ChonCon, The First *Chondria* Conference

Alien invasive species (AIS) pose a serious threat to Papahānaumokuākea, as without native predators keeping their population size in check, AIS can grow rapidly and outcompete other organisms. This disruptive unbalance in the ecosystem can lead to dramatic changes, something we've seen at Manawai (Pearl and Hermes Atoll), where thick blankets of *Chondria tumulosa* have grown over and smothered reefs. The remoteness of Papahānaumokuākea is part of what makes it special, but it also makes conducting research there challenging. *Chondria tumulosa* was first discovered in 2016, since then, there have been ~47 days in the monument available for hands-on work with *C. tumulosa*. This May (2024), we invited *Chondria* researchers and managers to the first *Chondria*-focused Conference, "ChonCon". The 76 participants (representing 17 different organizations) learned the most up to date information on how *C. tumulosa* grows, reproduces, and impacts the reef habitat under it and the reef fish around it. Experts in the field discussed different methods of control for not transporting *C. tumulosa* to new places, challenges linked to managing marine debris, and cutting edge early detection methods. There were exciting connections made across discrete fields of study, for example patterns identified in meta analyses of AIS distributions from one scientist aligned with oceanographic modeling of potential AIS origin locations from another scientist. In addition to 20 different talks, ChonCon included hands-on training with previously frozen *C. tumulosa* - the first time many managers and scientists have seen *C. tumulosa* in person. We ended the day with a discussion panel geared to link the science with manager needs, and to identify key knowledge gaps for advancing AIS management strategies. (Event summary by Dr. Chelsie Counsell)

This event was organized by PMNM's Resource Protection Specialist Brian Hauk, Research Coordinator Dr. Randy Kosaki, and CIMAR Research Supervisor Dr. Chelsie Counsell.
The event expenses were covered with support from the National Marine Sanctuary Foundation.

Photo credit: Brianna Evancoe, Colt Davis, & Brian Hauk

PAPAHĀNAUMOKUĀKEA MARINE NATIONAL MONUMENT

National Marine Sanctuary Foundation

ChonCon Research Symposium: Book of Abstracts

ChonCon Research Symposium: May 10, 2024 at Aloha Tower (Honolulu)

8:00 Registration, Coffee & Light Refreshments, Poster Session

9:00 Opening Protocol & Welcome: Dr. Randall Kosaki, PMNM Research Coordinator

9:15 Keynote Address: Invasive and non-indigenous seaweeds around the world: What have we learned? by Dr. Jennifer Smith, Scripps Institution of Oceanography

9:45 Introduction: An overview of *Chondria tumulosa* in Papahānaumokuākea Marine National Monument and the development of preliminary biosecurity protocols by Brian Hauk, PMNM Sanctuary Resource Protection Specialist

10:00 Morning Session I: Reproduction & Drivers

Phycological genetics: The good, the bad, and the ugly
Stacy Krueger-Hadfield, Solenn Stoeckel

Fragmentation structures populations of *Chondria tumulosa* in Papahānaumokuākea
Taylor Williams, Heather Spalding, Solenn Stoeckel, Brian Hauk, Randall Kosaki, Jonathan Plissner, Stacy Krueger-Hadfield

The role of oceanographic phenomena in the *Chondria* outbreak
Andrea Kealoha, Heather Spalding, Brian Hauk, Colt Davis, Brian Popp, Chris Wall

10:50 Break: 10 Minutes

11:00 Morning Session II: Mat Dynamics

***Chondria tumulosa* mat tissue nutrients and biomass in Papahānaumokuākea Marine National Monument**
Heather Spalding, Hannah Savage, Abigail Davis, Taylor Williams, Brian Hauk, Andrea Kealoha, Randall Kosaki

***Ecophysiology of the red alga *Chondria tumulosa*: A new cryptogenic invader in Papahānaumokuākea Marine National Monument, Hawai‘i**
Abigail Davis, Andrea Kealoha, Ian Rolfe, Heather Spalding

The microbiome of *Chondria tumulosa* and native algae at Manawai Atoll
Heather Fullerton, Evan Dunn, Kyle Macalincag, Abigail Davis, Taylor Williams, Heather Spalding

11:45 Identification techniques for *Chondria tumulosa* & similar species by Dr. Heather Spalding, College of Charleston

12:00 Lunch (provided), Poster Session, Hands-on Algae Identification

Posters:

Assessment of the spatial distribution of a nuisance macroalga at Manawai using high-resolution satellite imagery
Leiana Beyer, Tomoaki Miura, Ka‘ua‘oa Fraiola, Heather Spalding, Taylor Williams, Jonathan Martinez, Randall Kosaki, Keolohilani Lopes Jr.

