State of Hawaii
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Aquatic
Resources Honolulu, Hawaii
96813

June 25, 2021

Board of Land
and Natural Resources
Honolulu, Hawaii

Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National Monument Research Permit to Dr. Heather Spalding, College of Charleston, Department of Biology, for Access to State Waters to Conduct Research Activities on the Ecology, Physiology, and Diversity of Benthic Organisms Impacted by the Cryptogenic Alga *Chondria tumulosa* in the Northwestern Hawaiian Islands

The Division of Aquatic Resources (DAR) hereby submits a request for your authorization and approval for issuance of a Papahanaumokuakea Marine National Monument Research Permit to Dr. Heather Spalding, College of Charleston, Department of Biology, pursuant to §187 A-6, Hawai 'i Revised Statutes (HRS), Chapter 13-60.5, Hawai 'i Administrative Rules (HAR), and all other applicable laws and regulations.

The Research Permit, as described below, would allow entry and activities to occur in Papahanaumokuakea Marine National Monument, including the NWHI State Marine Refuge and the waters (0-3 nautical miles) surrounding the following sites:

- Nihoa Island
- Necker Island (Mokumanamana)
- French Frigate Shoals
- Gardner Pinnacles
- Laysan Island
- Lisianski Island
- Pearl and Hermes Atoll
- Midway Atoll
- Kure Atoll
- Maro Reef

The activities covered under this permit would be authorized to occur between July 2021 and June 2022.

INTENDED ACTIVITIES

Dr. Heather Spalding of the College of Charleston, Department of Biology (applicant), proposes to collect macroalgal, coral (for PMNM-2021-016 Kealoha - UHMCC), invertebrate, and water samples to study the ecology, physiology, and diversity of benthic organisms impacted or potentially impacted by the cryptogenic alga *Chondria tumulosa* (Chondria) as well as Chondria itself in the Northwestern Hawaiian Islands.

This research expedition is part of a larger ONMS cruise (covered under PMNM-2021-001 Co-Trustee Managers Permit and associated memo to file), and will be complimentary to both ONMS research activities and an additional research activity to be conducted on the same cruise under PMNM-2021-016, which will collect oceanographic data that will provide insight into the factors that contribute to the presence and distribution of Chondria at Pearl and Hermes Atoll (PHA) and will increase understanding for biogeochemical and oceanographic processes that may explain the presence and proliferation of Chondria.

Pearl and Hermes Atoll Biosecurity Measures and BMP

All researchers will adhere to the Pearl and Hermes Atoll Biosecurity Measures that were drafted (see attachment) to outline the mitigation steps ONMS cruise participants will follow to ensure adequate biosecurity measures are followed to mitigate the risk associated with accessing Manawai (Pearl and Hermes Atoll or "PHA") for research activities defined in permits: PMNM-2021-001 Co-Trustee Managers Permit (and associate memo to file), PMNM-2021-016 and PMNM-2021-019. These additional biosecurity measures were drafted to address research projects and activities involving *Chondria tumulosa* which fall outside of BMP 011 (Disease and Introduced Species Prevention Protocol for Permitted Activities in the Marine Environment, Papahānaumokuākea Marine National Monument) and the currently existing (or most recent version) draft BMP for PHA in BMP 011 (section D. Protocols For Conducting Operations at Pearl and Hermes Atoll).

Specific objectives are as followed:

- Characterize current distribution of Chondria, population structure and reproductive state
- Determine the role of oceanographic phenomena in the outbreak using algal and coral tissue as a metric
- Determine impacts to other marine life through the lens of the macroalgal microbiome of native species and Chondria
- Develop invasive algal mitigation and BMPs
- Ground-truth eDNA detection of Chondria through analyses of filtered water samples at varying distances from the outbreak
- Collect tissue for determination and analysis of the nuclear genome of Chondria tumulosa
- Examine primary production and growth by Chondria.

To accomplish these objectives, the researchers will collect macroalgae, coral, benthic invertebrate, and water samples via SCUBA diving or snorkeling at multiple sites within the Northwestern Hawaiian Islands. Collected samples will be used for macroalgal biodiversity studies, eDNA analyses, *Chondria*

physiological experiments on the boat while on site, macroalgal microbiome research contrasting native and cryptogenic/invasive species, *Chondria* population and reproductive state, mat-removal methodologies, fragment BMP studies, *Chondria* herbivore assessments, genome determination of *Chondria*, and macroalgal tissue nutrient and stable isotope analyses. The researchers will also collect coral samples for Kealoha (UHMCC) for stable isotope analyses. SCUBA and snorkeling surveys will be used to map the current distribution of *Chondria* as compared to 2019 at Manawai.

These activities will help the Monument by determining the current distribution of *Chondria* at Manawai, resolving oceanographic conditions via macroalgal and coral stable isotope and tissue nutrient analyses that may be influencing the abundance and distribution of *Chondria*, elucidating the connectivity and clonal state of present-day *Chondria* population(s), resolving the impact of *Chondria* on the health of the coral reef microbiome as compared to native algae, determining whether any invertebrate species are significant herbivores of *Chondria*, supporting ground-truthing of eDNA detection methodologies for *Chondria*, determining the genomic sequence of *Chondria* to compare to related species, as well as instantaneous measurement of photosynthesis for *Chondria* collected across the depth gradient from shallow to deep sites in its distribution, as initiating growth experiments that simulate those field conditions on the back deck of the research vessel. The outcomes will be targeted recommendations regarding *Chondria* containment, removal, detection, and management. This research is supported by the National Fish and Wildlife Foundation and National Science Foundation. The researchers plan to visit Manawai (PHA). However, other islands/atolls are a possibility depending on the research needs of other scientists on the ship. At each island/atoll, the researchers will collect water samples for eDNA, macroalgae, and coral tissue samples for isotopic analysis and ecology, physiology, and biodiversity analyses. The researchers will follow best management practices for boat operations and water/biological sampling. Although *Chondria* has been identified at Manawai, it is possible that the alga has spread to other locations. Therefore, if the researchers visit other locations with *Chondria*, it will be important to collect data for comparisons to Manawai.

The invasive-like, cryptogenic alga *Chondria tumulosa* was first noted as an "unknown red alga" in low occurrence in 2016 at Manawai. By July/August 2019, it had grown into an outbreak with patches covering thousands of meters of coral reefs on the northern, western, and eastern portions of Manawai. This alga was recently described as a new species (Sherwood et al. 2020), and little is known about its present-day ecology, physiology, reproduction, or the factors influencing its distribution and abundance. The researcher's goal is to provide the baseline data needed to properly manage and mitigate the spread of this alga to other locations within the Northwestern and Main Hawaiian Islands. The purpose of the proposed activities for *Chondria tumulosa* are to: a) characterize its current distribution (PI Spalding), population structure and reproductive state (PI Krueger-Hadfield), b) determine the role of oceanographic phenomena in the outbreak using algal and coral tissue as a metric (PI Spalding, PI Kealoha), c) determine impacts to other marine life through the lens of the macroalgal microbiome of native species and *Chondria* (PI Fullerton and PI Spalding), d) develop invasive algal mitigation and BMPs (PI Spalding), e) ground-truth eDNA detection of *Chondria* through analyses of filtered water samples at varying distances from the outbreak (PI Sherwood, in collaboration with P. Marko and P. Nichols), f) collect tissue for determination and analysis of the nuclear genome of *Chondria tumulosa* (PI Sherwood, in collaboration with B. Moore and T. Steele) and, g) examine primary production and growth by *Chondria*.

To do this, the researchers will collect small clumps of *Chondria* and/or native macroalgae every 1 m across a 100 m transect (estimated by fin kicks) at multiple sites of high and low *Chondria* abundance, and at sites without *Chondria* for comparison. Subsamples of the same collections will be used for multiple projects (population DNA analyses, reproductive state, stable isotopes and tissues nutrients,

physiology experiments, microbiome analyses, genome analyses) to maximize productivity and minimize disturbance. Coral samples for Kealoha will be collected at the same sites for combined analyses between macroalgal and coral stable isotopes. Water samples will be collected directly above, and at a series of distances away from *Chondria* mats, and filtered on board the ship for molecular analyses of the filters on Oahu to assess the efficacy of a newly developed eDNA *Chondria* detection protocol. Finally, plants collected from shallow and deep wild populations will be cleaned of epiphytes and initial readings of photosynthesis will be acquired on shipboard. These data will be coordinated with pigment analyses and tissue nitrogen from dried samples. Further work will include beginning experimental growth studies. At the end of growth experiments, small samples of plants will be extracted for Chlorophyll a.

For sampling purposes, researchers intend to target *Chondria tumulosa*; other macroalgae in the Rhodophyta, Chlorophyta, Phaeophyceae; Cyanobacteria; and corals (as described by Kealoha permit application). The primary goal of the researcher's proposed research is to determine the impact of *Chondria tumulosa* on coral reef communities at Manawai, and provide baseline data for its detection, management and possible mitigation. *Chondria* is a direct threat to the survival of coral reefs and their associated organisms in the Monument, particularly at Manawai. The researcher's mission is to only collect the specimens needed to fulfill the research objectives, and to dovetail these collections to maximize productivity and minimize impact or disturbance. Great care will be taken to minimize *Chondria* fragmentation. Collection equipment will be inspected and disinfected between sampling areas to mitigate the spread of *Chondria* and any other invasive species. Best efforts will be made to ensure that sample collection does not disturb marine life or resources in the surrounding environment. Other than the collected macroalgae (and any benthic invertebrates within the *Chondria* mats), corals, and water samples, the researchers will not touch any cultural or historical resources/artifacts within the Monument.

The researchers will collect small subsamples of macroalgae and coral colonies to limit disturbance. The information provided by this research will far outweigh the disturbance associated with macroalgal and coral collections. Managers will be provided with baseline data on *Chondria*'s ecology, physiology, genetic connectivity, and impact. This will allow managers to respond accordingly to the current algal outbreak, as well as potential future outbreaks across the archipelago. The eDNA detection protocol will allow managers to test for the presence of *Chondria* in a non-invasive manner at any of the locations at PMNM. Sampling and experimental design were optimized for physically small samples to support population genetics, microbiome, photosynthesis, growth, tissue nitrogen and pigment research.

There is no practicable alternative to the proposed activities. The only confirmed location of this alga is at Manawai, requiring these studies to be completed at Manawai and the surrounding islands and atolls. The researchers are also interested in comparing *Chondria* distributional data between 2019 and 2021, thus requiring surveys to be completed at Manawai for this temporal comparison. The fieldwork component of this research will be performed under the minimal time required to achieve the goals and objectives of the grant. Collaborators on the grant (but under a separate permit request – see Kealoha) will be on the same research cruise. The researchers will work together using previous algal surveys to select the best sites for the researcher's studies and to minimize time while at sea. Water sampling for eDNA and CTD casts will be conducted efficiently to maximize spatial resolution and minimize activity duration. Prior to the research cruise, the researchers will complete a comprehensive analysis of all available ecological, distributional, physiological, and other data to inform all field sampling.

<u>Research and Sampling</u>

The following types of research and sampling will occur at the following collection locations: Most collections will occur on Manawai (Pearl and Hermes Atoll) primarily (see attachments for maps of sampling sites), but other locations may include Nihoa, Necker, FFS, Garner, Lisianski, Laysan, Midway, Maro Reef and Kure Atoll.

1) Macroalgal collections

a. **Population genetics and reproduction** - The researchers will sample a patch of *Chondria* every 1 m along a 100 m transect. The length of the transect will be site dependent such that a mat of *Chondria* on a coral reef that is only 25m wide will be sampled along 4 25m transects to achieve 100 m with samples every 1 m. Moreover, the researchers will sample these 100m transects at 3 depths, but this depends on the site. The researchers will aim for two sites that have 3 depths. This type of sampling strategy has been used by Krueger-Hadfield et al. (2016 Molecular Ecology; 2017 Ecology and Evolution; 2018 Bioinvasion Records), Bonthond et al. (2020; 2021), Sotka et al. (2018), Flanagan et al. (2021) and is the only standardized way in which to sample free-floating or drifting algae that are also likely partially clonal (Krueger-Hadfield et al. 2021; Stoeckel et al. 2021). A sample of *Chondria* will likely contain multiple thalli that are interwoven. Thus, the researchers will subsample 3 thalli that are clearly separate but interwoven for genetic analyses to determine the clonal extent in the mats. Small pieces of the thalli will be added into pre-labeled tubes with silica gel (see SDS) from each site. Thus, the researchers will have 300 samples per transect. The reproductive status of each collected sample will be assessed visually under the microscope and via molecular analyses using chromosomal studies. These studies will determine the extent of clonality and population connectivity, and the reproductive state of individuals.

b. **Microbiome** - Representative samples will be collected in triplicates at each depth target of *Chondria* in addition to representative species from the Chlorophyta (green), Phaeophyceae (brown), and Rhodophyta (red) taxonomic groups. 50ml of seawater will be collected at each target depth to act as a control. Each thallus will be separated into two fractions for epibiont and endobiont sampling. Surface swabs will be taken to sample for the epibionts then each macroalgal sample will be surface sterilized to sample to endobionts. Swabs will be placed in 500ul of lysis buffer and frozen at -20°C. Macroalgal samples will then be surfaced sterilized with 1:1 mixture of sterile filtered Umonium Master and artificial seawater. Samples for endobionts will be placed in 500ul of RNA later for 24 hours and then frozen at -20°C. Water collected at depth will be filtered onto a 0.2µm filter and filters will be stabilized with RNA and frozen. These studies will determine how or if *Chondria* is changing the microbial communities on the reef, which are key for processes such as coral and macroalgal settlement and disease regulation.

c. **Stable isotopes and tissue nutrients** – Three representative samples of dominant algae from the beginning, middle, and end of the transect from each site will be collected, rinsed in DI water, and dried at 60 degrees C on the boat in a drying oven. These samples will be later ground to a fine powder, and analyzed for stable isotopes and tissue nutrients. Dried macroalgal samples brought back to the Main Hawaiian Islands will be dead. These studies will shed light on the widespread nutrient state and nutrient sources of *Chondria*, and determine if specific oceanographic processes are influencing its abundance and distribution.

d. ***Chondria* physiological ecology**- 100 individual specimens of *Chondria* will be collected from end points of the current *Chondria* distribution for immediate physiological characterization, tissue nutrient determinations and pigment extraction. Approximately half of those plants will be placed in minicosms - 1 liter glass vessels, supplied with an experimental mix of temperatures and nutrients to determine growth rates under simulated field conditions using equipment such as a Neslab Controlled temperature

water bath. At the end of growth experiments, plants will once again be subject to immediate physiological characterization, tissue nutrient determinations and pigment extraction. No live material will be returned to the Main Hawaiian Islands. Characterization of the potential invasive traits of red algae has been a long basis for experimental work in Smith's lab. These studies will augment the researcher's understanding substantially.

e. **Macroalgal biodiversity and *Chondria* genome** - Individual specimens of green, red, or brown macroalgae will be collected for characterization and identification. No more than three individuals per species will be collected to avoid over-collecting individual species. Specimens will be collected live, and a small portion of the individual will be preserved in silica gel for DNA analysis, and the remainder of the specimen frozen for pressing as a herbarium voucher on Oahu. No live material will be returned to the Main Hawaiian Islands. Characterization of the native macroalgal flora has been an ongoing research objective of the Sherwood Lab, and these opportunistic collections will support further description of newly discovered species from PMNM. Material for *Chondria* genome determination will be collected from a single location. 20 g of *Chondria* wet weight tissue will be preserved for this project.

2) Coral Collections – collected for Kealoha (see PMNM-2021-016 Kealoha - UHMCC)

3) Water samples – 30 water samples will be collected from locations around Manawai, ranging from directly over *Chondria* mats and away from mats. Samples can be collected either from the ship, or from the smaller boats. Water samples will be filtered aboard the ships, with filtered water returned overboard, and filters frozen on board to be brought back to the Sherwood lab on Oahu for analysis. No live material will be returned to the Main Hawaiian Islands.

4) *Chondria* distribution data - SCUBA and snorkeling in-water surveys of *Chondria* abundance will be compared with the 2019 *Chondria* distribution map. Similar positions will be snorkeled at ~20 ft depths by 2 persons to determine the presence of absence of Chondria around the perimeter and interior Lagoon of Manawai. SCUBA areas will involve estimates of abundance over 100 m long transects used for collections, and any other area available for swim surveys.

5) *Chondria* BMPs - Morphometrics for *Chondria* mats (i.e. mat depth and areal coverage of mats) will be completed at multiple depths and sites, and ease of removal (time for complete removal and amount of algal material remaining on reef) will be assessed. The researchers will expand and develop additional strategies to limit the spread of viable algal fragments based on previous studies with a 10% commercial bleach solution.

6) Chondria metabolites - Additional collections *Chondria tumulosa* will be collected for Dr. Karla McDermid, professor in the Marine Science Department at the University of Hawaii at Hilo, to determine what secondary metabolites in the Chondria tumulosa might be acting as herbivore deterrents. Collections (approximately 10 kg) will be made with SCUBA. Samples will be frozen in plastic bags or containers and stored in a freezer at no higher than 0° F (-18° C).

Justification of collection: Information from Pearl and Hermes, suggests that the distribution and abundance of *C. tumulosa* is not being influenced by herbivores (fish, invertebrates, or green turtles). Macroalgal species that are not readily grazed by marine herbivores often have anti-herbivores defense compounds in their tissues. An example of this is the sister genus to *Chondria*, *Laurencia*, which is well-known for its secondary metabolites, in fact, each *Laurencia* species has a distinct suite of brominated compounds. Other previously studied chemically defended species include *Portieria*

hornemanii, Plocamium sandvicensis, Asapragospsis spp., *Halimeda* spp., *Caulerpa* spp., and *Fucus* spp. An important reference for current knowledge of the chemical ecology of algae and the role of macroalgal chemical defense in structuring tropical marine communities is Amsler, C.D.. (ed.) 2008. Algal Chemical Ecology.
If sufficient quantities of secondary metabolites are extracted from *C. tumulosa*, they will be identified and used in feeding trials with known marine herbivores, such as nenue fish and sea urchins. Dr. Karla McDermid is certified by IUCAC to conduct research on vertebrates, such as fish. **Note:** The freezing process kills the alga - when the algae are defrosted, the ice crystals pierce and destroy cell membrane integrity as the melt (i.e. any metabolites sourced from the algae samples and used in feeding trials will not be viable in terms propagation).

Samples will be transported in the research ship's freezer to Honolulu, then the researcher will arrange to pick up the frozen material in Honolulu. From there, the frozen material will be shipped by air cargo or Fed Ex to Hilo.

Sample Sizes:
At each site, the researchers will collect a small clump (2-4 oz volume) of *Chondria* or abundant native algae every 1 m along a 100 m transect (determined by fin kicks). Each sample will be sealed in a plastic bag at depth and immediately processed on the boat for various analyses. The goal is to use these samples for all population genetics, reproductive state, stable isotopes and tissue nutrients, and microbiome analyses. Ideally, the researchers would be able to collect from 3 sites per day over a 10 day period, resulting in ~3000 samples for detailed analyses over a broad spatial scale around Manawai. The primary focus of intensive sampling will the Chondria. If another native species is found in high abundance, such as *Microdictyon* beds, then intensive sampling of this native species will occur for comparison to the Chondria. Otherwise, abundant native species will only be collected in replicates of three per site to avoid over-collecting individual species.

