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Filamentous mats proliferating at mesophotic depths off Rapa Nui: a new threat to local marine biodiversity?

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Through the use of a remotely operated vehicle (ROV) previously unexplored habitats (~40 to 350 m depth) around Rapa Nui (Easter Island) have been investigated. We have sighted vast fields of filamentous cyanobacteria-like mats, covering sandy bottoms, as well as over dead corals of mesophotic reefs (mainly composed by *Leptoseris* sp.). The highest coverage of these mats (up to ~100% of total seafloor surface), as well as major mortality of corals occur between 70 and 95 m depth off Hanga Roa, the main village of the island, located on its western side. Healthy *Leptoseris* sp. reefs were documented on the northern and southeastern sites of the island, which are also the least populated. A preliminary morphologic analysis of samples of the mats suggests that the assemblage is composed of at least four filamentous taxa, including two cyanobacteria (*Lyngbya* sp. and *Pseudoanabaena* sp.), a brown (*Ectocarpus* sp.), and a green algae (*Cladophora* sp.). An ongoing eutrophication process off the main town of the island, Hanga Roa village, is suggested as a potential driver of the proliferation of these filamentous mats.

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Abstract

Through the use of a remotely operated vehicle (ROV) previously unexplored habitats (~40 to 350 m depth) around Rapa Nui (Easter Island) have been investigated. We have sighted vast fields of filamentous cyanobacteria-like mats, covering sandy bottoms, as well as over dead corals of mesophotic reefs (mainly composed by *Leptoseris* sp.). The highest coverage of these mats (up to ~100% of total seafloor surface), as well as major mortality of corals occur between 70 and 95 m depth off Hanga Roa, the main village of the island, located on its western side. Healthy *Leptoseris* sp. reefs were documented on the northern and southeastern sites of the island, which are also the least populated. A preliminary morphologic analysis of samples of the mats suggests that the assemblage is composed of at least four filamentous taxa, including two cyanobacteria (*Lyngbya* sp. and *Pseudoanabaena* sp.), a brown (*Ectocarpus* sp.), and a green algae (*Cladophora* sp.). An ongoing eutrophication process off the main town of the island, Hanga Roa village, is suggested as a potential driver of the proliferation of these filamentous mats.

Introduction

Mesophotic coral reef ecosystems typically distribute from 30-40 m to over 150 m depth, they occur in tropical and subtropical waters and are formed mainly by light-dependent corals, but also including other typical reef organisms as sponge and macroalgae species (Baker et al., 2016). Those ecosystems are now recognized as ecologically distinct and independent from shallower counterparts, bearing a significant diversity of unique fauna, which is still unexplored in most parts of the world (Rocha et al., 2018). The insufficient knowledge is a product of the difficult access to those depths, where technical diving (e.g., rebreather system, trimix) or sophisticated submarine equipment (e.g., remotely operated vehicles, autonomous drop-cams and manned submersibles) are necessary. These ecosystems are vulnerable to a series of anthropogenic stressors, such as fishing, thermal stress, diseases, pollution, invasive species, marine aquarium trade, oil and gas exploration, cables and pipelines (Andradi-Brown et al., 2016).

Rapa Nui (Easter Island; 27° 07’ S, 109° 22’ W) is a remote island, formed ~0.8 Mya, and located at the westernmost side of the large chain of seamounts comprising the Salas y Gómez ridge, relatively close to the East Pacific Rise (Rodrigo, Díaz & González-Fernández, 2014). Located in the easternmost apex of the Polynesian triangle, it is recognized for the high overall endemism levels of its coastal marine fishes (~22%; Randal & Cea, 2010) and invertebrate taxa (4 to 34%, see Fernández et al., 2014). However, this unique marine biodiversity is severely threatened by several anthropogenic issues, such as coastal erosion, terrestrial runoff (Mieth & Bork, 2005), plastic pollution (Hidalgo-Ruz et al., 2021), exacerbated tourism (Figueroa & Rotarou, 2016), over-fishing (Zylich et al., 2014), and potential pollution by the percolation of domestic sewage and landfill into aquifers (Rosa 2013), among others issues.

Recently (2015-2018), through the use of a remotely operated vehicle (ROV) we have been able to access unexplored habitats (~40 to 350 m depth) around the island, as well as at nearby seamounts, allowing for a first approach to the biodiversity of mesophotic coral ecosystems and deeper sites (Easton et al., 2019), generating new records of fauna including fishes (e.g., Easton et al., 2017), echinoderms (Mecho et al., 2019) as well as reports of vast fields of the solitary mesophotic mushroom coral *Cycloseris vaughani* (Hoeksema, Sellanes & Easton, 2019).