Spatiotemporal observations of an invasive acting macroalga, *Chondria tumulosa*
Keolohilani Lopes Jr., Tomoaki Miura, Brian Hauk, Randall Kosaki, Jason Leonard, Cynthia Hunter

1:00 Afternoon Session I: Ecosystem Effects

Chemical characteristics of *Chondria tumulosa* (Rhodomelaceae, Rhodophyta) from the Northwestern Hawaiian Islands
Lauren Runnels, Justin Reinecke, Karla McDermid

***Reef fish community composition over a gradient o*f *Chondria tumulosa* cover**
Chelsie Counsell, Heather Ylitalo-Ward

Invertebrate communities differ between a cryptogenic invasive and a native macroalga in the Papahānaumokuākea Marine National Monument, Hawai'i
Ray Radick, Robert Podolsky, Kristina Hill-Spanik, Heather Spalding

Unraveling coral nutrition strategies in response to invasive macroalgae: Insights from compound-specific isotopic analysis in the Northwestern Hawaiian Islands
Mario Kaluhiokalani, Christopher Wall, Andrea Kealoha, Brian Popp, Heather Spalding, Natalie Wallsgrove, Brian Hauk

Remote sensing of *Chondria tumulosa*: Past, present, and future approaches
Tomoaki Miura, Keolohilani Lopes Jr., Leiana Beyer, Kaua Fraiola, Heather Spalding, Brian Hauk, Randall Kosaki

2:15 Break: 10 Minutes

2:25 Afternoon Session II: Management Tools

Environmental DNA detection of *Chondria tumulosa* in low-abundance
Patrick Nichols, Fraiola K.M.S., Alison Sherwood, Brian Hauk, Keolohilani Lopes Jr., Colt Davis, Jimmy Fumo, Chelsie Counsell, Taylor Williams, Heather Spalding, Peter Marko

Modeling the source location of *Chondria tumulosa
Jimmy Fumo, Patrick Nichols, Peter Marko, Brian Powell, Randall Kosaki, Heather Spalding, Taylor Williams, Brian Hauk, Alison Sherwood

****Chondria tumulosa* biosecurity experiments: Bleaching debris and decks**
Kimberly Fuller, Ian Rolfe, Brian Hauk, Heather Spalding

Addressing challenges in marine debris removal and nuisance algae mitigation within Papahānaumokuākea Marine National Monument
James Morioka, Kevin O'Brien, Kaylyn McCoy Vincente

Methods for the eradication of the macroalga *Acanthophora spicifera* may also work for *Chondria tumulosa
Angela Richards Donà, Celia Smith

3:20 Break: 10 Minutes

3:30 Panel Discussion: **The Next Frontier: An evaluation of future research & management practices for *Chondria tumulosa***
Panelists: Kimberly Fuller, Dr. Stacy Krueger-Hadfield, Brian Hauk, Patrick Nichols, Dr. Celia Smith, Dr. Jennifer Smith, Dr. Heather Spalding
Moderator: Dr. Chelsie Counsell, CIMAR PMNM Research Supervisor

4:30 Pau/Closing Remarks

5:00-7:00: Happy Hour

Keynote Address:

Invasive and non-indigenous seaweeds around the world: What have we learned?

Jennifer E. Smith (Scripps Institution of Oceanography, smithj@ucsd.edu)

The introduction of non-native species into new environments is often considered one of the largest threats to global biodiversity. Marine species invasions have occurred frequently around the globe and are considered to be a significant management challenge. Seaweeds are among the most common invaders into coastal habitats due to their ability to foul natural and anthropogenic surfaces, hitchhike on other taxa that are frequently cultivated in aquaculture, travel long distances on the hulls of ships, vegetatively propagate and their potential high fecundity once in a new environment. Several hundred species of seaweed have been introduced into areas that they are not considered native to. However, only a small percent of these non-indigenous taxa can be considered to be "invasive" where they are causing harm to the receiving communities. Here I review what is known about the impacts, ecological interactions and potential management of invasive and/or non-indigenous seaweeds around the world. This information can be used to contextualize and inform management options for the *Chondria tumulosa* outbreak in Papahānaumokuākea Marine National Monument.

Introduction:

An overview of *Chondria tumulosa* in Papahānaumokuākea Marine National Monument and the development of preliminary biosecurity protocols

Brian B. Hauk (PMNM Sanctuary Resource Protection Specialist, brian.hauk@noaa.gov), Heather L. Spalding, Taylor M. Williams, Randall K. Kosaki

Chondria tumulosa was first observed and collected in 2016 after it was found in small patches overgrowing coral reefs around the northeastern backreefs of Manawai (Pearl & Hermes Reef) in the Papahānaumokuākea Marine National Monument (PMNM). It was not until a research expedition in 2019 that the seriousness and extent of *Chondria* reef overgrowth and spread came to light. At that time, preliminary lethality experiments were conducted to inform management of the biosecurity protocols required to mitigate the risk of inadvertently spreading this cryptogenic species elsewhere in the PMNM or inhabited Hawaiian Islands. Further trials were conducted in 2022 to better expand managers' biosecurity mitigation options after the alga was discovered at the adjacent atoll of Kuaihelani (Midway Atoll) in 2021. In 2023, the species was identified at Hōlanikū (Kure Atoll) growing cryptically and is now considered to be present at the last three atolls of the Hawaiian Archipelago. It is imperative that managers understand the options available to prevent further spread of this species through vectors associated with anthropogenic activities and natural occurrences.