Water samples for eDNA analysis will be collected along a 100 m transect over *Chondria* mats and away from mats. 30 water samples will be collected, with each sample being 2-4 L in volume. Filtered water (samples will be filtered aboard the ship, on site) will be returned overboard. A total of 20 g of *Chondria* wet weight tissue will be preserved in RNAlater on board the ship, and frozen, for genome sequencing by colleagues at the Scripps Institution of Oceanography (B. Moore and T. Steele).

Individual specimens of green, red, or brown macroalgae will be collected for characterization and identification, in support of ongoing efforts to describe the macroalgal flora of PMNM. No more than three individuals per species will be collected to avoid over-collecting individual species. Specimens will be collected live and placed in individual plastic bags at depth, and then processed on board. A small portion of the individual will be preserved in silica gel for DNA analysis, and the remainder of the specimen frozen for pressing as an herbarium voucher on Oahu.

100 individual specimens of *Chondria* will be collected from end points of the current *Chondria* distribution for immediate physiological characterization, tissue nutrient determinations and pigment extraction. Approximately half of those plants will be placed in minicosms - 1 liter glass vessels, supplied with an experimental mix of temperatures and nutrients to determine growth rates under simulated field conditions. At the end of growth experiments, plants will once again be subject to immediate physiological characterization, tissue nutrient determinations and pigment extraction. No live material will be returned to the Main Hawaiian Islands.

Disposition of Specimens after Collection/Analysis:

Macroalgal herbarium specimens will be stored within the herbariums of Heather Spalding (CofC) and Alison Sherwood (UHM) while under study, and then submitted to the Bernice Pauahi Bishop Museum Herbarium (BISH) for final deposition upon publication. In cases where multiple isotypes can be established, one isotype will be submitted to the Smithsonian Institution to ensure redundancy of type information. Genetic sequences generated for all macroalgal and microbiome specimens will be published in online repositories (e.g. GenBank) as required for publication in peer-reviewed journals. DNA and silica-preserved specimens from macroalgae and microbiome specimens will be stored with the participating collaborators at their respective institutions (CofC, UHM, UH Maui College, UAB, and Scripps).

Filters of water samples for the eDNA project will be destroyed in the process of analysis, and DNA extracts resulting from the work will be stored in freezers in the lab of Peter Marko (UHM) or Alison Sherwood (UHM).

From the physiological ecology studies, samples will be dried at 60 deg C in an oven till dry, ground and submitted for tissue nitrogen determinations. Other samples will be extracted for Chlorophyll a in solvent using spectrophotometric analyses. Solvent samples will be part of hydrocarbon waste once they arrive at UHM and appropriately handled via Environmental Health and Safety protocols, UHM. All collected specimens of genetic, reproduction, microbial, and stable isotopes and tissue nutrient analyses will be processed immediately on the boat after collection and will be preserved for future analyses in various laboratories. The preservation of samples ensures that samples are not viable ("alive").

No organisms will be kept alive after collection of water samples for eDNA analysis, biodiversity characterization of macroalgae, or genome analysis. Water filters will be immediately frozen, and macroalgal samples will be placed in silica gel (desiccant), frozen damp, or frozen in RNAlater; all techniques ensure samples are not alive.

No organisms will be kept alive after collection and conclusion of the growth experiments. All tissues will be placed in closed system containers with lids and there is no outfall that would allow for escape of Chondria. No other organisms will be included in this study. No organisms or collected water will be released outside of their respective island or atoll of collection. Filtered water will be returned overboard, but without organisms.

The vessel will transport all collected samples and specimens out of the Monument. Upon arrival to Oahu, all specimens will be shipped via FedEx or another method of shipment to the laboratories of the researcher's respective collaborators, or hand-carried by car to laboratories at UHM.

All research occurring under this permit is highly collaborative, with the sharing of all specimens for multiple types of analyses. The same collected small "clumps" of algae will be used for genetic analyses to determine population-level connectivity, reproductive state, stable isotopes/tissue nutrients, physiology studies, the *Chondria* genome, and biodiversity from microscopic (microbiome) to macroscopic (macroalgal species) levels.

The activities proposed by the applicant directly support the Monument Management Plan's (PMNM MMP Vol. 1, 2008) priority management needs in the Marine Conservation Science action plan (3.1.1). Activities noted as part of these plans include monitoring and characterizing shallow and deep-water habitats:

• MCS-1: Continue and enhance research, characterization, and monitoring of marine ecosystems for the life of the plan, as appropriate.

The activities described above may require the following regulated activities to occur in State waters:

☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving monument resource
☒ Touching coral, living or dead
☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

The applicant would abide by the following PMNM Best Management Practices (BMPs) while conducting the aforementioned activities within the PMNM: Best Management Practices for Human Hazards to Seabirds (BMP#003); Boat Operations and Diving Activities (BMP #004); General Storage and Transport Protocols for Collected Samples (BMP#006); Special Conditions and Rules for Moving Between Islands/Atolls and Packing for Field Camps (BMP#007); Best Practices for Minimizing the Impact of Artificial Light on Sea Turtles (BMP#009); Marine Wildlife Viewing Guidelines (BMP #010); Disease and Introduced Species Prevention Protocol for Permitted Activities in the Marine Environment (BMP #011); and Best Management Practices for Maritime Heritage Sites (BMP#017).

REVIEW PROCESS:

The permit application was sent out for review and comment to the following scientific and cultural entities: Hawaii Division of Aquatic Resources, Hawaii Division of Forestry and Wildlife, Papahānaumokuākea Marine National Monument (NOAA/NOS), NOAA Pacific Islands Regional Office (NOAA-PIRO), United States Fish and Wildlife Service Hawaiian and Pacific Islands National Wildlife Refuge Complex Office, and the Office of Hawaiian Affairs (OHA). In addition, the permit application was posted on the Monument Web site in the spring of 2021, giving the public an opportunity to comment. The application was posted within 40 days of its receipt, in accordance with the Monument's Public Notification Policy

MMB Agency Reviewer Questions and Applicant Responses:

1. If not thoroughly done already, we ask that the applicant work closely with Monument staff who are familiar with the diverse array of activities that take place in the Monument in order to review the experimental design and ensure that the results of these studies accurately depict real-world situations. The results of this research will be invaluable to management.

a. We are working closely with the Monument (e.g. Randy Kosaki, Brian Hauk), DLNR, and other constituents in regards to experimental design and the management applications of this research. The existing scientific crew limitations (5 science crew) and diving restrictions (TBD) will limit the amount of research that will be realistically accomplished within the planned ~8-9 days of access at Manawai. However, we will do our best to be productive and efficient with whatever time is available.

2. Want to note that the Cultural Working Group (CWG) is interested in giving Chondria tumulosa a Hawaiian common name, so looking forward to learning more about this limu to assist in CWG's request.

a. We would be happy to work with the CWG on giving Chondria tumulosa a Hawaiian common name. Please let us know if there is specific information that you would like us to collect to accommodate this request during our upcoming cruise.

3. Please be aware working around Pearl and Hermes will require special conditions attached to your permit. Our team is in the process of finalizing a BMP on this subject and will be setting up a meeting in early May to discuss with you.

a. Mahalo. We will closely follow the rules of the BMP

4. What measures are you taking to comply with the U.S. Centers for Disease Control and Prevention current COVID-19 guidelines for keeping all listed personnel safe?

a. Access to the monument for permit activities will be occurring via a NOAA chartered vessel and in conjunction with Co-managers permit activities. All permittees will comply with measures presented by NOAA in their final COVID-19 policy for the cruise. All science personnel will also be fully vaccinated, wear masks while traveling, and will adhere to all CDC guidelines regarding COVID-19 safety protocols.

5. What measures are you taking to comply with the State of Hawaii's and City and County of Honolulu's current COVID-19 requirements, and the Board of Land and Natural Resources' current COVID-19 guidelines?
Papahānaumokuākea Marine National Monument Page 3 of 4 Permit App. #: PMNM-2021-019; Spalding
a. All science personnel travelling from the mainland will be fully vaccinated and will adhere to the current Mandatory State of Hawaii Travel and Health Form program (Safe Travels) recommendations.

6. What are your specific COVID-19 safety plans for departing from and/or returning to Hawaii?

a. All personnel departing from or returning to Hawaii will be fully vaccinated, wear a mask while traveling, and participate in the Mandatory State of Hawaii Travel and Health Form program. Current UNOLS regulations regarding COVID-19 testing and will be followed.

7. Does your proposed vessel have professionally trained medical personnel on board?

a. Yes, the medical representative will be the chamber operator from NOAA Dive Center.

8. What are your proposed vessel's capabilities/protocols in the event a medical evacuation is required?

a. Will defer to the medical evacuation protocols created by NOAA in the final cruise plan.

9. DAR requests that currently existing Pearl and Hermes protocols for BMP 011 (Disease And Introduced Species Prevention Protocol For Permitted Activities In The Marine Environment – Section D. Protocols For Conducting Operations at Pearl and Hermes Atoll) or the most recently updated

version of this protocol, be included in or referenced by the permit and implemented by the applicant during all activities conducted at Pearl and Hermes.

a. Noted

10. DAR requests to review and comment on any type of biosecurity plan or other similar plan that will be provided for the vessel research activities (on-board experiments) that include activities not covered under the current BMPs (e.g. handling of non-contained algae while on board to conduct experiments, transfer of preserved specimens back to MHI, etc.).

a. Noted

Environmental Compliance:

NEPA / HEPA: (check-one)

☒ Categorical Exclusion / Exempt Class: 5
☐ EA
☐ EIS

Other Consultations: (ESA/MMPA Section 7; NHPA Section 106, etc.)

- An informal review of all aforementioned activities following section 305(b) of the Magnuson-Stevens Fishery Conservation and Management Act (MSA; 16 U.S.C. 1855(b)) was completed on 5/11/2021. NMFS-prescribed conditions will be reflected in the PMNM permit, prior to issuance.

- On 5/4/2021, NMFS concurred that all proposed permit activities would be covered under PMNM's programmatic ESA Section 7 informal consultation. NMFS-prescribed conditions will be reflected in the PMNM permit, prior to issuance.

- To mitigate risk of spreading the *Chondria tumulosa* within the monument and main Hawaiian Islands discussions with subject matter experts occurred. Agreed upon *Chondria* mitigation language will be added into the final permit as special conditions.

The Department has made an exemption determination for this permit in accordance with Chapter 343, HRS, and Chapter 11-200.1, HAR. See Attachment ("DECLARATION OF EXEMPTION FROM THE PREPARATION OF AN ENVIRONMENTAL ASSESSMENT UNDER THE AUTHORITY OF CHAPTER 343, HRS AND CHAPTER 11-200.1 HAR, FOR PAPAHĀNAUMOKUĀKEA MARINE NATIONAL MONUMENT RESEARCH PERMIT TO DR. HEATHER SPALDING, COLLEGE OF CHARLESTON, FOR ACCESS TO STATE WATERS TO CONDUCT RESEARCH ACTIVITIES ON THE ECOLOGY, PHYSIOLOGY, AND DIVERSITY OF BENTHIC ORGANISMS IMPACTED BY THE CRYPTOGENIC ALGA *CHONDRIA TUMULOSA* IN THE NORTHWESTERN HAWAIIAN ISLANDS UNDER PERMIT PMNM-2021-019")

Has Applicant been granted a permit from the State in the past?		Yes ☐	No ☒
If so, please summarize past permits:			
Have there been any	a) violations:	Yes ☐	No ☒
	b) Late/incomplete post-activity reports:	Yes ☐	No ☒
Are there any other relevant concerns from previous permits?		Yes ☐	No ☒

STAFF OPINION:

DAR staff is of the opinion that Applicant has properly demonstrated valid justifications for their application and should be allowed to enter the NWHI State waters and to conduct the activities therein as specified in the application with certain special instructions and conditions, which are in addition to the Papahanaumokuakea Marine National Monument Conservation and Management Permit General Conditions. All suggested special conditions have been vetted through the legal counsel of the Co-Trustee agencies (see Recommendation section).

MONUMENT MANAGEMENT BOARD OPINION:

The MMB is of the opinion that the Applicant has met the findings of Presidential Proclamation 8031 and this activity may be conducted subject to completion of all compliance requirements. The MMB concurs with the special conditions recommended by NOAA, USFWS, ONMS, DAR, DOFAW and OHA staff.

RECOMMENDATION:
Based on the attached proposed declaration of exemption prepared by the department after consultation with and advice of those having jurisdiction and expertise for the proposed permit actions:

1. That the Board declare that the actions which are anticipated to be undertaken under this permit will have little or no significant effect on the environment and is therefore exempt from the preparation of an environmental assessment.

2. Upon the finding and adoption of the department's analysis by the Board, that the Board delegate and authorize the Chairperson to sign the declaration of exemption for purposes of recordkeeping requirements of chapter 343, HRS, and chapter 11-200.1, HAR.

3. That the Board authorize and approve a Research Permit to Dr. Heather Spalding, College of Charleston, for Access to State Waters to Conduct Research Activities on the Ecology, Physiology, and Diversity of Benthic Organisms Impacted by the Cryptogenic Alga *Chondria tumulosa* in the Northwestern Hawaiian Islands, with the following special conditions:

a. This permit is not to be used for nor does it authorize the sale of collected organisms. Under this permit, the authorized activities must be for noncommercial purposes not involving the use or sale of any organism, by-products, or materials collected within the Monument for obtaining patent or intellectual property rights.

b. The permittee may not convey, transfer, or distribute, in any fashion (including, but not limited to, selling, trading, giving, or loaning) any coral, live rock, or organism collected under this permit without the express written permission of the Co-Trustees.

c. To prevent introduction of disease or the unintended transport of live organisms, the permittee must comply with the disease and transport protocol attached to this permit.

d. Tenders and small vessels must be equipped with engines that meet EPA emissions requirements.

e. Refueling of tenders and all small vessels must be done at the support ships and outside the confines of lagoons or near-shore waters in the State Marine Refuge.

f. If there is any Hawaiian monk seal or any other protected species in the area when performing any permitted activity shall cease until the animal(s) depart the area, except as permitted for specific management of that species.

g. No fishing is allowed in State Waters except as authorized under State law for subsistence, traditional and customary practices by Native Hawaiians.

h. Permittee will adhere to currently existing Pearl and Hermes protocols for BMP 011 (Disease And Introduced Species Prevention Protocol For Permitted Activities In The Marine Environment – Section D. Protocols For Conducting Operations at Pearl and Hermes Atoll) or the most recently updated version of this protocol, during all activities conducted at Pearl and Hermes (note: condition language may differ in PMNM permit).

i. The permittee is required to follow all applicable Federal, State, and County laws with respect to the COVID-19 emergency response that apply at the time of departure and return. In issuance of this permit, the State of Hawaii is not otherwise monitoring or regulating permittee's compliance with COVID-19 laws and is not responsible for the health and safety of crew members, researchers or other occupants of the vessel associated with this permit.

Respectfully submitted,

Brian J. Neilson, Administrator
Division of Aquatic Resources

APPROVED FOR SUBMITTAL

Suzanne D. Case, Chairperson
Board of Land and Natural Resources

Attachments:

1) Declaration of Exemption ("DE") from the Preparation of an Environmental Assessment under the Authority of Chapter 343, HRS & Chapter 11-200.1 HAR
2) PMNM Application
3) CIS Form
4) Additional Pearl and Hermes Atoll Biosecurity Measures (supplemental to existing draft BMPs for PHA in BMP 011: Section D. Protocols For Conducting Operations at Pearl and Hermes Atoll).
5) Maps of Sampling Sites

DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

ROBERT K. MASUDA
FIRST DEPUTY

M. KALEO MANUEL
DEPUTY DIRECTOR - WATER

AQUATIC RESOURCES
BOATING AND OCEAN RECREATION
BUREAU OF CONVEYANCES
COMMISSION ON WATER RESOURCE MANAGEMENT
CONSERVATION AND COASTAL LANDS
CONSERVATION AND RESOURCES ENFORCEMENT
ENGINEERING
FORESTRY AND WILDLIFE
HISTORIC PRESERVATION
KAHOOLAWE ISLAND RESERVE COMMISSION
LAND
STATE PARKS

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

June 25, 2021

TO: Division of Aquatic Resources File

THROUGH: Suzanne D. Case, Chairperson

FROM: Brian J. Neilson, Administrator
Division of Aquatic Resources

SUBJECT:

DECLARATION OF EXEMPTION FROM THE PREPARATION OF AN ENVIRONMENTAL ASSESSMENT UNDER THE AUTHORITY OF CHAPTER 343, HRS AND CHAPTER 11-200.1 HAR, FOR A PAPAHĀNAUMOKUĀKEA MARINE NATIONAL MONUMENT RESEARCH PERMIT TO DR. HEATHER SPALDING, COLLEGE OF CHARLESTON, DEPARTMENT OF BIOLOGY, FOR ACCESS TO STATE WATERS TO CONDUCT RESEARCH ACTIVITIES ON THE ECOLOGY, PHYSIOLOGY, AND DIVERSITY OF BENTHIC ORGANISMS IMPACTED BY THE CRYPTOGENIC ALGA *CHONDRIA TUMULOSA* IN THE NORTHWESTERN HAWAIIAN ISLANDS UNDER PERMIT PMNM-2021-019.

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The following permitted activities are found to be exempted from preparation of an environmental assessment under the authority of Chapter 343, HRS and Chapter 11-200.1, HAR:

Project Title: Papahānaumokuākea Marine National Monument Research Permit to Dr. Heather Spalding, College of Charleston, Department of Biology, for Access to State Waters to Conduct Research Activities on the Ecology, Physiology, and Diversity of Benthic Organisms Impacted by the Cryptogenic Alga *Chondria tumulosa* in the Northwestern Hawaiian Islands.

Permit Number: PMNM-2021-019

Project Description: Dr. Heather Spalding of the College of Charleston, Department of Biology (applicant), proposes to collect macroalgal, coral (for PMNM-2021-016 Kealoha - UHMCC), invertebrate, and water samples to study the ecology, physiology, and diversity of benthic organisms impacted or potentially impacted by the cryptogenic alga *Chondria tumulosa* (Chondria) as well as Chondria itself in the Northwestern Hawaiian Islands.

The Research Permit, as described below, would allow entry and activities to occur in Papahanaumokuakea Marine National Monument, including the NWHI State Marine Refuge and the waters (0-3 nautical miles) surrounding the following sites:

- Nihoa Island
- Necker Island (Mokumanamana)
- French Frigate Shoals
- Gardner Pinnacles
- Laysan Island
- Lisianski Island
- Pearl and Hermes Atoll
- Midway Atoll
- Kure Atoll
- Maro Reef

The activities covered under this permit would be authorized to occur between July 2021 and June 2022.

INTENDED ACTIVITIES

This research expedition is part of a larger ONMS cruise (covered under PMNM-2021-001 Co-Trustee Managers Permit and associated memo to file), and will be complimentary to both ONMS research activities and an additional research activity to be conducted on the same cruise under PMNM-2021-016, which will collect oceanographic data that will provide insight into the factors that contribute to the presence and distribution of Chondria at Pearl and Hermes Atoll (PHA) and will increase understanding for biogeochemical and oceanographic processes that may explain the presence and proliferation of Chondria.