In those surveys, dense and extensive fields of filamentous mats, covering the seafloor and nearby reefs at mesophotic depths, have been also sighted around the island. It is known that cyanobacteria are a usual constituent of coral reef ecosystems (Stal, 2000; Bakker et al., 2017; Ford et al., 2017), having an important role in nitrogen fixation and primary production (Charpy et al., 2007).

However, under certain conditions, they can undergo massive proliferation, affecting the health of the ecosystem and these events have been associated with variations in irradiance, nutrient supply and other natural and anthropogenic disturbances (Ford et al., 2018). These proliferation events seem to be increasing at a global scale due to alterations in local biogeochemical cycles related to climate change (Paul, 2008, Paerl & Paul, 2012). Mats could develop into such dense blooms that could even wash ashore and accumulate in mass, as reported for Guam by Nagle and Paul (1999). At this place, benthic marine cyanobacterial blooms often occur in the presence of diverse assemblages of herbivorous fishes and urchins, but the underlying factors causing these proliferations, as well as the interaction mechanisms between grazers and these mats (since cyanobacteria are known to produce feed-deterrent compounds), are still poorly understood (Nagle& Paul, 1999, Cruz-Rivera & Paul 2000). Cyanobacteria have been also directly linked with ciguatera fish poisoning (CFP) outbreaks (Laurent et al., 2008), and mats could even generate suitable habitats for other toxic microalgae, including toxin-producing dinoflagellates, generating thus co-occurring blooms (Paerl & Otten, 2013). Although several species are not toxic, their growth could produce low oxygen conditions through organic matter accumulation and subsequent degradation processes in the bottom water, affecting also the benthic communities. In some environments mats form associations with sulfate-reducing bacteria, producing sulfide which is toxic for corals and establishing the black band disease (Charpy et al., 2012; Myers & Richardson, 2009).

It has been also reported that in littoral reefs green algae (i.e., Chlorophytes) are one of the most common indicator species of eutrophication (Barile, 2004). Most of the species in this group proliferate due to increased nutrient inputs and tolerate a wide range of environmental conditions, constituting an aggressive competitive species against sensible corals, and having sub-lethal effects over several biological functions of them (e.g., Koop et al., 2001; Fabricius 2005; Birrel et al., 2008).

In this context, the aims of the present study were: i) to assess the spatial coverage of filamentous mats in the benthic ecosystem around Rapa Nui, ii) to evaluate the extension of mesophotic coral reefs potentially impacted by these mats, and iii) to preliminary describe the taxonomic composition of these mats.

Materials and methods

A remotely operated vehicle (ROV), model Commander MKII (Mariscope Meerestechnik, Kiel, Germany), operated from local fishing boats, was deployed in 56 opportunities around the island (Fig. 1), 18 times each in January 2018 and 2019, and 20 during November/December 2019. The ROV was equipped with two laser pointers, separated by 10 cm, and a front-pointing at an angle of 45° HD video camera (Panasonic SD 909), recording with a resolution of 1920 × 1080 pixels at 30 fps. As mentioned above, some results of these ROV surveys have been presented elsewhere (e.g., Easton et al., 2017; Easton et al., 2019; Hoeksema, Sellanes & Easton, 2019; Mecho et al., 2019, for selected biotic components), but for the present study, the only focus was to analyze the presence of filamentous mats and overall health condition of mesophotic corals. The videos were analyzed at half their normal speed in GOM Player 2.3.19 (GOM & Company; https://www.gomlab.com/).

The presence and coverage of filamentous mats were assessed in a semi-quantitative way by observing the seafloor stepwise as the ROV advanced over the ground, in general, 10 to 20 min of video per site. For that, transects were cataloged in four groups: i) no presence of patches of filamentous mats, ii) low coverage, less than 50 % coverage in at least non-overlapping 5 frames of the video of this transect, iii) high coverage, 50 % to 75 % of coverage in at least 5 frames of the video of this transect, and iv) very high coverage, 100 % coverage in at least 5 frames.

Using the same methodological approximation, for assessing the health status of corals three criteria were considered: i) healthy reef with > 75% of all the corals alive, ii) 25-75% of the corals alive, and iii) only dead corals or fragments. Dead corals were easily identified by being in general of greenish or darker colors, and sometimes also covered by filaments.

