nodes putatively identified as palythine (ID 665), palythine-serine (ID 624), and palythine-threonine (ID 874). Matching fragment ions are highlighted in blue, while matching neutral losses are indicated in orange with dotted lines and arrows. The top section of each plot shows the modified cosine score and the number of matched signals.

Supplements legends

Fig. S1 Schematic scheme of the marine bottom cultivation system at San Andrés Beach, Pisco Province, Ica Region, Peru. The site is a shallow coastal habitat (0.5–1.0 m depth) with rocky substrates and interspersed sand. The system consists of horizontal mainlines anchored with moorings (nets filled with pebbles) and supporting 20 cultivation units each. Units are constructed from raffia to ensure neutral buoyancy, maintaining algae slightly above the seabed. Each was initially inoculated with ~40 g of juvenile red algal thalli and fixed at regular intervals. A surface buoy marks each line for retrieval. Illustration created with BioRender.com.

Fig. S2 Photographic overview of the cultivation site and macroscopic differentiation of gametophytes and sporophytes in *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis*.

Fig. S3 Supplementary details of the extraction protocol used for the isolation of mycosporine-like amino acids (MAAs) from lyophilized macroalgal samples.

Fig. S4 Clusters containing MAAs, with nodes labeled according to their corresponding feature IDs. Relative peak areas across different algal extracts are visualized as pie charts.

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Table 1. Physicochemical parameters recorded during spring and summer 2023-2024 at the cultivation site in Pisco, Peru, compared with historical seasonal and annual data from the Laboratorio Costero de Pisco (IMARPE). PAR = photosynthetically active radiation; DO = dissolved oxygen; SSTa = sea surface temperature anomaly. All values are expressed in SI units.

Parameter	Spring (06 Nov 2023)	Summer (05 Mar 2024)	2023 (range or mean ± SD)	2024 (range or mean ± SD)	Spring (Sept–Dec)	Summer (Dec–Mar)
Coordinates	S 13°48′25.57″ / W 76°15′6.64″		S 13°47′17.0″ / W 76°14′30.0″			
Sea Surface Temperature (°C)	19.50	21.50	18.84 – 23.61	17.84 – 23.94	18.84 – 21.80	21.24 – 23.94
Anomalies (ATSM)	–	–	0.48 ± 0.75	0.03 ± 0.56	0.28 ± 0.38	–0.80 ± 0.83
Dissolved Oxygen (DO, mg L^{-1})	10.46	7.93	4.44 ± 1.35	4.69 ± 1.24	5.06 ± 0.73	5.53 ± 0.43
Salinity (PSU)	36.10	34.30	33.99 ± 2.99	34.92 ± 0.25	35.08 ± 0.12	35.12 ± 0.13
pH	8.20	7.40	7.77 ± 0.30	7.71 ± 0.25	7.90 ± 0.32	7.67 ± 0.13
All sky surface total PAR (MJ m^{-2} day^{-1})	11.83	11.54	8.98 ± 2.26	9.08 ± 2.22	10.15 ± 1.17	11.24 ± 0.36
All sky surface UV Index (W m^{-2} × 40)	2.78	2.99	2.14 ± 0.63	2.26 ± 0.65	2.41 ± 0.39	2.93 ± 0.15

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Table 2. Relative abundance of secondary MAAs putatively identified by UHPLC–VWD–HRMS/MS followed by feature-based molecular networking in gametophytes and sporophytes of *Chondracanthus chamissoi* and *Gracilariopsis lemaneiformis* (spring 2023, summer 2024). Abundance categories: low (L) (< 1.0×10^6 mAU, green), medium (M) (1.0×10^6–1.0×10^7 mAU, yellow), and high (H) (> 1.0×10^7 mAU, red). *Porphyra-334 is classified as a primary MAAs only in *G. lemaneiformis* and is indicated in purple with the letter "P." m/z = mass-to-charge ratio; RT = retention time (min). Color coding is explained within the table for ease of interpretation.

m/z	RT (min)	MAA Type	*Chondracanthus chamissoi*				*Gracilariopsis lemaneiformis*		
			Spring		Summer		Spring	Summer	
			Gametophyte	Sporophyte	Gametophyte	Sporophyte	Gametophyte	Gametophyte	Sporophyte
347.14	2,26	Porphyra-334	L	L	M	M	P	P	P
259.13	2,32	Aplysiapalythine C	M	M	M	L	L	L	L
246.10	2,82	Mycosporine-glycine	L	L	L	L	M	L	M
303.15	3,02	Aplysiapalythine A	L	L	L	L	H	H	H
317.13	3,33	Mycosporine-glycine-alanine	M	M	L	L	L	L	L
273.14	4,77	Aplysiapalythine B	M	M	M	M	M	M	M
303.16	5,03	Mycosporine-methylamine-threonine	L	L	L	L	L	L	L
317.17	6,27	Mycosporine-lysine	M	M	M	M	L	L	L
285.14	8,70	Usujirene	L	M	M	M	H	M	H

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285,14	8,95	Palythene	L	L	M	M	H	M	H

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13. COPIAS DE CARTAS DE RESPUESTA A LAS OBSERVACIONES DE LOS REVISORES

Response to Reviewers

Manuscript title:
"Evaluation of UV-sunscreen mycosporine-like amino acids (MAAs) in two economically important red macroalgae from Peru"

***All changes in the original manuscript are highlighted in yellow.**

General revisions

Comment: Citations of multiple references should be in year (ascending)/authors order.

Response:
All in-text citations have been revised to follow the journal format, with references ordered chronologically and alphabetically where appropriate.

Example: (Geraldes and Pinto 2021; Jofre et al. 2020; Briani et al. 2018) → (Briani et al. 2018; Jofre et al. 2020; Geraldes and Pinto 2021)

Comment: Figure 1 is not necessary – just give the latitude and longitude.

Response:
Figure 1 has been removed. The sampling location is now described exclusively by latitude and longitude in the Materials and Methods section.

Comment: Figure 2 lacks tick marks on the axes.

Response:
Tick marks have been added to all axes in Figure 2 (Now Fig. 1).

Comment: Table 2: In English a decimal point (.) is used instead of a comma (,).

Response:
All decimal separators in Table 2 have been corrected to decimal points.

Comment: Figure 3: The y-axes lack tick marks.

Response:
Tick marks have been added to all y-axes in Figure 3 (Now Fig. 2).

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Reviewer 1

We thank Reviewer 1 for the thorough and constructive comments, which greatly helped to improve the clarity of the manuscript. All suggested revisions have been addressed and incorporated into the revised version, as detailed below.

Comment 1: Page 5, line 5: replace "shifts-conditions" with "shifts – conditions".

Response:
Corrected as suggested.

Comment 2: Page 6 – last line states: "three replicates per species". However, on page 9 – second paragraph of the "Statistical analysis section" states: "Samples corresponded to independent biological replicates, defined as different thalli randomly collected from the cultivation system until approximately 500 g of fresh biomass was obtained for each treatment combination. After lyophilization, dried material from each treatment was used to prepare the MAAs extract, which was made in three analytical (technical) replicates. Only technical replication was considered for statistical analyses (n = 3). On page 10 – lines 1 and 2 appears: "where n refers to the number of independent biological replicates (n = 3)."

Response:
Thank you for pointing out this inconsistency. We confirm that independent biological replicates were used.

For each treatment combination (species × season × life stage), approximately 500 g of fresh biomass was collected and three independent extracts were prepared after lyophilization of the fresh biomass. These extracts represent three independent biological replicates (n = 3).

To ensure consistency, the manuscript was revised as follows:

- **Page 6, last line**
  "three replicates per species" was replaced with "three independent biological replicates per treatment combination (species, season, and life stage)"
- **Page 9, Statistical analysis section**
  "three analytical (technical) replicates" was replaced with "three independent biological replicates (n = 3)"
- **Page 10, lines 1–2**
  The statement *"where n refers to the number of independent biological replicates (n = 3)"* is correct and was retained.

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Comment 3: Page 8 – third paragraph – lines 8 and 10: replace 60 000 with 60,000 and 15 000 with 15,000.

Response:
Corrected as suggested.

Comment 4: Page 10 – HPLC-DAD Quantification section: Was a statistical comparison performed between the total MAA concentrations among species? In my interpretation, the range for *G. lemaneiformis* is broader, but I do not agree with the statement that concentrations "were consistently higher in *G. lemaneiformis* than in *C. chamissoi*." I suggest revising this point.

Response:
We agree with the reviewer. While total MAAs were statistically compared between species, the original wording was overly strong. The text has been revised to state that *G. lemaneiformis* exhibited a broader range of total MAA concentrations and reached higher maximum values, rather than being consistently higher across all conditions.

Comment 5: Page 10 – UHPLC–VWD–HR-MS² and FBMN section: The paragraph "In brief, experimental MS2 … exhibit strong UV absorption." may be removed, as the same information is already provided in the Materials and Methods (last paragraph of the section UHPLC–VWD–HR-MS² Analysis and Feature-based Molecular Networking (FBMN)).

Response:
The paragraph was removed to avoid redundancy with the Materials and Methods section.

Comment 6: The file for Fig. 5 was corrupted; the comparative spectra of palythine and palythinethreonine cannot be properly visualized.

Response:
Figure 5 has been re-exported and uploaded again. The spectra can now be properly visualized.

Comment 7: Pages 11 and 12 – the three paragraphs " In *C. chamissoi*, ML and ..... profiles than gametophytes (Table 2): the information presented in these paragraphs refers to the interpretation of seasonal variations in MAAs. Therefore, they should be transferred to the following section, "Seasonal variations in MAAs production".

Response:
The indicated paragraphs were moved to the "Seasonal variations in MAAs production" subsection to improve logical flow and consistency.

Comment 8: Page 13: I suggest performing a PCA analysis to confirm the observation presented in the last paragraph of the Results section: "Taken together, … in *G. lemaneiformis*."

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Response:
We thank the reviewer for this suggestion. A PCA was carefully considered; however, it was not applied to the dataset referred to in this paragraph because it is not statistically appropriate for the variables involved. The observation in question is based on total MAA concentration, which represents a single quantitative response variable. PCA requires multiple quantitative variables to explore multivariate structure and covariance patterns, and applying PCA to a univariate dataset would not provide additional or meaningful information beyond univariate statistical analyses.

Accordingly, differences in total MAAs were evaluated using univariate statistical tests, which are more appropriate for assessing the effects of species, season, and life stage on overall MAA accumulation. Nevertheless, the text mentioned has been revised to avoid ambiguity and to ensure that interpretations are strictly supported by the applied statistical analyses.

Comment 9. Figures S1–S4 are not cited in the manuscript. Figures S1–S3 are very good and complementary to what is described in the Materials and Methods. Figure S4, however, seems unnecessary, as the information is already presented in Fig. 4.

Response:
Figures S1–S3 are now cited in the manuscript and integrated into the Materials and Methods section. Figure S4 has been removed, as its information duplicates Figure 4.

Comment 10. Page 18 – last paragraph: since statistical analyses were performed for all comparisons, avoid implying trends. If no significant difference was observed, it is better not to emphasize this information.

Response:
The text was revised to remove statements implying trends where no statistically significant differences were detected.

Comment 11. Caption of Fig. S1 – line 1: replace "scheme" with "illustration".

Response:
Corrected as suggested.

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Reviewer 2

We thank the reviewer for the thorough and constructive comments, which helped improve the clarity, precision, and biological interpretation of the manuscript. All changes have been incorporated into the revised version, as detailed below.

General comments

Comment: Standardize the order of articles in the same citation throughout the text. Usually, it starts with the oldest publications, following by alphabetic order. See the journal's guidance. I cite just one example, but there are others: Pellissier et al. 2023; Quiros-Guerrero et al. 2024; Hammerle et al. 2023 (Introduction).

Response:
All in-text citations have been standardized according to the journal's guidelines, with references ordered chronologically and, where applicable, alphabetically within the same year.

Comment: The supplementary figures were not cited in the text.

Response:
All supplementary figures are now explicitly cited at the appropriate locations in the text.

Comment: The authors cite Table S5, giving the impression that there are Tables 1-4, which is not the case. I suggest placing Table S5 in a separate file and naming it Table 1S.

Response:
Table S5 has been moved to a separate supplementary file called "Miyagi et al._Supplementary Table" and renamed Table S1, as suggested.

Comment: Considering what Figure S2 shows, authors worked with fertilized female gametophytes (cystocarpic plants), correct? Or they also identify male gametophytes, but not include them in the figure? It is important to specify exactly the type of gametophyte used in the study, as physiological differences have already been observed considering male gametophytes, unfertilized female gametophytes, and fertilized female gametophytes in other Rhodophyta species (e.g. Santelices & Varela 1995; Guimarães et al. 1999; Faria & Plastino 2023). Differences may also occur regarding mycosporines, especially considering quantity.

Response:
All gametophytes analyzed in this study correspond to fertilized female gametophytes. This information has now been explicitly stated in the Materials and Methods and clarified in the Figure S2. Male gametophytes were not included in the analyses.

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Comment: Sporophyte: I suggest replacing the term with tetrasporophyte (used for Rhodophyta) all long the text.

Response:
The term tetrasporophyte has been used consistently throughout the revised manuscript.

Results

MAAs profiles

Comment: The data presented in Fig. 2 were obtained from extracts taken during which period (spring or summer)? Specify in the legend.

Response:

The data presented in Fig. 2 (now Fig. 1) correspond to extracts obtained from spring gametophytes of each species. This information has been explicitly stated in the figure legend to avoid any ambiguity.

Comment: Complete Fig. 3: there are not letters above the bars in: *C. chamissoi* (total); *G. lemaneiformis* (asterina-330 - AS); and *C. chamissoi* gametophytes (spring B).

Response:

Figure 3 (now Fig. 2) has been revised to clarify the statistical notation. The figure legend now explicitly states that bars sharing the same letter are not significantly different, whereas bars with different letters, or bars without letters, indicate statistically significant differences. This clarification explains the absence of letters above certain bars and ensures that all statistical comparisons are unambiguous.

HPLC–DAD Quantification

Comment: "Total MAAS concentrations were consistently higher in *G. lemaneiformis* (1.73–3.69 mg g-1 DW) than in *C. chamissoi* (2.37–3.39 mg g-1 DW)" But is this statement based on statistical data? Which ones? Included it.

Response:
As also noted by Reviewer 1, this wording was overly strong. The text has been revised to indicate that *G. lemaneiformis* exhibited a broader range and higher maximum values of total MAAs, rather than consistently higher concentrations across all conditions.

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UHPLC–VWD–HR-MS² and FBMN

Comment (1st paragraph): I suggest moving some of the content from this paragraph to the Material and Methods section.

Response:
Redundant methodological content has been removed from the Results section and consolidated within the Materials and Methods.

Seasonal variations in MAAs production

Comment (3rd paragraph): - "…although the magnitude of the response is more pronounced in *G. lemaneiformis*." It is possible, but did you do a statistical test to affirm this? Where is the data?

Response:
We agree with the reviewer that the original wording implied a statistically tested difference in response magnitude between species, which was not directly evaluated. Accordingly, the text has been revised. The Results section now reports only statistically tested seasonal effects within each species and includes numerical comparisons of proportional seasonal increases. Specifically, seasonal increases ranged from 26–27% in *C. chamissoi* and reached 104% in *G. lemaneiformis* gametophytes. While no formal statistical test comparing response magnitude between species was performed, these proportional differences are now presented explicitly and interpreted cautiously, without implying statistical significance.

Comment (4th paragraph): "Several secondary MAAs, including PR, US, and PAE, were comparatively enriched in summer samples…." – for US, only to gametophyte. Make it clear in the text.

Response:
This has been clarified in the text. US enrichment is now explicitly described as restricted to gametophytes.

Comment (5th paragraph): "In *G. lemaneiformis,* all four primary MAAs…". As you did in the previous paragraph, include the statistical data here. Refer to Figure 3 and Table 2 in this paragraph.

Response:
Statistical results have been added, and the paragraph now explicitly refers to Fig. 3 (Now Fig. 2) and Table 2, in line with the structure used for *C. chamissoi*.

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Life stage influences on MAAs biosynthesis

Comment (4th paragraph): "...The secondary MAA profile was generally richer in sporophytes than gamethophytes during summer..." Of the 10 analyzed, this occurred in only three. Modify the language. Change "gamethophytes" by gametophytes.

Response:
The language has been revised to avoid overgeneralization. The text now states that differences were limited to specific compounds and conditions, rather than implying a general enrichment. The term "gamethophytes" has been corrected to gametophytes.

Comment (5th paragraph): "...whereas seasonal forcing exerted a stronger influence on MAAs composition in *G. lemaneiformis*."
Based on the results, I have doubts about that. Maybe not "stronger".

Response:
We agree. The wording has been softened to state that seasonal forcing appears to play a relatively greater role, without implying a quantitatively stronger effect.

