# A predictive framework for identifying source populations of non-native marine macroalgae: A case study of *Chondria tumulosa* in the Pacific Ocean (#115206)



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# A predictive framework for identifying source populations of non-native marine macroalgae: A case study of *Chondria tumulosa* in the Pacific Ocean

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The cryptogenic marine red alga *Chondria tumulosa* was first observed in 2016 in subtidal habitats at Manawai (Pearl and Hermes Atoll) in the Papahānaumokuākea Marine National Monument (PMNM), Hawai'i. This alga has since substantially increased in benthic cover and has been discovered on two additional atolls in PMNM: Kuaihelani (Midway) and Hōlanikū (Kure). It exhibits several characteristics indicative of non-native origins including putative prior absence in the region, persistence in high densities over nearly a decade, apparent lack of native herbivore pressure, and strong tetrasporophytic bias. Importantly, it is negatively impacting the culturally and ecologically important reefs of PMNM. The geographical origin of this putative invasion is unknown, and there are no published

reports of the species occurring anywhere other than PMNM. The central Pacific location of Hawai‘i allows a broad range of potential sources for the origin of *C. tumulosa*. Taxonomic ambiguities within the genus *Chondria* and challenges associated with sampling necessitate the development of a narrowed set of search locations and efficient search strategies to detect the species outside of PMNM. Attachment to floating debris is a potential introduction vector for *C. tumulosa* into PMNM, and an oceanographic model was used to identify the most likely source locations for this pathway between 2000 and 2015, including Japan in the western Pacific, Johnston Atoll, the Line Islands including Palmyra Atoll in the central Pacific, and Clipperton Atoll and the Galápagos Islands in the eastern Pacific. We used eDNA assays from three of the regions of interest to screen for *C. tumulosa* with no samples yielding positive detections. We provide a framework for investigating positive eDNA field detections using in-water surveys, microscopy, and DNA barcoding. A parallel sampling effort targeting preserved specimens stored in global herbaria is also presented. Identification of the native range of *C. tumulosa* is a critical step that will allow for an evaluation of its evolutionary ecology and any shifts that may have occurred that facilitated its putative invasion and subsequent spread, offering insights crucial for the development of mitigation strategies to safeguard PMNM against further risk.

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## Abstract

The cryptogenic marine red alga *Chondria tumulosa* was first observed in 2016 in subtidal habitats at Manawai (Pearl and Hermes Atoll) in the Papahānaumokuākea Marine National Monument (PMNM), Hawai‘i. This alga has since substantially increased in benthic cover and has been discovered on two additional atolls in PMNM: Kuaihelani (Midway) and Hōlanikū (Kure). It exhibits several characteristics indicative of non-native origins including putative prior absence in the region, persistence in high densities over nearly a decade, apparent lack of native herbivore pressure, and strong tetrasporophytic bias. Importantly, it is negatively impacting the culturally and ecologically valuable reefs of PMNM. The geographical origin of this putative invasion is unknown, and there are no published reports of the species occurring anywhere other than PMNM. The central Pacific location of Hawai‘i allows a broad range of potential sources for the origin of *C. tumulosa*. Taxonomic ambiguities within the genus *Chondria* and challenges associated with sampling necessitate the development of a narrowed set of search locations and efficient search strategies to detect the species outside of PMNM. Attachment to floating debris is a potential introduction vector for *C. tumulosa* into PMNM, and an oceanographic model was used to identify the most likely source locations for this pathway between 2000 and 2015, including Japan in the western Pacific, Johnston Atoll, the Line Islands including Palmyra Atoll in the central Pacific, and Clipperton Atoll and the Galápagos Islands in the eastern Pacific. We used eDNA assays from three of the regions of interest to screen for *C. tumulosa* with no samples yielding positive detections. We provide a framework for investigating positive eDNA field detections using in-water surveys, microscopy, and DNA barcoding. A parallel sampling effort targeting preserved specimens stored in global herbaria is also presented. Identification of the native range of *C. tumulosa* is a critical step that will allow for an evaluation of its evolutionary ecology and any shifts that may have occurred that facilitated its putative invasion and subsequent spread, offering insights crucial for the development of mitigation strategies to safeguard PMNM against further risk.


## Introduction

Papahānaumokuākea Marine National Monument (PMNM) is a protected marine region of over 1,500,000 $km^2$ surrounding the atolls and islands to the northwest of the populated Main Hawaiian Islands (MHI). It holds substantial value as a part of the cultural heritage of Hawai‘i with cosmological importance and symbolic meaning to Native Hawaiians (Kosaki, Chow, and Keenan 2009; Kikiloi et al. 2017). The vast nature of PMNM perpetuates long-standing oceanic activities, including traditional navigation (Wiener and Wagner 2013; Gaymer et al. 2014; Kikiloi et al. 2017). It was designated as a World Heritage Site in 2010 by the United Nations' Education, Scientific and Cultural Organization (UNESCO), and recognized as the first mixed site in the United States for its scientific and cultural importance (Abdulla et al. 2013). More recently, on January 16, 2025, the National Atmospheric and Oceanic Administration (NOAA)

designated the marine areas of PMNM as the Papahānaumokuākea National Marine Sanctuary making it the United States' 18th and largest National Marine Sanctuary (National Marine Sanctuaries 2025). PMNM is co-managed by several entities, including the State of Hawai‘i, the Fish and Wildlife Service, NOAA and the Office of Hawaiian Affairs with an incorporation of Native Hawaiian values, serving as an example for other large scale protected areas globally (Toonen et al. 2013; Kikiloi et al. 2017). The collaborative management approach not only safeguards the ecological integrity of PMNM, but fosters a harmonious relationship between conservation efforts and cultural heritage preservation.