To characterize the taxonomic composition of the filamentous mat assemblage, in May 2019, a small benthic trawl of 30 cm of horizontal aperture was deployed at a site off Hanga Roa, where patches with 100 % coverage were frequent. Mat samples were fixed with a 4 % aqueous solution of formaldehyde (ACS Reagent, Sigma-Aldrich, Merck Darmstadt, Germany). For morphological characterization, filaments were observed using an Olympus IX71 inverted microscope equipped with phase contrast and epifluorescence (Olympus Co, Tokyo, Japan). Micrographs were taken using a camera ProgRes C3 (JENOPTIK Optical Systems GmbH, Germany) and measurements of cells (length, width) were carried out using ProgRes® CapturePro (JENOPTIK Optical Systems GmbH, Germany) analytical software. For taxonomic identification of the algae composing the mats at least to genus, monographic publications, floristic studies and systematics articles were used (Santelices, 1989; Loiseaux-de Goër & Noailles, 2008; Cormaci, Furnari & Alongi, 2014; Ramirez et al., 2018). Likewise for cyanobacteria identification taxonomic guides and systematics articles were used (Komarek & Anagnostidis, 2007; Yu et al., 2015; Brocke et al., 2018; Zubia et al., 2019).

Sample collection was performed under permissions Res. Ext N°41/2016 and N°3314/2017 from SUBPESCA (National Fishing Authority of Chile) to Universidad Católica del Norte. This project was also presented to the local "Consejo del Mar de Rapa Nui" (Council of the Sea of Rapa Nui), which allowed underwater footage and sampling around the island.

Results

ROV transects around the island covered a range of 43 to 347 m depth. This allowed us to visualize the spatial and bathymetric distribution of filamentous mats (Fig. 1, 2) and mesophotic reefs around the island (Fig. 3). Mesophotic corals were represented by reef-forming *Porites lobata* and *Pocillopora* sp. at shallower depths (<60m), by *Leptoseris* sp. and *Cycloseris* sp. at depths between 70 and 117m depth, and by sea-whips (*Stichopathes* sp.) and other scleractinians between 127 and 327 m (Fig. 2).

Spatial distribution of the filamentous mats

In general, filamentous mats were absent or with a low presence in most of the studied sites around Rapa Nui (Fig. 1A), and medium to very high abundances were usually observed shallower than ~130 m and in the western side of the island (Fig. 1B). Indeed, high coverage was observed in the northwest corner (close to Hanga O'teo) at 123 m depth and high and very high coverages were observed mainly off Hanga Roa (Fig. 1B) from 70 to 95 m depth. In this area, mats were also observed covering sediments (Fig. 2A, B) and fringing recently discovered fields of the zooxanthellate mushroom coral *C. vaughani* (Fig. 2C, Fig. 3A; see Hoeksema, Sellanes & Easton, 2019), and nearby reefs of dead *Leptoseris* sp. (~80 m depth) overgrown, apparently by similar mats (Fig. 2D, Fig. 3B). However, healthy *Leptoseris* sp. reefs were documented in other sites around the island (e.g. Anakena) between 68 and 82 m depth (Figs. 2E, 3B).

Taxonomic characterization of the filamentous mat assemblage

Morphological analyses of samples of mats collected off Hanga Roa suggest that it is an assemblage composed of at least four taxa: one Chlorophyta (*Cladophora* sp.), one Ochrophyta (*Ectocarpu*s sp.) and two Cyanobacteria (*Lyngbia* sp. and *Pseudoanabaena* sp.) (Fig. 4) as follows:

Cladophora sp. (Fig. 4A, B): Thallus of green to light green branched uniseriate filaments with 2-3 cm in total length. Basal part of the filaments fixed in the substrate by a primary rhizoid. Presence of unilateral branches inserted laterally or obliquely the filament. Principal axis constituted by cylindrical cells with measures of 998.9 ± 69.2 µm in length and 223.3 ± 9.5 µm in diameter. The apical cells were cylindrical, round ended with a diameter of 250.0 ± 7.6 µm and length of 701.8± 76.0 µm. Zoosporangia were not observed.

Ectocarpus sp. (Fig. 4C, D): Thallus of light brown to olive sparingly branched filament with 0.1 – 0.5 cm in total length. The cells conform uniseriate filaments that ended in a rounded apical cell. Cells were barrel-shaped, 50.0 ± 7.3 µm in length and 14.1 ± 3.4 µm in diameter. Plurilocular sporangia were present, elongated with cylindroconical form with 80-130 µm in length and 20-30 in diameter.

Lyngbya sp. (Fig. 4E, F): Thallus caespitose, browning-red, filaments slightly curved, sheet colorless, lamellated with apices not attenuated at the end. Trichome not constricted at the cross-wall, cylindrical cells very short 3.5 ± 0.3 µm in length and 7.1 ± 0.1 µm in diameter, sheath 1.6 ± 0.3 µm, end cells rotund, calyptra absent.