Discussion

General suggestion:
I suggest starting with a paragraph that highlights the main results found.

Response:
A new introductory paragraph summarizing the main results has been added at the beginning of the Discussion.

Species differences in MAAs profiles

Comment (5th paragraph): "Tissue architecture…" Change to "Thallus" architecture.

Response:
Revised throughout.

Seasonal effects on MAAs composition

Comment (1st paragraph):"…Within the secondary MAAs set, PR, US and PAE increased in summer…" US, only for gametophytes. Tetrasporophytes remained stable.

Response:
Corrected accordingly.

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Comment:
- "*G. vermiculophylla*": change to *Gracilaria vermiculophylla*.

Response:
Corrected.

Life stage influences on MAAs biosynthesis

Comment (1st paragraph): Suggested rephrasing regarding triphasic life cycles and isomorph.

- "Both *C. chamissoi* and *G. lemaneiformis* exhibit isomorphic triphasic life cycles (Ávila et al. 2011; Kain and Destombe 1995), with gametophytes and sporophytes that are morphologically similar but can differ physiologically…"

Sugestion: Both *C. chamissoi* and *G. lemaneiformis* exhibit triphasic life cycles (Ávila et al. 2011; Kain and Destombe 1995), with isomorphic gametophytes and sporophytes, but they can differ physiologically…

- "tissue architecture" – change to thallus architecture.

Response:
The paragraph has been revised following the reviewer's suggested wording.

Comment (2nd paragraph): Do not state that tetrasporophytes are structurally more complex.

- "Under the conditions studied here, sporophytes, being diploid and structurally more complex, may require enhanced protection to ensure reproductive success under intense UV."

The morphology and anatomy are similar between gametophytes and tetrasporophytes. I believe it is not appropriate to say that the latter are structurally more complex than the former, except in relation to ploidy.

Response:
We agree. References to structural complexity have been removed. Differences are now discussed in terms of ploidy and physiological allocation, not morphology.

Comment (3rd paragraph): Do not state higher MAAs in tetrasporophytes when differences are not significant.

- "In *G. lemaneiformis*, sporophytes tended to have higher MAAs levels than gametophytes in summer, but differences were not statistically significant" Since authors used statistics, there is no sense in stating that tetrasporophytes had higher levels of MAAs than gametophytes. Statistical analysis clearly shows that they were similar!

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Response:
We agree with the reviewer that it is inappropriate to imply higher MAA levels between life stages when differences are not statistically significant. Accordingly, the text has been revised to remove any comparative language suggesting higher MAA concentrations in tetrasporophytes of *G. lemaneiformis*. The Results section now states that no statistically significant differences in total MAA concentrations were detected between life stages during summer, and that seasonal comparisons were not possible due to the absence of tetrasporophytes in spring. Consequently, no inference regarding life-stage-specific MAA regulation in *G. lemaneiformis* is made beyond the statistically supported results.

Life stage effects on MAAs composition

Comment: Morphology is similar between isomorphic phases; differences may relate to reproductive status.

2nd and 3th paragraphs
- "This enrichment is consistent with the thicker and structurally more complex morphology of sporophytes…"
- " the notion that life-stage-specific morphology modifies internal optical environments and…"

The structure of the thalus in Gracilaria and *Gracilariopsis* species (morphology and anatomy) is similar between gametophytes and tetrasporophytes (isomorphic phases). The difference is in the ploidy and in the reproduction structures produced. The presence or absence of these structures can lead to distinct physiological performances. Therefore, it is important to include information about the female gametophyte used in the study, i.e., fertilized female gametophytes (cystocarpic plants) or/and male gametophytes. The energy cost for cystocarp production is high, resulting in a decrease in growth rates, pigment content, etc. I believe this also helps explain the differences found between the two phases in this study (Fig. S2 shows cystocarpic plants...).

Response:
This important point has been incorporated. The Discussion now explicitly considers the energetic cost of cystocarp development in fertilized female gametophytes, which may influence physiological performance and MAA allocation. This interpretation is supported by the identification of cystocarpic plants in Fig. S2.

Conclusion

Comment: Include future studies on different gametophyte expressions.

3th paragraph
- "Continued research integrating multi-site comparisons, controlled cultivation experiments, and molecular approaches will be essential to ..."

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I suggest adding studies that evaluate the production of MAAs in the different gametophyte expressions (unfertilized female, fertilized female and male), since physiologically they can express different responses.

Response:
The Conclusion has been expanded to highlight the need for future studies evaluating MAAs production in male, unfertilized female, and fertilized female gametophytes, given their distinct physiological demands.

Reviewer 3

We thank the reviewer for the insightful comments, which helped improve the transparency and robustness of the methodological and interpretative aspects of the manuscript. Our responses are detailed below.

Comment 1: Clarification regarding the MAA standards used for quantification would be beneficial, particularly concerning their storage duration and stability that could affect quantitative results, as they may have been produced a long time ago (reference to previous studies).

Response:

We agree that clarification regarding the MAA standards is important. The standards used for HPLC–DAD quantification were prepared and characterized as described in Orfanoudaki et al. (2019) and have been stored under controlled conditions (−20 °C, protected from light) to minimize degradation. Stock solutions were regularly refreshed by re-isolation, and the stability and concentration of calibrator solutions were routinely checked prior to analysis. MAAs are known to be chemically stable under these storage conditions, as reported in previous studies. This information has now been explicitly added to the Materials and Methods section to address concerns regarding storage duration, stability, and potential effects on quantitative accuracy.

Comment 2: Morphological differences are mentioned as a factor potentially influencing MAA profiles, although this hypothesis requires further investigation.

Response:
Following this comment (and a similar concern raised by Reviewer 2), all interpretations attributing differences in MAAs profiles to morphological or structural complexity have been removed from the manuscript. The Discussion now explicitly acknowledges the isomorphic morphology and anatomy of gametophytes and tetrasporophytes in both species, and differences are instead interpreted in terms of ploidy, reproductive status, physiological regulation, and environmental forcing, with appropriate caution. No conclusions are now drawn based on morphology alone.

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Comment 3: One question remains regarding this new approach: how is the level of confidence associated with the results defining the three molecular clusters be assessed? In particular, Table S5 shows annotation differences between GNPS and in-house database (e.g. compound #22 annotated as either palythinol or aplysiapalythine), which would benefit from further explanation.

Response:
We thank the reviewer for this important question regarding annotation confidence in the FBMN analysis. The confidence associated with the definition of the three molecular clusters and individual annotations is based on a multi-criteria evaluation, which is now clarified in the Results section and Supplementary Material.

Annotation confidence was assessed through the combined consideration of the following complementary criteria:

1. Accurate mass and isotope patterns derived from high-resolution MS^1 data;
2. MS/MS spectral similarity within the GNPS molecular network, including cosine score and shared fragment ions;
3. UV absorbance at 330 nm, a diagnostic feature of MAAs;
4. Comparison with in-house reference spectra, obtained from authenticated standards and previously characterized algal extracts; and
5. Compound class prediction using SIRIUS and CANOPUS.

Differences between GNPS and in-house annotations (e.g., compound #22 annotated as either palythinol or aplysiapalythine) arise primarily because these approaches rely on different types of underlying information. GNPS annotations are generated by matching experimental MS^2 spectra against community reference libraries, where parameters such as minimum cosine score, number of matched fragments, and optional analog searches influence the range of suggested structures. Analog searches, in particular, may return structurally related but non-identical candidates.

In contrast, matching against the in-house database—implemented within MZmine during data preprocessing—is based exclusively on MS^1 information, where features are screened according to accurate mass within a defined error tolerance, without incorporating fragmentation data.

For this reason, individual annotations were not assigned based on a single criterion. Instead, all proposed hits were critically evaluated and consolidated by considering additional orthogonal information, including retention time (as a proxy for compound polarity and lipophilicity), MS/MS fragmentation coherence within clusters, UV absorption behavior, and chemotaxonomic consistency provided by SIRIUS/CANOPUS. Only features supported by multiple, independent lines of evidence were retained as putative MAAs.

This integrative strategy follows current best practices in untargeted metabolomics and ensures a transparent and robust assessment of annotation confidence while acknowledging the inherent limitations of each individual approach.

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14. VERSIÓN EN ESPAÑOL

Evaluación de aminoácidos tipo micosporinas (MAAs) con función fotoprotectora frente a UV en dos macroalgas rojas de importancia económica del Perú

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Resumen

Los aminoácidos tipo micosporinas (MAAs) son metabolitos multifuncionales con capacidad de absorción de radiación UV y creciente relevancia biotecnológica. Este estudio presenta la primera caracterización integral de los perfiles de MAAs en dos macroalgas rojas peruanas de importancia comercial, *Chondracanthus chamissoi* y *Gracilariopsis lemaneiformis*, cultivadas en Pisco (Ica, Perú). Se analizaron las diferencias estacionales (primavera vs. verano) y entre etapas del ciclo de vida (gametofito vs. tetrasporofito) mediante HPLC–DAD para cuantificación dirigida y UHPLC–HRMS/MS acoplado a redes moleculares basadas en características (feature-based molecular networking, FBMN) para el descubrimiento metabolómico no dirigido. Ambas especies fueron confirmadas como altas productoras de MAAs (> 2 mg g^{-1} peso seco) y compartieron un conjunto de 13 MAAs, aunque sus abundancias relativas variaron según especie, estación y etapa del ciclo de vida. *C. chamissoi* acumuló hasta 3.39 mg g^{-1} peso seco y presentó un perfil dominado por SH (shinorina), PA (palitina) y AS (asterina-330), complementado por diez MAAs adicionales de baja abundancia detectados mediante FBMN. *G. lemaneiformis* alcanzó concentraciones totales más elevadas (hasta 3.69 mg g^{-1} peso seco) y mostró un repertorio más amplio de MAAs primarios, incluyendo porphyra-334 (PR), junto con nueve metabolitos secundarios detectados a nivel traza. El forzamiento estacional produjo máximos pronunciados de MAAs totales en verano en ambas especies; sin embargo, la magnitud de inducción fue sustancialmente mayor en *G. lemaneiformis*, consistente con una estrategia fotoprotectora inducible y dependiente del hábitat, mientras que *C. chamissoi* mostró una respuesta más constitutiva, acorde con su exposición persistente a radiación UV en el intermareal. También se evidenciaron efectos asociados a la etapa del ciclo de vida: los tetrasporofitos de ambas especies generalmente acumularon mayores concentraciones totales de MAAs y exhibieron perfiles más diversos. El análisis mediante FBMN reveló varios MAAs previamente no reportados en ambos taxones, ampliando la diversidad química conocida de las Rhodophyta peruanas. En conjunto, estos resultados demuestran una fuerte modulación ambiental y ontogenética de la biosíntesis de MAAs y destacan a ambas especies —particularmente *G. lemaneiformis*— como fuentes prometedoras y sostenibles de compuestos fotoprotectores naturales con potencial aplicación cosmética, farmacéutica y biomédica.

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Palabras clave

Chondracanthus chamissoi, *Gracilariopsis lemaneiformis*, aminoácidos tipo micosporinas, FBMN, variación estacional, etapas del ciclo de vida, fotoprotección, Rhodophyta peruana

Introducción

El aumento de los niveles de radiación ultravioleta (UVR), impulsado por el cambio climático y el agotamiento del ozono estratosférico, representa un riesgo creciente para los ecosistemas acuáticos (Neale et al. 2023). Las regiones subtropicales de América del Sur, incluido el Perú, registran de manera constante algunos de los valores más altos del Índice UV a nivel mundial, lo que incrementa la vulnerabilidad de los organismos marinos frente a la radiación dañina (Robinson y Erickson 2015).

El Perú se ubica dentro de un hotspot global de biodiversidad modelado por el sistema de surgencia de borde oriental de la Corriente de Humboldt, uno de los ecosistemas marinos más productivos del mundo (Kämpf y Chapman 2016). A pesar de esta alta productividad, la elevada UVR asociada a las bajas latitudes impone un considerable estrés fisiológico y ecológico. Los organismos marinos, a diferencia de muchos taxones terrestres, carecen de estructuras protectoras o refugios sombreados, quedando directamente expuestos tanto a la UVR como a la radiación fotosintéticamente activa (PAR). En productores primarios como macroalgas y fitoplancton, la UVR puede inhibir la fotosíntesis, degradar pigmentos y afectar el transporte electrónico en el Fotosistema II (Vass et al. 2005), generando efectos en cascada sobre la productividad, las interacciones tróficas y la estabilidad del ecosistema (Häder et al. 2007; Williamson et al. 2019).

Para mitigar estos estresores, muchos productores primarios marinos sintetizan aminoácidos tipo micosporinas (MAAs), un grupo de metabolitos secundarios pequeños (<400 Da), hidrosolubles, que actúan como protectores solares naturales al absorber y disipar la UVR en forma de calor (Carreto y Carignan 2011; Muthumani et al. 2015; Geraldes y Pinto 2021). Los MAAs se generan principalmente a través de las vías del shikimato y de la pentosa fosfato (Spence et al. 2012) y presentan núcleos de ciclohexenona o ciclohexenimina con

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sustituyentes nitrogenados, lo que permite una fuerte absorción en el rango de 268–362 nm con coeficientes de extinción molar excepcionalmente altos (Balskus y Walsh 2010).

Más allá de la fotoprotección, los MAAs poseen propiedades antioxidantes y termoprotectoras, posicionándolos como compuestos multifuncionales frente al estrés en diversos ambientes marinos (Flaim et al. 2014; Torres et al. 2018). Su versatilidad ha despertado creciente interés industrial, particularmente en los sectores cosmético, farmacéutico y biomédico (Rosic et al. 2023). Como alternativas ecológicas a los filtros UV sintéticos, varios MAAs también presentan actividades inmunomoduladoras, antiinflamatorias y cicatrizantes (Vega et al. 2021; Görünmek et al. 2024).

Las macroalgas rojas (Rhodophyta) se encuentran entre los productores naturales más ricos en MAAs, con concentraciones que pueden alcanzar hasta 20 mg g^{-1} de peso seco (DW), e incluso superar este umbral en algunas especies (Lamare et al. 2004; Sun et al. 2020). Se han identificado más de 70 MAAs en distintos taxones marinos, de los cuales al menos 22 han sido reportados en Rhodophyta, incluyendo compuestos ubicuos como shinorina (SH), porphyra-334 (PR), palitina (PA), asterina-330 (AS) y palitinol (PAO) (Sun et al. 2020; Geraldes y Pinto 2021).

No obstante, la concentración y composición de MAAs en macroalgas es altamente variable y está determinada por una compleja interacción de factores ambientales e intrínsecos (Lalegerie et al. 2020). Entre los principales factores se incluyen la intensidad de UVR, PAR, ciclos estacionales, salinidad, temperatura, pH y disponibilidad de nutrientes, particularmente nitrógeno y fosfato (Briani et al. 2018; Jofre et al. 2020; Geraldes y Pinto 2021). Rasgos intrínsecos como la identidad de la especie, profundidad del hábitat, tipo de tejido, etapa del ciclo de vida y variabilidad genética también modulan la biosíntesis de MAAs (Hoyer et al. 2001; Marshall y Newman 2002; Boedeker y Karsten 2005; Carreto y Carignan 2011).

Los avances científicos recientes han ampliado notablemente la diversidad estructural conocida de los MAAs y mejorado las capacidades analíticas para su detección, particularmente en cuanto a variabilidad estructural, conectividad molecular y estereoquímica (Orfanoudaki et al. 2019, 2020a, 2023). Estos avances no solo han

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incrementado el número de compuestos fotoprotectores naturales conocidos, sino que también han impulsado el desarrollo de métodos analíticos especializados, desde protocolos validados de CE–DAD (Hartmann et al. 2017) hasta flujos de trabajo (U)HPLC–DAD optimizados para cuantificación (Orfanoudaki et al. 2020b; Zwerger y Ganzera 2022), permitiendo el perfilado confiable tanto de MAAs abundantes como de aquellos presentes en niveles traza.

Más recientemente, los enfoques metabolómicos no dirigidos, especialmente UHPLC–HRMS/MS combinados con redes moleculares basadas en características (FBMN; Nothias et al. 2020), se han convertido en herramientas poderosas en química de productos naturales para capturar huellas metabólicas completas e identificar familias de compuestos relacionados (Hammerle et al. 2023; Pellissier et al. 2023; Quiros-Guerrero et al. 2024). Acopladas a repositorios públicos de espectros MS^2 como GNPS y a herramientas de anotación estructural como SIRIUS 4 (Dührkop et al. 2019), estas estrategias permiten una anotación rápida y de alta confianza de MAAs conocidos y novedosos. Su aplicación en ficología está en expansión, incluyendo análisis recientes de macroalgas mediante el enfoque combinado UHPLC–HRMS/MS–FBMN (Zwerger et al. 2023) e incluso tejidos de peces ricos en MAAs (Hammerle et al. 2025).