Pearl and Hermes Atoll Biosecurity Measures and BMP

All researchers will adhere to the Pearl and Hermes Atoll Biosecurity Measures that were drafted (see attachment) to outline the mitigation steps ONMS cruise participants will follow to ensure adequate biosecurity measures are followed to mitigate the risk associated with accessing Manawai (Pearl and Hermes Atoll or "PHA") for research activities defined in permits: PMNM-2021-001 Co-Trustee Managers Permit (and associate memo to file), PMNM-2021-016 and PMNM-2021-019. These additional biosecurity measures were drafted to address research projects and activities involving *Chondria tumulosa* which fall outside of BMP 011 (Disease and Introduced Species Prevention Protocol for Permitted Activities in the Marine Environment, Papahānaumokuākea Marine National Monument) and the currently existing (or most recent version) draft BMP for PHA in BMP 011 (section D. Protocols For Conducting Operations at Pearl and Hermes Atoll).

Specific objectives are as followed:

- Characterize current distribution of Chondria, population structure and reproductive state

- Determine the role of oceanographic phenomena in the outbreak using algal and coral tissue as a metric
- Determine impacts to other marine life through the lens of the macroalgal microbiome of native species and Chondria
- Develop invasive algal mitigation and BMPs
- Ground-truth eDNA detection of Chondria through analyses of filtered water samples at varying distances from the outbreak
- Collect tissue for determination and analysis of the nuclear genome of Chondria tumulosa
- Examine primary production and growth by Chondria.

To accomplish these objectives, the researchers will collect macroalgae, coral, benthic invertebrate, and water samples via SCUBA diving or snorkeling at multiple sites within the Northwestern Hawaiian Islands. Collected samples will be used for macroalgal biodiversity studies, eDNA analyses, *Chondria* physiological experiments on the boat while on site, macroalgal microbiome research contrasting native and cryptogenic/invasive species, *Chondria* population and reproductive state, mat-removal methodologies, fragment BMP studies, *Chondria* herbivore assessments, genome determination of *Chondria*, and macroalgal tissue nutrient and stable isotope analyses. The researchers will also collect coral samples for Kealoha (UHMCC) for stable isotope analyses. SCUBA and snorkeling surveys will be used to map the current distribution of *Chondria* as compared to 2019 at Manawai.

These activities will help the Monument by determining the current distribution of *Chondria* at Manawai, resolving oceanographic conditions via macroalgal and coral stable isotope and tissue nutrient analyses that may be influencing the abundance and distribution of *Chondria*, elucidating the connectivity and clonal state of present-day *Chondria* population(s), resolving the impact of *Chondria* on the health of the coral reef microbiome as compared to native algae, determining whether any invertebrate species are significant herbivores of *Chondria*, supporting ground-truthing of eDNA detection methodologies for *Chondria*, determining the genomic sequence of *Chondria* to compare to related species, as well as instantaneous measurement of photosynthesis for *Chondria* collected across the depth gradient from shallow to deep sites in its distribution, as initiating growth experiments that simulate those field conditions on the back deck of the research vessel. The outcomes will be targeted recommendations regarding *Chondria* containment, removal, detection, and management. This research is supported by the National Fish and Wildlife Foundation and National Science Foundation.

The researchers plan to visit Manawai (PHA). However, other islands/atolls are a possibility depending on the research needs of other scientists on the ship. At each island/atoll, the researchers will collect water samples for eDNA, macroalgae, and coral tissue samples for isotopic analysis and ecology, physiology, and biodiversity analyses. The researchers will follow best management practices for boat operations and water/biological sampling. Although *Chondria* has been identified at Manawai, it is possible that the alga has spread to other locations. Therefore, if the researchers visit other locations with *Chondria*, it will be important to collect data for comparisons to Manawai.

The invasive-like, cryptogenic alga *Chondria tumulosa* was first noted as an “unknown red alga” in low occurrence in 2016 at Manawai. By July/August 2019, it had grown into an outbreak with patches covering thousands of meters of coral reefs on the northern, western, and eastern portions of Manawai. This alga was recently described as a new species (Sherwood et al. 2020), and little is known about its present-day

ecology, physiology, reproduction, or the factors influencing its distribution and abundance. The researcher's goal is to provide the baseline data needed to properly manage and mitigate the spread of this alga to other locations within the Northwestern and Main Hawaiian Islands.
The purpose of the proposed activities for *Chondria tumulosa* are to: a) characterize its current distribution (PI Spalding), population structure and reproductive state (PI Krueger-Hadfield), b) determine the role of oceanographic phenomena in the outbreak using algal and coral tissue as a metric (PI Spalding, PI Kealoha), c) determine impacts to other marine life through the lens of the macroalgal microbiome of native species and *Chondria* (PI Fullerton and PI Spalding), d) develop invasive algal mitigation and BMPs (PI Spalding), e) ground-truth eDNA detection of *Chondria* through analyses of filtered water samples at varying distances from the outbreak (PI Sherwood, in collaboration with P. Marko and P. Nichols), f) collect tissue for determination and analysis of the nuclear genome of *Chondria tumulosa* (PI Sherwood, in collaboration with B. Moore and T. Steele) and, g) examine primary production and growth by *Chondria*.

To do this, the researchers will collect small clumps of *Chondria* and/or native macroalgae every 1 m across a 100 m transect (estimated by fin kicks) at multiple sites of high and low *Chondria* abundance, and at sites without *Chondria* for comparison. Subsamples of the same collections will be used for multiple projects (population DNA analyses, reproductive state, stable isotopes and tissues nutrients, physiology experiments, microbiome analyses, genome analyses) to maximize productivity and minimize disturbance. Coral samples for Kealoha will be collected at the same sites for combined analyses between macroalgal and coral stable isotopes. Water samples will be collected directly above, and at a series of distances away from *Chondria* mats, and filtered on board the ship for molecular analyses of the filters on Oahu to assess the efficacy of a newly developed eDNA *Chondria* detection protocol. Finally, plants collected from shallow and deep wild populations will be cleaned of epiphytes and initial readings of photosynthesis will be acquired on shipboard. These data will be coordinated with pigment analyses and tissue nitrogen from dried samples. Further work will include beginning experimental growth studies. At the end of growth experiments, small samples of plants will be extracted for Chlorophyll a.

For sampling purposes, researchers intend to target *Chondria tumulosa*; other macroalgae in the Rhodophyta, Chlorophyta, Phaeophyceae; Cyanobacteria; and corals (as described by Kealoha permit application). The primary goal of the researcher's proposed research is to determine the impact of *Chondria tumulosa* on coral reef communities at Manawai, and provide baseline data for its detection, management and possible mitigation. *Chondria* is a direct threat to the survival of coral reefs and their associated organisms in the Monument, particularly at Manawai. The researcher's mission is to only collect the specimens needed to fulfill the research objectives, and to dovetail these collections to maximize productivity and minimize impact or disturbance. Great care will be taken to minimize *Chondria* fragmentation. Collection equipment will be inspected and disinfected between sampling areas to mitigate the spread of *Chondria* and any other invasive species. Best efforts will be made to ensure that sample collection does not disturb marine life or resources in the surrounding environment. Other than the collected macroalgae (and any benthic invertebrates within the *Chondria* mats), corals, and water samples, the researchers will not touch any cultural or historical resources/artifacts within the Monument.

The researchers will collect small subsamples of macroalgae and coral colonies to limit disturbance. The information provided by this research will far outweigh the disturbance associated with macroalgal and coral collections. Managers will be provided with baseline data on *Chondria*'s ecology, physiology, genetic connectivity, and impact. This will allow managers to respond accordingly to the current algal

outbreak, as well as potential future outbreaks across the archipelago. The eDNA detection protocol will allow managers to test for the presence of *Chondria* in a non-invasive manner at any of the locations at PMNM. Sampling and experimental design were optimized for physically small samples to support population genetics, microbiome, photosynthesis, growth, tissue nitrogen and pigment research.

There is no practicable alternative to the proposed activities. The only confirmed location of this alga is at Manawai, requiring these studies to be completed at Manawai and the surrounding islands and atolls. The researchers are also interested in comparing *Chondria* distributional data between 2019 and 2021, thus requiring surveys to be completed at Manawai for this temporal comparison. The fieldwork component of this research will be performed under the minimal time required to achieve the goals and objectives of the grant. Collaborators on the grant (but under a separate permit request – see Kealoha) will be on the same research cruise. The researchers will work together using previous algal surveys to select the best sites for the researcher's studies and to minimize time while at sea. Water sampling for eDNA and CTD casts will be conducted efficiently to maximize spatial resolution and minimize activity duration. Prior to the research cruise, the researchers will complete a comprehensive analysis of all available ecological, distributional, physiological, and other data to inform all field sampling.

Research and Sampling

The following types of research and sampling will occur at the following collection locations: Most collections will occur on Manawai (Pearl and Hermes Atoll) primarily (see attachments for maps of sampling sites), but other locations may include Nihoa, Necker, FFS, Garner, Lisianski, Laysan, Midway, Maro Reef and Kure Atoll.

1) Macroalgal collections

a. **Population genetics and reproduction** - The researchers will sample a patch of *Chondria* every 1 m along a 100 m transect. The length of the transect will be site dependent such that a mat of *Chondria* on a coral reef that is only 25m wide will be sampled along 4 25m transects to achieve 100 m with samples every 1 m. Moreover, the researchers will sample these 100m transects at 3 depths, but this depends on the site. The researchers will aim for two sites that have 3 depths. This type of sampling strategy has been used by Krueger-Hadfield et al. (2016 Molecular Ecology; 2017 Ecology and Evolution; 2018 Bioinvasion Records), Bonthond et al. (2020; 2021), Sotka et al. (2018), Flanagan et al. (2021) and is the only standardized way in which to sample free-floating or drifting algae that are also likely partially clonal (Krueger-Hadfield et al. 2021; Stoeckel et al. 2021). A sample of *Chondria* will likely contain multiple thalli that are interwoven. Thus, the researchers will subsample 3 thalli that are clearly separate but interwoven for genetic analyses to determine the clonal extent in the mats. Small pieces of the thalli will be added into pre-labeled tubes with silica gel (see SDS) from each site. Thus, the researchers will have 300 samples per transect. The reproductive status of each collected sample will be assessed visually under the microscope and via molecular analyses using chromosomal studies. These studies will determine the extent of clonality and population connectivity, and the reproductive state of individuals.

b. **Microbiome** - Representative samples will be collected in triplicates at each depth target of *Chondria* in addition to representative species from the Chlorophyta (green), Phaeophyceae (brown), and Rhodophyta (red) taxonomic groups. 50ml of seawater will be collected at each target depth to act as a control. Each thallus will be separated into two fractions for epibiont and endobiont sampling. Surface swabs will be taken to sample for the epibionts then each macroalgal sample will be surface sterilized to sample to endobionts. Swabs will be placed in 500ul of lysis buffer and frozen at -20°C. Macroalgal

samples will then be surfaced sterilized with 1:1 mixture of sterile filtered Umonium Master and artificial seawater. Samples for endobionts will be placed in 500ul of RNA later for 24 hours and then frozen at -20°C. Water collected at depth will be filtered onto a 0.2µm filter and filters will be stabilized with RNA and frozen. These studies will determine how or if *Chondria* is changing the microbial communities on the reef, which are key for processes such as coral and macroalgal settlement and disease regulation.

c. **Stable isotopes and tissue nutrients** – Three representative samples of dominant algae from the beginning, middle, and end of the transect from each site will be collected, rinsed in DI water, and dried at 60 degrees C on the boat in a drying oven. These samples will be later ground to a fine powder, and analyzed for stable isotopes and tissue nutrients. Dried macroalgal samples brought back to the Main Hawaiian Islands will be dead. These studies will shed light on the widespread nutrient state and nutrient sources of *Chondria*, and determine if specific oceanographic processes are influencing its abundance and distribution.

d. ***Chondria* physiological ecology**- 100 individual specimens of *Chondria* will be collected from end points of the current *Chondria* distribution for immediate physiological characterization, tissue nutrient determinations and pigment extraction. Approximately half of those plants will be placed in minicosms - 1 liter glass vessels, supplied with an experimental mix of temperatures and nutrients to determine growth rates under simulated field conditions using equipment such as a Neslab Controlled temperature water bath. At the end of growth experiments, plants will once again be subject to immediate physiological characterization, tissue nutrient determinations and pigment extraction. No live material will be returned to the Main Hawaiian Islands. Characterization of the potential invasive traits of red algae has been a long basis for experimental work in Smith's lab. These studies will augment the researcher's understanding substantially.

e. **Macroalgal biodiversity and *Chondria* genome** - Individual specimens of green, red, or brown macroalgae will be collected for characterization and identification. No more than three individuals per species will be collected to avoid over-collecting individual species. Specimens will be collected live, and a small portion of the individual will be preserved in silica gel for DNA analysis, and the remainder of the specimen frozen for pressing as a herbarium voucher on Oahu. No live material will be returned to the Main Hawaiian Islands. Characterization of the native macroalgal flora has been an ongoing research objective of the Sherwood Lab, and these opportunistic collections will support further description of newly discovered species from PMNM. Material for *Chondria* genome determination will be collected from a single location. 20 g of *Chondria* wet weight tissue will be preserved for this project.

2) Coral Collections – collected for Kealoha (see PMNM-2021-016 Kealoha - UHMCC)

3) Water samples – 30 water samples will be collected from locations around Manawai, ranging from directly over *Chondria* mats and away from mats. Samples can be collected either from the ship, or from the smaller boats. Water samples will be filtered aboard the ships, with filtered water returned overboard, and filters frozen on board to be brought back to the Sherwood lab on Oahu for analysis. No live material will be returned to the Main Hawaiian Islands.

4) *Chondria* distribution data - SCUBA and snorkeling in-water surveys of *Chondria* abundance will be compared with the 2019 *Chondria* distribution map. Similar positions will be snorkeled at ~20 ft depths by 2 persons to determine the presence of absence of Chondria around the perimeter and interior Lagoon

of Manawai. SCUBA areas will involve estimates of abundance over 100 m long transects used for collections, and any other area available for swim surveys.

5) *Chondria* BMPs - Morphometrics for *Chondria* mats (i.e. mat depth and areal coverage of mats) will be completed at multiple depths and sites, and ease of removal (time for complete removal and amount of algal material remaining on reef) will be assessed. The researchers will expand and develop additional strategies to limit the spread of viable algal fragments based on previous studies with a 10% commercial bleach solution.

6) Chondria metabolites - Additional collections *Chondria tumulosa* will be collected for Dr. Karla McDermid, professor in the Marine Science Department at the University of Hawaii at Hilo, to determine what secondary metabolites in the Chondria tumulosa might be acting as herbivore deterrents. Collections (approximately 10 kg) will be made with SCUBA. Samples will be frozen in plastic bags or containers and stored in a freezer at no higher than 0° F (-18° C).

Justification of collection: Information from Pearl and Hermes, suggests that the distribution and abundance of *C. tumulosa* is not being influenced by herbivores (fish, invertebrates, or green turtles). Macroalgal species that are not readily grazed by marine herbivores often have anti-herbivores defense compounds in their tissues. An example of this is the sister genus to *Chondria*, *Laurencia*, which is well-known for its secondary metabolites, in fact, each *Laurencia* species has a distinct suite of brominated compounds. Other previously studied chemically defended species include *Portieria hornemanii*, *Plocamium sandvicensis*, *Asapragospsis* spp., *Halimeda* spp., *Caulerpa* spp., and *Fucus* spp. An important reference for current knowledge of the chemical ecology of algae and the role of macroalgal chemical defense in structuring tropical marine communities is Amsler, C.D.. (ed.) 2008. Algal Chemical Ecology.

If sufficient quantities of secondary metabolites are extracted from *C. tumulosa*, they will be identified and used in feeding trials with known marine herbivores, such as nenue fish and sea urchins. Dr. Karla McDermid is certified by IUCAC to conduct research on vertebrates, such as fish. **Note:** The freezing process kills the alga - when the algae are defrosted, the ice crystals pierce and destroy cell membrane integrity as the melt (i.e. any metabolites sourced from the algae samples and used in feeding trials will not be viable in terms propagation).

Samples will be transported in the research ship's freezer to Honolulu, then the researcher will arrange to pick up the frozen material in Honolulu. From there, the frozen material will be shipped by air cargo or Fed Ex to Hilo.

Sample Sizes:
At each site, the researchers will collect a small clump (2-4 oz volume) of *Chondria* or abundant native algae every 1 m along a 100 m transect (determined by fin kicks). Each sample will be sealed in a plastic bag at depth and immediately processed on the boat for various analyses. The goal is to use these samples for all population genetics, reproductive state, stable isotopes and tissue nutrients, and microbiome analyses. Ideally, the researchers would be able to collect from 3 sites per day over a 10 day period, resulting in ~3000 samples for detailed analyses over a broad spatial scale around Manawai. The primary focus of intensive sampling will the Chondria. If another native species is found in high abundance, such as *Microdictyon* beds, then intensive sampling of this native species will occur for comparison to the

Chondria. Otherwise, abundant native species will only be collected in replicates of three per site to avoid over-collecting individual species.

Water samples for eDNA analysis will be collected along a 100 m transect over *Chondria* mats and away from mats. 30 water samples will be collected, with each sample being 2-4 L in volume. Filtered water (samples will be filtered aboard the ship, on site) will be returned overboard. A total of 20 g of *Chondria* wet weight tissue will be preserved in RNAlater on board the ship, and frozen, for genome sequencing by colleagues at the Scripps Institution of Oceanography (B. Moore and T. Steele).

Individual specimens of green, red, or brown macroalgae will be collected for characterization and identification, in support of ongoing efforts to describe the macroalgal flora of PMNM. No more than three individuals per species will be collected to avoid over-collecting individual species. Specimens will be collected live and placed in individual plastic bags at depth, and then processed on board. A small portion of the individual will be preserved in silica gel for DNA analysis, and the remainder of the specimen frozen for pressing as an herbarium voucher on Oahu.

100 individual specimens of *Chondria* will be collected from end points of the current *Chondria* distribution for immediate physiological characterization, tissue nutrient determinations and pigment extraction. Approximately half of those plants will be placed in minicosms - 1 liter glass vessels, supplied with an experimental mix of temperatures and nutrients to determine growth rates under simulated field conditions. At the end of growth experiments, plants will once again be subject to immediate physiological characterization, tissue nutrient determinations and pigment extraction. No live material will be returned to the Main Hawaiian Islands.

Disposition of Specimens after Collection/Analysis:
Macroalgal herbarium specimens will be stored within the herbariums of Heather Spalding (CofC) and Alison Sherwood (UHM) while under study, and then submitted to the Bernice Pauahi Bishop Museum Herbarium (BISH) for final deposition upon publication. In cases where multiple isotypes can be established, one isotype will be submitted to the Smithsonian Institution to ensure redundancy of type information. Genetic sequences generated for all macroalgal and microbiome specimens will be published in online repositories (e.g. GenBank) as required for publication in peer-reviewed journals. DNA and silica-preserved specimens from macroalgae and microbiome specimens will be stored with the participating collaborators at their respective institutions (CofC, UHM, UH Maui College, UAB, and Scripps).

Filters of water samples for the eDNA project will be destroyed in the process of analysis, and DNA extracts resulting from the work will be stored in freezers in the lab of Peter Marko (UHM) or Alison Sherwood (UHM).