Algal ID Session:

Identification techniques for *Chondria tumulosa* and similar species

Heather L. Spalding (College of Charleston, spaldinghl@cofc.edu)

Chondria tumulosa has been found growing cryptically in several locations within Papahānaumokuākea Marine National Monument and is challenging to identify morphologically in the field. Several other similar species, such as the red algal genera *Laurencia* and *Chondracanthus*, are easily confused with *Chondria*. This session will review some of the common identifying characteristics of *Chondria* in comparison to other genera to bolster early identification efforts. Following this session, participants will be encouraged to examine previously frozen *Chondria* mats and create personal herbaria of native algae for use in the field and laboratory.

*Presentations (in order of occurrence): *Denotes 5 minute vs 15 minute, Author name is the presenter*.

Morning Session I: Reproduction & Drivers

Phycological genetics: The good, the bad, and the ugly
Stacy A. Krueger-Hadfield (Virginia Institute of Marine Science, sakh@vims.edu), Solenn Stoeckel

Understanding a species' basic ecology and natural history is key to understanding evolutionary potential. This information, with a particular emphasis on connectivity and mating patterns, has long been lacking for haploid-diploid macroalgae. Macroalgal population genetic studies are few, likely due to the complexity of the typical algal life cycle. The majority of macroalgae exhibit life cycles in which there are two free-living phases – the gametophytes and sporophytes. Thus, the occurrence of both phases represents an inherent challenge of exploring macroalgal evolutionary ecology. For example, there is a general sampling rule of thumb of 30 individuals per population. However, haploid-diploid organisms differ fundamentally from diploid-dominant organisms and 30 samples is unlikely to be sufficient. Moreover, most macroalgae are partially clonal, engaging in simultaneous sexual and asexual reproduction. Yet, population genetic theory is based on exclusive sexuality or exclusive asexuality. The combined effects of proportion of gametophytes and the rate of clonality impacts the distributions of population genetic indices in ways not appreciated in other taxa. Appropriate sampling of macroalgae is critical to understand the consequences of anthropogenic climate change in algal-dominated communities as well as the eco-evolutionary impacts of introduced algae to novel ecosystems.

Fragmentation structures populations of *Chondria tumulosa* in Papahānaumokuākea
Taylor M. Williams (University of Alabama at Birmingham, tmwilli4@uab.edu), Heather L. Spalding, Solenn Stoeckel, Brian B. Hauk, Randall K. Kosaki, Jonathan H. Plissner, Stacy A. Krueger-Hadfield

Most marine red algae have free-living diploid tetrasporophytes and haploid male and female gametophytes, thereby complicating traditional population genetic sampling and analytical approaches. With recent theoretical and analytical developments, we can explore the population genetics of algae, including taxa undergoing rapid expansion. The cryptogenic red alga *Chondria tumulosa* occurs at three atolls in Papahānaumokuākea Marine National Monument: Manawai (Pearl and Hermes Atoll), Kuaihelani (Midway Atoll), and Hōlanikū (Kure Atoll). Here, we investigated the reproductive system and genetic structure to understand the rapid increase in biomass and connectivity among atolls in *C. tumulosa*. The reproductive system describes the relative rates of sexual and asexual reproduction in a population and has direct consequences for genetic structure. We collected 1,142 samples from Manawai (2019, 2021, and 2023), Kuaihelani (2022 and 2023), and Hōlanikū (2023), and examined each thallus under the microscope for the presence of reproductive structures. The observed thalli were vegetative (90%), tetrasporophytes (9%) and fertilized females (1%). We are currently exploring the relative rates of asexual reproduction and self-fertilization. These data are integral to understanding connectivity in Papahānaumokuākea and identifying potential approaches to limit algal invasions that threaten coral reef systems.