A pesar de la extensa investigación sobre MAAs en macroalgas de regiones templadas y polares, las regiones subtropicales como la costa peruana permanecen escasamente estudiadas (Hoyer et al. 2001; Briani et al. 2018; Sun et al. 2020). El Perú alberga una rica flora de Rhodophyta (Ávila-Peltroche y Padilla-Vallejos 2020); sin embargo, no existen estudios que caractericen los perfiles de MAAs de sus especies comerciales dominantes. Esta brecha es notable considerando el contexto oceanográfico único de la región: ensenadas someras y plataformas rocosas que transicionan abruptamente hacia zonas submareales profundas influenciadas por la intensa surgencia de la Corriente de Humboldt (Tarazona y Arntz 2001; Ávila-Peltroche y Padilla-Vallejos 2020). Estas condiciones generan marcados gradientes de luz, temperatura, nutrientes y turbidez en escalas espaciales reducidas (Gutiérrez et al. 2011), creando ambientes que difieren sustancialmente de las costas templadas más estudiadas.

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En consecuencia, las macroalgas peruanas están continuamente expuestas a radiación UV extrema, aguas frías ricas en nutrientes provenientes de surgencias y pronunciados cambios hidrográficos estacionales, condiciones que probablemente modelan sus estrategias biosintéticas de MAAs de manera distintiva. Estas características convierten a la región en un laboratorio natural ecológicamente relevante para el estudio de la biosíntesis de MAAs.

Actualmente, las macroalgas rojas de mayor importancia comercial en el Perú son *Chondracanthus chamissoi* ("yuyo") y *Gracilariopsis lemaneiformis* ("pelillo") (Rodríguez et al. 2018). *C. chamissoi* es recolectada para consumo humano y extracción de carragenina (Almeyda y Armas 2018; Arbaiza et al. 2019), mientras que *G. lemaneiformis* se cultiva para la producción de agar (Noriega 2016; Rodríguez et al. 2018). No obstante, la industria peruana de macroalgas continúa enfocada en la exportación de biomasa cruda —principalmente hacia China— con limitada inversión en bioproductos de valor agregado.

A pesar del crecimiento económico del sector, aún no existen estudios que caractericen los perfiles de MAAs de estas especies clave ni que examinen los factores ambientales que regulan su biosíntesis. Esta brecha de conocimiento limita el desarrollo de aplicaciones biotecnológicas sostenibles y de alto valor basadas en macroalgas peruanas.

Por lo tanto, este estudio tiene como objetivo caracterizar por primera vez los perfiles detallados de MAAs de *C. chamissoi* y *G. lemaneiformis* cultivadas en Ica, una de las regiones del Perú con mayores niveles de radiación UV (SENAMHI 2024). Mediante el análisis de la variación estacional (primavera vs. verano) y las diferencias entre etapas del ciclo de vida (gametofito vs. tetrasporofito), se busca dilucidar los factores ambientales y biológicos que modelan la producción de MAAs en estas especies. Finalmente, este trabajo establece una base para el desarrollo de compuestos fotoprotectores sostenibles de alto valor derivados de macroalgas peruanas y contribuye a una comprensión más amplia de la biosíntesis de MAAs en ecosistemas costeros subtropicales.

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Materiales y métodos

Recolección de macroalgas

Las muestras de *Chondracanthus chamissoi* (C. Agardh) Kützing y *Gracilariopsis lemaneiformis* (Bory) E.Y. Dawson, Acleto y Foldvik fueron recolectadas de un sistema de cultivo marino de fondo en la Playa La Puntilla, Paracas, Ica, Perú (13°48′25.57″S, 76°15′06.64″W). El sitio es gestionado por la Cooperativa de Trabajadores Pesqueros Artesanales Algas Marinas (COTRAPALMAR) en colaboración con el Laboratorio de Investigación en Cultivos Marinos (LICMA) (Figura suplementaria S1).

El muestreo se realizó en dos fechas: 6 de noviembre de 2023 (primavera) y 5 de marzo de 2024 (verano). Para cada especie, se recolectaron hasta 500 g de biomasa fresca de la zona intermareal (0–50 cm de profundidad), se colocaron en bolsas plásticas estériles y se transportaron en contenedores refrigerados al laboratorio de la Universidad Científica del Sur.

Para minimizar la variabilidad climática, las muestras fueron recolectadas en días soleados y dentro de una ventana horaria restringida (10:00–12:00 h), siguiendo las recomendaciones de Velasco-Charpentier et al. (2016) y Navarro et al. (2014). Se realizaron mediciones in situ de la temperatura superficial del agua de mar, oxígeno disuelto, salinidad y pH utilizando una sonda multiparamétrica YSI PLUS. Los datos oceanográficos históricos y estacionales (2023–2024) fueron obtenidos del Laboratorio Costero de Pisco, Instituto del Mar del Perú (IMARPE), como parte de su programa de monitoreo ambiental marino (https://www.imarpe.pe/imarpe/accesoinformacion/). Los parámetros relacionados con la luz, incluyendo la radiación fotosintéticamente activa (PAR) en superficie total y el índice UV, fueron obtenidos del NASA POWER Data Access Viewer (https://power.larc.nasa.gov/data-access-viewer/). Todos los parámetros fisicoquímicos se resumen en la Tabla 1.

Las especies fueron identificadas taxonómicamente con base en características morfológicas. Las identificaciones fueron verificadas por especialistas de LICMA y confirmadas formalmente en el Museo de Historia Natural de la Universidad Ricardo Palma. Las etapas

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de vida isomórficas se distinguieron con base en estructuras reproductivas, identificándose los gametofitos femeninos fecundados por la presencia de cistocarpos y los tetrasporofitos por la presencia de tetrasporangios (Figura suplementaria S2). Todos los gametofitos analizados en este estudio correspondieron exclusivamente a gametofitos femeninos fecundados (plantas cistocárpicas).

Extracción de MAAs

El material fresco fue enjuagado con agua destilada para eliminar epífitas, sal y detritos, luego fue liofilizado (-108 °C, 0.013 mbar, 24 h). Las muestras secas fueron molidas, tamizadas (< 250 µm) y almacenadas en frascos ámbar a -20 °C. Los MAAs fueron extraídos siguiendo a Karsten et al. (2009), con modificaciones. Diez miligramos de polvo de alga liofilizada (tres réplicas por combinación de tratamiento: especie, estación y etapa de vida) se mezclaron con 1 mL de metanol acuoso al 25%, se agitaron en vortex y se incubaron en un baño María a 45 °C durante 2 h en viales con tapa de rosca herméticamente sellados para permitir la extracción con solvente. Después de la incubación, las muestras se centrifugaron a 13,000 × g durante 5 min, y 800 µL del sobrenadante claro se transfirieron a nuevos viales y se evaporaron a sequedad bajo vacío. Los residuos secos se reconstituyeron en 800 µL de agua destilada, se agitaron en vortex, se centrifugaron nuevamente para eliminar partículas y finalmente se filtraron a través de filtros de membrana de 0.22 µm antes de transferirlos a viales de HPLC (Figura suplementaria S3).

Cuantificación e identificación de MAAs

El contenido de MAAs de los extractos fue determinado mediante cromatografía líquida de alta resolución con detector de arreglo de diodos (HPLC-DAD), siguiendo a Orfanoudaki et al. (2020b). Los análisis se realizaron en un instrumento Merck–Hitachi Elite La Chrom (Tokio, Japón) equipado con un organizador (SYSTEM O/B), una bomba cuaternaria (L-2100), un desgasificador (Degasys DG-2410; Uniflows Co., Ltd., Tokio, Japón), un automuestreador (L-2200), un horno de columna (L-2300) y un detector de arreglo de diodos (L-2455). Como fase estacionaria se utilizó una columna YMC-Pack ODS (250 mm × 4.60 mm, 5 µm; YMC Europe, Dinslaken, Alemania) en combinación con una precolumna adecuada. La fase móvil consistió en 20 mM de formiato de amonio y 0.25% (v/v) de ácido

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fórmico en agua (A) y metanol (B). El gradiente aplicado fue el siguiente: 100% A de 0 a 20 min, 80% A a los 30 min, 2% A a los 35 min, manteniéndose en esta composición durante 5 min (tiempo total de corrida de 40 min); seguido de 15 min de reequilibrio bajo las condiciones iniciales. El flujo, la temperatura y el volumen de inyección se ajustaron a 0.65 mL min^{-1}, 9 °C y 5.0 µL, respectivamente. Las longitudes de onda de detección se establecieron en 310, 330 y 350 nm. El área bajo la curva de los picos objetivo se determinó con el software EZChrom Elite utilizando los cromatogramas registrados a 330 nm.

Como se describió previamente (Bonin et al. 2024; Hammerle et al. 2025), la calibración se realizó utilizando soluciones patrón de shinorina (SH), palitina (PA), asterina-330 (AS) y porphyra-334 (PR), originalmente aisladas y caracterizadas en trabajos anteriores (Orfanoudaki et al. 2020b). Estos estándares, junto con compuestos de referencia adicionales incluyendo mycosporine-glycine (MG), mycosporine-glycine-alanine (MGA), aplysiapalythine A (APA), aplysiapalythine B (APB), mycosporine-methylamine-threonine (MMT), usujirene (US) y palythene (PAE), se almacenaron a −20 °C y protegidos de la luz para minimizar la degradación. Las soluciones patrón se renovaron periódicamente mediante re-aislamiento, y la estabilidad y concentración de las soluciones calibradoras se verificaron rutinariamente antes del análisis. Se sabe que los MAAs son químicamente estables bajo estas condiciones de almacenamiento, como se documenta en estudios previos (Orfanoudaki et al. 2019).

Análisis UHPLC–VWD–HR-MS^2 y Redes Moleculares Basadas en Características (FBMN)

Los análisis UHPLC–HRMS/MS se llevaron a cabo en colaboración con la Universidad de Innsbruck. Los extractos, blancos (agua ultrapura) y estándares de referencia (Orfanoudaki et al. 2020b) se analizaron siguiendo los flujos de trabajo de Zwerger et al. (2023) y Hammerle et al. (2025), con modificaciones menores.

Las separaciones cromatográficas se realizaron en un sistema Vanquish UHPLC (Thermo Scientific, EE.UU.), compuesto por una bomba binaria (VC-P10-A), un automuestreador (VC-A12-A), un horno de columna (VC-C10-A) y un detector de longitud de onda variable (VC-D40-A) conectado a una unidad HRMS Orbitrap Exploris 120 de Thermo Scientific. Se

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utilizó como fase estacionaria una columna Luna Omega C18 100 Å (100 mm × 2.1 mm; tamaño de partícula 1.6 µm; Phenomenex, Torrance, EE.UU.), protegida por una precolumna SecurityGuard ULTRA C18. La fase móvil comprendió agua con 0.25% de ácido fórmico y 20 mM de formiato de amonio (A) y acetonitrilo (B). El programa de gradiente fue: 0–10 min, 0–5% B; 10–11 min, 5–90% B; 11–13 min, 90% B; seguido de 20 min de reequilibrio bajo condiciones iniciales. El flujo, la temperatura del horno de columna, la temperatura del automuestreador y el volumen de inyección se ajustaron a 0.3 mL min^{-1}, 17 °C, 20 °C y 1 µL, respectivamente. La detección UV se realizó a 330 y 350 nm, con una tasa de adquisición de datos de 2.0 Hz, tiempo de respuesta de 2.0 s y ancho de pico de 0.2 min.

El sistema fue controlado por el software Thermo Scientific Xcalibur 4.4. La calibración del analizador de masas se realizó mediante la mezcla de calibración propietaria de Thermo Scientific y la función automática correspondiente. Los parámetros espectrométricos fueron los siguientes: fuente de ionización heated-ESI, voltaje de pulverización estático (positivo: 3500 V), gas de nebulización (N_2): 30 unidades arbitrarias, gas auxiliar (N_2): 17 unidades arbitrarias, gas de barrido (N_2): 0 unidades arbitrarias. La temperatura del tubo de transferencia iónica y del vaporizador se ajustó a 370 y 420 °C, respectivamente. Los datos MS (rango 70–1000 m/z) se registraron de 0 a 20 min con una resolución de 60,000 FWHM para MS1. El parámetro RF lens se estableció en 70%. Se realizaron experimentos dependientes de datos con modo de energía de colisión escalonada y tipo de energía de colisión normalizada usando energías HCD de 15, 30 y 45% con una resolución de 15,000 FWHM. El número de escaneos dependientes se fijó en 3. Se emplearon los siguientes filtros: umbral de intensidad (1.0E5), exclusión dinámica (automática), exclusión de isótopos (asignada), estado de carga (realizar escaneos dependientes solo en precursores con carga simple) y filtro de ápice (ventana deseada de ápice: 75%). Además, se creó una lista de exclusión específica para la medición utilizando agua grado HPLC como extracto de fondo mediante un cuaderno IODA Mass Spec (Zuo et al. 2021).

Los datos crudos se convirtieron usando MSConvert (Chambers et al. 2012) y se procesaron en MZmine 4.3.0 (Schmid et al. 2023). Las tablas de características y los espectros MS^2 se enviaron posteriormente al flujo de trabajo FBMN en GNPS (Nothias et al. 2020) para generar redes moleculares, que se visualizaron en Cytoscape (Shannon et al. 2003). La

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anotación de metabolitos se realizó utilizando SIRIUS 6.1.1 (Dührkop et al. 2015, 2019, 2021), y las predicciones de clases de compuestos mediante CANOPUS (Kim et al. 2021) se utilizaron para caracterizar adicionalmente los grupos de características. Los parámetros detallados de procesamiento y anotación se proporcionan en el Material Suplementario.

Para identificar clústeres que potencialmente contuvieran MAAs, se siguió el flujo de trabajo descrito por Zwerger et al. (2023), aplicando una estrategia de anotación de múltiples criterios. Los espectros MS^2 experimentales se compararon primero con una base de datos interna de referencia de MAAs durante el preprocesamiento de datos en MZmine, utilizando masas exactas $[M+H]^+$ dentro de una tolerancia de error de masa definida. En paralelo, los espectros MS^2 se buscaron en la biblioteca espectral comunitaria de GNPS, permitiendo coincidencias basadas en similitud espectral y búsquedas análogas. Las predicciones de fórmula molecular se refinaron adicionalmente utilizando SIRIUS, y las anotaciones de clase de compuesto se asignaron con CANOPUS para visualizar categorías quimiotaxonómicas NPC (Kim et al. 2021) dentro de la red molecular, resaltando características clasificadas como “mycosporines and mycosporine-like amino acids”.

Además, se aplicó un filtro UV330 compatible con Cytoscape a la red para identificar características MS que coeluían con picos cromatográficos que presentaban fuerte absorbancia a 330 nm, una longitud de onda diagnóstica para MAAs. Solo se retuvieron como MAAs putativos aquellas características respaldadas por múltiples líneas independientes de evidencia —incluyendo masa exacta, similitud espectral MS/MS, comportamiento de absorción UV, tiempo de retención y consistencia quimiotaxonómica—.

Análisis estadístico

Todos los análisis estadísticos se realizaron utilizando IBM SPSS Statistics v30 (IBM Corp., Armonk, NY, EE.UU.). Para cada especie (*C. chamissoi* y *G. lemaneiformis*), se evaluaron los efectos de la estación (primavera vs. verano) y la etapa de vida (gametofito vs. tetrasporofito) sobre (i) la concentración total de MAAs y (ii) las concentraciones de los MAAs principales individuales (SH, PA, AS y PR).

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Las muestras correspondieron a réplicas biológicas independientes, definidas como diferentes talos recolectados aleatoriamente del sistema de cultivo hasta obtener aproximadamente 500 g de biomasa fresca para cada combinación de tratamiento. Después de la liofilización, el material seco de cada tratamiento se utilizó para preparar el extracto de MAAs, el cual se realizó en tres réplicas independientes (n = 3).

Los datos se evaluaron para normalidad mediante la prueba de Shapiro–Wilk y para homogeneidad de varianzas mediante la prueba de Levene. Cuando se cumplieron los supuestos, se aplicó un análisis de varianza de dos vías (ANOVA) con estación y etapa de vida como factores fijos. Cuando se detectaron efectos significativos, se utilizó la prueba post hoc de diferencia honestamente significativa (HSD) de Tukey para comparaciones por pares.

Para comparaciones específicas seleccionadas, se aplicaron adicionalmente pruebas t de muestras independientes para explorar diferencias entre grupos particulares. La significancia estadística se estableció en $p < 0.05$, y se reportan valores exactos de p siempre que fue posible.

Los resultados se expresan como media ± desviación estándar (SD), donde n se refiere al número de réplicas biológicas independientes (n = 3). Dado el pequeño tamaño de muestra, los resultados estadísticos se interpretaron con cautela y se apoyaron en tendencias consistentes entre estaciones y etapas de vida. Para *G. lemaneiformis*, los tetrasporofitos estuvieron ausentes del sistema de cultivo durante la primavera; por lo tanto, no se realizaron comparaciones estacionales para esta etapa de vida.