From the physiological ecology studies, samples will be dried at 60 deg C in an oven till dry, ground and submitted for tissue nitrogen determinations. Other samples will be extracted for Chlorophyll a in solvent using spectrophotometric analyses. Solvent samples will be part of hydrocarbon waste once they arrive at UHM and appropriately handled via Environmental Health and Safety protocols, UHM.

All collected specimens of genetic, reproduction, microbial, and stable isotopes and tissue nutrient analyses will be processed immediately on the boat after collection and will be preserved for future analyses in various laboratories. The preservation of samples ensures that samples are not viable ("alive").

No organisms will be kept alive after collection of water samples for eDNA analysis, biodiversity characterization of macroalgae, or genome analysis. Water filters will be immediately frozen, and macroalgal samples will be placed in silica gel (desiccant), frozen damp, or frozen in RNAlater; all techniques ensure samples are not alive.

No organisms will be kept alive after collection and conclusion of the growth experiments. All tissues will be placed in closed system containers with lids and there is no outfall that would allow for escape of Chondria. No other organisms will be included in this study. No organisms or collected water will be released outside of their respective island or atoll of collection. Filtered water will be returned overboard, but without organisms.

The vessel will transport all collected samples and specimens out of the Monument. Upon arrival to Oahu, all specimens will be shipped via FedEx or another method of shipment to the laboratories of the researcher's respective collaborators, or hand-carried by car to laboratories at UHM.

All research occurring under this permit is highly collaborative, with the sharing of all specimens for multiple types of analyses. The same collected small "clumps" of algae will be used for genetic analyses to determine population-level connectivity, reproductive state, stable isotopes/tissue nutrients, physiology studies, the *Chondria* genome, and biodiversity from microscopic (microbiome) to macroscopic (macroalgal species) levels.

The activities proposed by the applicant directly support the Monument Management Plan's (PMNM MMP Vol. 1, 2008) priority management needs in the Marine Conservation Science action plan (3.1.1). Activities noted as part of these plans include monitoring and characterizing shallow and deep-water habitats:

• MCS-1: Continue and enhance research, characterization, and monitoring of marine ecosystems for the life of the plan, as appropriate.

The activities described above may require the following regulated activities to occur in State waters:

☒ Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving monument resource

☒ Touching coral, living or dead

☒ Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

The applicant would abide by the following PMNM Best Management Practices (BMPs) while conducting the aforementioned activities within the PMNM: Best Management Practices for Human Hazards to Seabirds (BMP#003); Boat Operations and Diving Activities (BMP #004); General Storage and Transport Protocols for Collected Samples (BMP#006); Special Conditions and Rules for Moving Between

Islands/Atolls and Packing for Field Camps (BMP#007); Best Practices for Minimizing the Impact of Artificial Light on Sea Turtles (BMP#009); Marine Wildlife Viewing Guidelines (BMP #010); Disease and Introduced Species Prevention Protocol for Permitted Activities in the Marine Environment (BMP #011); and Best Management Practices for Maritime Heritage Sites (BMP#017).

REVIEW PROCESS:

The permit application was sent out for review and comment to the following scientific and cultural entities: Hawaii Division of Aquatic Resources, Hawaii Division of Forestry and Wildlife, Papahānaumokuākea Marine National Monument (NOAA/NOS), NOAA Pacific Islands Regional Office (NOAA-PIRO), United States Fish and Wildlife Service Hawaiian and Pacific Islands National Wildlife Refuge Complex Office, and the Office of Hawaiian Affairs (OHA). In addition, the permit application was posted on the Monument Web site in the spring of 2021, giving the public an opportunity to comment. The application was posted within 40 days of its receipt, in accordance with the Monument's Public Notification Policy

MMB Agency Reviewer Questions and Applicant Responses:

1. If not thoroughly done already, we ask that the applicant work closely with Monument staff who are familiar with the diverse array of activities that take place in the Monument in order to review the experimental design and ensure that the results of these studies accurately depict real-world situations. The results of this research will be invaluable to management.

a. We are working closely with the Monument (e.g. Randy Kosaki, Brian Hauk), DLNR, and other constituents in regards to experimental design and the management applications of this research. The existing scientific crew limitations (5 science crew) and diving restrictions (TBD) will limit the amount of research that will be realistically accomplished within the planned ~8-9 days of access at Manawai. However, we will do our best to be productive and efficient with whatever time is available.

2. Want to note that the Cultural Working Group (CWG) is interested in giving Chondria tumulosa a Hawaiian common name, so looking forward to learning more about this limu to assist in CWG's request.

a. We would be happy to work with the CWG on giving Chondria tumulosa a Hawaiian common name. Please let us know if there is specific information that you would like us to collect to accommodate this request during our upcoming cruise.

3. Please be aware working around Pearl and Hermes will require special conditions attached to your permit. Our team is in the process of finalizing a BMP on this subject and will be setting up a meeting in early May to discuss with you.

a. Mahalo. We will closely follow the rules of the BMP

4. What measures are you taking to comply with the U.S. Centers for Disease Control and Prevention current COVID-19 guidelines for keeping all listed personnel safe?

a. Access to the monument for permit activities will be occurring via a NOAA chartered vessel and in conjunction with Co-managers permit activities. All permittees will comply with measures presented by NOAA in their final COVID-19 policy for the cruise. All science personnel will also be fully vaccinated, wear masks while traveling, and will adhere to all CDC guidelines regarding COVID-19 safety protocols.

5. What measures are you taking to comply with the State of Hawaii's and City and County of Honolulu's current COVID-19 requirements, and the Board of Land and Natural Resources' current COVID-19 guidelines?
Papahānaumokuākea Marine National Monument Page 3 of 4 Permit App. #: PMNM-2021-019; Spalding
a. All science personnel travelling from the mainland will be fully vaccinated and will adhere to the current Mandatory State of Hawaii Travel and Health Form program (Safe Travels) recommendations.

6. What are your specific COVID-19 safety plans for departing from and/or returning to Hawaii?

a. All personnel departing from or returning to Hawaii will be fully vaccinated, wear a mask while traveling, and participate in the Mandatory State of Hawaii Travel and Health Form program. Current UNOLS regulations regarding COVID-19 testing and will be followed.

7. Does your proposed vessel have professionally trained medical personnel on board?

a. Yes, the medical representative will be the chamber operator from NOAA Dive Center.

8. What are your proposed vessel's capabilities/protocols in the event a medical evacuation is required?

a. Will defer to the medical evacuation protocols created by NOAA in the final cruise plan.

9. DAR requests that currently existing Pearl and Hermes protocols for BMP 011 (Disease And Introduced Species Prevention Protocol For Permitted Activities In The Marine Environment – Section D. Protocols For Conducting Operations at Pearl and Hermes Atoll) or the most recently updated version of this protocol, be included in or referenced by the permit and implemented by the applicant during all activities conducted at Pearl and Hermes.

a. Noted

10. DAR requests to review and comment on any type of biosecurity plan or other similar plan that will be provided for the vessel research activities (on-board experiments) that include activities not covered under the current BMPs (e.g. handling of non-contained algae while on board to conduct experiments, transfer of preserved specimens back to MHI, etc.).

a. Noted

Environmental Compliance:

NEPA / HEPA: (check-one)

☒ Categorical Exclusion / Exempt Class: 5
☐ EA
☐ EIS

Other Consultations: (ESA/MMPA Section 7; NHPA Section 106, etc.)

- An informal review of all aforementioned activities following section 305(b) of the Magnuson-Stevens Fishery Conservation and Management Act (MSA; 16 U.S.C. 1855(b)) was completed on 5/11/2021. NMFS-prescribed conditions will be reflected in the PMNM permit, prior to issuance.

- On 5/4/2021, NMFS concurred that all proposed permit activities would be covered under PMNM's programmatic ESA Section 7 informal consultation. NMFS-prescribed conditions will be reflected in the PMNM permit, prior to issuance.

- To mitigate risk of spreading the *Chondria tumulosa* within the monument and main Hawaiian Islands discussions with subject matter experts occurred. Agreed upon *Chondria* mitigation language will be added into the final permit as special conditions.

Has Applicant been granted a permit from the State in the past? Yes ☐ No ☒

If so, please summarize past permits:

Have there been any a) violations: Yes ☐ No ☒
b) Late/incomplete post-activity reports: Yes ☐ No ☒

Are there any other relevant concerns from previous permits? Yes ☐ No ☒

Consulted Parties: The permit application was sent out for review and comment to the following scientific and cultural entities: Hawaii Division of Aquatic Resources, Hawaii Division of Forestry and Wildlife, Papahānaumokuākea Marine National Monument (NOAA/NOS), NOAA Pacific Islands Regional Office (NOAA-PIRO), United States Fish and Wildlife Service Hawaiian and Pacific Islands National Wildlife Refuge Complex Office, and the Office of Hawaiian Affairs (OHA). In addition, the permit application has been posted on the Monument Web site, giving the public an opportunity to comment. The application was posted within 40 days of its receipt, in accordance with the Monument's Public Notification Policy.

Exemption Determination: After reviewing §11-200.1-15, HAR, including the criteria used to determine significance under §11-200.1-13, HAR, DLNR has concluded that the activities under this permit would have minimal or no significant effect on the environment and that issuance of the permit is categorically exempt from the requirement to prepare an environmental assessment based on the following analysis:

1. All activities associated with this permit have been evaluated as a single action. Since this permit involves an activity that is precedent to a later planned activity, i.e., the same methodology used throughout the permit period, the categorical exemption determination here will treat all planned activities as a single action under §11-200.1-10, HAR.

2. The General Exemption Type #5 for Basic Data Collection, Research and Experimental Management with no Serious or Major Environmental Disturbance Appears to Apply. §11-200.1-16 (a) (1) and §11-200.1-16 (a) (2), HAR, exempts the class of actions that involve "basic data collection, research, experimental management, and resource evaluation activities which do not result in a serious or major disturbance to an environmental resource." This exemption type has been interpreted to include the collection of the limited amounts of macroalgal, coral, invertebrate, and water samples, such as those being proposed.

The proposed activities here appear to fall squarely under the general exemption type identified under HAR §11-200.1-16 (a) (1) and §11-200.1-16 (a) (2), as described under the revised 2020 DLNR Exemption List (Concurred on by the Environmental Council on November 10, 2020), under the general exemption type #5 (Part 1), items #13 and #15 and (Part 2), item #4, which includes, respectively, "research that the Department declares is designed specifically to monitor, conserve, or enhance native species or native species' habitat", "game and non-game wildlife surveys, vegetation and rare plant surveys, aquatic life surveys, inventory studies, new transect lines, photographing, recording, sampling, collection, culture, and captive propagation" and "experimental management actions that the Department declares are designed specifically to monitor, conserve, or enhance native species or native species' habitat."

The applicant would abide by the following PMNM Best Management Practices (BMPs) while conducting the aforementioned activities within the PMNM: Best Management Practices for Human Hazards to Seabirds (BMP#003); Boat Operations and Diving Activities (BMP #004); General Storage and Transport Protocols for Collected Samples (BMP#006); Special Conditions and Rules for Moving Between Islands/Atolls and Packing for Field Camps (BMP#007); Best Practices for Minimizing the Impact of Artificial Light on Sea Turtles (BMP#009); Marine Wildlife Viewing Guidelines (BMP #010); Disease and Introduced Species Prevention Protocol for Permitted Activities in the Marine Environment (BMP #011); and Best Management Practices for Maritime Heritage Sites (BMP#017).

As discussed below, no significant disturbance to any environmental resource is anticipated. Thus, so long as the below considerations are met, the general exemption types should include the action now contemplated.

3. Cumulative Impacts of Actions in the Same Place and Impacts with Respect to the Potentially Particularly Sensitive Environment Will Not be Significant. Even where a categorical exemption appears to include a proposed action, the action cannot be declared exempt if "the cumulative impact of planned successive actions in the same place, over time, is significant, or when an action that is normally insignificant in its impact on the environment may be significant in a particularly sensitive environment." §11-200.1-15 (d), HAR. To gauge whether a significant impact or effect is probable, an exempting agency must consider every phase of a proposed action, any expected primary and secondary consequences, the

long-term and short-term effects of the action, the overall and cumulative effect of the action, and the sum effects of an action on the quality of the environment. §11-200.1-13, HAR.

The researchers on this project will collect small subsamples of macroalgae and coral colonies to limit disturbance. The information provided by this research will far outweigh the disturbance associated with macroalgal and coral collections. Managers will be provided with baseline data on *Chondria*'s ecology, physiology, genetic connectivity, and impact. This will allow managers to respond accordingly to the current algal outbreak, as well as potential future outbreaks across the archipelago. The eDNA detection protocol will allow managers to test for the presence of *Chondria* in a non-invasive manner at any of the locations at PMNM. Sampling and experimental design were optimized for physically small samples to support population genetics, microbiome, photosynthesis, growth, tissue nitrogen and pigment research. With that in mind, significant cumulative impacts are not anticipated as a result of this activity, and numerous safeguards further ensure that the potentially sensitive environment of the project area will not be significantly affected. All activities will be conducted in a manner compatible with the management direction of the Monument Proclamation in that the activities do not diminish monument resources, qualities, and ecological integrity, or have any indirect, secondary, cultural, or cumulative effects. The joint permit review process did not reveal any anticipated indirect or cumulative impacts that would occur as a result of these activities.

Since no significant cumulative impacts or significant impacts with respect to any particularly sensitive aspect of the project area are anticipated, the categorical exemptions identified above should remain applicable.

4. Overall Impacts will Probably have a Minimal or No Significant Effect on the Environment. Any foreseeable impacts from the proposed activity will probably be minimal, and further mitigated by general and specific conditions attached to the permit. Specifically, all research activities covered by this permit will be carried out with strict safeguards for the natural, historic, and cultural resources of the Monument as required by Presidential Proclamation 8031, other applicable law and agency policies and standard operating procedures.

Conclusion. Upon consideration of the permit to be approved by the Board of Land and Natural Resources, the potential effects of the above listed project as provided by Chapter 343, HRS and Chapter 11-200.1 HAR, have been determined to be of probable minimal or no significant effect on the environment and exempt from the preparation of an environmental assessment.

Papahānaumokuākea Marine National Monument

RESEARCH Permit Application

NOTE: ***This Permit Application (and associated Instructions) are to propose activities to be conducted in the Papahānaumokuākea Marine National Monument. The Co-Trustees are required to determine that issuing the requested permit is compatible with the findings of Presidential Proclamation 8031. Within this Application, provide all information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Papahānaumokuākea Marine National Monument (Monument).***

ADDITIONAL IMPORTANT INFORMATION:

- Any or all of the information within this application may be posted to the Monument website informing the public on projects proposed to occur in the Monument.

- In addition to the permit application, the Applicant must either download the Monument Compliance Information Sheet from the Monument website OR request a hard copy from the Monument Permit Coordinator (contact information below). The Monument Compliance Information Sheet must be submitted to the Monument Permit Coordinator after initial application consultation.

- Issuance of a Monument permit is dependent upon the completion and review of the application and Compliance Information Sheet.

INCOMPLETE APPLICATIONS WILL NOT BE CONSIDERED

Send Permit Applications to:
NOAA/Inouye Regional Center
NOS/ONMS/PMNM/Attn: Permit Coordinator
1845 Wasp Blvd, Building 176
Honolulu, HI 96818
nwhipermit@noaa.gov
PHONE: (808) 725-5800 FAX: (808) 455-3093

SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT REQUIRED. FOR ADDITIONAL SUBMITTAL INSTRUCTIONS, SEE THE LAST PAGE.

Papahānaumokuākea Marine National Monument
Permit Application Cover Sheet

This Permit Application Cover Sheet is intended to provide summary information and status to the public on permit applications for activities proposed to be conducted in the Papahānaumokuākea Marine National Monument. While a permit application has been received, it has not been fully reviewed nor approved by the Monument Management Board to date. The Monument permit process also ensures that all environmental reviews are conducted prior to the issuance of a Monument permit.

Summary Information

Applicant Name: Heather Spalding

Affiliation: Department of Biology, College of Charleston

Permit Category: Research
Proposed Activity Dates: June 1 – August 30, 2021 depending on vessel availability
Proposed Method of Entry (Vessel/Plane): Vessel
Proposed Locations: Northwestern Hawaiian Islands, including Nihoa, FFS, LIS, LAY, PHA, MID, KUR, Gardner, Maro and Mokumanamana

Estimated number of individuals (including Applicant) to be covered under this permit: 14 individuals

Estimated number of days in the Monument: 44 days

Description of proposed activities: (complete these sentences):

a.) The proposed activity would collect macroalgal, coral (for Kealoha, UHMCC), invertebrate, and water samples to study the ecology, physiology, and diversity of benthic organisms impacted or potentially impacted by the cryptogenic alga *Chondria tumulosa* (*Chondria*) as well as *Chondria* itself in the Northwestern Hawaiian Islands.

b.) To accomplish this activity, we would collect macroalgae, coral, benthic invertebrate, and water samples via SCUBA diving or snorkeling at multiple sites within the Northwestern Hawaiian Islands. Collected samples will be used for macroalgal biodiversity studies, eDNA analyses, *Chondria* physiological experiments on the boat while on site, macroalgal microbiome research contrasting native and cryptogenic/invasive species, *Chondria* population and reproductive state, mat-removal methodologies, fragment BMP studies, *Chondria* herbivore assessments, genome determination of *Chondria*, and macroalgal tissue nutrient and stable isotope analyses. We will also collect coral samples for Kealoha (UHMCC) for stable isotope analyses. SCUBA and snorkeling surveys

will be used to map the current distribution of *Chondria* as compared to 2019 at Manawai.

c.) These activities would help the Monument by determining the current distribution of *Chondria* at Manawai, resolving oceanographic conditions via macroalgal and coral stable isotope and tissue nutrient analyses that may be influencing the abundance and distribution of *Chondria*, elucidating the connectivity and clonal state of present-day *Chondria* population(s), resolving the impact of *Chondria* on the health of the coral reef microbiome as compared to native algae, determining whether any invertebrate species are significant herbivores of *Chondria*, supporting ground-truthing of eDNA detection methodologies for *Chondria*, determining the genomic sequence of *Chondria* to compare to related species, as well as instantaneous measurement of photosynthesis for *Chondria* collected across the depth gradient from shallow to deep sites in its distribution, as initiating growth experiments that simulate those field conditions on the back deck of the research vessel. The outcomes will be targeted recommendations regarding *Chondria* containment, removal, detection, and management.

Other information or background:
This research is supported by the National Fish and Wildlife Foundation and National Science Foundation.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Spalding, Heather L.