Legal protection and the remoteness of PMNM have helped preserve this region, which is relatively pristine in comparison to the MHI and other populated areas worldwide, and is largely shielded from direct anthropogenic stressors, such as overfishing, eutrophication, sedimentation, and until recently, the presence of invasive species (Selkoe et al. 2009; Sherwood et al. 2020; Carlton and Schwindt 2024). Fish communities of PMNM are dominated by apex predators (Friedlander and DeMartini 2002; Holzwarth et al. 2006) and atolls are characterized by low rates of coral disease (Kenyon et al. 2006), healthy benthic communities (Vroom and Braun 2010), and large populations of birds and turtles (Harrison 1990; Balazs and Chaloupka 2004; Dale, Meyer, and Clark 2011). The relatively pristine state of PMNM provides a baseline for the study of coral ecosystems globally, and those in the MHI. For example, the relative dominance of macroalgae in PMNM diverges from the paradigm of coral dominance in "healthy" reef systems, indicating that strategies for reef health assessment need revision (Vroom and Braun 2010). Despite the overall robust ecosystem health of PMNM reefs, the region is far from immune to global challenges, such as climate change (ocean warming and acidification), sea level rise, and marine debris (Selkoe et al. 2009; Royer et al. 2023).

The origin of Hawai‘i's marine animal biodiversity has been studied in some detail (Briggs and Bowen 2012; Briggs and Bowen 2013). Hawai‘i's marine animal fauna is derived from multiple source regions; however, biodiversity is both received and exported from the archipelago (Bowen et al. 2013). Biogeographic connectivity of these groups is mainly with the Indo-Polynesian province (Briggs and Bowen 2012) where intermediary islands including Johnston Atoll and the Line Islands can act as stepping stones (Bowen et al. 2013). Connectivity also exists with Japan in the western Pacific via the Kuroshio Current and the North Pacific Subtropical Gyre as demonstrated by the 2011 Tōhoku earthquake, which generated substantial amounts of marine debris that rafted to the Hawaiian Islands carrying marine organisms (Carlton et al. 2017; 2018). Marine debris facilitates the dispersal of organisms, overcoming natural dispersal barriers and connecting regions of distinct biodiversity. Entanglement in human-made marine debris enables essentially indefinite dispersal for certain organisms provided their physiological requirements for light, nutrients, and temperature are met (Simkanin et al. 2019). Marine debris is increasingly recognized as a vector for introductions, and has the potential to facilitate transit across exceptional distances from source to sink locations (Carlton et al. 2018;

Chong et al. 2023; Haram et al. 2023; Carlton and Schwindt 2024). Further, PMNM is impacted by an abundance of large, floating, abandoned, lost, or discarded fishing gear, which acts as a habitat substitute in the open ocean with the capacity for non-native species transport and introduction (Coleman et al. 2014; Royer et al. 2023; Benadon et al. 2024). Manawai Atoll (Pearl and Hermes Atoll) leads the PMNM in volume of marine debris, with 505 metric tons removed over the last 40 years (Baker et al. 2024). Fewer connections exist between the central Pacific and the Eastern Tropical Pacific (ETP) due to the large open ocean distances separating the two regions (Briggs and Bowen 2013). Occasional connections across this soft boundary do occur in animal groups and these events disperse genetic material in both directions (Lessios and Robertson 2006; Wood et al. 2016; Romero-Torres et al. 2018).

As compared to animals, the Indo-Pacific biogeographic patterns in the red algae remain an enigma. The historical biogeography of the brown alga *Lobophora* J.Agardh (Vieira et al. 2017) and the green algal family Udoteaceae J.Agardh (Lagourgue et al. 2022) supports patterns of dispersal from the western Indo-Pacific to the central Pacific islands, followed by limited diversification. One of the few studies to examine red algal biogeography across the Indo-Pacific focused on the genus *Portieria* Zanardini (Leliaert et al. 2018). The findings suggest that diversity in Hawai‘i may have been imported from the southwestern Pacific (i.e., Tonga, Fiji, New Caledonia, and the east coast of Australia). Additionally, diversity within the Hawaiian archipelago appears to be relatively low, with no evidence of export from the region (Leliaert et al. 2018). Yet, red algal propagules are short lived (Hoffman and Camus 1989), non-flagellated (Santelices 1990), negatively buoyant (Okuda and Neushul 1981; Hoffman and Camus 1989), and attach rapidly to nearby substrata when released (Okuda and Neushul 1981; Fletcher and Callow 1992). Moreover, female gametes do not disperse and are retained on the female gametophyte (Searles 1980). Thus, red algae are unlikely to disperse via spores or gametes over long distances, exhibiting patterns of isolation by distance from small to large spatial scales (e.g., Engel et al. 2004; Krueger-Hadfield et al. 2013; Krueger-Hadfield et al. 2017). Given these limitations, marine debris may be a vector of importance in moving red algae through the Pacific Ocean.