Todos los gráficos se generaron utilizando Microsoft Excel 2021 y muestran valores medios con barras de error que representan ± SD. Letras diferentes sobre las barras indican diferencias estadísticamente significativas entre grupos según las pruebas post hoc HSD de Tukey ($p < 0.05$), mientras que las barras que comparten la misma letra no difieren significativamente.

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Resultados

Perfiles de MAAs

Los análisis cromatográficos revelaron patrones tanto compartidos como específicos de especie en la acumulación de MAAs en *C. chamissoi* y *G. lemaneiformis* (Fig. 1). El HPLC–DAD cuantificó los MAAs primarios —definidos aquí como los principales contribuyentes al contenido total de MAAs—, mientras que el UHPLC–HRMS/MS combinado con FBMN amplió la diversidad química detectable al resolver compuestos de baja abundancia, denominados aquí como MAAs secundarios. En ambas especies se anotaron putativamente 13 MAAs: *C. chamissoi* presentó tres MAAs primarios y diez secundarios, mientras que *G. lemaneiformis* presentó cuatro MAAs primarios y nueve secundarios (Fig. 2; Tabla 2).

Cuantificación por HPLC–DAD

El análisis utilizando cuatro calibradores y siete estándares de referencia mostró que *C. chamissoi* estuvo dominada por SH, PA y AS, mientras que *G. lemaneiformis* exhibió un perfil primario más amplio que también incluyó PR (Fig. 2). Las concentraciones totales de MAAs difirieron entre especies, mostrando *G. lemaneiformis* un rango más amplio de valores y alcanzando concentraciones máximas más elevadas (1.73–3.69 mg g^{-1} DW) en comparación con *C. chamissoi* (2.37–3.39 mg g^{-1} DW), lo que indica diferencias específicas de especie en la capacidad biosintética.

UHPLC–VWD–HR-MS^2 y FBMN

El análisis UHPLC–VWD–HRMS/MS de extractos algales y blancos, seguido del procesamiento de datos y la generación de redes moleculares basadas en características en el entorno GNPS, produjo una red molecular compuesta por 881 nodos y 1,367 aristas, organizados en 45 clústeres que contenían tres o más nodos. Para identificar características potencialmente correspondientes a MAAs, se aplicó el flujo de trabajo establecido por Zwerger et al. (2023), incluyendo filtrado por absorbancia UV a 330 nm y similitud espectral MS/MS. Aproximadamente el 40% de todos los nodos exhibieron características de absorción UV consistentes con MAAs. Con base en estos criterios, se identificaron tres

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clústeres y tres nodos individuales como características putativas relacionadas con MAAs (Fig. 3A).

Además de los MAAs cuantificados mediante HPLC–DAD (SH, PA, AS y PR), el análisis LC–MS/MS reveló una diversidad química más amplia, con 13 MAAs putativamente identificados detectados en ambas especies, incluyendo varios compuestos presentes solo a niveles traza (Tabla 2). La comparación con espectros de referencia internos obtenidos de múltiples estudios previos permitió la anotación putativa con alta confianza de APC, MG, APA, MGA, APB, MMT, mycosporine-lysine (ML), US y PAE (Fig. 3B). También se observaron nodos adicionales correspondientes a análogos menos comunes, como palythine-serine, palythine-threonine y shinorine methyl ester (Fig. 4).

El Clúster 1 contenía 42 nodos, ocho de los cuales fueron clasificados como MAAs a nivel de clase de compuesto mediante SIRIUS, incluyendo compuestos bien conocidos como SH, PA, AS y varias aplysiapalythinas. MAAs estructuralmente relacionados, incluyendo el par cis–trans US y PAE, así como el grupo compuesto por SH, AS y APA, formaron subregiones distintivas dentro de este clúster. El Clúster 2 comprendió 11 características, incluyendo PR y MMT, que comparten estrecha similitud estructural, siendo MMT el análogo descarboxilado de PR. El Clúster 3 estuvo compuesto por MG junto con iones de baja masa estrechamente relacionados (m/z 246 y 188), indicativos de derivados estructuralmente relacionados.

Los resultados detallados de anotación se presentan en el Material Suplementario (Tabla S1). La confianza en la anotación fue evaluada mediante una estrategia de múltiples criterios que combinó masa exacta y patrones isotópicos, similitud espectral MS/MS dentro de la red molecular GNPS, absorbancia UV diagnóstica a 330 nm, comparación con datos de referencia internos y predicciones de clase de compuesto generadas por SIRIUS/CANOPUS. Las discrepancias entre las anotaciones GNPS y las internas reflejan diferencias en las fuentes de datos subyacentes (coincidencias basadas en MS^2 versus MS^1) y se resolvieron mediante la evaluación integrada de toda la información ortogonal disponible, de acuerdo con las mejores prácticas actuales para la presentación de metabolómica no dirigida (Alseekh et al. 2021).

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Con base en este análisis, se identificaron perfiles secundarios de MAAs claramente específicos de especie. En *C. chamissoi*, el perfil secundario estuvo dominado por ML y aplysiapalythinas, particularmente APC y APB, que generalmente ocurrieron en abundancia media (10^6–10^7 mAU). En contraste, *G. lemaneiformis* exhibió un perfil secundario más amplio, caracterizado por la abundancia consistentemente alta de APA (>1.0 × 10^7 mAU), mientras que APB fue detectado a niveles medios en todas las muestras.

Variaciones estacionales en la producción de MAAs

Las concentraciones totales de MAAs difirieron significativamente entre estaciones en ambas especies, registrándose valores consistentemente más altos en verano (Fig. 2). En *C. chamissoi*, los gametofitos aumentaron de 2.37 ± 0.06 mg g^{-1} DW en primavera a 3.01 ± 0.05 mg g^{-1} DW en verano (ANOVA de dos vías, Tukey HSD, $p = 0.0009$), mientras que los tetrasporofitos aumentaron de 2.68 ± 0.06 a 3.39 ± 0.22 mg g^{-1} DW en el mismo período ($p = 0.0004$).

G. lemaneiformis mostró un incremento estacional pronunciado en el contenido total de MAAs. Las concentraciones totales en gametofitos se duplicaron con creces, pasando de 1.73 ± 0.02 mg g^{-1} DW en primavera a 3.53 ± 0.17 mg g^{-1} DW en verano (ANOVA de dos vías, Tukey HSD, $p = 4 \times 10^{-5}$). Los tetrasporofitos en verano alcanzaron 3.69 ± 0.11 mg g^{-1} DW; sin embargo, los tetrasporofitos estuvieron ausentes del sistema de cultivo durante la primavera, lo que impidió comparaciones estacionales para esta etapa de vida.

En conjunto, estos resultados demuestran que las condiciones de verano promueven fuertemente la acumulación de MAAs en ambas especies. En *C. chamissoi*, los incrementos estacionales oscilaron entre 26–27% en ambas etapas de vida, mientras que los gametofitos de *G. lemaneiformis* exhibieron un incremento relativo marcadamente mayor (104%). Estas diferencias proporcionales indican una respuesta estacional más fuerte en *G. lemaneiformis*.

También se evidenciaron cambios estacionales en la composición de MAAs. En *C. chamissoi*, SH permaneció como el compuesto primario dominante y aumentó significativamente durante el verano (ANOVA de dos vías, $p < 0.01$), mientras que PA y AS no mostraron variación estacional significativa (Fig. 2). Varios MAAs secundarios,

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incluyendo PR, US (restringido a gametofitos) y PAE, estuvieron comparativamente enriquecidos en muestras de verano, mientras que MGA fue más abundante en primavera. APC disminuyó específicamente en tetrasporofitos de verano, indicando regulación específica por etapa (Tabla 2).

En *G. lemaneiformis*, los cuatro MAAs primarios (SH, PA, AS y PR) aumentaron más del doble de primavera a verano (ANOVA de dos vías, Tukey HSD, $p < 0.00001$), convirtiéndose SH y PR en los principales constituyentes durante el verano (Fig. 2). También se observaron cambios estacionales entre los MAAs secundarios en gametofitos, donde MG, US y PAE fueron más abundantes en primavera (Tabla 2).

En conjunto, ambas especies exhibieron mayor abundancia de MAAs durante el verano, reflejando una acumulación incrementada de compuestos primarios acompañada por patrones específicos de especie en la composición de MAAs secundarios bajo condiciones de alta irradiancia.

Influencia de la etapa de vida en la biosíntesis de MAAs

La etapa de vida influyó significativamente en la acumulación de MAAs en *C. chamissoi*. Los tetrasporofitos presentaron consistentemente concentraciones totales de MAAs más altas que los gametofitos en ambas estaciones (ANOVA de dos vías, $p < 0.05$; Fig. 2), lo que indica una capacidad dependiente de la etapa para la producción de metabolitos fotoprotectores. En contraste, *G. lemaneiformis* no mostró diferencias significativas en la concentración total de MAAs entre etapas de vida durante el verano. Las comparaciones estacionales para la primavera no fueron posibles debido a la ausencia de tetrasporofitos en el sistema de cultivo durante este período.

Los efectos de la etapa de vida sobre la composición de MAAs difirieron entre especies (Fig. 2). En *C. chamissoi*, las concentraciones de PA y AS fueron consistentemente más altas en tetrasporofitos (ANOVA de dos vías, $p < 0.001$), mientras que SH no mostró variación significativa entre etapas de vida. Entre los MAAs secundarios, APC disminuyó específicamente en tetrasporofitos de verano, mientras que US fue más abundante en tetrasporofitos de primavera que en gametofitos (Tabla 2).

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En *G. lemaneiformis*, los cuatro MAAs primarios fueron detectados en ambas etapas de vida (Fig. 2), pero solo AS estuvo significativamente enriquecido en tetrasporofitos en comparación con gametofitos durante el verano (ANOVA de dos vías, $p < 0.01$). Las diferencias entre etapas de vida en los MAAs secundarios estuvieron restringidas a compuestos específicos (Tabla 2). MG, US y PAE fueron más abundantes en tetrasporofitos durante el verano, mientras que los demás MAAs secundarios mostraron abundancias comparables entre etapas de vida. En contraste, durante la primavera, MG, US y PAE presentaron mayores abundancias relativas en gametofitos.

En general, estos resultados indican que los efectos de la etapa de vida sobre la biosíntesis y composición de MAAs son específicos de especie. Dichos efectos fueron claramente detectables en *C. chamissoi*, mientras que en *G. lemaneiformis* no fueron estadísticamente significativos o estuvieron restringidos a compuestos específicos y dependieron del contexto estacional.

Discusión

El análisis integrado mediante HPLC–DAD y UHPLC–HRMS/MS acoplado a FBMN reveló diferencias dependientes de especie, estación y etapa de vida en la concentración y composición de MAAs en *C. chamissoi* y *G. lemaneiformis* cultivadas bajo condiciones idénticas. Se anotaron putativamente un total de 13 MAAs en ambas especies (Fig. 2; Tabla 2); sin embargo, *C. chamissoi* exhibió un perfil primario más restringido y un perfil secundario dominado por aplysiapalythinas y ML, mientras que *G. lemaneiformis* presentó un perfil primario más amplio que incluyó PR y alcanzó concentraciones máximas totales de MAAs más altas. La producción de MAAs estuvo fuertemente modulada por la estacionalidad, registrándose concentraciones totales significativamente mayores en verano en ambas especies. En contraste, los efectos de la etapa de vida en la acumulación total de MAAs fueron específicos de especie y evidentes únicamente en *C. chamissoi*, donde los tetrasporofitos acumularon consistentemente mayores cantidades totales de MAAs que los gametofitos.

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Diferencias específicas de especie en los perfiles de MAAs

Según Sun et al. (2020), tanto *C. chamissoi* como *G. lemaneiformis* se clasifican como productoras altas de MAAs (>2 mg g^{-1} DW), aunque difieren notablemente en sus capacidades de MAAs y diversidad química. En *C. chamissoi*, las concentraciones totales de MAAs (2.37–3.39 mg g^{-1} DW) alcanzan el rango superior reportado para poblaciones naturales chilenas de la misma especie (0.8–3.3 mg g^{-1} DW; Véliz et al. 2019). Los valores más altos observados en este estudio probablemente reflejan el cultivo a menor profundidad (~1 m) y, por tanto, mayor irradiancia que los sitios semi-protegidos a 3–4 m muestreados por Véliz et al. (2019). En *G. lemaneiformis*, las concentraciones totales de MAAs (1.73–3.69 mg g^{-1} DW) superan las documentadas para congéneres, incluyendo *G. longissima* (0.69–1.6 mg g^{-1} DW; Álvarez-Gómez et al. 2019; Chaves-Peña et al. 2019) y *G. tenuifrons* (<1.5 mg g^{-1} DW incluso bajo alta PAR; Torres et al. 2016), demostrando una fuerte capacidad intrínseca para la acumulación de MAAs.

La composición de MAAs primarios también difirió entre especies. *C. chamissoi* estuvo dominada por SH, seguida por PA y AS —un patrón típico de Rhodophyta intermareales (Sun et al. 2020) y parcialmente consistente con poblaciones chilenas de *C. chamissoi*, que presentan SH y PA (Véliz et al. 2019). El UHPLC–HRMS/MS amplió el perfil de MAAs previamente reportado para poblaciones chilenas de esta especie al revelar diez MAAs adicionales de baja abundancia (Tabla 2), subrayando el valor de los flujos de trabajo de alta resolución para resolver metabolitos coeluyentes o presentes en trazas (Hartmann et al. 2015; Zwerger y Ganzera 2022). Las diferencias observadas entre poblaciones peruanas y chilenas de la misma especie cuestionan la noción de perfiles de MAAs estrictamente fijos por especie (Boedeker y Karsten 2005) y sugieren, en cambio, que la plasticidad impulsada por el ambiente moldea huellas químicas específicas de población. Estudios comparativos a lo largo de gradientes Perú–Chile ayudarían a disentanglar las contribuciones genéticas de las ambientales.

En contraste, *G. lemaneiformis* presentó un espectro de MAAs más rico y amplio, compuesto por cuatro MAAs primarios (SH, PA, AS, PR) y nueve metabolitos secundarios, proporcionando el primer perfil detallado para esta especie. Informes previos se basaron

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únicamente en absorbancia alrededor de ~325 nm (Zheng y Gao 2009), lo que impidió la identificación a nivel de compuesto. El perfil resuelto coincide parcialmente con patrones generales de Gracilariaceae (por ejemplo, *G. longissima*: SH, PA, AS, PR, palythinol; Álvarez-Gómez et al. 2019; *G. tenuifrons*: AS, palythinol, US, PAE; Torres et al. 2016) y respalda la existencia de un núcleo biosintético conservado modulado por ajustes específicos de especie.

Los MAAs secundarios identificados mediante FBMN enfatizaron aún más las diferencias interespecíficas. *C. chamissoi* estuvo enriquecida en ML y aplysiapalythinas (notablemente APC y APB), formando un clúster clásico compuesto por ciclohexeniminas sustituidas con glicina (Clúster 1), característico de Rhodophyta (Zwerger et al. 2023). En contraste, *G. lemaneiformis* exhibió un perfil secundario más abundante, incluyendo niveles consistentemente altos de APA, junto con US, PAE y MG (Clústeres 2–3), indicativos de mayor acumulación metabólica. MAAs menos comunes como palythine-serine, palythine-threonine y shinorine methyl ester fueron identificados putativamente en ambas especies, destacando el poder del MS/MS de alta resolución combinado con FBMN para capturar metabolitos fotoprotectores raros que de otro modo permanecerían indetectados.

La consideración combinada del contexto ecológico, los rasgos morfológicos y el trasfondo filogenético proporciona un marco coherente para explicar estos contrastes interespecíficos bajo condiciones de cultivo idénticas. *G. lemaneiformis* habita típicamente ambientes turbios de fondo blando, semi-protegidos, hasta aproximadamente 6 m de profundidad, donde los campos de luz subacuáticos son espacial y espectralmente heterogéneos debido a la turbidez y la atenuación variable (Obrador y Pretus 2008; Carbajal et al. 2019). Tales condiciones son consistentes con la expresión de sistemas fotoprotectores inducibles y composicionalmente más amplios, incluyendo perfiles enriquecidos en PR. En contraste, *C. chamissoi* coloniza predominantemente sustratos rocosos expuestos al oleaje en el intermareal inferior y submareal somero (hasta ~8 m) (Carbajal et al. 2019), donde los organismos experimentan irradiancia y exposición a UV consistentemente altas. Estos ambientes se asocian con perfiles de MAAs constitutivamente elevados pero menos variables en composición, dominados por SH, reflejando una estrategia de fotoprotección sostenida bajo condiciones estables de alta luz.