Title: Assistant Professor

1a. Intended field Principal Investigator (See instructions for more information):
Spalding, Heather L

2. Mailing address (street/P.O. box, city, state, country, zip):

SEE ORIGINAL APPLICATION FOR CONTACT INFO

Phone: SEE ORIGINAL APPLICATION FOR CONTACT INFO

Fax:

Email: SEE ORIGINAL APPLICATION FOR CONTACT INFO

3. Affiliation (institution/agency/organization directly related to the proposed project):
Department of Biology, College of Charleston
School of Life Sciences, University of Hawaii at Manoa
Department of Biology, University of Alabama Birmingham
University of Hawaii Maui College (collecting for Kealoha permit)

4. Additional persons to be covered by permit. List all personnel roles and names (if known at time of application) here (e.g. John Doe, Research Diver; Jane Doe, Field Technician):
1) Heather Spalding, PI/Research Diver, College of Charleston
2) Taylor Williams, Research Diver, College of Charleston
3) Heather Fullerton, Scientist, College of Charleston
4) Gabbie Kuba, Field Assistant, College of Charleston
5) Stacy Krueger-Hadfield, Scientist, University of Alabama Birmingham
6) Andrea Kealoha, UHMCC (see Kealoha permit for information)
7) Alison Sherwood, Scientist, University of Hawaii at Manoa
8) Jimmy Fumo, Research Diver and Scientist, University of Hawaii at Manoa
9) Peter Marko, Scientist, University of Hawaii at Manoa

NOTE: SEE ORIGINAL APPLICATION FOR CONTACT INFO FOR ALL RESEARCHERS

10) Patrick Nichols, Research Diver and Scientist, University of Hawaii at Manoa
11) Bradley Moore, Scientist, Scripps Institution of Oceanography
12) Taylor Steele, Scientist, Scripps Institution of Oceanography
13) Celia Smith, Scientist, University of Hawaii at Manoa
14) Jane Doe, Physiological ecology research assistant

NOTE: SEE ORIGINAL APPLICATION FOR CONTACT INFO FOR ALL RESEARCHERS

Section B: Project Information

5a. Project location(s):

		Ocean Based	
X Nihoa Island	☐ Land-based	X Shallow water	☐ Deep water
X Necker Island (Mokumanamana)	☐ Land-based	X Shallow water	☐ Deep water
X French Frigate Shoals	☐ Land-based	X Shallow water	☐ Deep water
X Gardner Pinnacles	☐ Land-based	X Shallow water	☐ Deep water
X Maro Reef			
X Laysan Island	☐ Land-based	X Shallow water	☐ Deep water
X Lisianski Island, Neva Shoal	☐ Land-based	X Shallow water	☐ Deep water
X Pearl and Hermes Atoll	☐ Land-based	X Shallow water	☐ Deep water
X Midway Atoll	☐ Land-based	X Shallow water	☐ Deep water
X Kure Atoll	☐ Land-based	X Shallow water	☐ Deep water
☐ Monument Expansion Area			
☐ Other			

NOTE: Shallow water is defined by water less than 100 meters in depth.

☐ Remaining ashore on any island or atoll (with the exception of Sand Island, at Midway Atoll and field camp staff on other islands/atolls) between sunset and sunrise.

NOTE: There is a fee schedule for people visiting Midway Atoll National Wildlife Refuge via vessel and aircraft.

Location Description:

We plan to visit Manawai (PHA). However, other islands/atolls are a possibility depending on the research needs of other scientists on the ship. At each island/atoll, we will collect water samples for eDNA, macroalgae, and coral tissue samples for isotopic analysis and ecology, physiology, and biodiversity analyses. We will follow best management practices for boat operations and water/biological sampling. Although *Chondria* has been identified at Manawai, it is possible that the alga has spread to other locations. Therefore, if we visit other locations with *Chondria*, it will be important to collect data for comparisons to Manawai.

5b. Check all applicable regulated activities proposed to be conducted in the Monument:

X Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving Monument resource

☐ Drilling into, dredging, or otherwise altering the submerged lands other than by anchoring a vessel; or constructing, placing, or abandoning any structure, material, or other matter on the submerged lands

☐ Anchoring a vessel

☐ Deserting a vessel aground, at anchor, or adrift

☐ Discharging or depositing any material or matter into the Monument
X Touching coral, living or dead
☐ Possessing fishing gear except when stowed and not available for immediate use during passage without interruption through the Monument
☐ Attracting any living Monument resource
☐ Sustenance fishing (Federal waters only, outside of Special Preservation Areas, Ecological Reserves and Special Management Areas)
☐ Subsistence fishing (State waters only)
X Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

6. Purpose/Need/Scope ***State purpose of proposed activities:***
The invasive-like, cryptogenic alga *Chondria tumulosa* was first noted as an "unknown red alga" in low occurrence in 2016 at Manawai. By July/August 2019, it had grown into an outbreak with patches covering thousands of meters of coral reefs on the northern, western, and eastern portions of Manawai. This alga was recently described as a new species (Sherwood et al. 2020), and little is known about its present-day ecology, physiology, reproduction, or the factors influencing its distribution and abundance. Our goal is to provide the baseline data needed to properly manage and mitigate the spread of this alga to other locations within the Northwestern and Main Hawaiian Islands.

The purpose of the proposed activities for *Chondria tumulosa* are to: a) characterize its current distribution (PI Spalding), population structure and reproductive state (PI Krueger-Hadfield), b) determine the role of oceanographic phenomena in the outbreak using algal and coral tissue as a metric (PI Spalding, PI Kealoha), c) determine impacts to other marine life through the lens of the macroalgal microbiome of native species and *Chondria* (PI Fullerton and PI Spalding), d) develop invasive algal mitigation and BMPs (PI Spalding), e) ground-truth eDNA detection of *Chondria* through analyses of filtered water samples at varying distances from the outbreak (PI Sherwood, in collaboration with P. Marko and P. Nichols), f) collect tissue for determination and analysis of the nuclear genome of *Chondria tumulosa* (PI Sherwood, in collaboration with B. Moore and T. Steele) and, g) examine primary production and growth by *Chondria*.

To do this, we will collect small clumps of *Chondria* and/or native macroalgae every 1 m across a 100 m transect (estimated by fin kicks) at multiple sites of high and low *Chondria* abundance, and at sites without *Chondria* for comparison. Subsamples of the same collections will be used for multiple projects (population DNA analyses, reproductive state, stable isotopes and tissues nutrients, physiology experiments, microbiome analyses, genome analyses) to maximize productivity and minimize disturbance. Coral samples for Kealoha will be collected at the same sites for combined analyses between macroalgal and coral stable isotopes. Water samples will be collected directly above, and at a series of distances away from *Chondria* mats, and filtered on board the ship for molecular analyses of the filters on Oahu to assess the efficacy of a newly developed eDNA *Chondria* detection protocol. Finally, plants collected from shallow and deep wild populations will be cleaned of epiphytes and initial readings of photosynthesis will be acquired on shipboard.These data will be coordinated with pigment analyses and tissue nitrogen from dried samples. Further work will include beginning experimental growth studies. At the end of growth experiments, small samples of plants will be extracted for Chlorophyll a.

*Considering the purpose of the proposed activities, do you intend to film / photograph federally protected species beyond the protocols provided in PMNM Best Management Practices (https://www.papahanaumokuakea.gov/permit/bestmanagement.html)? Yes ☐ No X

If so, please list the species you specifically intend to target.
Chondria tumulosa; other macroalgae in the Rhodophyta, Chlorophyta, Phaeophyceae; Cyanobacteria; and corals (as described by Kealoha permit application)

For a list of terrestrial species protected under the Endangered Species Act visit:
http://www.fws.gov/endangered/
For a list of marine species protected under the Endangered Species Act visit:
http://www.nmfs.noaa.gov/pr/species/esa/
For information about species protected under the Marine Mammal Protection Act visit:
http://www.nmfs.noaa.gov/pr/laws/mmpa/

7. Answer the Findings below by providing information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historic, and cultural resources of the Monument:

The Findings are as follows:

a. How can the activity be conducted with adequate safeguards for the cultural, natural and historic resources and ecological integrity of the Monument?

The primary goal of our proposed research is to determine the impact of *Chondria tumulosa* on coral reef communities at Manawai, and provide baseline data for its detection, management and possible mitigation. *Chondria* is a direct threat to the survival of coral reefs and their associated organisms in the Monument, particularly at Manawai. Our mission is to only collect the specimens needed to fulfill our research objectives, and to dovetail these collections to maximize productivity and minimize impact or disturbance. Great care will be taken to minimize *Chondria* fragmentation. Collection equipment will be inspected and disinfected between sampling areas to mitigate the spread of *Chondria* and any other invasive species. Best efforts will be made to ensure that sample collection does not disturb marine life or resources in the surrounding environment. Other than the collected macroalgae (and any benthic invertebrates within the *Chondria* mats), corals, and water samples, we will not touch any cultural or historical resources/artifacts within the Monument.

b. How will the activity be conducted in a manner compatible with the management direction of this proclamation, considering the extent to which the conduct of the activity may diminish or enhance Monument cultural, natural and historic resources, qualities, and ecological integrity, any indirect, secondary, or cumulative effects of the activity, and the duration of such effects?

We will collect small subsamples of macroalgae and coral colonies to limit disturbance. The information provided by this research will far outweigh the disturbance associated with macroalgal and coral collections. Managers will be provided with baseline data on *Chondria*'s ecology, physiology, genetic connectivity, and impact. This will allow managers to respond accordingly to the current algal outbreak, as well as potential

future outbreaks across the archipelago. The eDNA detection protocol will allow managers to test for the presence of *Chondria* in a non-invasive manner at any of the locations at PMNM. Sampling and experimental design were optimized for physically small samples to support population genetics, microbiome, photosynthesis, growth, tissue nitrogen and pigment research.

c. Is there a practicable alternative to conducting the activity within the Monument? If not, explain why your activities must be conducted in the Monument.

There is no practicable alternative to the proposed activities. The only confirmed location of this alga is at Manawai, requiring these studies to be completed at Manawai and the surrounding islands and atolls. We are also interested in comparing *Chondria* distributional data between 2019 and 2021, thus requiring surveys to be completed at Manawai for this temporal comparison.

d. How does the end value of the activity outweigh its adverse impacts on Monument cultural, natural and historic resources, qualities, and ecological integrity?

The management implications and value of this research far outweighs the adverse impact on Monument resources and ecological integrity. The Manawai ecosystem has been devastated by the *Chondria* outbreak, and its spread to other islands/atolls in the Hawaiian Archipelago is unknown. The information generated by the proposed activities will help scientists and managers better understand *Chondria*'s impact and the mechanisms enhancing its success at Manawai. These data may help to predict and/or prevent the spread of *Chondria* within the Monument and across the Hawaiian Archipelago.

e. Explain how the duration of the activity is no longer than necessary to achieve its stated purpose.

The fieldwork component of this research will be performed under the minimal time required to achieve the goals and objectives of the grant. Collaborators on the grant (but under a separate permit request – see Kealoha) will be on the same research cruise. We will work together using previous algal surveys to select the best sites for our studies and to minimize time while at sea. Water sampling for eDNA and CTD casts will be conducted efficiently to maximize spatial resolution and minimize activity duration. Prior to the research cruise, we will complete a comprehensive analysis of all available ecological, distributional, physiological, and other data to inform all field sampling.

f. Provide information demonstrating that you are qualified to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

Dr. Heather Spalding is an Assistant Professor at the College of Charleston and received her Ph.D. from the University of Hawaii at Manoa on Hawaiian macroalgal ecology, physiology, and biodiversity. She will be the PI leading the field collections for

this permit in coordination with Kealoha. Spalding has a thorough understanding of the logistical and permitting needs to work in this location and has partnered with Dr. R. Kosaki (PMNM) to share specimens and data under the same permit on past cruises. Spalding has conducted research on macroalgal diversity in the Northwestern Hawaiian Islands since 2010, and has over 25 years of diving and field experience. She has served as Chief Scientist on over 20 NOAA-funded research cruises in California and Hawaiʻi, and was the lead phycologist on the 2019 NOAA cruise that documented *Chondria* at Manawai. Spalding has worked with cultural practitioners in naming new Hawaiian species and has conducted extensive community outreach in Hawaiʻi, including partnerships with KUA, Malama Maunalua, GK-12 outreach in public and private schools, and curriculum development (e.g. Spalding et al. 2009, 2010). Spalding has co-authored 45 peer-reviewed journal articles and book chapters on macroalgal ecology, diversity, and mesophotic coral ecosystems. She is currently the co-Chair of the Gordon Research Conference on mesophotic coral ecosystems, and is working with three graduate students (MS) studying macroalgal dominated ecosystems in the Main and Northwestern Hawaiian Islands.

Dr. Heather Fullerton is an Assistant Professor at the College of Charleston and will be the PI leading the microbial community research. Fullerton has over 15 years of experience using molecular biology approaches to understanding microbial communities and microbial metabolisms, with a focus on diverse environments and the microbial impacts on biogeochemical cycling. She has mentored 12 undergraduate and seven master's students in microbial ecology and bioinformatics. Fullerton's research utilizes culture-independent high-throughput sequencing approaches to reconstruct community structure and examine metabolic pathways. Fullerton has participated on a number of NSF-funded multidisciplinary oceanographic research cruises and conducts research on microbial communities from Lōʻihi Seamount in the Main Hawaiian Islands. Fullerton is actively engaged in mentoring and recruitment. The students in her lab, all women and other underrepresented minorities in STEM, regularly present and publish their research at international conferences and in peer-reviewed journals.

Dr. Stacy Krueger-Hadfield is an Assistant Professor at the University of Alabama at Birmingham and will be the PI leading invasive algal population genetics and reproductive phenology. Krueger-Hadfield has over 15 years of experience as an evolutionary ecologist, with an emphasis on algae. She has mentored 41 early-career scientists since 2009 (3 post-docs, 5 PhD students, 9 MS students, 15 BS students, 4 postbac, 3 high school). Krueger-Hadfield has developed novel tools for studying the population genetics of organisms with complex life cycles (i.e., more than one free-living stage) and with mixed reproductive systems. She was recognized as the second Norma J. Lang Early Career Fellow by the Phycological Society of America to further this work in 2018. She has published 41 peer and 2 editor reviewed journal articles, with 7 manuscripts in review or in revision after review (17 as first author, 10 as senior author). She is an internationally recognized science communicator: (i) she has written over 100 popular science articles and blog posts (e.g., >85 posts for The Molecular Ecologist,

including documenting and chronicling field work that will be continued under the auspices of the proposed research); (ii) she has provided opportunities for >30 students to write for both The Molecular Ecologist through the #StudentSciComm series and the American Genetics Association Blog; (iii) she serves as the Editor of Social Media for the American Genetics Association; (iv) she was invited by the Ecological Society of America as a #MySciComm contributor; and (v) she was profiled by the Society of the Study of Evolution New Faculty series.

Dr. Alison Sherwood is a Professor in the School of Life Sciences at the University of Hawaii at Manoa, and the Interim Associate Dean of the College of Natural Sciences at the University of Hawaii at Manoa. She is a leading world expert on algal diversity and the description of novel species, with 100 publications (approximately 80 based on the Hawaiian flora), and 20 years of experience working with Hawaiian algae. Her diverse background includes research on the systematics, phylogeography, and genomics of algae, with an emphasis on Hawaiian species. Dr. Sherwood has trained dozens of undergraduate students, graduate students, and postdoctoral fellows in molecular and morphological characterization of marine and freshwater algae, and has held numerous leadership roles in scientific societies, as well as associate editorships of scientific journals. She is leading the eDNA assay development and deployment, and a phylogeographic analysis of *Chondria* across the tropical Pacific. The Sherwood Laboratory has undertaken three NSF-funded algal biodiversity surveys, which included characterization of thousands of diverse algal collections. The lab group has worked collaboratively for a number of years with colleagues at NOAA-PMNM, the Bishop Museum, and the College of Charleston.

Dr. Celia Smith is a Professor in the School of Life Sciences at the University of Hawaii at Manoa, and the Co-Director of the Marine Biology Graduate Program, at the University of Hawaii at Manoa. She is a leading world expert in algal physiological ecology and invasive species studies for the Main Hawaiian Islands, and as PI via multiple NOAA-funded Aquarius missions in the Florida Keys, examining potential algal overgrowth of reefs in both systems. Smith was PI for 12 yrs of biofouling research (Office of Naval Research) with the target of developing model systems to test anti-fouling coatings, using invasive algae. Smith was team lead (UHM, DLNR, TNC-Hawaii) and PI (NOAA ECoHAB) in the innovation of several of the programs now in place to control invasive species managed by the Division of Aquatic Resources, Department of Land and Natural Resources, State of Hawai‘i. Her collaborative work has led to over 100 papers, and 30 years of experience working with native vs invasive algal issues in tropical ecosystems. Smith‘s latest project is as lead for a state-wide assessment of cesspools as sources for wastewater in Hawaiian coastal regions, as a driver for algal blooms in the Main Hawaiian Islands. Smith’s background includes research into the balance between nutrient supplies and herbivory as factors that determine benthic communities in coral reef regions with collateral impacts of wastewater pollution linked to upper trophic levels as in the diet of the green sea turtle. Dr. Smith has trained dozens of undergraduate and graduate students and postdocs. Her lab group has

worked collaboratively for a number of years with colleagues at NOAA Turtle Program, Monterey Bay Aquarium, and the Bishop Museum.

g. Provide information demonstrating that you have adequate financial resources available to conduct and complete the activity and mitigate any potential impacts resulting from its conduct.

This project is supported by NFWF grants to PI H. Spalding and A. Sherwood. Fullerton, Krueger-Hadfield, Smith, and Kealoha are co-PI's on these grants. Sufficient funds have been provided for the collection and analyses described in this permit.

h. Explain how your methods and procedures are appropriate to achieve the proposed activity's goals in relation to their impacts to Monument cultural, natural and historic resources, qualities, and ecological integrity.

The methods used in this study have been successfully used by all permittees for all of the described analyses in this permit using macroalgal, coral, and water samples collections in the Hawaiian Archipelago.

i. Has your vessel been outfitted with a mobile transceiver unit approved by OLE and complies with the requirements of Presidential Proclamation 8031?

Yes, the vessel will be equipped with the appropriate mobile transceiver unit.

j. Demonstrate that there are no other factors that would make the issuance of a permit for the activity inappropriate.

There are no other factors that would make the issuance of this permit inappropriate.