*Chondria tumulosa* Sherwood and Huisman, first observed at Manawai in 2016 (Sherwood et al. 2020) is a red alga that negatively impacts reefs of the northernmost atolls of PMNM for around a nearly a decade (Sherwood et al. 2020; Lopes et al. 2023). Entangled thalli form mats up to 18 cm thick, which smother the existing benthic subtidal community in patches of several thousand square meters (Sherwood et al. 2020). Mats have been observed attached, loosely adhered, tumbling along the benthos, and acting as flotsam in PMNM (Sherwood et al. 2020; Lopes et al. 2023). The prolific growth of these mats is a threat to the biodiversity and abundance of native benthic species that are overgrown. Mats or clumps of the alga break away and disperse along the benthos, and more rarely disperse along the surface of the ocean; presumably, these thallus fragments can establish and grow in new areas via secondary reattachments and asexual

processes (Sherwood et al. 2020; Lopes et al. 2023; Williams et al. 2024). Accumulations of fragmented clumps, which tend to be negatively buoyant, gather in regions of bathymetric depressions and form dark patches visible from high resolution satellites (Lopes et al. 2023). Moreover, the sites sampled at Manawai in 2019 were dominated by tetrasporophytes – a hallmark of range expansions and asexual reproduction in haploid-diploid algae (Krueger-Hadfield 2020) – as no gametophytic thalli were observed using microscopy or microsatellite genotyping and there are genetic signatures of asexual reproduction (e.g., repeated genotypes) (Williams et al. 2024). Observations from authors during in-water surveys suggest growth forms of *C. tumulosa* can vary from robust and mat-forming to cryptic and epiphytic, yet the drivers of these diverse growth habits remain unknown.

Though the status of *C. tumulosa* has not been confirmed as non-native, there are multiple indications that the species is introduced to PMNM. Although the first observation of *C. tumulosa* at Manawai was in 2016 (Sherwood et al. 2020), satellite observations of accumulations of fragmented clumps revealed the species was likely present since at least 2015 (Lopes et al. 2023). Spatial analysis of these accumulations showed a 115-fold increase in surface area coverage from 2015-2021 at Manawai (Lopes et al. 2023). In 2021, the alga was observed ~150 km to the northwest at Kuaihelani (Midway Atoll) and in low abundance in 2023 at Hōlanikū (Kure Atoll), a further ~100 km to the northwest and the northwestern-most atoll in PMNM. Lacking a morphological or DNA barcoding sequence match in any publicly available database, a new species was described and declared cryptogenic (Sherwood et al. 2020). The growth habit of *C. tumulosa* (Sherwood et al. 2020), its persistence in high densities over nearly a decade (Lopes et al. 2023), tetrasporophytic dominance facilitated by asexual thallus fragmentation (Williams et al. 2024), signatures of clonality not expected in a native species (Williams et al. 2024), putative prior absence in the region, regionally restricted distribution, anthropogenic dispersal potential (Lopes et al. 2023; Fumo et al. 2024), as well as its apparent lack of native herbivore pressure (Lopes et al. 2023), collectively indicate a recent introduction to PMNM (see also Carlton and Schwindt 2024). Additionally, the species has been observed rafting on flotsam in PMNM, indicating that this may be the dispersal vector not only for the recent spread of the species within PMNM, but perhaps also into the region (Fumo et al. 2024). While there are other potential means of introduction, drift material may be considered the most plausible vector for the introduction of *C. tumulosa* to PMNM because it is unlikely that propagules could survive the long distances required to reach PMNM through other transport mechanisms (Fumo et al. 2024), the species has been observed rafting on flotsam (Lopes et al. 2023), and marine debris strongly influences PMNM (Royer et al. 2023). Observations of trans-oceanic rafting events, including those of *C. tumulosa* in PMNM, demonstrate that taxa once confined to their respective biogeographic zones can overcome long established dispersal barriers via episodic rafting events (Theil and Haye 2006; Carlton et al. 2017; Lopes et al. 2023; Benadon et al. 2024). Further, as demonstrated by the prolific red algal invader *Gracilaria vermiculophylla* (Ohmi) Papenfuss, which occurs in warmer waters throughout its non-native

range compared to its source populations (Sotka et al. 2018), species can establish, persist, and spread into novel environmental conditions, complicating efforts to pinpoint their origins. Thus, the potential source regions of *C. tumulosa* and the vectors for transport into PMNM are broad, necessitating mechanisms to strategically sample regions of interest in an efficient manner.