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La arquitectura del talo puede contribuir adicionalmente a las diferencias interespecíficas observadas. *G. lemaneiformis* forma talos cilíndricos, carnosos y altamente ramificados (>1 m) que generan gradientes internos de luz donde absorbentes complementarios de UV-A/UV-B son ventajosos (Hoyer et al. 2001; Karsten et al. 2005). En contraste, *C. chamissoi* desarrolla láminas membranosas y aplanadas (0.3–2.5 cm de ancho, hasta 50 cm de largo) con alta relación superficie-volumen, exponiendo la mayoría de las células directamente a la radiación incidente. Esta arquitectura es consistente con la dependencia de un conjunto más limitado de MAAs, como SH y derivados relacionados (Carbajal et al. 2019). Sin embargo, los vínculos directos entre morfología del talo, campos de luz internos y asignación de MAAs siguen poco resueltos y requieren enfoques experimentales dedicados para establecerse de manera concluyente. Filogenéticamente, las Gracilariaceae (por ejemplo, *G. lemaneiformis*) tienden a exhibir mayor inducibilidad de las vías de MAAs que las Gigartinaceae (por ejemplo, *C. chamissoi*) (Sun et al. 2020). En conjunto, estos factores ecológicos, morfológicos y filogenéticos ayudan a explicar por qué *G. lemaneiformis* alcanzó tanto mayores concentraciones como un espectro más amplio de MAAs que *C. chamissoi* bajo las mismas condiciones de cultivo.

En general, ambas especies emergen como fuentes prometedoras de MAAs. Notablemente, *G. lemaneiformis* combina alto contenido total de MAAs con una composición amplia de MAAs primarios y secundarios, lo que puede resultar particularmente ventajoso para aplicaciones biotecnológicas dirigidas a diferentes ventanas espectrales.

Factores ambientales y estacionales que impulsan la producción de MAAs

En la latitud subtropical, los contrastes estacionales de la TSM son modestos ($\Delta TSM \approx 2$ °C), sin embargo, la radiación solar permanece intensa durante todo el año, con índices UV en pleno verano que superan 12 (SENAMHI, 2024). Esto crea un contexto de estrés UV crónico para macroalgas intermareales y submareales someras. El sistema de afloramiento Pisco–San Juan (Perú) opera durante todo el año, pero alcanza su máxima intensidad a finales del invierno y primavera, introduciendo aguas frías y ricas en nutrientes en la zona costera e influyendo fuertemente en la disponibilidad de nitrógeno y en la claridad del agua (Gutiérrez et al. 2011). Estos pulsos son críticos porque los MAAs son metabolitos ricos en nitrógeno y

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su biosíntesis es sensible al suministro de este nutriente. Además, episodios de agotamiento de la capa de ozono a inicios de la primavera incrementan adicionalmente la radiación UVR biológicamente efectiva (Williamson et al. 2019; Barnes et al. 2022), añadiendo un factor estacional de estrés que puede estimular respuestas fotoprotectoras.

Nuestro período de estudio fue contemporáneo con el Niño Costero 2023–2024, el cual se superpuso a un evento El Niño de escala oceánica mayor. El evento comenzó a inicios de 2023, produjo anomalías regionales de TSM y alteró el afloramiento normal a lo largo de la costa peruana. Las anomalías de TSM en el Pacífico oriental superaron +2 °C durante noviembre–diciembre de 2023 antes de disminuir en abril de 2024 (Peng et al. 2024, 2025). Aunque se observó un calentamiento significativo a escala de cuenca, las anomalías térmicas locales en Pisco permanecieron modestas (Tabla 1), condición probablemente mitigada por el afloramiento costero persistente. Esta acción amortiguadora restringió el calentamiento extremo de la superficie, pero alteró en consecuencia el suministro de nutrientes, la estructura de la columna de agua y la estabilidad hidrográfica. Tales efectos indirectos pueden influir sustancialmente en el metabolismo algal, particularmente en vías dependientes de nitrógeno como la biosíntesis de MAAs, donde la interacción entre luz y disponibilidad de nutrientes modula la acumulación.

En conjunto, el incremento estacional tanto en la concentración como en la complejidad composicional de MAAs observado en *C. chamissoi* y *G. lemaneiformis* se explica mejor por la convergencia de múltiples factores ambientales (Briani et al. 2018). En el presente estudio, estos factores pueden incluir la irradiancia máxima de verano, la recuperación parcial de la disponibilidad de nutrientes tras la limitación primaveral y anomalías ambientales episódicas, más que la irradiancia por sí sola, que suele enfatizarse en la literatura (Sun et al. 2020).

Efectos estacionales sobre la concentración de MAAs

Ambas especies alcanzaron sus niveles máximos de MAAs totales en verano (Fig. 2). A pesar de la variabilidad estacional relativamente pequeña en TSM, oxígeno disuelto, salinidad, pH, PAR e índice UV registrada en el sitio (Tabla 1), la acumulación de MAAs aumentó marcadamente, lo que indica que ningún parámetro medido individualmente explica por sí solo el patrón observado. Por lo tanto, proponemos que la limitación de nutrientes durante la

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primavera —asociada con un afloramiento reducido o irregular al inicio del Niño Costero 2023–2024— restringió la biosíntesis de MAAs en ambas especies. Para el verano, la disponibilidad de nutrientes se recuperó parcialmente mientras que los niveles de UV y PAR permanecieron altos, creando condiciones favorables para una mayor producción de MAAs. Esta interacción luz–nutriente es consistente con evidencia experimental que demuestra que la biosíntesis de MAAs no está impulsada únicamente por la luz, sino que también depende de la disponibilidad de nitrógeno, ya que precursores aminoacídicos ricos en nitrógeno se incorporan directamente en las vías biosintéticas de MAAs (Korbee et al. 2005; Álvarez-Gómez et al. 2017).

C. chamissoi exhibió un aumento estacional modesto y constante en los MAAs totales, consistente con su adaptación a condiciones crónicamente altas de UV y requerimientos fotoprotectores comparativamente estables. Este patrón complementa observaciones en Chile donde los gradientes geográficos y latitudinales predominaban sobre la estacionalidad (Véliz et al. 2019). En contraste, *G. lemaneiformis* mostró una respuesta más drástica: los gametofitos de verano duplicaron con creces su contenido de MAAs en comparación con la primavera, lo que indica una fuerte inducibilidad. Esto es consistente con la alta plasticidad documentada en especies de Gracilariaceae, donde la combinación de UVR y enriquecimiento nutricional incrementa significativamente la acumulación de MAAs (Álvarez-Gómez et al. 2017). Dicha inducibilidad probablemente refleja la ecología de la especie; al habitar ambientes turbios y variables en Perú, se beneficia de una estrategia metabólica flexible que incrementa rápidamente la fotoprotección durante periodos de alto estrés.

En conjunto, estos resultados indican estrategias estacionales contrastantes de fotoprotección. *C. chamissoi* presenta una respuesta más conservadora y constitutiva al forzamiento estacional, mientras que *G. lemaneiformis* depende de una estrategia más inducible, mostrando mayor sensibilidad a la variabilidad estacional e hidrográfica. Estas estrategias complementarias resaltan la diferenciación ecológica en la regulación de MAAs en macroalgas rojas y tienen implicancias para su resiliencia y potencial biotecnológico.

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Efectos estacionales sobre la composición de MAAs

La modulación específica por compuesto reflejó los cambios en el contenido total de MAAs. En *C. chamissoi*, SH permaneció dominante en ambas estaciones y aumentó significativamente en verano, mientras que PA y AS permanecieron comparativamente estables (Fig. 2). Este patrón sugiere una regulación preferencial al alza de SH, consistente con su fuerte absorción UV (λmax ≈ 332–334 nm), capacidad antioxidante y fotostabilidad (Karsten et al. 1998; Hartmann et al. 2015; Orfanoudaki et al. 2019; Geraldes y Pinto 2021; Rosic et al. 2023). Dentro del conjunto de MAAs secundarios, PR, US (solo en gametofitos) y PAE aumentaron en verano, mientras que MGA fue más abundante en primavera.

En *G. lemaneiformis*, los perfiles de verano se desplazaron hacia la dominancia de SH y PR, mientras que en primavera la distribución fue más equilibrada entre SH, PA, AS y PR. Esta asignación preferencial hacia absorbentes de amplio espectro (SH y PR) bajo condiciones de alta irradiancia se asemeja a patrones reportados para otras Gracilariaceae (Pliego-Cortés et al., 2019) y para *Gracilaria vermiculophylla*, en la que el momento y la magnitud de los máximos estacionales de MAAs varían entre localidades (Barceló-Villalobos et al., 2017). En contraste, varios MAAs secundarios (MG, US y PAE) fueron más abundantes en gametofitos de primavera que en verano. Debido a que la radiación UV y la PAR fueron comparables entre estaciones, es poco probable que este patrón esté impulsado únicamente por la irradiancia y más plausiblemente refleje diferencias estacionales en la disponibilidad de nutrientes vinculadas al afloramiento intensificado en primavera y a la variabilidad hidrográfica temprana asociada a El Niño. Tales condiciones pueden favorecer la síntesis de MAAs secundarios específicos, mientras que los perfiles de verano parecen priorizar MAAs primarios de amplio espectro (SH y PR).

En conjunto, estos cambios indican que la estacionalidad modula no solo los niveles totales de MAAs, sino también el balance relativo entre tipos de MAAs, ampliando así el repertorio fotoprotector durante periodos de mayor estrés ambiental.

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Influencia de la etapa del ciclo de vida en la biosíntesis de MAAs

Tanto *C. chamissoi* como *G. lemaneiformis* presentan ciclos de vida trifásicos con gametofitos y tetrasporofitos isomorfos (Kain y Destombe 1995; Ávila et al. 2011). A pesar de su similitud morfológica, las etapas del ciclo de vida pueden diferir fisiológicamente como consecuencia del nivel de ploidía, el estado reproductivo y la asignación metabólica, factores conocidos por influir en la producción de metabolitos secundarios, incluidos los MAAs (Hoyer et al. 2001; Boedeker y Karsten 2005; Korbee et al. 2005; García-Jiménez y Robaina 2015; Vieira et al. 2021).

En el presente estudio, todos los gametofitos analizados correspondieron exclusivamente a gametofitos femeninos fertilizados. Los altos costos energéticos y nutricionales asociados con el desarrollo del cistocarpo están bien documentados en algas rojas y se sabe que reducen el crecimiento y el contenido de pigmentos (Guillemin et al. 2013). Tales compensaciones reproductivas pueden restringir indirectamente la biosíntesis de MAAs o modificar la asignación entre compuestos, como se ha reportado para otros metabolitos secundarios (Kain y Destombe 1995).

En *C. chamissoi*, los tetrasporofitos acumularon concentraciones totales de MAAs significativamente más altas que los gametofitos en ambas estaciones. Esto contrasta con observaciones en poblaciones chilenas, donde se reportó que los gametofitos femeninos dominaban la acumulación de MAAs (Véliz et al. 2019), lo que indica que los patrones asociados a la etapa del ciclo de vida dependen del contexto y están modulados por condiciones ambientales como la profundidad de cultivo y la exposición a UV. Bajo irradiancia somera y consistentemente alta, los niveles más elevados de MAAs en tetrasporofitos probablemente reflejan regulación fisiológica relacionada con la ploidía y asignación metabólica específica de la etapa del ciclo de vida, mientras que los gametofitos cistocárpicos pueden priorizar la inversión reproductiva sobre la producción de metabolitos secundarios, más que diferencias morfológicas per se.

En *G. lemaneiformis*, no se detectaron diferencias estadísticamente significativas en las concentraciones totales de MAAs entre etapas del ciclo de vida durante el verano, y los tetrasporofitos estuvieron ausentes en el sistema de cultivo durante la primavera, lo que

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impidió comparaciones estacionales. En consecuencia, los efectos de la etapa del ciclo de vida sobre la acumulación de MAAs no pudieron evaluarse de manera robusta para esta especie. La ausencia de tetrasporofitos durante la primavera coincidió con condiciones atípicas asociadas al Niño Costero 2023–2024, lo que pudo haber influido en la expresión del ciclo de vida, ya que se han reportado cambios estacionales y dependientes de la temperatura en las proporciones de ploidía en Gracilariaceae (Feng et al. 2022).

En conjunto, estos resultados indican que la etapa del ciclo de vida es un factor significativo que impulsa la acumulación de MAAs en *C. chamissoi* bajo las condiciones estudiadas, mientras que efectos comparables no pudieron confirmarse para *G. lemaneiformis*, donde la variabilidad de MAAs parece estar más influenciada por el forzamiento estacional.

Efectos de la etapa del ciclo de vida sobre la composición de MAAs

Las diferencias ontogenéticas también influyeron en la composición de MAAs en ambas especies. En *C. chamissoi*, PA y AS estuvieron enriquecidos en tetrasporofitos, mientras que SH no mostró una variación clara específica de la etapa del ciclo de vida. Dada la morfología isomorfa de las etapas del ciclo de vida, este efecto se atribuye más plausiblemente a regulación fisiológica dependiente de la ploidía y asignación metabólica que a diferencias estructurales (Kain y Destombe 1995). PA absorbe principalmente en el rango UV-B (λmax ≈ 320 nm) y presenta actividad antioxidante, mientras que AS absorbe en el rango UV-A (λmax ≈ 330 nm), ampliando conjuntamente la fotoprotección espectral y complementando el papel de amplio espectro de SH (Karsten et al. 1998; de la Coba et al. 2019).

En *G. lemaneiformis*, los cuatro MAAs primarios (SH, PA, AS y PR) estuvieron presentes en ambas etapas del ciclo de vida; sin embargo, AS fue significativamente más abundante en tetrasporofitos durante el verano. Dado que las diferencias morfológicas entre etapas del ciclo de vida también son mínimas en este género, este patrón probablemente refleja regulación fisiológica asociada con la ploidía y/o el estado reproductivo más que rasgos anatómicos.

Los MAAs secundarios exhibieron variación dependiente de la etapa del ciclo de vida, aunque las diferencias estuvieron restringidas a compuestos específicos en lugar de indicar un perfil generalmente más complejo en tetrasporofitos. En conjunto, estos hallazgos

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sugieren que la modulación de la composición de MAAs según la etapa del ciclo de vida opera a través de regulación selectiva a nivel de compuesto, impulsada por restricciones fisiológicas y reproductivas, interactuando con factores estacionales y específicos de especie para moldear el metabolismo fotoprotector.

Conclusión

Este estudio proporciona la primera caracterización integral de MAAs en dos macroalgas rojas comercialmente importantes del Perú, *C. chamissoi* y *G. lemaneiformis*, integrando la cuantificación dirigida mediante HPLC–DAD con metabolómica no dirigida basada en UHPLC–HRMS/MS y FBMN. Ambas especies fueron confirmadas como altas productoras de MAAs y compartieron el mismo conjunto de 13 MAAs; sin embargo, difirieron marcadamente en sus estrategias bioquímicas y regulatorias.

La estacionalidad emergió como el principal impulsor de la acumulación y composición de MAAs en ambas especies, observándose concentraciones consistentemente más altas en verano. La etapa del ciclo de vida moduló adicionalmente la biosíntesis de MAAs en *C. chamissoi*, donde los tetrasporofitos acumularon mayores concentraciones totales de MAAs y exhibieron patrones composicionales distintos, lo que indica una regulación ontogenética específica de la especie. En contraste, no se pudieron confirmar efectos comparables de la etapa de vida en *G. lemaneiformis*, lo que sugiere que la variabilidad de MAAs en esta especie está más restringida por el forzamiento estacional y ambiental.

La aplicación de FBMN amplió sustancialmente el repertorio conocido de MAAs en ambas especies, permitiendo la detección de derivados de palythine de baja abundancia y raros que probablemente permanecerían sin resolverse utilizando únicamente enfoques dirigidos. Estos resultados subrayan el valor de integrar la metabolómica no dirigida para revelar quimiodiversidad oculta con relevancia para la fotoprotección y el descubrimiento de productos naturales.

En general, *C. chamissoi* y *G. lemaneiformis* representan fuentes prometedoras y sostenibles de compuestos naturales fotoprotectores contra la radiación UV. En particular, la fuerte

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inducibilidad estacional observada en *G. lemaneiformis* resalta su potencial para estrategias de acuicultura optimizadas orientadas a aumentar los rendimientos de MAAs y ajustar la cobertura espectral. Fortalecer el conocimiento base del Perú sobre compuestos bioactivos algales, junto con la promoción del procesamiento y valorización local de la biomasa de macroalgas en lugar de su exportación como materia prima, podría apoyar el desarrollo de una bioeconomía nacional centrada en metabolitos fotoprotectores de alto valor.

Investigaciones futuras que integren comparaciones de campo en múltiples sitios, experimentos de cultivo controlado, enfoques moleculares y análisis de la arquitectura del talo, junto con la evaluación dirigida de la producción de MAAs en diferentes etapas del ciclo de vida —particularmente entre las expresiones gametofíticas (masculina, femenina no fecundada y femenina fecundada)— serán esenciales para desentrañar los impulsores ambientales, genéticos y reproductivos de la biosíntesis de MAAs y para aprovechar plenamente el potencial biotecnológico de estas Rhodophyta clave del Perú.