8. Procedures/Methods:

1) Macroalgal collections

a. **Population genetics and reproduction** - We will sample a patch of *Chondria* every 1 m along a 100 m transect. The length of the transect will be site dependent such that a mat of *Chondria* on a coral reef that is only 25m wide will be sampled along 4 25m transects to achieve 100 m with samples every 1 m. Moreover, we will sample these 100m transects at 3 depths, but this depends on the site. We will aim for two sites that have 3 depths. This type of sampling strategy has been used by Krueger-Hadfield et al. (2016 Molecular Ecology; 2017 Ecology and Evolution; 2018 Bioinvasion Records), Bonthond et al. (2020; 2021), Sotka et al. (2018), Flanagan et al. (2021) and is the only standardized way in which to sample free-floating or drifting algae that are also likely partially clonal (Krueger-Hadfield et al. 2021; Stoeckel et al. 2021). A sample of *Chondria* will likely contain multiple thalli that are interwoven. Thus, we

will subsample 3 thalli that are clearly separate but interwoven for genetic analyses to determine the clonal extent in the mats. Small pieces of the thalli will be added into pre-labeled tubes with silica gel (see SDS) from each site. Thus, we will have 300 samples per transect. The reproductive status of each collected sample will be assessed visually under the microscope and via molecular analyses using chromosomal studies. These studies will determine the extent of clonality and population connectivity, and the reproductive state of individuals.

b. **Microbiome** - Representative samples will be collected in triplicates at each depth target of *Chondria* in addition to representative species from the Chlorophyta (green), Phaeophyceae (brown), and Rhodophyta (red) taxonomic groups. 50ml of seawater will be collected at each target depth to act as a control. Each thallus will be separated into two fractions for epibiont and endobiont sampling. Surface swabs will be taken to sample for the epibionts then each macroalgal sample will be surface sterilized to sample to endobionts. Swabs will be placed in 500ul of lysis buffer and frozen at -20°C. Macroalgal samples will then be surfaced sterilized with 1:1 mixture of sterile filtered Umonium Master and artificial seawater. Samples for endobionts will be placed in 500ul of RNA later for 24 hours and then frozen at -20°C. Water collected at depth will be filtered onto a 0.2µm filter and filters will be stabilized with RNA and frozen. These studies will determine how or if *Chondria* is changing the microbial communities on the reef, which are key for processes such as coral and macroalgal settlement and disease regulation.

c. **Stable isotopes and tissue nutrients** – three representative samples of dominant algae from the beginning, middle, and end of the transect from each site will be collected, rinsed in DI water, and dried at 60 degrees C on the boat in a drying oven. These samples will be later ground to a fine powder, and analyzed for stable isotopes and tissue nutrients. Dried macroalgal samples brought back to the Main Hawaiian Islands will be dead. These studies will shed light on the widespread nutrient state and nutrient sources of *Chondria*, and determine if specific oceanographic processes are influencing its abundance and distribution.

a. ***Chondria* physiological ecology**- 100 individual specimens of *Chondria* will be collected from end points of the current *Chondria* distribution for immediate physiological characterization, tissue nutrient determinations and pigment extraction. Approximately half of those plants will be placed in minicosms - 1 liter glass vessels, supplied with an experimental mix of temperatures and nutrients to determine growth rates under simulated field conditions using equipment such as a Neslab Controlled temperature water bath. At the end of growth experiments, plants will once again be subject to immediate physiological characterization, tissue nutrient

determinations and pigment extraction. No live material will be returned to the Main Hawaiian Islands. Characterization of the potential invasive traits of red algae has been a long basis for experimental work in Smith's lab. These studies will augment our understanding substantially.

b. **Macroalgal biodiversity and *Chondria* genome** - Individual specimens of green, red, or brown macroalgae will be collected for characterization and identification. No more than three individuals per species will be collected to avoid over-collecting individual species. Specimens will be collected live, and a small portion of the individual will be preserved in silica gel for DNA analysis, and the remainder of the specimen frozen for pressing as a herbarium voucher on Oahu. No live material will be returned to the Main Hawaiian Islands. Characterization of the native macroalgal flora has been an ongoing research objective of the Sherwood Lab, and these opportunistic collections will support further description of newly discovered species from PMNM. Material for *Chondria* genome determination will be collected from a single location. 20 g of *Chondria* wet weight tissue will be preserved for this project.

2) **Coral Collections** – collected for Kealoha (see Kealoha permit)

3) **Water samples** – 30 water samples will be collected from locations around Manawai, ranging from directly over *Chondria* mats and away from mats. Samples can be collected either from the ship, or from the smaller boats. Water samples will be filtered aboard the ships, with filtered water returned overboard, and filters frozen on board to be brought back to the Sherwood lab on Oahu for analysis. No live material will be returned to the Main Hawaiian Islands.

4) *Chondria* distribution data

a. SCUBA and snorkeling in-water surveys of *Chondria* abundance will be compared with the 2019 *Chondria* distribution map. Similar positions will be snorkeled at ~20 ft depths by 2 persons to determine the presence of absence of Chondria around the perimeter and interior Lagoon of Manawai. SCUBA areas will involve estimates of abundance over 100 m long transects used for collections, and any other area available for swim surveys.

5) *Chondria* BMPs

a. Morphometrics for *Chondria* mats (i.e. mat depth and areal coverage of mats) will be completed at multiple depths and sites, and ease of removal (time for complete removal and amount of algal material remaining on reef) will be assessed. We will expand and develop additional strategies to limit the spread of viable algal fragments based on previous studies with a 10% commercial bleach solution.

NOTE: If land or marine archeological activities are involved, contact the Monument Permit Coordinator at the address on the general application form before proceeding.

9a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name:
Macroalgae (see Kealoha permit for coral collection methodologies and species)

Scientific name:
Chondria tumulosa, Rhodophyta, Chlorophyta, Phaeophyceae, Cyanobacteria

& size of specimens:
At each site, we will collect a small clump (2-4 oz volume) of *Chondria* or abundant native algae every 1 m along a 100 m transect (determined by fin kicks). Each sample will be sealed in a plastic bag at depth and immediately processed on the boat for various analyses. Our goal is use these samples for all population genetics, reproductive state, stable isotopes and tissue nutrients, and microbiome analyses. Ideally, we would be able to collect from 3 sites per day over a 10 day period, resulting in ~3000 samples for detailed analyses over a broad spatial scale around Manawai. The primary focus of intensive sampling will the Chondria. If another native species is found in high abundance, such as *Microdictyon* beds, then intensive sampling of this native species will occur for comparison to the Chondria. Otherwise, abundant native species will only be collected in replicates of three per site to avoid over-collecting individual species.

Water samples for eDNA analysis will be collected along a 100 m transect over *Chondria* mats and away from mats. 30 water samples will be collected, with each sample being 2-4 L in volume. Filtered water (samples will be filtered aboard the ship, on site) will be returned overboard.

A total of 20 g of *Chondria* wet weight tissue will be preserved in RNAlater on board the ship, and frozen, for genome sequencing by colleagues at the Scripps Institution of Oceanography (B. Moore and T. Steele).

Individual specimens of green, red, or brown macroalgae will be collected for characterization and identification, in support of ongoing efforts to describe the macroalgal flora of PMNM. No more than three individuals per species will be collected to avoid over-collecting individual species. Specimens will be collected live and placed in individual plastic bags at depth, and then processed on board. A small portion of the individual will be preserved in silica gel for DNA analysis, and the remainder of the specimen frozen for pressing as an herbarium voucher on Oahu.

100 individual specimens of *Chondria* will be collected from end points of the current *Chondria* distribution for immediate physiological characterization, tissue nutrient determinations and pigment extraction. Approximately half of those plants will be placed in minicosms - 1 liter glass vessels, supplied with an experimental mix of temperatures and nutrients to determine growth rates under simulated field conditions. At the end of growth experiments, plants will once again be subject to immediate physiological characterization, tissue nutrient determinations and pigment extraction. No live material will be returned to the Main Hawaiian Islands.

Collection location:
Manawai primarily, but other locations may include Nihoa, Necker, FFS, Garner, Lis, Laysan, Midway, Maro, Kure

☐ Whole Organism X Partial Organism

9b. What will be done with the specimens after the project has ended?

Macroalgal herbarium specimens will be stored within the herbariums of Heather Spalding (CofC) and Alison Sherwood (UHM) while under study, and then submitted to the Bernice Pauahi Bishop Museum Herbarium (BISH) for final deposition upon publication. In cases where multiple isotypes can be established, one isotype will be submitted to the Smithsonian Institution to ensure redundancy of type information. Genetic sequences generated for all macroalgal and microbiome specimens will be published in online repositories (e.g. GenBank) as required for publication in peer-reviewed journals. DNA and silica-preserved specimens from macroalgae and microbiome specimens will be stored with the participating collaborators at their respective institutions (CofC, UHM, UH Maui College, UAB, and Scripps).

Filters of water samples for the eDNA project will be destroyed in the process of analysis, and DNA extracts resulting from the work will be stored in freezers in the lab of Peter Marko (UHM) or Alison Sherwood (UHM).

From the physiological ecology studies, samples will be dried at 60 deg C in an oven till dry, ground and submitted for tissue nitrogen determinations. Other samples will be extracted for Chlorophyll a in solvent using spectrophotometric analyses. Solvent samples will be part of hydrocarbon waste once they arrive at UHM and appropriately handled via Environmental Health and Safety protocols, UHM.

9c. Will the organisms be kept alive after collection? ☐ Yes X No

All collected specimens of genetic, reproduction, microbial, and stable isotopes and tissue nutrient analyses will be processed immediately on the boat after collection and will be preserved for future analyses in various laboratories. The preservation of samples ensures that samples are not viable (“alive”).

No organisms will be kept alive after collection of water samples for eDNA analysis, biodiversity characterization of macroalgae, or genome analysis. Water filters will be immediately frozen, and macroalgal samples will be placed in silica gel (desiccant), frozen damp, or frozen in RNAlater; all techniques ensure samples are not alive.

No organisms will be kept alive after collection and conclusion of the growth experiments.

• General site/location for collections:
Manawai primarily, but other locations may include Nihoa, Necker, FFS, Garner, Lis, Laysan, Midway, Maro, Kure

• Is it an open or closed system? ☐ Open X Closed
Closed: All tissues will be placed in closed system containers with lids.

• Is there an outfall? ☐ Yes X No
No: There is no outfall that would allow for escape of Chondria.

• Will these organisms be housed with other organisms? If so, what are the other organisms?
No other organisms will be included in this study.

• Will organisms be released?

No organisms or collected water will be released outside of their respective island or atoll of collection. Filtered water will be returned overboard, but without organisms.

10. If applicable, how will the collected samples or specimens be transported out of the Monument?
The vessel will transport all collected samples and specimens out of the Monument. Upon arrival to Oahu, all specimens will be shipped via FedEx or another method of shipment to the laboratories of our respective collaborators, or hand-carried by car to laboratories at UHM.

11. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research:
All research occurring under this permit is highly collaborative, with the sharing of all specimens for multiple types of analyses. The same collected small “clumps” of algae will be used for genetic analyses to determine population-level connectivity, reproductive state, stable isotopes/tissue nutrients, physiology studies, the *Chondria* genome, and biodiversity from microscopic (microbiome) to macroscopic (macroalgal species) levels.

12a. List all specialized gear and materials to be used in this activity:
SCUBA and typical scientific diving gear
Underwater cameras, housings, and strobes

Flower art drying silica gel
Herbarium pressing supplies
LiCOR irradiance profiling unit to measure light attenuation
Syringes for collecting water at depth
Motorized pump and water filtration system to filter water samples
-20 deg C freezer for storing specimens
Liquid nitrogen dewar for storing water filters after water filtration
Plastic bins to contain 1 L Mason jars as mini-cosms for growth studies
Controlled temperature water bath to pump to cool water to simulate deeper water temperatures (Neslab Controlled temperature water bath)
Walz Jr PAM fluorometer and PC computer to measure photosynthesis of plants

12b. List all Hazardous Materials you propose to take to and use within the Monument:

1) Commercial bleach
2) Liquid nitrogen
3) RNAlater for preservation of Chondria tissue for genome determination
4) Dimethyl Formamide for pigment extractions (100 ml) stored in refrigerator (an alternative solvent for pigment extractions would be acetone)
5) Umonium
6) Lysis Buffer

13. Describe any fixed installations and instrumentation proposed to be set in the Monument:

None

14. Provide a time line for sample analysis, data analysis, write-up and publication of information:

Sample and data analyses, and write-up/publication will be completed within 2 years of sample collection

15. List all Applicants' publications directly related to the proposed project:

Heather Spalding

Cabrera FC, Huisman JL, **Spalding HL**, Kosaki RK, Sherwood AR (2021) Diversity of Kallymeniaceae (Gigartinales, Rhodophyta) associated with Hawaiian mesophotic reefs. *European Journal of Phycology*. doi.org/10.1080/09670262.2021.1891462

Strait N**, **Spalding HL**. (2021) Mind your methods: Acidification degrades total nitrogen and stable isotopic values within calcified marine macroalgae. *Phycologia* DOI:10.1080/00318884.2020.1865713
****Graduate student mentee**

Sherwood AR, Paiano MO, Cabrera FC, **Spalding HL**, Hauk BB, Kosaki RK (2021) *Ethelia hawaiiensis* (Etheliaceae, Rhodophyta), a new mesophotic marine alga from Manawai (Pearl & Hermes Atoll), Papahānaumokuākea Marine National Monument, Hawai'i, U.S.A. *Pacific Science*

Sherwood AR, Paiano MO, Wade RM, Cabrera FC, **Spalding HL**, Kosaki RK (2021) Biodiversity of Hawaiian Peyssonneliales (Rhodophyta). 1. Two new species in the genus *Ramicrusta* from Lehua Island. *Pacific Science*

Paiano MO, Huisman JL, Cabrera FC, **Spalding HL**, Kosaki RK, Sherwood AR (2020) *Haraldiophyllum hawaiiensis* sp. nov. (Delesseriaceae, Rhodophyta): a new genus record for the Hawaiian Islands. *Algae*

Sherwood AR, Paiano MO, **Spalding HL**, Kosaki RK (2020) Biodiversity of Hawaiian Peyssonneliales (Rhodophyta): *Sonderophycus copusii* sp. nov., a new species from the Northwestern Hawaiian Islands. *Algae* 35(2): 145-155

Sherwood AR, Huisman JM, Paiano MO, Williams T, Kosaki RK, Smith CM, Giuseffi L, **Spalding HL** (2020) Taxonomic determination of the cryptogenic red alga, *Chondria tumulosa* sp. nov., (Rhodomelaceae, Rhodophyta) from Papahānaumokuākea Marine National Monument, Hawai'i, USA: a new species displaying invasive characteristics. *PLoS ONE* 15: e0234358

Tsuda RT, Abbott IA, **Spalding HL**, Guiseffi LM, Okano R, Kennedy BH, Sherwood AR (2020) Marine benthic algae from Ni'ihau and Adjacent Lehu'a Islet, Main Hawaiian Islands. *Bishop Museum Occasional Papers* 129: 93-107

Veazey L**, Williams O, Wade R, Toonen RJ, **Spalding HL** (2019) Present-day distribution and potential spread of invasive green alga *Avrainvillea amadelpha*. *Frontiers in Marine Science*. doi: 10.3389/fmars.2019.00402, ***Graduate student mentee***

Sherwood AR, Showe-Mei L, Wade RM, **Spalding HL**, Smith CM, Kosaki RK (2019) Characterization of *Martensia* (Delesseriaceae; Rhodophyta) from shallow and mesophotic habitats in the Hawaiian Islands: description of four new species. *European Journal of Phycology* 55: 172-185

Padilla-Gamiño JL, Roth MS, Rodrigues LJ, Bradley CJ, Bidigare RR, Gates RD, Smith CM, **Spalding HL**. (2019) Ecophysiology of mesophotic reef building corals in Hawai'i is influenced by symbiont-host associations, photoacclimatization, trophic plasticity, and adaption. *Limnology and Oceanography* doi:10.1002/lno.11164

Foster A*, **Spalding HL**, Cox TE, La Valle F, Philippoff J. (2019) The invasive green alga *Avrainvillea* sp. transforms native epifauna and algal communities on a hard substrate reef. *Phycological Research* 67: 164-169. http://dx.doi.org/10.1111/pre.12359 ***Undergraduate mentee***

Wade R, **Spalding H**, Peyton K, Foster K, Sauvage T, Ross M, Sherwood A (2018) A new record of *Avrainvillea* cf. *erecta* (Berkeley) A. Gepp & E. S. Gepp (Bryopsidales, Chlorophyta) from urbanized estuaries in the Hawaiian Islands. *Biodiversity Data Journal* 6: e21617

Wainwright BJ, Zahn LZ, **Spalding HL**, Sherwood AR, Smith CM, Amend AS (2017) Fungi associated with mesophotic macroalgae from the 'Au'au Channel, west Maui are differentiated by host and overlap with terrestrial communities. *PeerJ* 5: e3532 ***Top 5 most viewed article in subject area for 2017***

Cox TE, **Spalding HL**, Foster MS (2017) Spatial and temporal variation of diverse intertidal algal assemblages in Southwest O'ahu. *Marine Ecology* 38: e12429

Langston R, **Spalding HL** (2017) A survey of fishes associated with Hawaiian deep-water *Halimeda kanaloana* (Bryopsidales: Halimedaceae) and *Avrainvillea* sp. (Bryopsidales: Udoteaceae) meadows. *PeerJ* 5: e3307.

Sansone FJ, **Spalding HL**, Smith CM (2017) Sediment biogeochemistry in mesophotic meadows of calcifying macroalgae. *Aquatic Biogeochemistry*: 1-24. doi:10.1007/s10498-017-9315-9

Spalding HL, O'Kelly CJ, Smith CM, Sherwood AR (2016) New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian Archipelago. *Journal of Phycology*. 52: 40-53 ***Nominated for Provasoli Award for Best Paper***

Pyle RL, Boland R, Bolick H, Bowen BW, Bradley CJ, Kane C, Kosaki RK, Langston R, Longenecker K, Montgomery AD, Parrish FA, Popp BN, Rooney J, Smith CM, Wagner D, **Spalding HL** (2016) A comprehensive investigation of mesophotic coral ecosystems in the Hawaiian Archipelago. *PeerJ* 4:e2475

Wagner D, Barkman A, **Spalding HL**, Calcinai B, Godwin SL (2016) A photographic guide to the benthic flora and fauna from mesophotic coral ecosystems in the Papahānaumokuākea Marine National Monument. *Marine Sanctuaries Conservation Series* ONMS-16-04

Tsuda RT, **Spalding HL**, Sherwood AR (2015) New species records of marine benthic algae in the Papahānaumokuākea Marine National Monument (Northwestern Hawaiian Islands). *Bishop Museum Occasional Papers.* 116: 41-47

Costa B, Kendall M, Parrish F, Rooney J, Boland R, Chow M, Lecky J, Montgomery A, and **Spalding H** (2015) Identifying suitable locations for mesophotic hard corals offshore of Maui, Hawai'i. *PLoS one* 10(7): e0130285

Ainsworth TD, Bridge T, Torda G, Raina JB, Gates R, Padilla-Gamiño J, **Spalding HL**, Smith C, Woosley ES, Krause L, Bourne D G , Bongaerts P, Hoegh-Guldberg O, and Leggat W (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME*. 17 April 2015; doi:10.1038/ismej.2015.39

Pochon X, Forsman ZH, **Spalding HL**, Padilla-Gamino J, Smith CM, Gates RD (2015) Depth specialization in mesophotic corals (*Leptoseris* spp.) and associated algal symbionts in Hawai'i. *Royal Society Open Science.* 2:140351

Roth MS, Padilla-Gamiño J, Pochon X, Bidigare RR, Gates RD, Smith CS, **Spalding HL** (2015) Fluorescent protein expression in mesophotic reef-building corals. *Marine Ecology Progress Series*. 521: 63-79

Wagner D, Kosaki RK, **Spalding HL**, Whitton R, Pyle RL, Sherwood AR, Tsuda RT, Calcinai B (2014) Mesophotic surveys of the flora and fauna at Johnston Atoll, Central Pacific Ocean. *Marine Biodiversity Records* 7:1-10

Zechman FW, Verbruggen H, Leliaert F, Ashworth M, Buccheim MA, Fawley MW, **Spalding H**, Pueschel CM, Buccheim JA, Verghese B, Hanisak MD (2010) An unrecognized lineage of green plants persists in deep marine waters. *Journal of Phycology* 46: 1288-1295. ***Provasoli Award for Best Paper***

Rooney J, Donham E, Montgomery A, **Spalding HL**, Parrish FA, Boland R, Fenner D, Gove J, Vetter O (2010) Mesophotic coral ecosystems in the Hawaiian Archipelago. *Coral Reefs* 29: 361-367

Sansone FJ, **Spalding H**, Smith CM (2008) Submersible-operated porewater sampler for permeable sediments. *Limnol. Oceanogr: Methods* 6: 119-125

Verbruggen H, De Clerck O, N'Yeurt ADR, **Spalding H**, Vroom PS (2006) Phylogeny and taxonomy of *Halimeda incrassata*, including the description of *H. kanaloana* and *H. heteromorpha* spp. nov. (Bryopsidales, Chlorophyta). *European Journal of Phycology* 41: 337-362

Webster JM, Clague DA, Braga JC, **Spalding H**, Renema W, Kelley C, Applegate B, Smith J, Paull CK, Moore JG, Potts D (2006) Drowned coralline algal dominated deposits off Lāna'i'i, Hawai'i: carbon accretion and vertical tectonics over the last 30 ka. *Marine Geology* 225: 223-246

Stacy Krueger-Hadfield

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Bonthond G, T Bayer, **SA Krueger-Hadfield**, N Stärk, G Wang, M Nakaoka, S Künzel, F Weinberger (2021) Increased host promiscuity may have promoted invasiveness of a macroalgal holobiont. *ISME* https://doi.org/10.1038/s41396-020-00878-7

Cahill R, **SA Krueger-Hadfield**, WH Ryan (2021) The effects of stress on the bacterial community associated with the sea anemone *Diadumene lineata*. *Journal of Emerging Investigators* 3: 1-7.