The role of oceanographic phenomena in the *Chondria* outbreak
Andrea K. Kealoha (University of Hawai'i at Mānoa, andreake@hawaii.edu), Heather L. Spalding, Brian B. Hauk, Colt A. Davis, Brian N. Popp, Chris B. Wall

Following the discovery of *Chondria tumulosa* at Manawai in 2016, research has focused efforts to better understand the drivers behind its presence, distribution and growth. Promoters of successful spread of marine invasive species include ship traffic or floating debris transported over long distances by oceanic currents, but oceanographic and biogeochemical processes may also facilitate aquatic invasions. We combined hydrographic measurements with isotopic analyses of coral tissues to investigate the role of oceanographic phenomena in the spread of *C. tumulosa*. In 2020, ~160 Manawai sites were sampled for temperature, salinity, nutrients, carbonate chemistry, chlorophyll a, and stable isotope signatures of particulate organic matter. Coral tissue samples (n=40) were collected across 14 sites. Several oceanographic features were identified, including upwelling and hotspots of productivity. Preliminary data

from repeat surveys in 2023 reveal these oceanographic features are likely transient. Although the spatial distribution of *C. tumulosa* does not appear correlated with any of the measured parameters, the comprehensive biogeochemical dataset collected through these efforts provide valuable insight into the role of oceanographic processes in influencing coral reef ecosystem health.

Morning Session II: Mat Dynamics

Chondria tumulosa mat tissue nutrients and biomass in Papahānaumokuākea Marine National Monument

Heather L. Spalding (College of Charleston, spaldinghl@cofc.edu), Hannah Savage, Abigail Davis, Taylor M. Williams, Brian B. Hauk, Andrea K. Kealoha, Randall K. Kosaki

The Papahānaumokuākea Marine National Monument supports a high diversity and abundance of native marine flora and fauna. In 2019, a new cryptogenic red alga, *Chondria tumulosa*, was observed forming mats that overgrew native corals and algae at Manawai. By 2023, 40% of sites surveyed at Manawai contained *Chondria* mats in the lagoon, back reef, and fore reef from 1 to 21 m depths. *Chondria* percent cover was up to 95% over a 25 x 1 m area, with canopy heights from 1 to 21 cm. *Chondria* mat biomass was similar or less than other invasive mat-forming algae, but its dense mats absorbed 98-99% of available light depending on the canopy height. Stable isotope analyses of *Chondria* tissue δ15N ranged from 2.2-3.4‰, suggesting its distribution was not influenced by anthropogenic or shore-based processes (like seabird guano). However, the %N in algal tissue ranged from 0.95-2.75%, suggesting enrichment by other processes is occurring. This alga poses a serious threat to coral reefs in the Pacific because of its dense mat-forming morphology, fragmentation, attachment to nets and other algae, and high abundance in nearly pristine, nutrient-poor environments.

***Ecophysiology of the red alga _Chondria tumulosa_: A new cryptogenic invader in Papahānaumokuākea Marine National Monument, Hawai‘i**

Abigail Davis (College of Charleston, davisac7@g.cofc.edu), Andrea K. Kealoha, Ian Rolfe, Heather L. Spalding

A new cryptogenic macroalga, *Chondria tumulosa*, recently invaded the Papahānaumokuākea Marine National Monument in the Northwestern Hawaiian Islands. This mat-forming species overgrows and smothers native algae and corals and is spreading rapidly. However, little is known about the ecophysiological traits contributing towards its high abundance. The goal of this study was to examine the influence of abiotic factors (in situ nutrients and irradiance) on the photosynthetic efficiency of dense mats at 11 sites at Manawai (Pearl and Hermes Atoll). At each site, three *Chondria* samples were collected along a 25 meter transect at 1.5 to 12 m depths. Three replicates of photosynthetic efficiency were measured from the top and bottom of each mat. Water samples from within and directly above the mats were collected for nutrient analyses. Light (lux) was measured above and below *Chondria* mats ranging from 3 to 11 cm in height. Water nutrients were significantly greater from within as compared to above the mats, suggesting that mat formation increases the microscale nutrient environment in otherwise oligotrophic waters. *Chondria* mats absorbed over 99% of light and the lower mat portions were still photosynthetic, suggesting that *Chondria* has the potential to occur in low-light environments, such as the mesophotic.

The microbiome of _Chondria tumulosa_ and native algae at Manawai Atoll

Heather Fullerton, Evan Dunn (College of Charleston, dunne@g.cofc.edu), Kyle A. Macalincag, Abigail Davis, Taylor M. Williams, Heather L. Spalding

The ocean houses diverse and unique ecosystems which can vary by depth and distance from land. In many of these habitats, macroalgae are primary producers, habitat formers, and indicator organisms for overall ecosystem health. All organisms form close relationships with bacteria and archaea and this microbiome can be indicators for host health. Representative, abundant macroalgae from the Chlorophyta, Rhodophyta, and Phaeophyceae were collected from 16 sites in 2021 for microbiome analyses. A previous study with limited collections of the invasive-like alga, *Chondria tumulosa*, noted it had a distinct microbiome

compared to other Rhodophyta in the same location. This study seeks to expand the comparative microbiome analysis between *C. tumulosa* and native macroalgae at Manawai Atoll. These results will provide an understanding of microbial-macroalgal interactions and how such relationships influence the microbial communities of each associated macroalgae. The increase in abundance of *C. tumulosa* and its resulting decrease in macroalgal and coral species may negatively impact the diversity of macroalgal microbiomes in this coral reef environment.