Agradecimientos

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identificación de especies. Se reconoce a Akemi Watanabe por su apoyo en el procesamiento de muestras en laboratorio, incluyendo la liofilización, y a Azam Omidi por el apoyo en la realización de los análisis estadísticos.

Asimismo, reconocemos el apoyo de los siguientes grupos de investigación: CTS Group—Caracterización, Transformación y Sostenibilidad de los Recursos Naturales (Perú); LICMA—Grupo de Investigación en Acuicultura Sostenible (Perú); y el Grupo de Ecología Aplicada y Ficología, Instituto de Biociencias, Universidad de Rostock (Alemania).

Contribuciones de los autores

F.M.: Investigación, metodología, visualización, redacción—borrador original.
J.F.: Supervisión, redacción—revisión y edición.
F.H.: Metodología, visualización, redacción—borrador original, redacción—revisión y edición.
M.G.: Obtención de financiamiento, redacción—revisión y edición.
B.B.: Visualización, redacción—borrador original, redacción—revisión y edición.
O.R.: Supervisión, metodología, redacción—revisión y edición.
U.K.: Supervisión, redacción—borrador original, redacción—revisión y edición, obtención de financiamiento.

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Disponibilidad de datos

Los conjuntos de datos generados y analizados durante este estudio están disponibles a solicitud razonable al autor de correspondencia.

Conflicto de intereses

Los autores declaran que no tienen conflictos de interés.

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Leyendas de figuras

Fig. 1 Cromatogramas representativos de iones extraídos (EICs) obtenidos por UHPLC–HRMS monitoreados a 330 nm para los aminoácidos tipo micosporinas (MAAs) anotados en *Chondracanthus chamissoi* y *Gracilariopsis lemaneiformis*. Los cromatogramas corresponden a extractos obtenidos de gametofitos de primavera de cada especie. Los picos principales están etiquetados como shinorina (SH), palitina (PA), asterina-330 (AS), porphyra-334 (PR), aplysiapalythine A (APA), aplysiapalythine B (APB), usujireno (US) y paliteno (PAE). Los MAAs adicionales detectados en menor abundancia se listan en la Tabla 2.

Fig. 2 Concentración y composición de los aminoácidos tipo micosporinas (MAAs) primarios y contenido total de MAAs (mg g^{-1} PS), determinados mediante HPLC–DAD en

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gametofitos y tetrasporofitos de *Chondracanthus chamissoi* y *Gracilariopsis lemaneiformis* recolectados en primavera 2023 y verano 2024. Los valores representan medias ± desviación estándar (DE) de tres réplicas biológicas independientes (n = 3). Las barras que comparten la misma letra no son significativamente diferentes; las barras con letras diferentes o sin letra indican diferencias estadísticamente significativas (Tukey HSD, $p < 0.05$). Abreviaturas: SH, shinorina; PA, palitina; AS, asterina-330; PR, porphyra-334. Los tetrasporofitos de *G. lemaneiformis* estuvieron ausentes en primavera.

Fig. 3 A | Red molecular basada en características (feature-based molecular network) generada a partir de los extractos de *C. chamissoi* y *G. lemaneiformis*. Los clústeres que contienen MAAs están resaltados con recuadros rojos y etiquetas correspondientes. Los nodos anotados como posibles MAAs mediante SIRIUS se muestran en rojo. El tamaño del nodo refleja las características de absorción UV, donde los nodos más grandes indican características que coeluyen con un pico a 330 nm (filtro UV330: 1). El grosor del borde del nodo representa el tiempo de retención, aumentando con una elución más tardía. B | Vista detallada de los clústeres que contienen MAAs. La abundancia relativa de cada característica en los extractos algales se muestra como un gráfico circular en el nodo correspondiente. Los nodos anotados putativamente como MAAs por SIRIUS se representan como octágonos. Los resultados de anotación se muestran a la derecha, incluyendo estructuras moleculares, valores m/z observados, nombres triviales y tiempos de retención correspondientes. El tamaño del nodo nuevamente representa la variable del filtro UV, donde nodos más grandes indican absorción a 330 nm. La numeración de los compuestos anotados corresponde a las etiquetas de los nodos en la red.

Fig. 4 Espectros de fragmentación en formato mirror plot de nodos identificados putativamente como palitina (ID 665), palitina-serina (ID 624) y palitina-treonina (ID 874). Los iones fragmento coincidentes están resaltados en azul, mientras que las pérdidas neutras coincidentes se indican en naranja con líneas punteadas y flechas. La parte superior de cada gráfico muestra el puntaje de coseno modificado y el número de señales coincidentes.

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Leyendas de material suplementario

Fig. S1 Ilustración esquemática del sistema de cultivo de fondo marino en la Playa San Andrés, provincia de Pisco, región Ica, Perú. El sitio corresponde a un hábitat costero somero (0.5–1.0 m de profundidad) con sustratos rocosos y arena intercalada. El sistema consiste en líneas principales horizontales ancladas con fondeos (redes llenas de guijarros) que sostienen 20 unidades de cultivo cada una. Las unidades están construidas con rafia para asegurar flotabilidad neutra, manteniendo las algas ligeramente por encima del fondo marino. Cada unidad fue inoculada inicialmente con ~40 g de talos juveniles de algas rojas y fijada a intervalos regulares. Una boya superficial marca cada línea para su recuperación. Ilustración creada con BioRender.com.

Fig. S2 Vista fotográfica general del sitio de cultivo y diferenciación macroscópica de gametofitos femeninos fecundados y tetrasporofitos en *Chondracanthus chamissoi* y *Gracilariopsis lemaneiformis*.

Fig. S3 Detalles suplementarios del protocolo de extracción utilizado para el aislamiento de aminoácidos tipo micosporinas (MAAs) a partir de muestras de macroalgas liofilizadas.

Fig. S4 Clústeres que contienen MAAs, con nodos etiquetados de acuerdo con sus identificadores de característica correspondientes. Las áreas relativas de pico en los diferentes extractos algales se visualizan como gráficos circulares.

Tabla 1. Parámetros fisicoquímicos registrados durante primavera y verano 2023–2024 en el sitio de cultivo en Pisco, Perú, comparados con datos históricos estacionales y anuales del Laboratorio Costero de Pisco (IMARPE). PAR = radiación fotosintéticamente activa; DO = oxígeno disuelto; SSTa = anomalía de la temperatura superficial del mar. Todos los valores están expresados en unidades del SI.

Tabla 2. Abundancia relativa de MAAs secundarios identificados putativamente mediante UHPLC–VWD–HRMS/MS seguido de redes moleculares basadas en características (FBMN) en gametofitos y tetrasporofitos de *Chondracanthus chamissoi* y *Gracilariopsis lemaneiformis* (primavera 2023, verano 2024). Categorías de abundancia: baja (L) (< 1.0 ×

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10^6 mAU, verde), media (M) (1.0×10^6–1.0×10^7 mAU, amarillo) y alta (H) ($> 1.0 \times 10^7$ mAU, rojo). *Porphyra-334 se clasifica como MAA primario únicamente en G. lemaneiformis y se indica en púrpura con la letra "P". m/z = relación masa/carga; RT = tiempo de retención (min). La codificación por colores se explica dentro de la tabla para facilitar la interpretación.*

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15. ABREVIATURAS

List of Abbreviations (English)

Analytical Techniques & Instrumentation

- **HPLC–DAD** – High-Performance Liquid Chromatography with Diode Array Detection
- **UHPLC–HRMS/MS** – Ultra-High-Performance Liquid Chromatography coupled to High-Resolution Tandem Mass Spectrometry
- **UHPLC–VWD–HRMS/MS** – Ultra-High-Performance Liquid Chromatography with Variable Wavelength Detector coupled to High-Resolution Tandem Mass Spectrometry
- **CE–DAD** – Capillary Electrophoresis with Diode Array Detection
- **ESI** – Electrospray Ionization
- **HCD** – Higher-energy Collisional Dissociation
- **MS** – Mass Spectrometry
- **MS^1** – First-stage Mass Spectrometry
- **MS^2** – Tandem Mass Spectrometry
- **EIC** – Extracted Ion Chromatogram
- **FBMN** – Feature-Based Molecular Networking
- **GNPS** – Global Natural Products Social Molecular Networking
- **MZmine** – Mass Spectrometry Data Processing Software
- **SIRIUS** – Software for Molecular Formula and Structure Elucidation
- **CANOPUS** – Compound Class Prediction Tool
- **NPC** – Natural Product Classification
- **UV330 filter** – Ultraviolet absorbance filter at 330 nm

Chemical Compounds (MAAs and Related)

- **MAAs** – Mycosporine-like Amino Acids
- **SH** – Shinorine
- **PA** – Palythine
- **AS** – Asterina-330
- **PR** – Porphyra-334
- **APA** – Aplysiapalythine A
- **APB** – Aplysiapalythine B
- **APC** – Aplysiapalythine C
- **MG** – Mycosporine-glycine
- **MGA** – Mycosporine-glycine-alanine
- **MMT** – Mycosporine-methylamine-threonine
- **ML** – Mycosporine-lysine
- **US** – Usujirene
- **PAE** – Palythene
- **PAO** – Palythinol

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Environmental & Ecophysiological Parameters

- **UVR** – Ultraviolet Radiation
- **UV-A / UV-B** – Ultraviolet A / Ultraviolet B
- **PAR** – Photosynthetically Active Radiation
- **SST** – Sea Surface Temperature
- **SSTa** – Sea Surface Temperature Anomaly
- **DO** – Dissolved Oxygen
- **PSU** – Practical Salinity Units
- **ATSM** – Anomalía de Temperatura Superficial del Mar (Spanish acronym used in table)

Units & Statistical Terms

- **DW** – Dry Weight
- **mAU** – milli-Absorbance Units
- **m/z** – Mass-to-Charge Ratio
- **RT** – Retention Time
- **FWHM** – Full Width at Half Maximum
- **ANOVA** – Analysis of Variance
- **HSD** – Honestly Significant Difference
- **SD** – Standard Deviation
- **n** – Number of Biological Replicates
- **p** – Probability value

Institutions & Programs

- **IMARPE** – Instituto del Mar del Perú
- **SENAMHI** – Servicio Nacional de Meteorología e Hidrología del Perú
- **LICMA** – Laboratorio de Investigación en Cultivos Marinos
- **CTS Group** – Characterization, Transformation and Sustainability of Natural Resources Group
- **PRODUCE** – Ministry of Production of Peru
- **ABSCH** – Access and Benefit-Sharing Clearing-House

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Lista de Abreviaturas (Español)

Técnicas Analíticas e Instrumentación

- **HPLC–DAD** – Cromatografía Líquida de Alta Eficiencia con Detector de Arreglo de Diodos
- **UHPLC–HRMS/MS** – Cromatografía Líquida de Ultra Alta Eficiencia acoplada a Espectrometría de Masas de Alta Resolución en tándem
- **UHPLC–VWD–HRMS/MS** – Cromatografía Líquida de Ultra Alta Eficiencia con Detector de Longitud de Onda Variable acoplada a Espectrometría de Masas de Alta Resolución en tándem
- **CE–DAD** – Electroforesis Capilar con Detector de Arreglo de Diodos
- **ESI** – Ionización por Electrospray
- **HCD** – Disociación por Colisión de Alta Energía
- **MS / MS^1 / MS^2** – Espectrometría de Masas (simple / primera etapa / en tándem)
- **EIC** – Cromatograma de Iones Extraídos
- **FBMN** – Redes Moleculares Basadas en Características
- **GNPS** – Red Social Global de Productos Naturales
- **UV330** – Filtro de absorbancia ultravioleta a 330 nm

Compuestos Químicos (MAAs)

- **MAAs** – Aminoácidos tipo micosporina
- **SH** – Shinorina
- **PA** – Palythina
- **AS** – Asterina-330
- **PR** – Porphyra-334
- **APA** – Aplysiapalythina A
- **APB** – Aplysiapalythina B
- **APC** – Aplysiapalythina C
- **MG** – Micosporina-glicina
- **MGA** – Micosporina-glicina-alanina
- **MMT** – Micosporina-metilamina-treonina
- **ML** – Micosporina-lisina
- **US** – Usujireno
- **PAE** – Palythene
- **PAO** – Palythinol

Parámetros ambientales y ecofisiológicos

- **UVR** – Radiación ultravioleta
- **UV-A / UV-B** – Ultravioleta A / Ultravioleta B
- **PAR** – Radiación fotosintéticamente activa
- **SST** – Temperatura superficial del mar
- **SSTa** – Anomalía de la temperatura superficial del mar
- **DO** – Oxígeno disuelto

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• **PSU** – Unidades prácticas de salinidad
• **ATSM** – Anomalía de Temperatura Superficial del Mar

Unidades y términos estadísticos

• **DW** – Peso seco
• **mAU** – miliunidades de absorbancia
• **m/z** – Relación masa/carga
• **RT** – Tiempo de retención
• **FWHM** – Ancho completo a mitad del máximo
• **ANOVA** – Análisis de varianza
• **HSD** – Diferencia honestamente significativa
• **SD** – Desviación estándar
• **n** – Número de réplicas biológicas
• **p** – Valor de probabilidad

Instituciones y programas

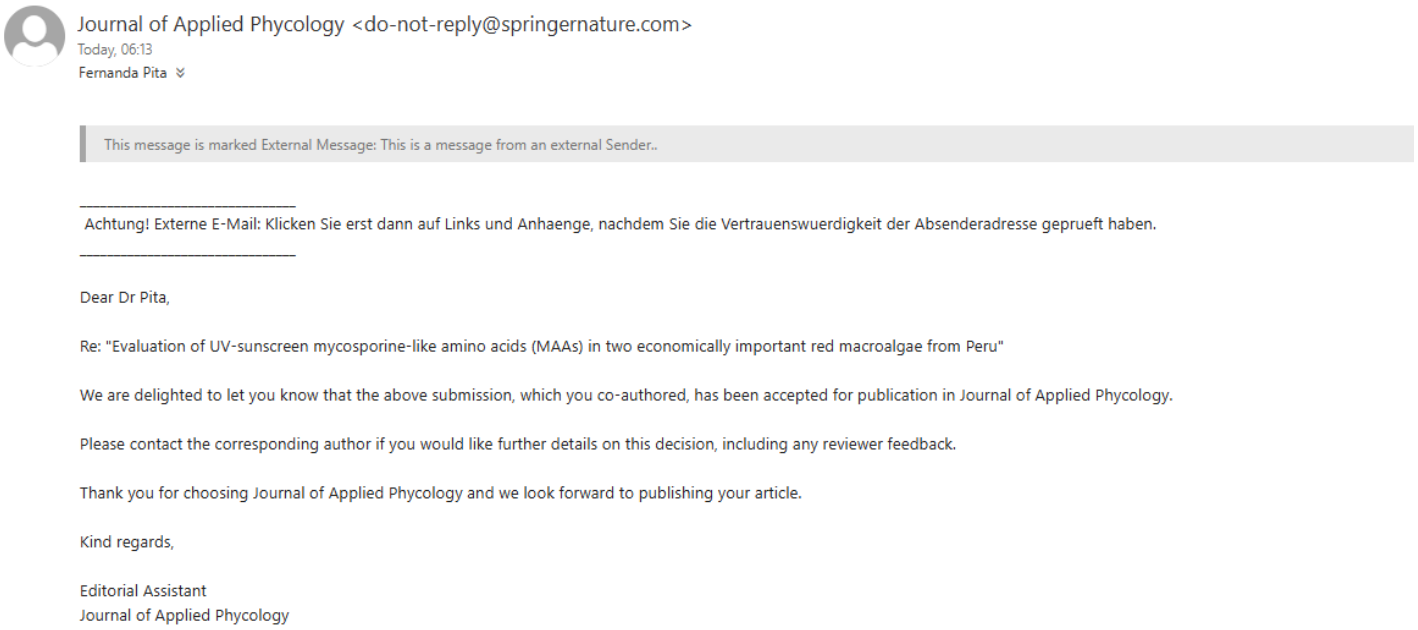
• **IMARPE** – Instituto del Mar del Perú
• **SENAMHI** – Servicio Nacional de Meteorología e Hidrología del Perú
• **LICMA** – Laboratorio de Investigación en Cultivos Marinos
• **CTS Group** – Grupo de Caracterización, Transformación y Sostenibilidad de Recursos Naturales
• **PRODUCE** – Ministerio de la Producción del Perú
• **ABSCH** – Centro de Intercambio de Información sobre Acceso y Participación en los Beneficios

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16. ANEXOS:

Anexo 1. Carta de aceptación del artículo científico en la revista *Journal of Applied Phycology* (Springer).