Pseudoanabaena sp. (Fig. 4G, H): Trichomes solitary or crowded in clusters, straight or almost straight, pale blue-green, cells. Barrel-shaped, 2.8 ± 0.8 µm in length and 1.2 ± 0.1 µm in diameter, intensely constricted at cross walls, not heterocysts or sheath, end cells round.

Discussion

Although we only have a very preliminary taxonomic characterization of the filamentous mats covering sandy areas and dead-mesophotic reefs off Rapa Nui, based on morphology, it is suggested that they are compossed of at least to cyanobacteria. It is known that cyanobacteria-dominated microbial mats are typical inhabitants of coral reef systems and often undergo massive proliferation (Stal, 2000). These events have been associated with natural processes (e.g., variations in irradiance), but mostly with anthropogenic disturbances that generate an increase in the nutrient supply to the marine environment (Ford et al., 2017). Highest coverage of mats was observed mainly off Hanga Roa village, which concentrates the largest part of the islands' population (7,750 habitants; www.ine.cl) and where most tourists develop their recreational activities (~150,000 visitants during 2019; www.sernatur.cl). In this sense, the over-tourism, the absence of wastewater treatment (predominant use of cesspools) and the unlined landfill (Rosa, 2013) could be a great threat, due to the potential input of organic matter, nutrients and contaminants to the adjacent marine environment. Pollutants could reach the sea by runoff or through percolation to aquifers that eventually discharge into the sea. At Rapa Nui, submarine groundwater discharges are ubiquitous in intertidal environments around the island (Brosnan, Becker & Lipo, 2018), and hypothetically could also seep through deeper sediments (Montgomery & Associates, 2011), potentially conducting nutrients of anthropogenic origin directly to mesophotic habitats. Additionally, the permanent coastal erosion around the island and terrestrial runoff during rainy seasons could also increase the nutrient concentrations in the coastal environments (e.g., ammonia, nitrate and silicate) and potentially reaching mesophotic depths (P. Muñoz, 2019, unpublished data). However, the volcanic origin of Rapa Nui and their elevated erosion could also increase the iron concentration in the marine ecosystem, which added to a decrease in the N:P ratio (Ford et al., 2018), could stimulate the proliferation of benthic cyanobacteria in other places around the island.

The damage of mesophotic corals as *Leptoseris* sp. is extensive and evident off Hanga Roa village (also covering the edges of a *C. vaughani* field), in contrast to other sites where healthy corals were documented (e.g. Anakena, La Perouse and Vinapú). Unfortunately, although no previous detailed information about the health condition and community structure of these degraded mesophotic reefs exists, a previous record, obtained in November 1999 during the first ROV survey at the island (Gorny & Retamal, 2000), suggests a healthy status of the *Leptoseris* reef off Hanga Roa (Fig. 2D) ~20 years ago. At that opportunity, the tourist number reached ~20.000 visitants per year (Figueroa & Rotarou, 2016). Despite this circumstantial indication of the status of the mesophotic reefs at Rapa Nui a few decades ago, the ecological impacts on the biodiversity and ecosystem functions associated with anthropogenic causes are still unknown and need to be further addressed in the short-term.

Conclusions

We report for the first time filamentous mats covering sandy areas and dead-mesophotic reefs (*Leptoseris* sp.) off Rapa Nui. A preliminary morphological analysis suggests the presence of at least four filamentous taxa, including two cyanobacteria (*Lyngbya* sp. and *Pseudoanabaena* sp.), a brown (*Ectocarpus* sp.), and a green algae (*Cladophora* sp.). Catastrophic damage was observed at mesophotic *Leptoseris* sp. reefs off the main village of the island. Further research should address a more detailed taxonomic characterization of these mats, e.g., through molecular techniques, the assessment of the seasonal, spatial and structural patterns of the assemblage, their eventual role in reef deterioration, recognition of eutrophication mechanisms, and long-term monitoring of nutrient dynamics. These studies are encouraged to implement effective and integrated sea-land management actions, including the implementation of waste-water treatment This information should be key to inform the implementation of management strategies (land-sea integrate) of the recently created Marine Protected Area of multiple uses (MPA-MU) of Rapa Nui, currently the largest in Latin America with ~579,000 km^2 (Paredes et al., 2019) and aiming to protect this unique world biodiversity heritage place. Finally, this study will serve also as a baseline for future studies of changes over the mesophotic ecosystem off Rapa Nui after the island has been closed for tourism, from March 2019 to date, due to the COVID 19 pandemic.

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Figure 1

Study area showing different ROV dive sites around Rapa Nui and coverage of filamentous mats at them

A) Dive sites and levels of coverage of filamentous mats on the benthic ecosystems. B) Depth range of ROV transect for the different categories of filamentous mats coverage used in the study. Green: no mats observed, Yellow: low coverage, Orange: high coverage, and Red: very high coverage. Box plots indicate median and 1 Standard Deviation, whiskers indicate depth range for this category.