Afternoon Session I: Ecosystem Effects

Chemical Characteristics of *Chondria tumulosa* (Rhodomelaceae, Rhodophyta) from the Northwestern Hawaiian Islands

Lauren Runnels (University of Hawai'i at Hilo, lrunnels@hawaii.edu), Justin Reinecke, Karla McDermid (University of Hawai'i at Hilo, mcdermid@hawaii.edu)

The macroalga, *Chondria tumulosa* (Phylum Rhodophyta), is found in the remote Northwestern Hawaiian Islands (NWHI). Scientific divers have noted a lack of herbivory on *C. tumulosa* which suggests that the alga may have secondary metabolites that deter herbivores. Other species of *Chondria*, as well as closely related species of *Laurencia* and *Chondrophycus* in the red algal family Rhodomelaceae, are known for their diverse, bioactive natural chemicals. *Chondria tumulosa* samples collected from Pearl and Hermes Atoll in 2021 and 2023 were frozen, shipped to O'ahu, flown to Hawai'i Island, cleaned, freeze-dried, and stored at 20°C until chemical extraction. Solvent extractions utilized methanol, dichloromethane/methanol (8:2), and hexane. Extractions were filtered followed by solvent removal. Twenty-six compounds were identified from the major peaks after manual inspection of the Liquid Chromatography Mass-Spectrometry (LC-MS) analysis of the methanol crude extract. Fractionation of the methanol crude extract was accomplished with column chromatography. In Kirby-Bauer Disk Diffusion Susceptibility Test Protocol analyses, the methanol crude extract of *C. tumulosa* did not inhibit growth of *E. coli* nor *Bacillus cereus* at full or half strength concentration. The extracted compounds and their bioactivities may contribute to the chemical ecology of *C. tumulosa* in the NWHI.

***Reef fish community composition over a gradient of *Chondria tumulosa* cover at Manawai**

Chelsie W. W. Counsell (CIMAR PMNM Research Supervisor, chelsie.counsell@noaa.gov), Heather Ylitalo-Ward

The balance between coral and macroalgae within reef ecosystems is of particular interest with phase shifts in the dominant benthic cover occurring on some reefs. *Chondria tumulosa* is a red macroalgae that can form mat-like structures covering other substrates including corals. *C. tumulosa* was not previously known; however, in the last 8 years, it has been found to dominate some reefs within the northern region of Papahānaumokuākea. Using SCUBA based fish surveys paired with 3D models of the reef habitat (created from structure for motion benthic photos), we assessed changes in the reef fish community along a gradient of *C. tumulosa* cover at 28 sites around Manawai. At sites with higher abundance of *C. tumulosa* we anticipate a decline in reef fish abundance and biomass. We expect that this trend will be particularly notable for coral-associated fishes (*C. tumulosa* smothers coral) and herbivorous fishes (*C. tumulosa* outcompetes native algae and seems unpalatable). Apex predators are less likely to show a change in their abundance (*C. tumulosa* mats are patchy on the scale of apex predator mobility). Understanding the impacts of *C. tumulosa* on reef fish is key to informing management on the broader impacts of this invasively-behaving macroalgae in Papahānaumokuākea.

Invertebrate communities differ between a cryptogenic invasive and a native macroalga in the Papahānaumokuākea Marine National Monument, Hawai‘i

Ray M. Radick (College of Charleston, radickr@g.cofc.edu), Robert D. Podolsky, Kristina M. Hill-Spanik, Heather L. Spalding

Habitat alteration by a newly established species can change community abundances and diversity, especially in relatively undisturbed ecosystems such as Papahānaumokuākea Marine National Monument (PMNM) in the Northwestern Hawaiian Islands. The red alga *Chondria tumulosa* was first discovered in PMNM at the atolls Manawai and Kuaihelani in 2016 and 2021, respectively. This cryptogenic invader forms dense mats up to 21 cm in height that overgrow native macroalgae and corals, which may impact associated invertebrate communities. The goals of this study were to characterize and compare invertebrate abundance and diversity in *C. tumulosa* mats as compared to native *Microdictyon setchellianum* beds. Samples of *C. tumulosa* (n=275, 6 sites) and *M. setchellianum* (n=100, 2 sites) were collected from Kuaihelani in July 2022. Invertebrates from each sample were sorted to morphospecies using a dissecting microscope, and DNA barcoding was used to identify representative specimens. Each alga was associated with distinct invertebrate communities based on a PERMANOVA and nonmetric multidimensional scaling plot. This study provides the first comparison of invertebrate communities in native and cryptogenic algae in PMNM and establishes a baseline to better understand how the continued spread of this habitat-altering alga might further influence invertebrate community structure.