Journal of Applied Phycology <do-not-reply@springernature.com>
Today, 06:13
Fernanda Pita

This message is marked External Message: This is a message from an external Sender..

Achtung! Externe E-Mail: Klicken Sie erst dann auf Links und Anhaenge, nachdem Sie die Vertrauenswuerdigkeit der Absenderadresse geprueft haben.

Dear Dr Pita,

Re: "Evaluation of UV-sunscreen mycosporine-like amino acids (MAAs) in two economically important red macroalgae from Peru"

We are delighted to let you know that the above submission, which you co-authored, has been accepted for publication in Journal of Applied Phycology.

Please contact the corresponding author if you would like further details on this decision, including any reviewer feedback.

Thank you for choosing Journal of Applied Phycology and we look forward to publishing your article.

Kind regards,

Editorial Assistant
Journal of Applied Phycology

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Anexo 2. Documento de aprobación del Comité de Ética.

COMITÉ INSTITUCIONAL DE ÉTICA EN INVESTIGACIÓN CON ANIMALES Y BIODIVERSIDAD

UNIVERSIDAD CIENTÍFICA DEL SUR

CONSTANCIA N° 126-CIEI-AB-CIENTÍFICA-2023

El presidente del Comité Institucional de Ética en Investigación con Animales y Biodiversidad de la Universidad Científica del Sur (CIEI-AB-CIENTÍFICA) da constanciaque el proyecto de investigación, cuyos datos se registran a continuación, ha sido **aprobado**.

Código de registro:	**PRE-5-2023-00455**
Título:	**Evaluación de aminoácidos tipo micosporinas (maas) en *Chondracanthus chamissoi Kützing* 1843 y *Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 procedentes de la actividad acuícola (Pisco-Perú)"**
Investigador(a):	**Fernanda Etsuko Miyagi Pita**

La aprobación del proyecto de investigación implica que el documento presentado a evaluación cumple con los lineamientos de Universidad en materia de investigación y ética, los cuales se sustentan en la normativa internacional vigente.

En tal sentido, esta aprobación carecerá de valor si es que el proyecto de investigación arriba indicado es modificado de cualquier forma. Toda enmienda, añadido, eliminacióno eventualidad (eventos adversos, etc.) posterior a la fecha de aprobación debe ser reportado al CIEI-AB-CIENTÍFICA.

La vigencia de la aprobación de este documento es de dieciocho (18) meses (**hasta el 10 de abril de 2025**), periodo en el que puede desarrollarse el proyecto. Cualquier trámite para su renovación deberá ser enviado antes del término de la vigencia de estedocumento, debiendo suspenderse todo desarrollo hasta que la renovación sea aprobada.

Villa El Salvador, 11 de octubre de 2023

UNIVERSIDAD CIENTÍFICA DEL SUR - UCSUR - Comité Institucional de Ética en Investigación con Animales y Biodiversidad

Méd. Vet. Milena Paola Montenegro Veja
Presidenta (e)
Comité Institucional de Ética en
Investigacióncon Animales y Biodiversidad

Asesoría al postulante
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Anexo 3. Resolución directoral de aprobación del proyecto de tesis.

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RESOLUCIÓN DIRECTORAL ACADÉMICA DE CARRERA N°088-DACBM-DAFCVB-U. CIENTÍFICA-2023

Lima, 13 de octubre de 2023

Visto el proyecto de tesis presentado por la estudiante de la Carrera de Biología Marina, se expide la presente Resolución:

CONSIDERANDO:

- Que, para optar el título profesional en Biología Marina, de la Carrera de Biología Marina de la Facultad de Ciencias Veterinarias y Biológicas de la Universidad Científica del Sur, es necesario la realización y sustentación de una tesis.
- Que, el Reglamento de Tesis pertinente, dispone que el Proyecto de Tesis debe ser presentado a la Carrera de acuerdo con el perfil aprobado, el cual debe ser dictaminado por el Comité de Investigación.
- Que, el estudiante ha cumplido con presentar su proyecto de tesis, bajo la asesoría de su asesor.
- Que la Comisión de Grados y Títulos de la Dirección ha emitido una evaluación favorable y recomienda la inscripción del proyecto citado.

Se expide la resolución:

ESTUDIANTE:	**Fernanda Etsuko Miyagi Pita**
TÍTULO:	Evaluación de aminoácidos tipo *micosporinas (maas)* en *Chondracanthus chamissoi Kützing* 1843 *y Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 procedentes de la actividad acuícola (Pisco-Perú)
ASESOR:	Juan Carlos Francia Quiróz
N° REGISTRO:	PRE-5-2023-00455
N° CONSTANCIA ÉTICA:	126-CIEI-AB-CIENTÍFICA-2023

Estando a lo dispuesto por el Reglamento General de Grados y Títulos de la Universidad Científica del Sur, el Reglamento de Tesis de la Carrera y a las normas vigentes, en uso de las atribuciones conferidas a la Carrera,

SE RESUELVE:

1º **APROBAR** el mencionado Proyecto de Tesis para optar el título profesional en Biología Marina.

2° Declarar al estudiante expedito para la realización del Proyecto de Tesis aprobado.

3° Nombrar al docente mencionado como Asesor del Proyecto de Tesis, que realizara el estudiante citado.

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4º Disponer la inscripción del proyecto de tesis aprobado en el Registro de Tesis para optar el Título Profesional de la Carrera de Biología Marina de la CIENTÍFICA.

Regístrese, comuníquese y archívese.



MG. NATHALY MARINA VARGAS LOPEZ
DIRECTORA ACADÉMICA
CARRERA BIOLOGÍA MARINA

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RESOLUCIÓN DIRECTORAL ACADÉMICA DE CARRERA N°183-2025-DACBM-DAFCVB-U. CIENTÍFICA

Lima, 27 de noviembre de 2025

Visto:

La solicitud presentada por: **Fernanda Etsuko Miyagi Pita**, con el fin de extender la fecha de la aprobación del proyecto de tesis titulado "Evaluación de aminoácidos tipo micosporinas (maas) en *Chondracanthus chamissoi* Kützing 1843 y *Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 procedentes de la actividad acuícola (Pisco-Perú)" **previamente** fijada para el día: **13 de octubre de 2025.**

CONSIDERANDO:

Que, de acuerdo con lo dispuesto en los reglamentos de pre y posgrado de la Universidad Científica del Sur (artículos 93 y 46, respectivamente) el tesista tiene como máximo un (1) año y medio para la ejecución y sustentación de la tesis, pudiendo solicitar una prórroga a la Dirección Académica de Carrera hasta por seis (6) meses, previa justificación".

Que, recibida la justificación del tesista en donde indica los motivos que sustentan su solicitud a la extensión del plazo para la sustentación de tesis; y en el que menciona que requiere la extensión del plazo por seis (6) meses

SE RESUELVE:

Aprobar la solicitud para la extensión del plazo de la aprobación del proyecto de tesis titulada: "Evaluación de aminoácidos tipo micosporinas (maas) en *Chondracanthus chamissoi* Kützing 1843 y *Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 procedentes de la actividad acuícola (Pisco-Perú)", señalando como **fecha inicial del plazo: 13 de octubre de 2025** y como fecha final para la presentación del **informe final de tesis el día: 13 de abril 2026.**

Regístrese, comuníquese y archívese.



MG. NATHALY MARINA VARGAS LOPEZ
DIRECTORA ACADÉMICA
CARRERA BIOLOGÍA MARINA

T // (511) 610 6738
informes@cientifica.edu.pe
cientifica.edu.pe

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UNIVERSIDAD CIENTÍFICA DEL SUR

Anexo 4. Carta de invitación de la Universidad de Rostock.

Universität Rostock Traditio et Innovatio

IfBi

University of Rostock | Faculty of Natural Sciences

Institute of Biological Sciences
Applied Ecology & Phycology

Prof. Dr. Ulf Karsten

Albert-Tel.: +49 (0)381/498Einstein-Strasse 3, D--180596090; RostockFAX: -6072, Germany
e-mail: ulf.karsten@uni-rostock.de

Rostock, 2nd October 2023

Fernanda Etsuko Miyagi Pita
Marine Biology Student
Universidad Científica del Sur
Peru

Re: Invitation for a research stay at the University of Rostock, Germany

Dear Fernanda,

in my capacity as full Professor and chair of Applied Ecology & Phycology at the University of Rostock, Germany I herewith like to warmly invite you to work in my group as guest researcher. I herewith confirm that based on your application to your home university for financial support (travel money, salary) I can provide laboratory space, office space including PC as well as all consumables without any extra cost for your project on UV-sunscreen compounds in aquaculture red algae from Peru.
Your project will be fully compatible to the research in my group, since we are working on UVsunscreen compounds such as mycosporine-like amino acids (MAAs) in marine and terrestrial algae from all over the world with a focus on elucidation of new chemical structures along with functional aspects. My lab and that of my closest collaborators can provide state-of-the-art analytical facilities for your samples, and all costs are covered by running projects.

The proposed dates for your visit are for a period of at least six months in 2024.

In case of any problems please don't hesitate to contact me.

Best wishes and see you soon.

(Prof. Dr. Ulf Karsten)

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TRADUCCIÓN AL ESPAÑOL:

Universidad de Rostock
Instituto de Ciencias Biológicas
Ecología Aplicada y Ficología
Prof. Dr. Ulf Karsten
Albert-Tel: +49 (0)381/498Einstein-Strasse 3, D-18059 Rostock; Fax: -6072, Alemania
e-mail: ulf.karsten@uni-rostock.de
Rostock, 2 de octubre de 2023

Fernanda Etsuko Miyagi Pita
Estudiante de Biología Marina
Universidad Científica del Sur
Perú

Asunto: Invitación para una estancia de investigación en la Universidad de Rostock, Alemania

Estimada Fernanda:

En mi calidad de profesor titular y director de Ecología Aplicada y Ficología en la Universidad de Rostock, Alemania, me gustaría invitarte cordialmente a trabajar en mi grupo como investigadora invitada. Confirmo que, basado en tu solicitud a tu universidad de origen para apoyo financiero (dinero para viaje, salario), puedo proporcionarte espacio de laboratorio, espacio de oficina incluyendo PC, así como todos los consumibles sin ningún costo adicional para tu proyecto sobre compuestos de protección UV en algas rojas de acuicultura de Perú.

Tu proyecto será completamente compatible con la investigación en mi grupo, ya que estamos trabajando en compuestos de protección UV como los aminoácidos tipo micosporina (MAAs) en algas marinas y terrestres de todo el mundo, con un enfoque en la elucidación de nuevas estructuras químicas junto con aspectos funcionales. Mi laboratorio y el de mis colaboradores más cercanos pueden proporcionar instalaciones analíticas de vanguardia para tus muestras, y todos los costos están cubiertos por proyectos en curso.

Las fechas propuestas para tu visita son por un período de al menos seis meses en 2024.

En caso de cualquier problema, no dudes en ponerte en contacto conmigo.

Mis mejores deseos y hasta pronto.

(Prof. Dr. Ulf Karsten)

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Anexo 5. Carta de colaboración de investigación con la Universidad de Innsbruck.

Ministry of Production
Directorate of Climate Change and Fisheries & Aquaculture Biodiversity
Calle Uno Oeste 060 - Urbanización Córpac
Lima - 15036
PERU

universität innsbruck

Prof. Dr. Markus Ganzera
University of Innsbruck
6020
Tel: +43 512 507 – 58406
Email: markus.ganzera@uibk.ac.at

Commitment Letter

Innsbruck, Austria, March 28, 2025

Dear Noe Augusto Balbín Inga,

Through this letter, the University of Innsbruck commits to handling the genetic material collected during the scientific activities of the research project titled:

"Evaluation of Mycosporine-Like Amino Acids (MAAs) in *Chondracanthus chamissoi* **Kützing 1843 and** *Gracilariopsis lemaneiformis* **E.Y. Dawson, Acleto & Foldvik 1964 from Aquaculture Activities (Pisco-Peru)"**

exclusively in accordance with the procedures described in the contract with the Ministry of Production and in compliance with Peruvian legislation. This material will not be transferred to any laboratory or third party that is not explicitly stated in the contract with the Ministry of Production. Furthermore, no ownership will be claimed over the material itself or its derived products.

All publications, research, and results will include proper recognition of the Peruvian origin of the accessed genetic resources or their derivatives. Additionally, any remaining genetic material will be disposed of, or if necessary, additional analyses, such as molecular taxonomic identification, may be conducted to enhance the research findings. Should any further analyses be performed in the future, PRODUCE will be duly notified before carrying out any procedures outside the scope of the access authorization, especially if new collaborations are included to strengthen the research.

Moreover, the validity and effectiveness of this commitment letter will be subject to the authorization of access for non-commercial purposes by PRODUCE.

Sincerely,

Prof. Dr. Markus Ganzera
Research Collaborator
University of Rostock

Innsbruck, Austria, March 28, 2025

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Anexo 6. Carta del Ministerio de la Producción (PRODUCE) en relación con los permisos de exportación de muestras biológicas.

PERÚ Ministerio de la Producción | DIRECCIÓN GENERAL DE PESCA PARA CONSUMO HUMANO DIRECTO E INDIRECTO

"Decenio de la Igualdad de Oportunidades para Mujeres y Hombres"
"Año del Bicentenario, de la consolidación de nuestra Independencia, y de la conmemoración de las heroicas batallas de Junín y Ayacucho"

Lima, 18/03/2024

CARTA Nº 00000117-2024-PRODUCE/DGPCHDI

Señor(a):
FERNANDA ETSUKO MIYAGI PITA
Calle Ocharan 444 dtpa 401A, Miraflores
Lima.-
Correo: 100044757@cientifica.edu.pe / fernanda.miyagi24@hotmail.com

Asunto : AUTORIZACIÓN PARA LA EXPORTACIÓN O IMPORTACION DE ESPECIES HIDROBIOLÓGICAS VIVAS CON FINES DE INVESTIGACIÓN, RECREACION Y/O DIFUSIÓN CULTURAL, EXCEPTUANDO EXPORTACIÓN DE PECES ORNAMENTALES

Referencia : Escrito con registro N° 00092050-2023.

Me dirijo a usted con relación al documento de la referencia, mediante el cual solicitó, en el marco del ordenamiento pesquero vigente, una autorización para la exportación de especies hidrobiológicas vivas con fines de investigación, recreacion y/o difusión cultural, exceptuando exportación de peces ornamentales" con el objeto de exportar muestras de diferentes especies de macroalgas en estado liofilizado con el fin de ser empleadas con fines científicos e investigativos como parte integral de una tesis de graduación en la Universidad de Rostock, Alemania.

Al respecto, con Carta Nº 00000166-2024-PRODUCE/DGPCHDI se comunicó que no era posible atender su solicitud debido a que no eran especies hidrobiológicas vivas. Adicionalmente, en atención al requerimiento antes descrito, con fecha 4 de marzo se atendió la audiencia solicitada por la administrada precisándose en la misma que esta Dirección General otorga autorizaciones en casos de exportación o importación de especies hidrobiológicas vivas con fines de investigación, recreación y/o difusión cultural y cuando se lleve a cabo investigaciones pesqueras especializadas en los casos en los cuales se utilicen embarcaciones, extraiga recursos hidrobiológicos, usen espacios acuáticos públicos u operen plantas de procesamiento.

En ese contexto, considerando que las muestras objeto de exportación corresponden a un producto que ha pasado por un proceso de transformación (liofilizado), mediante Oficio N° 000025-2024-PRODUCE/DGPCHDI de fecha 04/03/2024 se trasladó la presente solicitud al Organismo Nacional de Sanidad Pesquera (SANIPES) a efectos que sea atendido en el marco de sus competencias. Dicha entidad, mediante Oficio N° 217-2024-SANIPES/DHC puso de conocimiento a la administrada que *"la exportación de muestras de macroalgas liofilizadas con fines de investigación no es obligatoria la certificación sanitaria; salvo requerimiento de la autoridad sanitaria del país de destino, para lo cual se recomienda realizar la consulta directamente con la Universidad de Rostock de Alemania sobre los requisitos para exportar dichas muestras para investigación"*.

Por lo que, dado que el producto a exportar con fines de investigación corresponde a muestras anteriormente colectadas y ya procesadas (liofilizadas) en un equipo liofilizador ubicado dentro de las instalaciones de la Universidad Científica del Sur (UCSUR), del cual obtuvo cantidades de aproximadamente 50 gramos por cada especie de macroalgas, se concluye que no requiere autorización por parte de este despacho, lo que se pone a su conocimiento para los fines correspondientes.

Atentamente,

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Entidad: Ministerio de la Producción
Motivo: Autor del documento
Fecha: 2024/03/18 20:11:03-0500

BALBIN INGA, NOE AUGUSTO
DIRECTOR GENERAL
DIRECCIÓN GENERAL DE PESCA PARA CONSUMO HUMANO DIRECTO E INDIRECTO

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BICENTENARIO DEL PERÚ 2021 - 2024

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Con Punche Perú

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Anexo 7. Carta de SANIPES en relación con los permisos de exportación de muestras biológicas.