Given the broad range of potential origins and varied pathways by which *C. tumulosa* could have arrived in PMNM, connectivity modeling may help identify key areas to prioritize in sampling efforts and enable more efficient and targeted searches. Connectivity modeling facilitates investigations on species population connectivity (Paris et al. 2013; Criales et al. 2019) and provides insights into how environmental and biological factors influence dispersal (Swearer et al. 2019; Fumo et al. 2024). By integrating oceanographic data with biological processes, connectivity models can predict patterns of species distributions, identify barriers to dispersal, and predict the phylogeography of marine organisms (Vaz 2012; Paris et al. 2013; Wren et al. 2016; Hixon et al. 2022). Congruence between modeled and observed connectivity, both globally (e.g., Fraser et al. 2022) and in Hawai‘i (e.g., Counsell et al. 2022), highlights the utility of these models in various applications, including fisheries management (Hixon et al. 2022), conservation planning (Schill et al. 2015), and assessing dispersal across oceanic distances (Wood et al. 2016). Moreover, connectivity modeling helps in the examination of the spread and arrival of invasive species (Treml et al. 2008; Johnston and Purkis 2011; Johnston and Purkis 2013) and serves as a cost-effective tool for studying connectivity (Swearer et al. 2019), contributing to an integrated understanding of species dispersal in marine environments.

Accurate and sensitive detection methods are crucial for understanding the dispersal of nuisance taxa across oceanic basins; however, the resources required to visually survey all potential source locations render this option unfeasible. Instead, molecular detection methods, such as environmental DNA (eDNA)– any genetic material obtained from the environment– may be better suited for rapid and widespread detection of rare aquatic taxa (Darling and Blum 2007; Goldberg et al. 2013; Doyle, McKinnon, and Uthicke 2017; Mauvisseau et al. 2018; Keller et al. 2022; Gargan et al. 2022). eDNA can circumvent the need for resource-intensive visual surveys (Dejean et al. 2012; Smart et al. 2015) and has recently been developed for *C. tumulosa* (Nichols et al. 2025). For aquatic nuisance taxa, eDNA can be isolated from the environment by filtering water through porous membranes (Taberlet et al. 2014). Quantitative polymerase chain reaction (qPCR) amplification of eDNA using species-specific assays can then be used to detect rare taxa and increase detection over conventional direct observation (Harvey et al. 2009, Smart et al. 2015, Burian et al. 2021, Keller et al. 2022). The concentration of target DNA can also be reliably quantified, presuming eDNA concentrations exceed the assay's limits of detection (LOD, where 95% of technical replicates amplify) and quantification (LOQ, the lowest initial DNA concentration quantifiable with a coefficient of variation below 35%), respectively (Klymus et al. 2020). In this manner, eDNA detections can provide information regarding a population's relative abundance and be used to spur more intensive non-molecular surveys (Jerde

et al. 2011). Thus, an assay detecting *C. tumulosa* eDNA in seawater samples can assist in uncovering the origins of this cryptogenic alga (Nichols et al. 2025).

The morphological and genetic complexities of distinguishing species of *Chondria* and related genera highlight the crucial role of molecular analysis in confirming species identity and range. There are currently 78 accepted species names for *Chondria* with 68 having been placed in synonymy (Guiry and Guiry 2024). *Chondria tumulosa* is morphologically distinct from other species in the genus in having large, robust thalli, a mat-forming tendency, and terete, bluntly rounded axes with decreasing diameter with each level of branching (Sherwood et al. 2020). The mat-forming habit of the species is a result of multicellular haptera attaching and reattaching the thallus to the substratum, and a gradient of colors exists from golden-brown to dark brown or purple from the upper to lower sections of the mat where irradiance is limited by self-shading (Sherwood et al. 2020). Morphologically distinguishing *Chondria* species is challenging, especially from pressed material. Furthermore, many species have not been sequenced, limiting the utility of genetic comparisons for the identification of the source location of *C. tumulosa*. The Universal Plastid Amplicon (UPA) provides a useful DNA barcode because it exhibits high amplification and sequencing success rates from degraded DNA; however, it is a relatively conserved region in comparison to other commonly used markers (Sherwood et al. 2010). Despite the conserved nature of UPA, no published matches have yet been identified for *C. tumulosa* outside of PMNM, implying this barcode can be used for initial screening of specimens. However, as many species of *Chondria* have yet to be sequenced using the UPA barcode, there may be species which share exceptionally similar sequences. Thus, further sequencing of the nuclear SSU rDNA, mitochondrial COI, and plastidial *rbc*L DNA barcodes can be used to bolster identifications of *C. tumulosa* (Sherwood et al. 2020). The full plastid genome of *C. tumulosa* is also available on GenBank under accession MW309501 (Paiano et al. 2021).