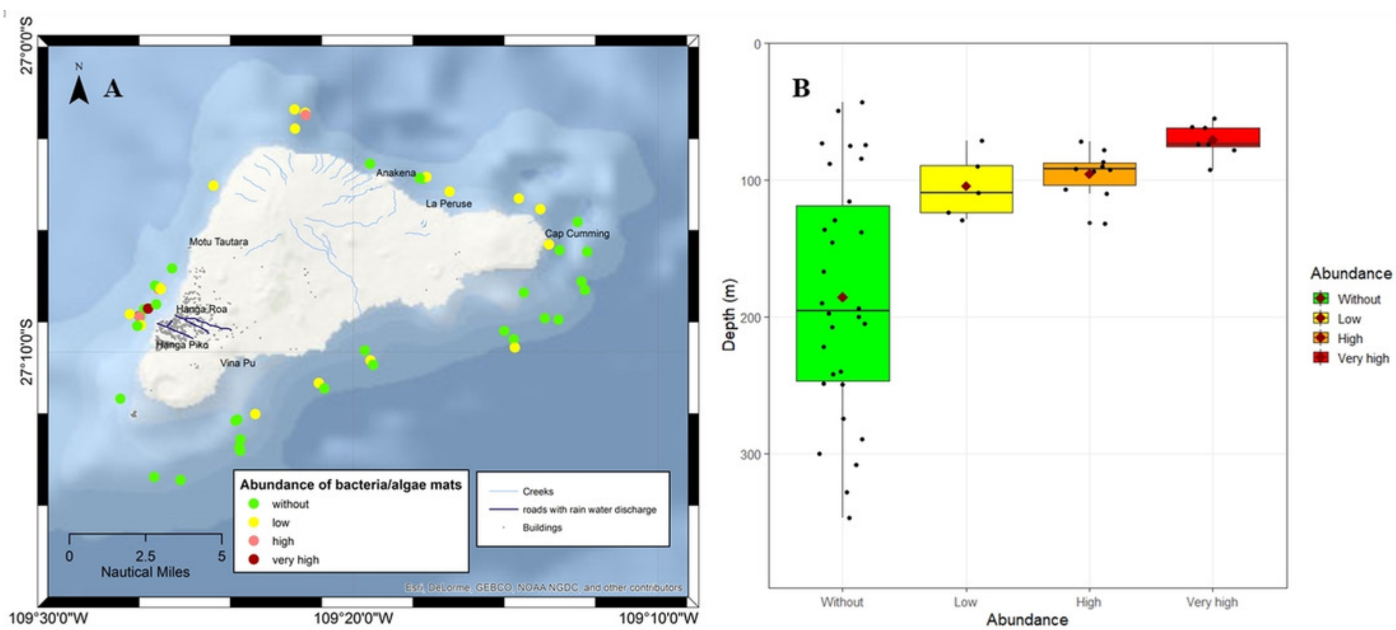


Figure 2

ROV images of the filamentous mats and mesophotic reefs off Rapa Nui. A) filamentous mats field ~80 m depth off Hanga Roa, Rapa Nui.

B) Close up of the filaments. C) Filaments among *C. vaughani* individuals. D) Dead *Leptoseris* sp. reef ca. 80 m depth overgrown by filaments. E) Healthy *Leptoseris* reef off Anakena ~ 80 m depth. F) Healthy *Leptoseris* reef off Hanga Roa filmed during prospective ROV surveys in “CIMAR-5 Islas” cruise made in 1999. Scale bars: 10 cm (A, B, C), 25 cm (D, E, F). Images: Matthias Gorny, OCEANA.