Unraveling coral nutrition strategies in response to invasive macroalgae: Insights from compound-specific isotopic analysis in the Northwestern Hawaiian Islands

Mario Kaluhiokalani (University of Hawai‘i at Mānoa, mariok@hawaii.edu), Christopher Wall, Andrea K. Kealoha, Brian Popp, Heather L. Spalding, Natalie Wallsgrove, Brian B. Hauk

Coral reefs confront escalating threats from climate change, including invasive species proliferation. Understanding coral nutrition responses to environmental stressors is vital for predicting reef resilience. This study explores the impact of macroalgal cover, particularly the invasive red alga *Chondria tumulosa* (*C. tumulosa*), on coral nutritional strategies in the Northwestern Hawaiian Islands (NWHI). Coral samples were collected pre- and post-invasion, employing compound-specific isotopic analysis of amino acids (CSIA-AA) to assess trophic dynamics. CSIA-AA revealed significant separation in essential amino acid δ13C values among coral hosts, symbionts, plankton, and *C. tumulosa*. Trophic position, assessed by comparing δ15N values, suggested heterotrophic bacteria hosted by *C. tumulosa* and trophic plasticity in *Pocillopora spp*. Obtaining nutrition directly from *C. tumulosa* was improbable for *Pocillopora spp*., but essential amino acid δ13C values could identify organisms preying on *C. tumulosa*. These findings stress the importance of understanding coral-algal interactions amid environmental change and highlight CSIA-AA's utility in elucidating complex trophic dynamics within coral reef ecosystems. This research pioneers isotopic identification and tracing of *C. tumulosa*, offering insights for future research and management practices. Further studies should consider expanding sample sizes and environmental variables to enhance comprehension of coral-algal interactions and reef resilience.

Remote sensing of *C. tumulosa*: Past, present, and future approaches

Tomoaki Miura (University of Hawai‘i at Mānoa, tomoakim@hawaii.edu), Keolohilani Lopes, Jr., Leiana Beyer, Kaua Fraiola, Heather L. Spalding, Brian B. Hauk, Randall K. Kosaki

One fundamental to the management of an alien invasive species is knowing its presence and spatial extent. In the past, our lab investigated the temporal and spatial distribution of *C. tumulosa* by visual analysis of high resolution satellite images (Friaola et al., 2023; Lopes et al., 2023; and Beyer et al., in review). As *C. tumulosa* has low reflectance, their presence can be identified as 'dark patches' in satellite images. Our analysis resulted in a better and comprehensive understanding of the potential spatial extent of *C. tumulosa* across the forereefs, backreefs, and lagoons in Manawai Atoll. Presently, several additional approaches are being examined and developed for the improved detection and mapping of *C. tumulosa*. We are analyzing hyperspectral reflectance data acquired in the July 2023 field season for the improved

separability. Several atmosphere-water correction methods that can bring the benthic bottoms to the surface level in satellite images are being evaluated. An uncrewed marine system (UMS) that utilizes computer vision, spectrometer, and eDNA to independently confirm the presence of *C. tumulosa* is being developed by the Queensland University of Technology in collaboration. Field testing of this robotics system is scheduled to begin on the week of May 13, 2024.

Afternoon Session II: Management Tools

Environmental DNA detection of *Chondria tumulosa* in low-abundance

Patrick K. Nichols (University of Hawai'i at Mānoa, pkn@hawaii.edu), Fraiola K.M.S., Alison R. Sherwood, Brian B. Hauk, Keolohilani Lopes, Jr., Colt A. Davis, Jimmy T. Fumo, Chelsie W. W. Counsell, Taylor M. Williams, Heather L. Spalding, Peter B. Marko

Early detection of nuisance species is crucial for the conservation and management of threatened ecosystems, reducing the risk of widespread establishment. Environmental DNA (eDNA) data can increase the sensitivity of biomonitoring programs with reduced cost and effort. Yet, eDNA analyses have inherent errors that can complicate integration of molecular survey methods into existing management frameworks. Therefore, it is crucial for eDNA studies to consider imperfect detections and estimate error rates accordingly. We developed an assay to detect the nuisance marine alga using eDNA collected from surface seawater samples, maximizing detection sensitivity by filtering large volumes of seawater. The existing protocol, while necessary for the assay's development, is resource-intensive, requiring specialized equipment and laboratory space for sample filtration. Here, we employ a simplified passive method for eDNA collection, bypassing the need for processing water samples and enabling the expansion of monitoring efforts. Occupancy modeling was used to infer *Chondria tumulosa* presence-absence and estimate associated error rates. The passive eDNA assay can reliably detect *C. tumulosa* in extreme low abundance (< 1%) along its colonization front. eDNA analyses enhance surveillance and inform timely management interventions by providing a reliable, cost-effective tool for detecting low-abundance species.