This study develops a framework for identifying the source of *C. tumulosa* in PMNM through oceanographic modeling, eDNA surveys, in-water collections, molecular barcoding of specimens collected from the field, literature review, and by analyzing preserved collections from global herbaria. Through a combination of the techniques described here we (i) identify potential source locations of *C. tumulosa*, (ii) describe an efficient method of detecting this species in the field, and, (iii) identify species that remain targets for sequencing from herbarium collections. The methods used here are relevant for other cryptogenic algae for which information is lacking as in *C. tumulosa*.

## Materials & Methods

### Oceanographic modeling

Oceanographic information was acquired from the Hybrid Coordinate Ocean Model (HYCOM) with the Navy Coupled Ocean Data Assimilation 1/12° Global Ocean Forecasting System 3.1

41-layer Reanalysis GLBv0.08 Experiment 53.X daily snapshot (Cummings 2005; Cummings and Smedstad 2013). The dataset consisted of surface currents from January 1, 2000 to December 30, 2015 between the coordinates 90ºE-55ºW and 80ºS-90ºN, exceeding the bounds of the entirety of the Pacific Ocean and predating the earliest detection of *C. tumulosa* at Manawai by over a decade.

HYCOM oceanographic information was passed to the individual-based biophysical stochastic lagrangian dispersal simulator, the Connectivity Modeling System (CMS) (Paris et al. 2013). CMS can run a backtracking module that traces a particle found at a particular place and time backwards to its estimated source location (Paris et al. 2013). Model runs released 100 particles in daily releases from Manawai (27.8333 ºN, 175.8333 ºW) for each day from January 1, 2000 to December 30, 2015. While *C. tumulosa* is present at all three of the northernmost atolls of PMNM, Manawai was selected as the modeled release location because it has the highest recorded abundance and biomass of the alga among the atolls of PMNM (Nichols et al. 2025) and was the earliest documented location of its detection (Sherwood et al. 2020). Each particle was restricted to the surface ocean (flag upperlevelsurface=true) and resolved at daily intervals. Potential release areas were created by extracting 1º hexagonal grids intersecting with the Global Territorial Sea 12 nm shapefile from the Pacific Data Hub (Flanders Marine Institute 2023; QGIS 2023). Hexagonal grid settlement regions better capture the contours of coastlines and are more suitable than rectangular grids for individual based modeling (Birch, Oom, and Beecham 2007). Hexagonal settlement regions encompassing the Hawaiian archipelago were removed from the shapefile prior to inclusion in the CMS runs to prevent the possibility of self-recruitment back to the region of origin. All particles were given a horizontal diffusivity of 10 $m^2/s$ (Okubo 1971; Fig. S1).

Data downloads and model runs were conducted using the CMS functions getdata and cms, respectively on the University of Hawai‘i’s High Performance Computing (HPC) cluster (https://datascience.hawaii.edu/hpc). The number of particles arriving in each hexagon (landings) was mapped and higher-resolution mapping of each region displaying increased likelihood of settlement was conducted using the sf (Pebesma 2018; Pebesma and Bivand 2023) and mapdata (Becker 2022) libraries in R (R Core Team 2023). Regions of interest were determined based on the likelihood of landings from the backward trajectory start point, Manawai (Fig. S2). Bounding boxes were established around high-likelihood source regions (regions of interest): Japan’s Eastern coast from the Izu Islands to Hokkaidō (29-43.5 ºN; 135-150 ºE), The central Pacific encompassing Johnston Atoll and the Line Islands including Palmyra (0.5-18 ºN; 155-172 ºW), and the Eastern Tropical Pacific islands of Clipperton and the Galápagos Archipelago (2.5 ºS-12 ºN; 88.5-110 ºW). The drift time to each region of interest was assessed through pairwise comparisons using Wilcoxon rank sum tests with Bonferroni correction (R Core Team 2023). The timing of arrivals to Manawai, categorized by regions of interest, was analyzed in relation to the phase of the El Niño Southern Oscillation (ENSO) and by season of the year. Seasons were

defined as September-October-November, December-January-February, March-April-May, and June-July-August. These analyses were plotted and tested for significance using a Wilcoxon paired rank sum test with Bonferroni correction (R Core Team 2023). ENSO phase conditions in each month over the modeled period were downloaded using the rsoi library (Albers 2023) in R.

Diffusivity value optimization was conducted through comparison of targeted CMS runs with real-world drifter data. Marine debris at Manawai was tagged using Satlink solar-powered satellite buoys weighing 13.7 kg with a 40.6 cm diameter and 36.8 cm height in September-October 2018. Units were set to record their position once every four hours. Of the six tracking devices attached to marine debris, two loggers became detached and escaped the atoll on February 1, 2020 and December 14, 2020, respectively. Both loggers stopped recording in September 2021 and drifted a considerable distance from Manawai. For comparison with CMS output, we utilized HYCOM data from February 1, 2020 to September 30, 2021 for surface currents between the coordinates 150ºE-160ºW and 15-40ºN. CMS runs were conducted in backtracking mode independently for each logger with 1000 releases from the final GPS coordinate and timestamp of each receiver. Each release was replicated five times with each iteration varying only in horizontal diffusivity which was set to 0.5, 5, 10, 20, or 50 $m^2/s$. The optimum diffusivity value for drifting nets in the backtracking model was assessed by calculating the minimum distance that each particle drifted from Manawai, the true release location of the loggers, using the geosphere library (Hijmans 2022) in R. The performance of each turbulence value was evaluated through a pairwise Wilcoxon rank sum test with Bonferroni correction implemented in R (R Core Team 2023) (Fig. S1).