PERÚ Ministerio de la Producción — SANIPES Organismo Nacional de Sanidad Pesquera

"Decenio de la Igualdad de Oportunidades para Mujeres y Hombres"
"Año del Bicentenario, de la consolidación de nuestra Independencia, y de la conmemoración de las heroicas batallas de Junín y Ayacucho"

Lima, 06 de marzo de 2024

OFICIO N° 217-2024-SANIPES/DHC

Señora
FERNANDA ETSUKO MIYAGI PITA
Calle Ocharan 444 dpto 401A, Miraflores, Lima – Perú
e-mail: 100044757@cientifica.edu.pe, fernanda.miyagi24@hotmail.com
Presente. -

Asunto : Sobre solicitud de exportación de muestras de diferentes especies de macroalgas en estado liofilizado.

Referencias : OFICIO N° 00000025-2024-PRODUCE/DGPCHDI (HT 62352024)

Tengo el agrado de dirigirme a usted para saludarla cordialmente, y en atención al documento de la referencia, mediante la cual presentó a la Dirección General de Pesca para Consumo Humano Directo e Indirecto del Ministerio de la Producción, una solicitud de autorización para la exportación de muestras de diferentes especies de macroalgas en estado liofilizado con fines científicos e investigativos como parte integral de una tesis de graduación en la Universidad de Rostock, Alemania, las cuales fueron procesadas empleando un equipo liofilizador ubicado dentro de las instalaciones de la Universidad Científica del Sur.

Al respecto, se informa que, el Organismo Nacional de Sanidad Pesquera – SANIPES es la Autoridad Sanitaria competente de normar, supervisar y fiscalizar las actividades de sanidad e inocuidad pesquera y acuícola, así como realizar la vigilancia y control de los productos de origen pesquero y acuícola, incluido los piensos aditivos y productos veterinarios destinados a la acuicultura.

Asimismo, para la exportación de recursos y productos hidrobiológicos, el SANIPES en el marco de sus funciones, a través de su órgano de línea, la Dirección de Habilitaciones y Certificaciones - DHC emite certificado sanitario con fines de exportación, según exigencia del país de destino y/o importador, no siendo obligatoria, de acuerdo a lo señalado en el artículo 3 del Decreto Supremo N° 017-2020-PRODUCE, *"que aprueba la prestación de servicios brindados en exclusividad por el Organismo Nacional de Sanidad Pesquera (SANIPES), vinculados a la exportación de recursos y productos hidrobiológicos, y piensos de uso en acuicultura"*.

Es necesario precisar que, toda mercancía a exportar y certificar sanitariamente debe provenir de un establecimiento habilitado sanitariamente por SANIPES, de acuerdo con lo señalado en el D.S. 017-2020-PRODUCE; las muestras antes citadas no cumplirían con dicho requisito.

En ese sentido, para la exportación de muestras de macroalgas con fines de investigación, no es obligatoria la certificación sanitaria; salvo requerimiento de la autoridad sanitaria del país de destino, para lo cual se recomienda realizar la consulta directamente con la Universidad de Rostock de Alemania sobre los requisitos para exportar dichas muestras para investigación.

Hago propicia la oportunidad para expresarle los sentimientos de mi especial consideración y es personal.

Atentamente,

FIRMA DIGITAL
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Motivo: Soy el autor del documento
Fecha: 07/03/2024 17:40:40-0500

(Firmado digitalmente)
ARTHUR JUAN MELÉNDEZ ALCÁZAR
Dirección de Habilitaciones y Certificaciones
Organismo Nacional de Sanidad Pesquera

V°B°
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Anexo 8. Resolución directoral del permiso de acceso a recursos genéticos.

RESOLUCIÓN DIRECTORAL

Nº 00290-2024-PRODUCE/DGAAMPA

10/12/2024

VISTOS: Los escritos con registro N° 00057088-2024-E de fecha 25 de julio de 2024, N° 00076756-2024-E de fecha 07 de octubre de 2024, y N° 00079789-2024-E de fecha 17 de octubre de 2024; el Informe Técnico-Legal N° 00000020-2024-PRODUCE/DCCBPA-magonzales de la Dirección de Cambio Climático y Biodiversidad Pesquera y Acuícola de la Dirección General de Asuntos Ambientales Pesqueros y Acuícolas; y,

CONSIDERANDO:

1. Mediante escritos con registro N° 00057088-2024-E, N° 00076756-2024-E, y N° 00079789-2024-E, la señora FERNANDA ETSUKO MIYAGI PITA identificada con DNI N° 76391804 (en adelante, la administrada), solicitó ante la Dirección de Cambio Climático y Biodiversidad Pesquera y Acuícola (DCCBPA) la Autorización de Acceso a los Recursos Genéticos y sus Derivados de las Especies Hidrobiológicas Sin Fines Comerciales, en el marco del Reglamento de Acceso a los Recursos Genéticos y sus Derivados, aprobado con Decreto Supremo N° 019-2021-MINAM, en concordancia con la Decisión 391 - Régimen Común sobre Acceso a los Recursos Genéticos; para el proyecto de investigación denominado "EVALUACIÓN DE AMINOÁCIDOS TIPO MICOSPORINAS (MAAS) EN Chondracanthus chamissoi Kützing 1843 Y Gracilariopsis lemaneiformis E.Y. Dawson, Acleto & Foldvik 1964 PROCEDENTES DE LA ACTIVIDAD ACUÍCOLA (PISCO-PERÚ)";

2. Con el Decreto Supremo N° 019-2021-MINAM se aprueba el Reglamento de Acceso a los Recursos Genéticos y sus Derivados (en adelante, el Reglamento de Acceso), el cual tiene por objeto regular las disposiciones referidas al acceso a los recursos genéticos y sus derivados establecidas en la Decisión 391 de la Comisión del Acuerdo de Cartagena de la Comunidad Andina (CAN), Régimen Común sobre Acceso a los Recursos Genéticos, en concordancia con lo establecido por el Protocolo de Nagoya sobre el Acceso a los Recursos Genéticos y Participación Justa y Equitativa en los Beneficios que se deriven de su utilización al Convenio sobre la Diversidad Biológica;

3. Conforme al literal c) del artículo 12 del Reglamento de Acceso, el Ministerio de la Producción (PRODUCE) es la Autoridad Nacional Competente para los recursos genéticos y sus derivados de las especies hidrobiológicas de aguas marinas y continentales y sus microorganismos asociados, que se encuentran en el territorio nacional, incluyendo las áreas naturales protegidas de administración nacional, áreas de conservación regional y áreas de conservación privada. Esta competencia abarca los recursos genéticos y sus derivados contenidos en todo o parte del ejemplar;

4. El numeral 27.1 del artículo 27 del Reglamento de Acceso, referido a los Requisitos de la solicitud de acceso, para las autorizaciones de acceso a los recursos genéticos y sus derivados sin fines comerciales, establece: "El procedimiento se inicia con la presentación ante la Autoridad

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Anexo 9. Certificado internacional del permiso de acceso a recursos genéticos.

UN environment
Convention on Biological Diversity

Access and Benefit-Sharing Clearing-House (ABSCH)

ABSCH-IRCC-PE-276990-1
Internationally recognized certificate of compliance constituted from information on the permit or its equivalent made available to the Access and Benefit-sharing Clearing-House

In accordance with Article 17, paragraph 2, of the Nagoya Protocol on Access and Benefit-sharing, a permit or its equivalent issued in accordance with Article 6, paragraph 3 (e) and made available to the Access and Benefit-sharing Clearing-House, shall constitute an internationally recognized certificate of compliance.

General Information

Issuing country

PERU

Verification link (view latest version)

https://absch.cbd.int/database/ABSCH-IRCC-PE-276990

ABS-CH Unique Identifier (UID)

ABSCH-IRCC-PE-276990-1

Issuing Authority

- COMPETENT NATIONAL AUTHORITY: | ABSCH-CNA-PE-254922-2

COMPETENT NATIONAL AUTHORITY:

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Anexo 10. Resolución directoral de la Beca Cabieses – Fondos para Tesistas de Pregrado.

UNIVERSIDAD CIENTÍFICA DEL SUR

RESOLUCIÓN DIRECTORAL No. 013-DGIDI-CIENTIFICA-2023-2

Villa El Salvador, 11 de octubre de 2023

CONSIDERANDO:

Que, la Universidad Científica del Sur considera que la investigación es una de sus funciones fundamentales.

Que, el Reglamento de Investigación indica en su artículo 19:

> La Universidad pone a disposición fondos concursables anuales para tesistas de posgrado, profesores e investigadores, así como bianuales, para tesistas de pregrado. Las bases de cada concurso son aprobadas y difundidas a la comunidad universitaria por la Dirección General de Investigación. (p. 9)

Que, mediante la Resolución Directoral N° 11-DGIDI-CIENTÍFICA-2023 se aprobaron las bases del "Concurso de fondos Beca Cabieses – Proyectos de Tesis de pregrado y postgrado 2023-2", el cual concede apoyo a investigaciones con fondos propios.

Que, la misma Resolución Directoral menciona que el financiamiento a cada proyecto de tesis puede ser de hasta s/ 3 000 (tres mil soles) para pregrado y de hasta s/ 6 000 (seis mil soles) para postgrado.

Que, luego de la evaluación de los proyectos participantes en el Concurso se ha establecido la lista de ganadores que fueron seleccionados en base a su mérito científico. En esta resolución se detalla la lista de ganadores de las categorías pregrado y posgrado:

Ganadores del "Concurso de Proyectos de Tesis de Posgrado 2023-2"

Autor	Título	Programa Académico
Lourdes Mercedes Figueroa Eche	Diversidad molecular y filogeografía de Lebiasina bimaculata (Characiformes: Lebiasinidae), una especie ampliamente distribuida en las cuencas transandinas de Perú (079-2023-PRO99)	Doctorado en Ciencias Biológicas
Susan Mariana Villafuerte Pumarayme	Eficacia y seguridad del monitoreo continuo de glucosa en niños y adolescentes con diabetes mellitus tipo 1. Revisión sistemática con metaanálisis (POS-50-2023-00659)	Maestría en EpidemiologíaClínica y Estadística
Jean Pierre Carrion Arcela	Valor pronóstico del índice leucoglucémico en pacientes con infarto agudo de miocardio st elevado en el desarrollo intrahospitalario de falla cardiaca y mortalidad (POS-50-2023-00583)	Maestría de Epidemiología y Bioestadística

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informes@cientifica.edu.pe
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UNIVERSIDAD CIENTÍFICA DEL SUR

Autor	Título	Facultad	Carrera
GANADORES			
Romina Gozalo Boggio / Abigail Cecilia Borja Gómez	Desarrollo de nuggets de pollo con adición de cáscara de pitahaya (Hylocereus undatus) como fuente de fibra dietética sobre la calidad nutricional y protección contra la lipoperoxidación del producto, Lima - Perú (PRE-17-2022-00576)	Ciencias de la Salud	Nutrición y Dietética
Pilar Estefani Atalaya Benites	Aplicaciones móviles relacionadas con alimentación y nutrición en el Perú: análisis de las características, evaluación de calidad e influencia en la adquisición de hábitos alimentarios saludables (1062-2021-PRE17)	Ciencias de la Salud	Nutrición y Dietética
Glendys Alarcon Talaverano / Alfredo Sebastián Neyra Najar	Evaluación del cumplimiento de los mensajes de las Guías Alimentarias para la Población Peruana: Análisis de la Encuesta Nacional de Hogares 2019, 2020 y 2021 (PRE-17-2023-00638)	Ciencias de la Salud	Nutrición y Dietética
Elizabeth Buitron Huillca	Burnout parental, perfeccionismo y autocompasión en una muestra de padres y madres de Lima Metropolitana (PRE-18-2023-00113)	Ciencias de la salud	Psicología
Leny Nicol Minaya Alberca / Vivian Santillan Maslucan	Bienestar psicológico y estrés académico en cadetes del cuarto año de una escuela militar del Distrito de Chorrillos (PRE-18-2022-00579)	Ciencias de la salud	Psicología
Angely Nicole Rodriguez Arroyo	Publicidad y discapacidad: análisis de tres campañas publicitarias sobre el síndrome de down (PRE-6-2023-00141)	Ciencias Humanas	Comunicación y Publicidad
Fernanda Etsuko Miyagi Pita	Evaluación de aminoácidos tipo micosporinas (maas) en chondracanthus chamissoi y gracilariopsis lemaneiformis procedentes de la actividad acuícola (pisco-perú) (PRE-5-2023-00455)	Ciencias Veterinarias y Biológicas	Biología Marina

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Autor	Título	Facultad	Carrera
GANADORES			
Lucía Belén Armas Gástulo	Caracterización de la infección por Aeromonas hydrophila y determinación de la DL50-96h en Colossoma macropomum (PRE-5-2022-00332)	Ciencias Veterinarias y Biológicas	Biología Marina
Milagros Linnet Loza Diburga / Manuel Alberto Feria Zevallos	Caracterización del microbioma bacteriano cultivable asociado a la piel de telmatobius brachydactylus en el lago Junín (mayo a julio del 2024) (PRE-5-2023-00400)	Ciencias Veterinarias y Biológicas	Biología Marina
Widny Sujanett Tovar Denegri	Actualización y análisis comparativo de los impulsores de cambio en humedales costeros de Lima (Perú) (PRE-5-2023-00664)	Ciencias Veterinarias y Biológicas	Biología Marina
Valeria Alessandra Talavera Arquiñigo	Evaluación del efecto de un hongo entomopatógeno (beauveria bassiana) como controlador biológico de escarabajos (alphitobius diaperinus) en una granja de ponedoras hysex brown, en Ica, Perú (040-2023-PREB16)	Ciencias Veterinarias y Biológicas	Medicina Veterinaria
Anahí Otero Balaguer	Características funcionales basales del espermatozoide de caballo peruano de paso en Lima – Perú (PRE-16-2022-00575)	Ciencias Veterinarias y Biológicas	Medicina Veterinaria

SE RESUELVE:

ARTÍCULO ÚNICO. Declarar ganadores a los proyectos de investigación seleccionados en el Concurso de fondos Beca Cabieses – Proyectos de Tesis de pregrado y posgrado 2023-2, aprobado mediante Resolución Directoral N°011-DGIDI- CIENTÍFICA-2023, cuya lista sedetalla en este documento y estipular el plazo hasta el 17 de noviembre de 2023 para presentar la constancia del comité de ética y resolución de aprobación del proyecto para cobrar el fondo.

Regístrese, comuníquese y archívese.

UNIVERSIDAD CIENTÍFICA DEL SUR - UCSUR - Dirección General de Investigación, Desarrollo e Innovación

Carlos B. P. Zavalaga Reyes, Ph.D.
DIRECCIÓN GENERAL DE INVESTIGACIÓN, DESARROLLO E INNOVACIÓN

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Anexo 10. Resolución de Facultad – Modificación de título

UNIVERSIDAD CIENTÍFICA DEL SUR

RESOLUCIÓN DE FACULTAD N°0083-FCCAA-U. CIENTÍFICA-2026

Lima, 20 de febrero de 2026

VISTA:

La solicitud presentada por: Fernanda Etsuko Miyagi Pita con el fin de modificar el título de la tesis: EVALUACIÓN DE AMINOÁCIDOS TIPO MICOSPORINAS (MAAS) EN *Chondracanthus chamissoi* Kützing 1843 Y *Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 PROCEDENTES DE LA ACTIVIDAD ACUÍCOLA (PISCO-PERÚ).

CONSIDERANDO:

Que, de acuerdo con lo dispuesto en las Normas y Procedimientos de los Trabajos de Investigación para la Obtención de Títulos Profesionales y Grados Académicos, se admite el cambio del título de la tesis dados motivos que justifican el cambio.

Que, de acuerdo con la normativa vigente de la Universidad Científica del Sur, en uso de las atribuciones conferidas al decano de la facultad.

Que, habiendo recibido la justificación del cambio de título y, luego de su evaluación por parte de la carrera profesional, esta se considera satisfactoria.

SE RESUELVE:

Aprobar el cambio del título de la tesis: "EVALUACIÓN DE AMINOÁCIDOS TIPO MICOSPORINAS (MAAS) EN *Chondracanthus chamissoi* Kützing 1843 Y *Gracilariopsis lemaneiformis* E.Y. Dawson, Acleto & Foldvik 1964 PROCEDENTES DE LA ACTIVIDAD ACUÍCOLA (PISCO-PERÚ)" por "Evaluación de aminoácidos tipo micosporina (MAAs) con función fotoprotectora frente a la radiación UV en dos macroalgas rojas de importancia económica del Perú".

Regístrese, comuníquese y archívese.

Marie Anne Gálvez Escudero
Decana de Facultad
de Ciencias Ambientales

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