Ryan WH, J Aida, **SA Krueger-Hadfield** (2021) The contribution of clonality to population genetic structure in the sea anemone *Diadumene lineata*. *Journal of Heredity* esaa050

Krueger-Hadfield SA, C Destombe, ML Guillemin, M Valero, S Stoeckel (2021) Exploring the genetic consequences of clonality in haplodiplontic taxa. *Journal of Heredity* esaa063

Stoeckel S, S Arnaud-Haond, **SA Krueger-Hadfield** (2021) The combined effect of haplodiplonty and partial clonality in population genetics. *Journal of Heredity* esaa062

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Heather Fullerton

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Alison Sherwood

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Celia Smith (select papers)

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With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury of that the information I have provided on this application form is true and correct. I agree that the Co-Trustees may post this application in its entirety on the Internet. I understand that the Co-Trustees will consider deleting all information that I have identified as "confidential" prior to posting the application.

Heather Spalding 15 March 2021

Signature Date

SEND ONE SIGNED APPLICATION VIA MAIL TO THE MONUMENT OFFICE BELOW:

NOAA/Inouye Regional Center
NOS/ONMS/PMNM/Attn: Permit Coordinator
1845 Wasp Blvd, Building 176
Honolulu, HI 96818
FAX: (808) 455-3093

DID YOU INCLUDE THESE?
X Applicant CV/Resume/Biography
X Intended field Principal Investigator CV/Resume/Biography
X Electronic and Hard Copy of Application with Signature
NA Statement of information you wish to be kept confidential
X Material Safety Data Sheets for Hazardous Materials

Papahānaumokuākea Marine National Monument
Compliance Information Sheet

1. Updated list of personnel to be covered by permit. List all personnel names and their roles here:

Participant	Contact info	Affiliation	Position/Role
Brian Hauk	808-232-6379 Brian.hauk@noaa.gov	JIMAR/PMNM	Cruise 1:Chief Scientist, Cox'n, diver
Keo Lopes	808-352-2702 keolohilani.lopes@noaa.gov	JIMAR/PMNM	Cruise 1 & 2: Cox'n, diver, UAS pilot
Jason Leonard	808-436-8725 jason.leonard@noaa.gov	NOAA/PMNM	Cruise 1: Cox'n, diver, UAS pilot Cruise 2: Chief Scientist
LTJG Luke Evancoe	412-841-6680 luke.evancoe@noaa.gov	NOAA/PMNM	Cruise 1 & 2: Cox'n, diver
Celia Smith	celia@hawaii.edu	UH Mānoa	Cruise 1: NFWF *C. tumulosa* growth experiments
Allison Sherwood	asherwoo@hawaii.edu	UH Manoa	CO-PI on Spalding Permit, shore based work
Jimmy Fumo	jfumo@hawaii.edu	UH Mānoa-Sherwood Lab	Cruise 1: NFWF eDNA & genetic studies, diver
Andrea Kealoha	andreake@hawaii.edu	UH Maui	PI-Kealoha Permit, shore based work
Arik Dadez	arikdadez@gmail.com	UH Maui-Kealoha Lab	Cruise 1: oceanographic water sample processing
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Kim Jeffries	808-253-9215 kjj@hawaii.edu	UH Manoa	Videography, Outreach
*TBD	206-526-6460 xo.ndc@noaa.gov	NOAA/NDC	NDC Chamber operator, Divemaster
Ship's Crew (6)	808-526-9311 marcy@pmtugs.com	HRG	Charter Vessel Crew

2. Specific Site Location(s): (Attach copies of specific collection locations):
See attached 2021 Survey sites

3. Other permits (list and attach documentation of all other related Federal or State permits): Kealoha PMNM-2021-016 & Spalding PMNM-2021-019

3a. For each of the permits listed, identify any permit violations or any permit that was suspended, amended, modified or revoked for cause. Explain the circumstances surrounding the violation or permit suspension, amendment, modification or revocation. N/A

4. Funding sources (Attach copies of your budget, specific to proposed activities under this permit and include funding sources. See instructions for more information): All NOAA and NFWF funds

5. Time frame:
Activity start: July 8, 2021
Activity completion: August 20, 2021

Dates actively inside the Monument:
1st Cruise From: July 10 to 25
2nd Cruise From: August 3 to18

Describe any limiting factors in declaring specific dates of the proposed activity at the time of application: Weather, ship mechanical delays and/or COVID effects

Personnel schedule in the Monument: listed above in #1

6. Indicate (with attached documentation) what insurance policies, bonding coverage, and/or financial resources are in place to pay for or reimburse the Monument trustees for the necessary search and rescue, evacuation, and/or removal of any or all persons covered by the permit from the Monument: See attached insurance policy for HRG vessel. All permittees entering the monument have liability and workers compensation insurance provide by their employers

7. Check the appropriate box to indicate how personnel will enter the Monument:

☒ Vessel
☐ Aircraft

Provide Vessel and Aircraft information: M/V Imua

8. The certifications/inspections (below) must be completed prior to departure for vessels (and associated tenders) entering the Monument. Fill in scheduled date (attach documentation):

☒ Rodent free, Date: July 7, 2021
☒ Tender vessel, Date: July 7, 2021
☐ Ballast water, Date: N/A Fresh water only
☒ Gear/equipment, Date: July 8, 2021
☒ Hull inspection, Date: June 15, 2021

9. Vessel information (NOTE: if you are traveling aboard a National Oceanic and Atmospheric Administration vessel, skip this question):
Vessel name: M/V Imua
Vessel owner: Hawaii Resource Group/PM Tugs
Captain's name: Hans Bishop
IMO#: 8968193
Vessel ID#:1117720
Flag: USA
Vessel type: Cargo / Supply Vessel
Call sign: WDK2768
Embarkation port: Pearl Harbor
Last port vessel will have been at prior to this embarkation: Kewalo Basin
Length: 185’
Gross tonnage: 92 GRT
Total ballast water capacity volume (m3): 126997 gallons (fresh/potable water)
Total number of ballast water tanks on ship: 9

Total fuel capacity: 124,640
Total number of fuel tanks on ship: 8
Marine Sanitation Device: Yes
Type: II

Explain in detail how you will comply with the regulations regarding discharge in the Monument.
IMUA has adequate holding capacity to remain discharge free while transiting and working in the Monument Zone. IMUA has been approved to make several voyages for Fish and Wildlife Service through the monument in the last 18 months. IMUA is keel cooled, so there is no engine water overboard discharge. The crew on IMUA are extremely familiar with the rules and regulations regarding discharges in the Monument.

Describe in detail. If applicable, attach schematics of the vessel's discharge and treatment systems:
IMUA will not need to discharge or treat any discharge overboard while in the Monument.

Other fuel/hazardous materials to be carried on board and amounts: gasoline for small boats (500 gallons)

Provide proof of a National Oceanic and Atmospheric Administration (NOAA) Office of Law Enforcement-approved Vessel Monitoring System (VMS). Provide the name and contact information of the contractor responsible for installing the VMS system. Also describe VMS unit name and type: Thrane & Thrane TT-3026D installed by Oceantronics

VMS Email: IMUA@skyfile
Inmarsat ID#: 881651443085

* Individuals MUST ENSURE that a type-approved VMS unit is installed and that its automatic position reports are being properly received by the NOAA OLE system prior to the issuance of a permit. To make sure your VMS is properly configured for the NOAA OLE system, please contact NOAA OLE at (808) 725-6110 or (808) 725-6100.

* PERMITS WILL NOT BE ISSUED TO INDIVIDUALS ENTERING THE MONUMENT VIA VESSEL UNTIL NOAA OLE HAS CONTACTED THE MONUMENT PERMIT COORDINATOR WITH A ‘POSITIVE CHECK’ READING.

10. Tender information:

On what workboats (tenders) will personnel, gear and materials be transported within the Monument? List the number of tenders/skiffs aboard and specific types of motors: (2) two NOAA/PMNM 19' Safeboats with twin 90hp Honda motors.

Additional Information for Land Based Operations

11. Proposed movement of personnel, gear, materials, and, if applicable, samples: N/A

12. Room and board requirements on island: N/A

13. Work space needs: N/A

DID YOU INCLUDE THESE?
☒ Map(s) or GPS point(s) of Project Location(s), if applicable
☐ Funding Proposal(s)
☐ Funding and Award Documentation, if already received
☒ Documentation of Insurance, if already received
☐ Documentation of Inspections
☐ Documentation of all required Federal and State Permits or applications for permits

Pearl and Hermes Atoll Biosecurity Measures
PMNM-2021-001 (ONMS), PMNM-2021-016 and PMNM-2021-019

The following document outlines the mitigation steps ONMS cruise participants will follow to ensure adequate biosecurity measures are followed to mitigate the risk associated with accessing Manawai (Pearl and Hermes Atoll) for research activities defined in permits: PMNM-2021-001 Co-Trustee Managers Permit (and associate memo to file), PMNM-2021-016 and PMNM-2021-019.

Manawai is the sole known location of *Chondria tumulosa* and therefore provides the only option for conducting the management critical research outlined by these permitted activities. This research is essential for management to develop science-based protocols for preventing further spread and loss of resources.

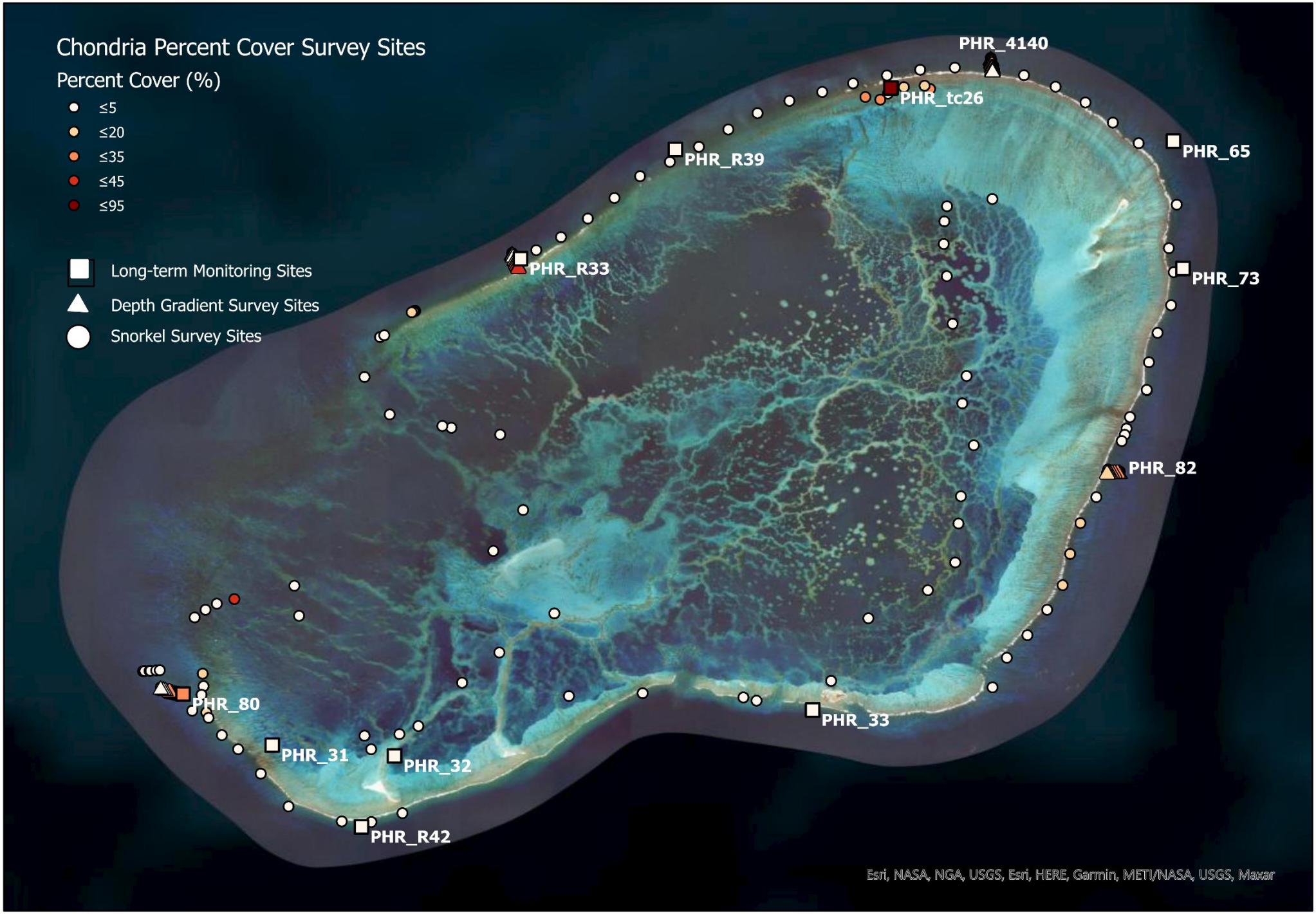
The steps outlined below apply to research projects and activities involving *Chondria tumulosa* which fall outside of BMP 011 (Disease and Introduced Species Prevention Protocol for Permitted Activities in the Marine Environment, Papahānaumokuākea Marine National Monument) and draft BMP XX (Best Management Practices for Activities at Pearl and Hermes Atoll). Draft BMP XX will be referred to as BMP PHA for the remainder of the document.

A HACCP analysis was undertaken to thoroughly assess steps and associated activities for the research activities below to ensure all steps had been evaluated.

Research Activities:	Supplemental Mitigation Steps:
SCUBA Surveys/Collections	Will follow all SOPs outlined in BMP 011 and BMP PHA pertaining to dive and sampling equipment. All disinfection procedures defined by BMP 011 will utilize bleach concentrations referenced in BMP PHA. Equipment, divers and small boat will be inspected daily and will be disinfected and thoroughly dried before returning to Honolulu
Water samples for nutrients and eDNA	Will follow all SOPs outlined in BMP 011 and BMP PHA regarding sampling equipment and laboratory disinfection. All disinfection procedures defined by BMP 011 will utilize bleach concentrations referenced in BMP PHA. Water samples will be stored in secondary containment aboard ship and all collections will be preserved in a biosecure manner (e.g. frozen or chemically preserved). Excess water from eDNA filtration will be disposed of overboard at PHA or in ships grey water tank which will be pumped once outside 50nm original boundaries.

Algal samples for stable isotope and genetic analyses	Researchers will follow all SOPs outlined in BMP 011 and BMP PHA regarding disinfection of sampling equipment and laboratory spaces. All disinfection procedures defined by BMP 011 will utilize bleach concentrations referenced in BMP PHA. Algal samples will be double bagged in the field before being placed in tertiary (3 layers) containment in the small boat. Lab areas will have confinement trays to prevent water or algal fragment distribution and all samples will be stored in secondary containment aboard ship. All collections will be preserved in a biosecure manner (e.g. frozen or chemically preserved) and no live samples will leave PHA.
Shipboard tank-based PAM fluorometry and growth experiments	Researchers will follow all SOPs outlined in BMP 011 and BMP PHA regarding disinfection of collection equipment, laboratory spaces, and growth experiment equipment upon completion of experimentation. All disinfection procedures defined by BMP 011 will utilize bleach concentrations referenced in BMP PHA. Algal samples will be double bagged in the field before being placed in tertiary containment in the small boat. Algal samples will then be transferred to secure grow out containers (1 liter mason jars) for replicant growth experiments. All replicants will have secondary containment measures in place and will be secured on the ship. Additionally, all specimens will be frozen (to ensure no viable fragments) and disposed of before leaving PHA. PAM fluorometry experiments will take place within a controlled environment utilizing secondary containment for all samples. PAM Laboratory measurement spaces will be disinfected per BMP protocols.
Water Quality Sampling near and around Chondria	Researchers will follow all SOPs outlined in BMP 011 and BMP PHA regarding sampling equipment and laboratory disinfection. All disinfection procedures defined by BMP 011 will utilize bleach concentrations referenced in BMP PHA. Water samples will be stored in secondary containment aboard ship and all collections will be preserved in a biosecure manner (e.g. frozen or chemically preserved). Any excess water will be disposed of overboard at PHA or in ships grey water tanks which will be pumped once outside 50nm original boundaries.

UAS Survey Flights	UAS operations will follow all requirements provided by ONMS and OMAO leadership. There are no known risks with these airborne activities. As a precaution, UAS and associated equipment will be wiped down with disinfection wipes, inspected and thoroughly dried before being stored for transit back to Honolulu. If UAS makes contact with water, it will be thoroughly cleaned and disposed of properly if deemed dysfunctional.
Ship Based Operations	The NOAA charter vessel *Imua* will not be anchoring and will be in constant motion for the duration it is offshore of PHA. Time at PHA is not expected to exceed 9 days. The vessel will have undergone a hull inspection prior to its departure to ensure below water surfaces have adequate anti-fouling coatings. The *Imua* has no ballast tanks. All deck spaces surrounding small boats storage, ship entry/egress areas, crane operation zones, dive equipment storage, lab spaces and associated areas will be disinfected per BMP protocols and washed down before departing PHA at required distances offshore.
Small Boat Usage	Both PMNM vessels utilized during this trip will be thoroughly disinfected per BMPs and will not be utilized at any location upon departing PHA. There is a possibility to recover an acoustic buoy on the transit back that would utilize the contract vessel's small boat, which would have not been in contact with the PHA environment.