We accessed high-resolution monthly satellite measurements from the National Oceanic and Atmospheric Administration's Optimum Interpolation Sea Surface Temperature (OISST) dataset, with a spatial resolution of 1/4° latitude by 1/4° longitude (Reynolds et al., 2007). For each high-likelihood polygon and Manawai, we retrieved the OISST measurement from the grid cell nearest the centroid of the polygon for the dates January 1, 2000 to December 30, 2015 for a total of 193 monthly values at each location. In the cases where numerous high likelihood polygons were adjacent to each other (i.e., Izu Islands, Johnston Atoll, and the northwestern corner of the Galápagos archipelago) one measurement was accessed. Mean, median, and minimum monthly measurements were calculated and recorded (Table 2).

**eDNA collection and screening**

Three potential source regions identified by oceanographic modeling were sampled for *C. tumulosa* eDNA during 2024: Kiritimati Atoll of the Line Islands (n=22), Johnston Atoll (n=40), and Japan’s Okinawa Island (n=20) (Fig. S3; Table S1). At each site within a region, 2-L replicate seawater samples were collected from within 1 m of the bottom. Each 2-L biological sample and a control were shaken, filtered through mixed cellulose ester filters (Millipore; diameter: 47 mm; pore size: 0.22 µm) on a peristaltic pump (Cole-Parmer, USA), and frozen in

liquid nitrogen. DNA was extracted (DNeasy Blood & Tissue kit, Qiagen, USA) from thawed filters, and cut in half as described in Nichols and Marko (2019) and Nichols, Timmers, and Marko (2022). Water samples for eDNA analysis were collected under United States Fish and Wildlife Service Special Use Permit 12543-24001 (Johnston Atoll), a Research Consent Certificate from the Ministry of Fisheries and Marine Resources Development (Kiribati), and with the permission of the University of the Ryukyus (Okinawa).

To increase screening efficiency, eDNA from each individual replicate water sample was pooled within each region and amplified using a qPCR assay for *C. tumulosa* (Nichols et al. 2025). To ensure that pooling multiple samples during the screening process did not create false-negative detections (due to dilution of presumably low concentrations of *C. tumulosa* eDNA), a second pool was also created per region, with an addition of 1 µL eDNA from a known positive site at Kuaihelani, PMNM, where *C. tumulosa* was observed in 1% relative abundance (hereafter referred to as the "field positive"). Conceptually, pooled regions with spiked positive eDNA (hereafter referred to as the "positive pool") should produce amplifications using the assay, signaling the presence of *C. tumulosa.* Pooled regions without the spiked eDNA (hereafter referred to as the "sample pool") should only amplify if *C. tumulosa* is locally present.

Reactions were run using triplicate technical replicates consisting of 4.5 µL SYBR green SSo Advanced Supermix (Bio-Rad), 3 µL ultrapure $H_2O$ (Growcells), 0.5 µL bovine serum albumin (20 mg/mL, ThermoFisher Scientific), 0.5 µL (10 µM) of each of the forward (5'-GCCGTGAATCGTTCTATTGC-3') and reverse (5'-TCAGCTCTTTCGTACATATTCTCC-3') primers (Nichols et al. 2025) for a fragment of *rbc*L, and 1 µL sample pool eDNA. Amplifications were run on a CFX96 Touch Real-Time PCR Detection System and CFX Manager software (Bio-Rad). The CFX96 calculated critical thresholds of fluorescence (above which detections can be discriminated from background noise), averaged across amplification plates. A positive DNA starting quantity (calculated automatically in CFX Manager against a tenfold serial dilution of standards with known DNA concentrations) in any technical replicate was considered a positive detection. Amplification curves were plotted using ggplot2 (Wickham 2016) in R (R Core Team 2023) and fitted with generalized additive model (GAM) smoothers. Positive detections were considered reliable if the GAM smoother exceeded the mean threshold of fluorescence, demonstrating precision among replicates.

All laboratory surfaces and equipment were routinely decontaminated using a 10% bleach solution. Water collection containers were sterilized with 10% bleach for 12 h, air dried, and rinsed with surface seawater at each new location prior to sampling. Equipment contamination was monitored using bleach-sterilized filtration equipment and a 1-L tap water or DI water equipment blank (EB) filtered prior to field samples, at least once per day, which served as a negative control for both the filtration and DNA extraction steps. PCR contamination was monitored with triplicate PCR negatives (no-template controls, NTC) per 96-well plate.