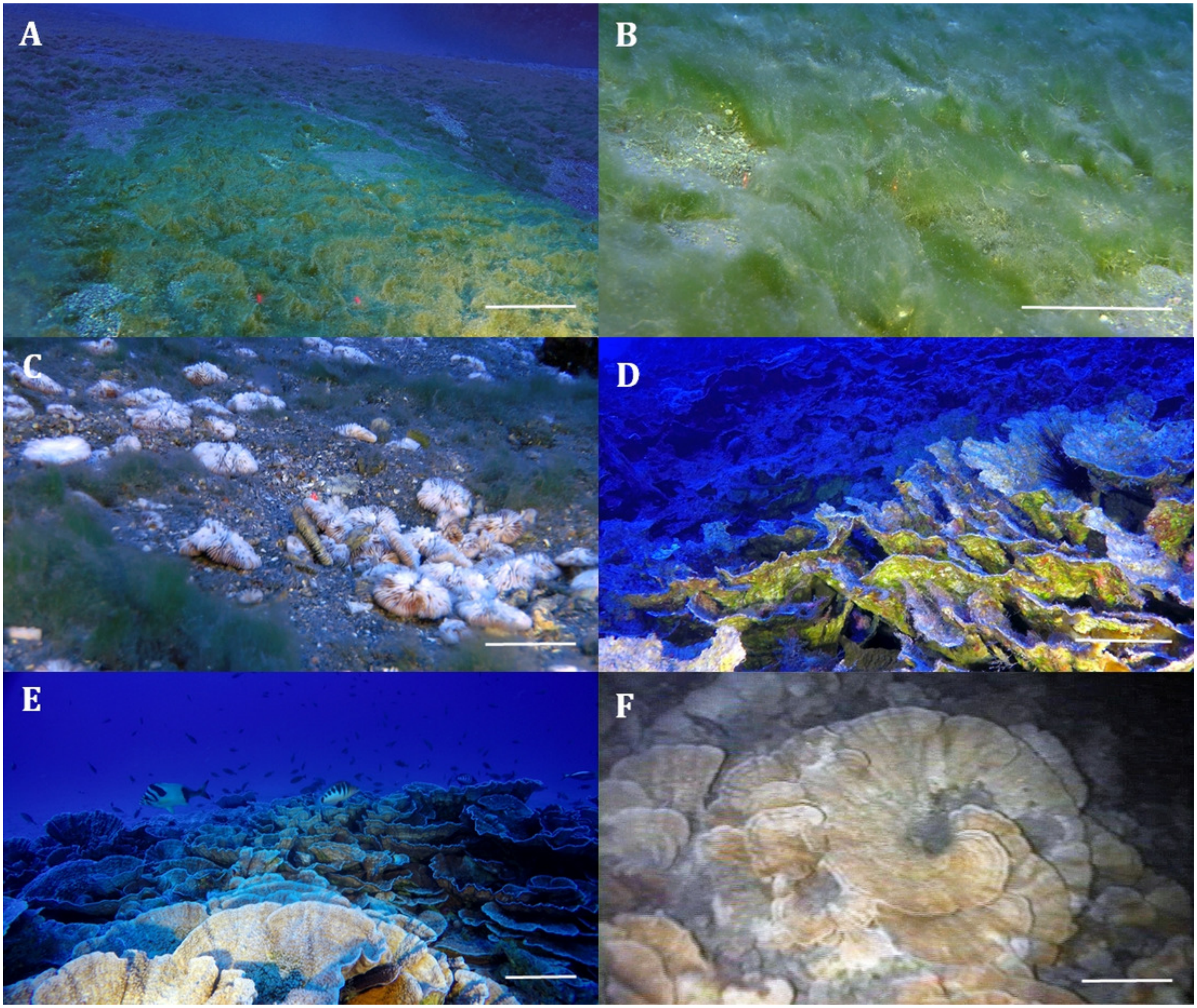
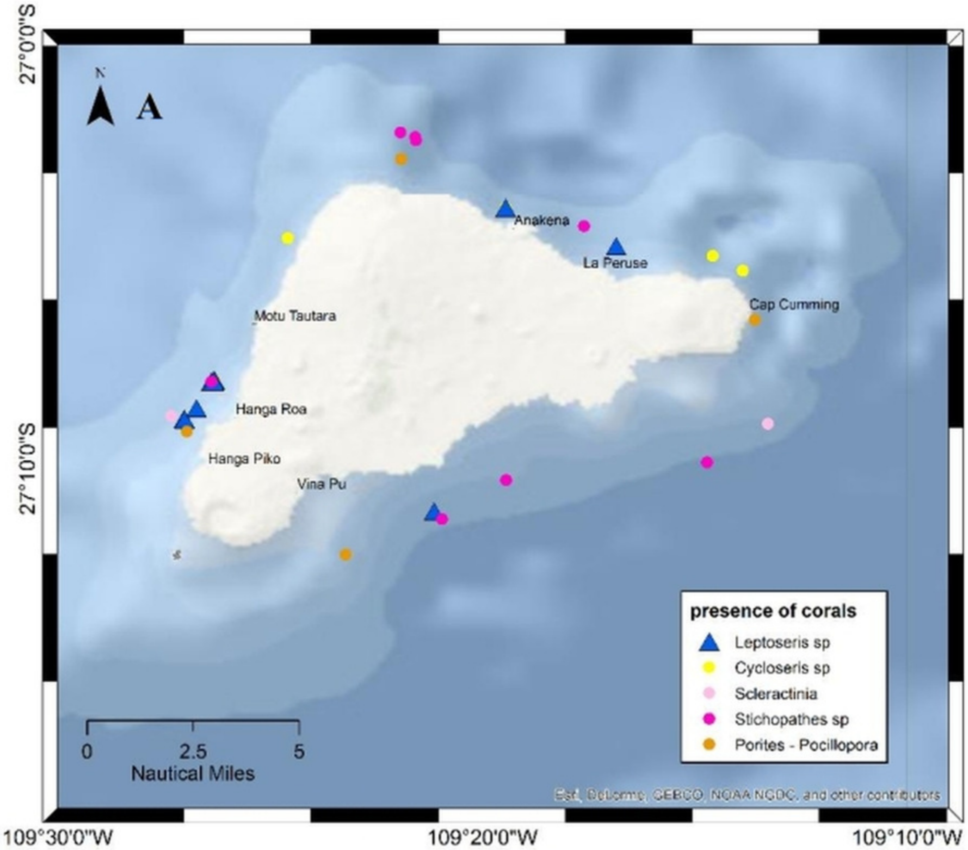