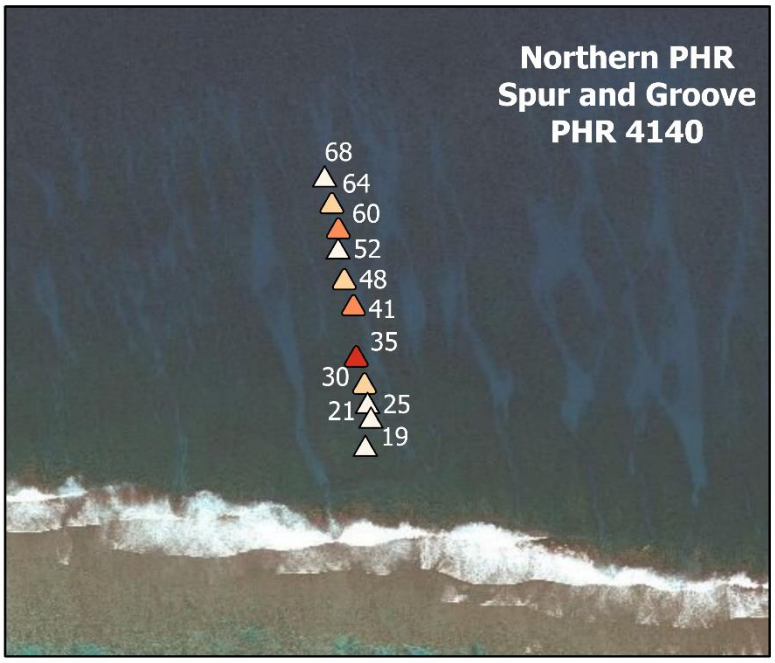


Abundance and Distribution of *Chondria tumulosa*

Pearl and Hermes Atoll 2019

0 0.5 1 2 Miles

N



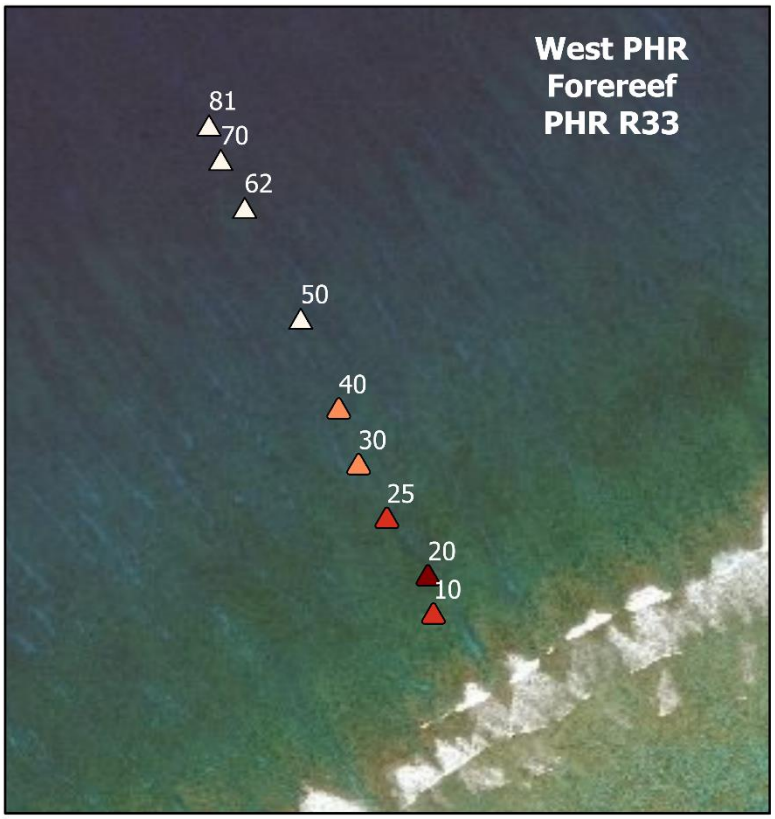
Chondria tumulosa Depth Survey
Pearl and Hermes Atoll 2019

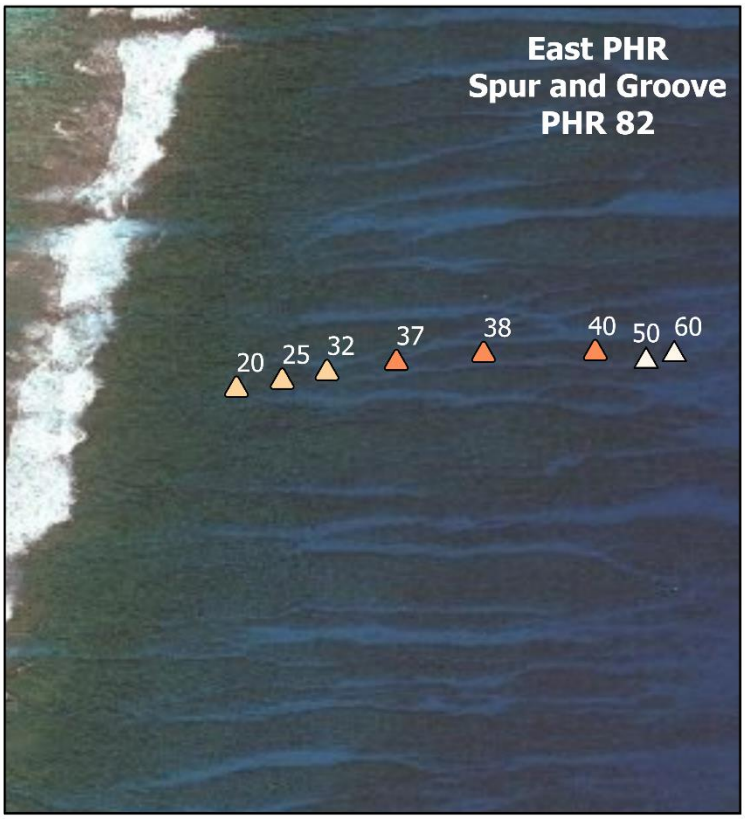
Chondria Depth Survey Sites

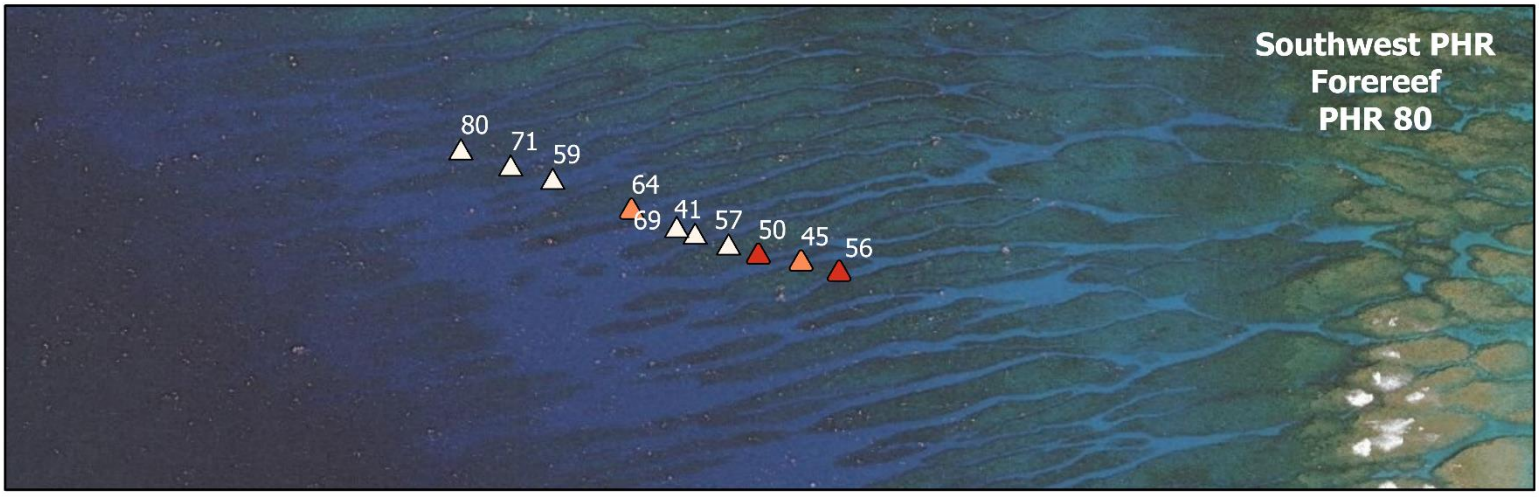
Percent Cover (%)

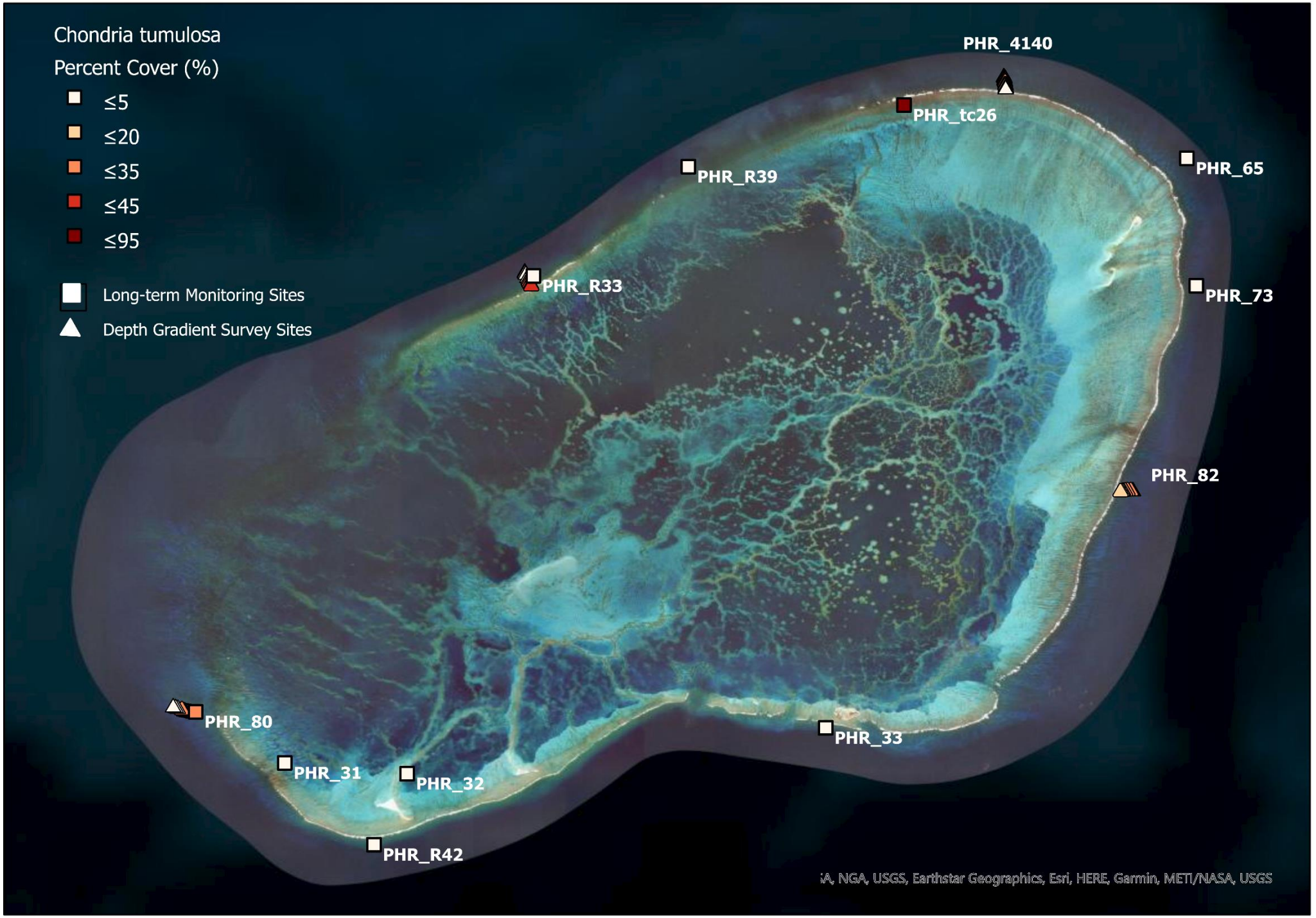
- △ ≤5
- △ ≤20
- △ ≤45
- △ ≤75
- △ ≤95

*** Depth in fsw are labeled next to each site**









Priority Chondria tumulosa Survey Sites 2021

Pearl and Hermes Atoll, NWHI

0 0.75 1.5 3 Miles

N

Long-term Monitoring Sites for Coral Health

Site	Chondria_%	Lat	Long	Depth(fsw)	Reef_Zone	Pins
PHR_31	1.95	27.775752	-175.973484	25	Backreef	NO
PHR_32	0	27.772788	-175.939602	25	Backreef	YES
PHR_33	0	27.78547	-175.82375	40	Forereef	YES
PHR_65	0	27.94305998	-175.72381	66	Forereef	NO
PHR_73	0	27.90783	-175.72118	62	Forereef	NO
PHR_80	28.3	27.78992	-175.99815	40	Forereef	NO
PHR_R33	1.75	27.91051667	-175.9046667	38	Forereef	YES
PHR_R39	0	27.940767	-175.86175	35	Forereef	YES
PHR_R42	0	27.753133	-175.948767	48	Forereef	YES
PHR_tc26	70.7	27.9578	-175.80208	5	Backreef	YES

****Please see *Chondria tumulosa* data excel sheet for field notes about each long-term site.

Email kpascoe@hawaii.edu or johnhr@hawaii.edu for questions

Depth Survey Sites in 2019

Site	Lat	Long	Depth_fsw	Chondria_%
PHR_80	27.79004507	-175.9989996	56	60
PHR_80	27.79019041	-175.9995386	45	30
PHR_80	27.79028672	-176.000145	50	70
PHR_80	27.79041848	-176.0005664	57	1
PHR_80	27.79057112	-176.0010409	41	1
PHR_80	27.79066558	-176.0013012	69	5
PHR_80	27.79092475	-176.0019424	64	30
PHR_80	27.79134829	-176.0030582	59	0
PHR_80	27.79153554	-176.0036547	71	0
PHR_80	27.79176688	-176.0043603	80	0
PHR_R44	27.91209639	-175.9070383	81	0
PHR_R44	27.91182113	-175.9069445	70	0
PHR_R44	27.91143849	-175.9067535	62	0
PHR_R44	27.91055487	-175.9063072	50	0
PHR_R44	27.90983998	-175.9060043	40	35
PHR_R44	27.90939557	-175.9058457	30	40
PHR_R44	27.90896785	-175.9056208	25	75
PHR_R44	27.90850919	-175.905293	20	95
PHR_R44	27.90820476	-175.9052493	10	65
PHR_82	27.8515384	-175.7386908	60	0
PHR_82	27.85148618	-175.7389153	50	1
PHR_82	27.85154628	-175.7393205	40	35
PHR_82	27.85153178	-175.7402111	38	40
PHR_82	27.85146656	-175.7409064	37	30
PHR_82	27.8513871	-175.7414596	32	15
PHR_82	27.8513177	-175.7418164	25	20
PHR_82	27.85125375	-175.7421811	20	15
PHR_4140	27.96611667	-175.774446	68	1
PHR_4140	27.96578147	-175.774356	64	15
PHR_4140	27.96546858	-175.7742744	60	30
PHR_4140	27.96521695	-175.7742778	52	5
PHR_4140	27.9648344	-175.7742014	48	10
PHR_4140	27.96451195	-175.7740865	41	45
PHR_4140	27.96387342	-175.7740525	35	60
PHR_4140	27.96353538	-175.7739496	30	20
PHR_4140	27.96329582	-175.7739113	25	5
PHR_4140	27.96311201	-175.773869	21	1
PHR_4140	27.96276198	-175.7739391	19	0

Proposed Sites

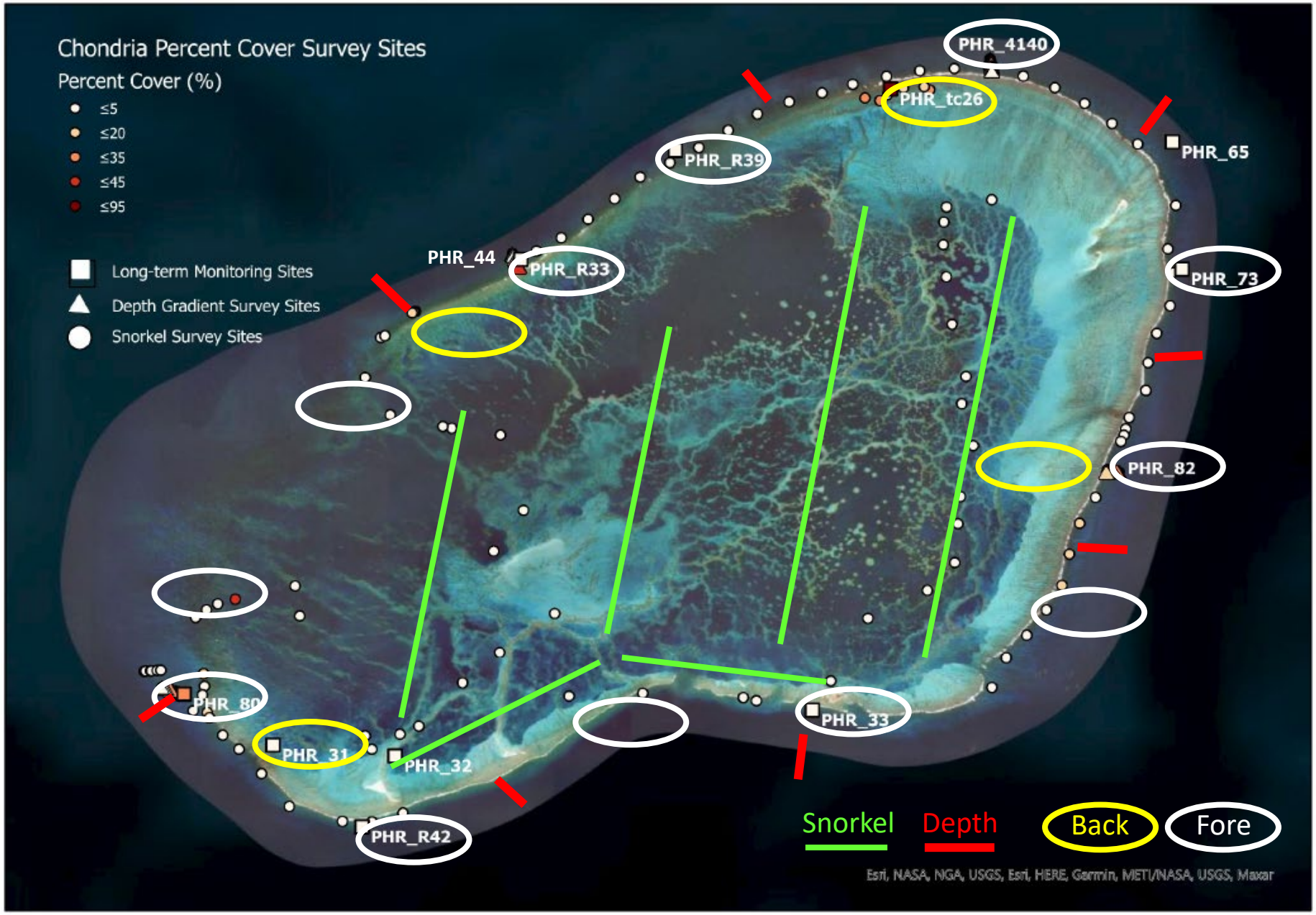
July 2021 Cruise - Manawai

FFS – Shake down dives

- Rapture Reef
  - Collect algae and images of algal/coral successional patterns
  - Images for MEGA lab

Manawai – 8 days of diving @ 3 dives/day

- Forereef (n=12 dives)
  - Dives spread around the perimeter
  - 30-60 ft depth target
  - Use permanent coral sites when available
- Backreef (n=4 dives)
  - 2 permanent backreef sites
  - 2 new sites to the West and East
- Depth gradient dives (n=8)
  - ~70-80 ft start to 10 ft depths
- Lagoon (Snorkel)
  - Visual transects snorkeling to ~20 ft
  - Free dives to assess %cover



Abundance and Distribution of *Chondria tumulosa*

Pearl and Hermes Atoll 2019

0 0.5 1 2 Miles

N

Placement is approximate