**DNA barcoding of herbarium specimens**

Sequencing was attempted from 156 preserved specimens including 60 pressed algal specimens from the *Herbarium Pacificum* of the Bernice P. Bishop Museum in Honolulu, Hawai‘i, USA (BISH), 39 from the herbarium of Meise Botanic Garden (BR) (formally from Ghent University, GENT), 26 from the Environmental Research Institute, São Paulo, Brazil (SP), 14 from the National Institute of Water and Atmospheric Research, Wellington, New Zealand (NIWA), 7 from the University of Malaya in Kuala Lampur, Malaysia (KLU), 4 from the Prince of Songkla University, Hat Yai, Thailand (PSU), and six from *C. tumulosa* collected in PMNM (Table S2).

Genomic DNA was extracted following a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1989) with a CTAB buffer and β-mercaptoethanol as a grinding solution as described in Fumo and Sherwood (2023). The Universal Plastid Amplicon (UPA) region (Presting 2006) was amplified following Sherwood and Presting (2007) with the primer pair p23SrV_f1 (5’-GGACAGAAAGACCCTATGAA-3’) and p23SrV_r1 (5’-TCAGCCTGTTATCCCTAGAG-3’). Cycling conditions followed those of Sherwood and Presting (2007) with successful PCR amplifications confirmed using gel electrophoresis and purified with ExoSAP-IT (Affymetrix, Santa Clara, California, United States) before submission to GENEWIZ (Azenta Corporation South Plainfield, New Jersey, United States) for Sanger sequencing. Sequences generated in this study were uploaded to GenBank and assigned accession numbers PV036782 - PV036852 (Table S2).

Forward and reverse reads were assembled in Geneious Prime 2024.0.3 (https://www.geneious.com) and aligned with a reference database. This database was constructed by retrieving other closely related sequences using an existing *C. tumulosa* UPA sequence (NC_057618), which was compared using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to recover the 100 nearest matches from GenBank. While obvious contaminants were removed, no further taxonomic reassignment was performed beyond the GenBank name or the original collector’s identification. The goal of this analysis was not to identify each sequence as a species but rather to determine whether any sequences matched *C. tumulosa*. Additional UPA sequences for *C. tumulosa* were generated using the extracted DNA generated during the description of the species (Sherwood et al. 2020).

All Rhodomelacean sequences generated from the BLAST search (n=84) were aligned with those from the successful amplifications of *C. tumulosa* from PMNM (n=6) and those generated from material stored in herbaria (n=66) using MUSCLE v5.1 in Geneious (Edgar 2022) to create a final alignment of 159 UPA sequences of 421 bp in length. Percent identity was recorded for each sequence in comparison to NC_057618 (Paiano et al. 2021) and organized according to similarity (Table S2). Additional BLAST searches for the DNA sequences of the SSU, COI, and

*rbc*L *C. tumulosa* barcoding markers were conducted to ensure no matching sequences had been uploaded since the initial description of the species by Sherwood et al. (2020).

### Geographic distributions of *Chondria* species

All entities classified under the genus *Chondria* C.Agardh were investigated for range overlap with the regions of interest outlined by model results. Entities that had been synonymized with taxa in other genera were included in this analysis unless the nomenclatural change removed them from the family Rhodomelaceae altogether. Detailed distribution information was obtained from AlgaeBase (Guiry and Guiry 2024). For each entity the GenBank NCBI database was queried and the DNA barcodes available for each were recorded.

## Results

### Oceanographic modeling

Landing totals for 584,300 particles tracked backwards in time (Table S3) indicated that the most likely regions of origin for *C. tumulosa* in PMNM suggested by CMS were (i) Japan (27.4% of released particles), (ii) the central Pacific islands of Johnston Atoll, the Line Islands including Palmyra Atoll (23.8%), and (iii) the ETP islands of Clipperton and the Galápagos archipelago (14.3%) (Fig. 1). Additional landings occurred throughout the regions outside of these boundaries in the central, western, and eastern Pacific at lower frequency. Landings in individual hexagons were generally low with 18 of the 3782 modeled polygons supplying ≥ 1% of particles to Manawai (Fig. S2).

The six individual landing polygons with the highest likelihood of supplying *C. tumulosa* to Manawai were geographically concentrated in four locations. Two polygons were located in Japan: Cape Inubo with 44,336 particles (7.6%), and the Izu islands to the south of Tokyo with 24,415 (4.2%). Two polygons were at Johnston Atoll: the western polygon with 43,248 (7.4% of released particles) and the northeastern polygon with 28,405 (4.9%). The remaining two polygons were in the Galápagos archipelago: the northwestern corner, centered around the islands of Darwin and Wolf, with 32,133 (5.5%), and the second most northwestern polygon between Isabela and Wolf Islands with 23,149 (4.0%).

Modeled landings were observed along Japan's eastern coastline with a peak in origin likelihood (landings) occurred at Cape Inubo (Fig. 1). Johnston Atoll is relatively small in comparison to the surrounding hexagonal grids with high landings across the entire area and an apparent peak in landings on the western edge of the region. The Line Islands including Palmyra Atoll exhibited lower landings overall with a local peak in the northern ends of the islands and island chain (Fig. 1). The Galápagos Islands in the ETP showed a peak in landings at the northwestern end of the archipelago in the grids surrounding the islands of Darwin and Wolf while Clipperton Island showed relatively low likelihood throughout the region (Fig. 1).