Modeling the source location of *Chondria tumulosa

Jimmy T. Fumo (University of Hawai'i at Mānoa, jfumo@hawaii.edu), Patrick K. Nichols, Peter B. Marko, Brian Powell, Randall K. Kosaki, Heather L. Spalding, Taylor M. Williams, Brian B. Hauk, Alison R. Sherwood

The red alga *Chondria tumulosa* is a cryptogenic species of likely non-native status causing harm to the culturally and ecologically important reefs of Papahānaumokuākea Marine National Monument (PMNM). First seen in 2016 at Manawai (Pearl and Hermes Atoll), the species has since proliferated to alarming levels of benthic cover and has spread to two more atolls in PMNM. Despite several indications of non-native status and comparisons with global databases the species remains unknown from any other part of the world. The broad spectrum of potential source regions, taxonomic ambiguities in the genus *Chondria*, and challenges associated with sampling necessitate the development of a narrowed set of search locations and efficient search strategies. This study used an oceanographic model to determine that the most likely source locations for *C. tumulosa* are: Japan, Johnston Atoll, the Line Islands and Palmyra Atoll, and the Galápagos Islands. We suggest a framework for searching within those regions using eDNA, in-water surveys, microscopy, barcoding, and ultimately genomic sequencing. A coincident sampling effort targeting pressed material stored in global herbaria is also suggested. Identifying the original habitat of *C. tumulosa* will allow for an evaluation of its ecological behavior in its natural environment, offering insights crucial for safeguarding PMNM against potential risks.

****Chondria tumulosa* biosecurity experiments: Bleaching debris and decks**

Kimberly Fuller (State of Hawai'i, Department of Land and Natural Resources, Division of Aquatic Resources, kimberly.h.fuller@hawaii.gov), Ian Rolfe, Brian B. Hauk, Heather L. Spalding

Federal, state, and academic partners have developed biosecurity protocols for operations in Papahānaumokuākea Marine National Monument (PMNM) Nuisance Algae Management Zones (NAMZ).

The biosecurity protocols are outlined in PMNM Best Management Practices (BMP) # 20 and involve spraying down all surfaces on the ship deck and small boats, twice, with 10% bleach solution. Marine debris in areas with visible *Chondria tumulosa* growth is not currently collected but debris has been collected from NAMZ in areas *C. tumulosa* is not observed. The Papahānaumokuākea Marine Debris Project (PMDP) has additionally devised a protocol in which the nets collected in NAMZs can be bleached in 10% bleach for a range of 4-72 hours. We performed experiments to inform deck and debris bleach biosecurity protocols in July of 2023.

Addressing challenges in marine debris removal and nuisance algae mitigation within Papahānaumokuākea Marine National Monument

James Morioka (Papahānaumokuākea Marine Debris Project, james@pmdphawaii.org), Kevin O'Brien, Kaylyn McCoy Vincente

Chondria tumulosa, a nuisance algae, has been observed at three out of the seven islands and atolls significantly affected by marine debris within the Papahānaumokuākea Marine National Monument (PMNM). These areas, designated as Nuisance Algae Mitigation Zones (NAMZ), include Manawai (Pearl and Hermes Atoll), Kuaihelani (Midway Atoll), and Hōlanikū (Kure Atoll). The presence of marine debris has a significant negative impact on the protected wildlife of PMNM, particularly the endangered Hawaiian monk seal, threatened Hawaiian green sea turtle, various seabird species, and sensitive habitats like coral reefs. The Papahānaumokuākea Marine Debris Project (PMDP), a Hawai'i-based nonprofit organization, is dedicated to protecting PMNM from the threats posed by marine debris. The removal of marine debris from NAMZ presents considerable challenges, including the identification and removal of debris associated with *C. tumulosa*, implementation of comprehensive biosecurity protocols such as soaking debris in highly concentrated bleach solution, and safe transportation and disposal of debris in Honolulu, Hawai'i. Current biosecurity protocols pose risks to PMDP staff involved in debris treatment, and the remote nature of operation presents logistical limitations on chartered vessel operations. Models of ocean currents and wind patterns have shown that it is possible for floating debris from PMNM to reach the Main Hawaiian Islands. Therefore, failure to remove marine debris may facilitate the spread of *C. tumulosa* to the MHI, underscoring the urgency of prioritizing debris removal. A project in 2018 showed that floating debris can move throughout PMNM, as debris was tracked moving within an atoll, as well as from atoll to atoll (Manawai to Hōlanikū), risking further spread of the nuisance algae.To address these challenges, PMDP advocates for the development of new marine debris treatment methods in NAMZ that prevent the spread of *C. tumulosa* while ensuring the safety of staff conducting the work. This imperative reflects the need to safeguard both the marine environment and those dedicated to its preservation.