A
B
C
D
E
F

Figure 3

Location of mesophotic reefs off Rapa Nui in this study.

A) Map showing the position of reefs and main taxa constituting them recorded with ROV between 40-350 m. B) Map showing the different health statuses of *Leptoseris* sp. reefs. Green: Healthy (no noticeable impact), Yellow: Some damage (25-75% of corals damaged), and Red: Destroyed (only dead corals or fragments).


27°0'0"S
A
Anakena
La Peruse
Cap Cumming
Motu Tautara
Hanga Roa
Hanga Piko
Vina Pu
27°10'0"S
presence of corals
Leptoseris sp
Cycloseris sp
Scleractinia
Stichopathes sp
Porites - Pocillopora
0 2.5 5
Nautical Miles
109°30'0"W
109°20'0"W
109°10'0"W

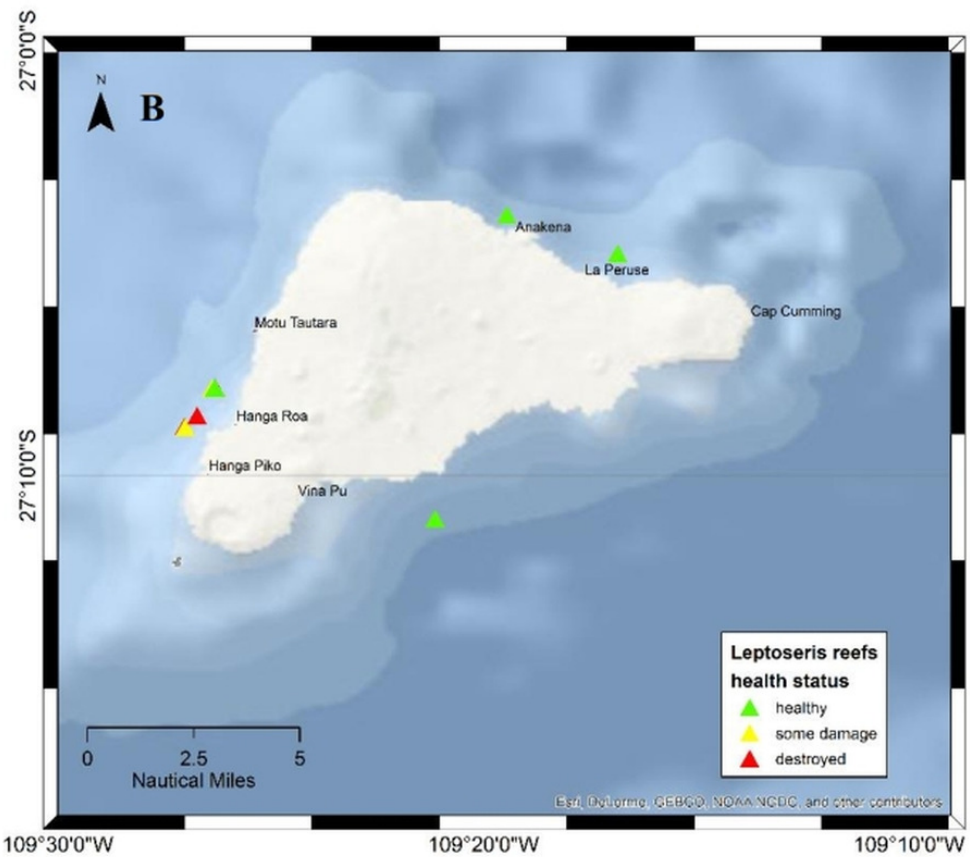
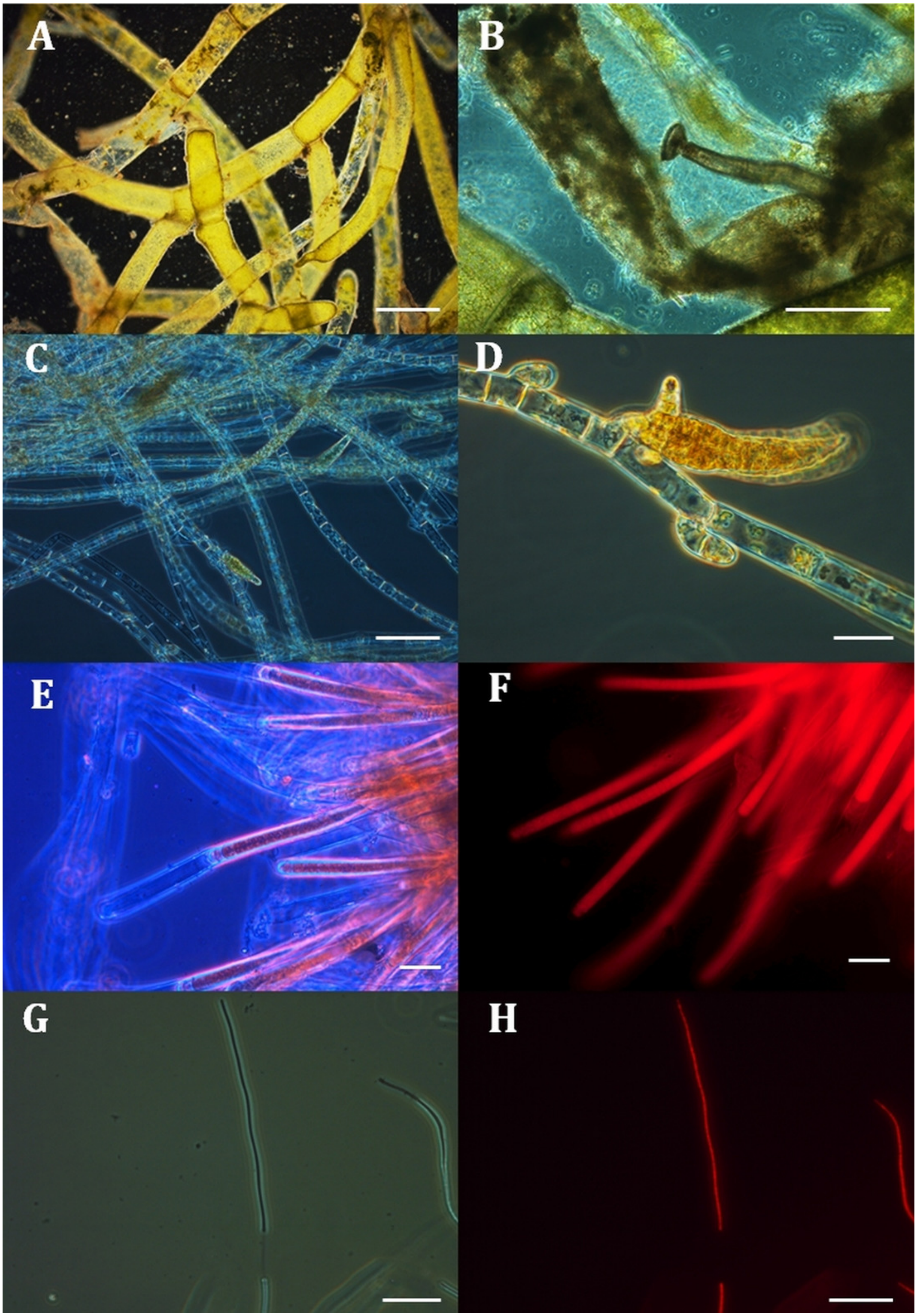

27°0'0"S
B
Anakena
La Peruse
Cap Cumming
Motu Tautara
Hanga Roa
Hanga Piko
Vina Pu
27°10'0"S
Leptoseris reefs
health status
healthy
some damage
destroyed
0 2.5 5
Nautical Miles
109°30'0"W
109°20'0"W
109°10'0"W

Figure 4

Micrographs of the four filamentous taxa collected off Hanga Roa, Rapa Nui at mesophotic depths using phase-contrast (A-E, and G) and epifluorescence (F and H) techniques.

(A, B) *Cladophora* sp. (C, D) *Ectocarpus* sp. (E, F) *Lyngbya* sp. (G, H) *Pseudoanabaena* sp.

Scale bars = A: 500 µm, B: 200 µm, C: 100 µm, D: 30 µm, E and F: 20 µm, G and H: 30 µm.


A
B
C
D
E
F
G
H