Methods for the eradication of the macroalga *Acanthophora spicifera* may also work for *Chondria tumulosa

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Informed by recently acquired physiological data for *Acanthophora spicifera*, we ran experiments to test the efficacy of several methods to kill this invasive alga. We used heating and a combination of heating and soaking in common household solutions to disrupt photosynthesis and ultimately kill the algae. Our primary goals were to determine the most effective and definitive method to achieve complete and unequivocal death of the plant, and evaluate how the method could be scaled up to eradicate the growing population at Midway. Using A. spicifera as a proxy species for *Chondria tumulosa*, we also set out to determine whether the most potent method could potentially be used during marine debris collection trips in Papahānaumokuākea Marine National Monument (PMNM) to kill algal fragments attached to ghost nets and other floating flotsam. Among the promising methods: 30-minute soaks in 10% solutions of Dawn dishwashing detergent, or baking soda, followed by 3 hours of desiccation in a drying oven at ~45 °C. These solutions are non-toxic and safer to use shipboard than the current use of 10% bleach and could possibly be used during the upcoming debris collection season.

(Poster) **Assessment of the spatial distribution of a nuisance macroalga at Manawai in Papahānaumokuākea Marine National Monument using high-resolution satellite imagery**

Leiana Beyer (Fish and Wildlife Service, leiana_beyer@fws.gov), Tomoaki Miura, Ka‘ua‘oa Fraiola, Heather L. Spalding, Taylor M. Williams, Jonathan Martinez, Randall K. Kosaki, Keolohilani H. Lopes Jr.

In 2019, a new red algal species, *Chondria tumulosa*, was discovered overgrowing native coral and algal species and changing the benthic communities of Manawai in Papahānaumokuākea Marine National Monument (PMNM). The algae's invasive characteristics suggest it may pose a threat to the ecological structure of PMNM and could spread south to the Main Hawaiian Islands. The main objective of this study was to assess the spatial distribution of *C. tumulosa* at Manawai using remote sensing to support management efforts. WorldView-2 and -3 commercial high-resolution satellite images were obtained for a 12-year period from 2010 to 2021. Time-series animations were created from the 12-year satellite image dataset and overlaid with geo-located field survey data of *C. tumulosa* cover collected in 2019-2021. Previous studies reported that *C. tumulosa* appeared as distinctively dark features in satellite imagery. Thus, animations were visually inspected to detect dark patches suspected to be *C. tumulosa* with the field survey data as a reference. Using those dark features as a reference and a machine learning algorithm, the latest high-resolution satellite images from 2019 through 2021 were classified into a distribution map of distinctive dark patches suspected to be *C. tumulosa* with an overall average accuracy of 76%. The estimated distribution indicated the highest percent cover of the suspected dark patches in the western and northern regions of Manawai. This study provides resource managers with information on the potential distribution and abundance of *C. tumulosa* to further monitor for long-term management.

(Poster) **Spatiotemporal observations of an invasive acting macroalga, *Chondria tumulosa***

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In 2019, a newly described, invasive-acting marine macroalga, *Chondria tumulosa*, was observed growing attached to the forereef, encompassing large areas of Manawai atoll in Papahānaumokuākea (AKA Northwestern Hawaiian Islands). In 2021, researchers observed dislodged *C. tumulosa* on the seafloor rolling like “tumbleweeds”, aggregating in a network of meandering accumulations. We refer to these accumulations as *Chondria* trails. These *Chondria* trails were dark and distinct against the sandy backdrop of the lagoon bottom. This made it observable from the ocean surface, uncrewed aerial systems (drones), and satellite imagery. We quantified the geographic extent of this unique phenomenon. The length and area of these *Chondria* trails were calculated from a satellite image data set spanning ten-years (2011, 2015, 2017 to 2021). Our results show the *Chondria* trail first became visible in 2015 with a length of ~736 m covering ~882 m in area. The length and area expanded to ~41 km and ~101 km , respectively, in 2021. The average increase was just under ~7 km/yr. in length and just under ~17 km /yr. in area. This once isolated alga has since been observed (July, 2023) at the two northernmost atolls in the Hawaiian archipelago, Kuaihelani and Hōlanikū. This research provides a historical snapshot of the proliferation of the *Chondria* trail and establishes a foundation for the further remote sensing of this species.

Panel Discussion:

The Next Frontier: An evaluation of future research and management practices for *Chondria tumulosa*

Panelists: Kimberly Fuller, Brian Hauk, Dr. Stacy Krueger-Hadfield, Patrick Nichols, Dr. Celia Smith, Dr. Jennifer Smith, Dr. Heather Spalding

Moderator: Dr. Chelsie Counsell