Particles landing in Japan were generally tracked on a northerly route from Japan through the North Pacific Subtropical Gyre (i.e., Kuroshio, North Pacific, California, and North Equatorial Currents) to Manawai, while particles landing in the ETP largely traveled through the central Pacific via the North Equatorial Current before landing at Manawai. Central Pacific particles generally traveled to the north and west, taking a more direct path towards Manawai. The combined locations of all tracks centers around Manawai, tapering to the west towards Japan and towards the south and east towards the ETP with an extension towards the southwest and nearer Micronesia (Fig. 2; Fig. S4). The mean drift time for landing in each region from the backward trajectory start point differed significantly at 420, 552, and 1007 days for the central Pacific, Japan, and the ETP, respectively (Wilcoxon p=2.2e-16 in all cases) (Fig. S5). The number of particles landing per month varied considerably through time, yet ENSO phase was not correlated with landing likelihood of particles in the ETP and Line Islands including Palmyra (Wilcoxon p>0.24 and shown in Table S4). Neutral period landings from Japan were significantly higher in comparison to El Niño and La Niña landings (p=0.038; p=0.003, respectively). There were no significant differences in landings by season from any of the source regions of interest (Wilcoxon p>0.41 in all cases shown in Table S5).

The modeled minimum passing distance of particles released from the final correspondence point of marine debris tagged at Manawai were compared between trials where diffusivity values were equal to 0.5, 5, 10, 20, and 50 $m^2/s$. Mean (median) minimum passing distances for each diffusivity value were 160.7 (136.2), 133.5 (119.3), 125.9 (113.7), 129.2 (113.6), and 140.6 (117.1) km, respectively and the central tendencies of the groups varied significantly (Kruskal-Wallis p=2.2e-16). A Wilcoxon signed rank test indicated that the minimum passing distance in group 0.5 $m^2/s$ differed from all other groups (Table S6). Groups 5 $m^2/s$, 10 $m^2/s$, 20 $m^2/s$, and 50 $m^2/s$ did not differ (Fig. S1; Table S6). Thus, the passing distance was not significantly affected by diffusivity values between 5-50 $m^2/s$ and the lowest passing distance was observed in the group 10 $m^2/s$ and selected as the best fit value for the backtracking model run (Fig. S1).

The SST at Manawai, Hawai‘i, one of the locations affected by *C. tumulosa* and the model backtracking start point, exhibited a minimum of 18.7 ºC, a median of 23.9 ºC, a mean of 24.0 ºC, and a maximum of 28.7 ºC (Table 2). Comparatively, the SST values at the other locations varied widely. Cape Inubo, Japan, had the lowest minimum SST value at 11.9 ºC, which was 6.8 ºC lower than Manawai, while the maximum SST at Cape Inubo was 26.0 ºC, 2.7 ºC below that of Manawai. The Izu Islands, Japan, were more similar overall to Manawai yet still cooler, with a minimum of 16.2 ºC; however, this point matched the maximum of 28.7 ºC. Johnston Atoll had a notably higher SST, with a minimum of 24.7 ºC, exceeding Manawai by 6.0 ºC. The median and mean values at Johnston Atoll were both 26.6 ºC, notably higher than the corresponding values for Manawai. Palmyra Atoll and Clipperton Atoll had SST values that were consistently higher than Manawai as well, with the median and mean SST at Palmyra at 28.3 ºC and 28.2 ºC, respectively, both over 4 ºC above that of Manawai. The Galápagos Islands of Darwin and Wolf

exhibited SST values that were closer to those of Manawai, with a minimum of 23.1 ºC and a maximum of 28.7 ºC.

**Genetic analyses of eDNA and herbarium specimens**

For eDNA screening at Kiritimati, Line Islands (n=22), Johnston Atoll (n=40), and Okinawa Island, Japan (n=20) (Table S1), all the sample pools were presumed negative, lacking any discernible traces of *C. tumulosa* eDNA (Fig. 3a). Target eDNA was, however, detected in all positive pools, with concentrations below 10 copies per reaction (Fig. 3b).

Specimens bearing a superficial morphological resemblance to *C. tumulosa* were sampled from preserved pressed specimens from different herbaria (n=156). Attempts to amplify the UPA marker were successful for 65 of these (Table S2) and none returned BLAST matches to *C. tumulosa*. The nearest match to *C. tumulosa* based on the UPA marker was accession number ARS 11026 (BISH 682104) with a 97.5% similarity. This specimen, labeled *Chondrophycus cartilagineus* (Yamada) Garbary & J.T.Harper, was collected from Pohnpei, Federated States of Micronesia in 1996 (Table S2). This similarity exceeds the nearest match available on NCBI GenBank (HQ421169- *Chondria dangeardii* E.Y.Dawson from Hawai‘i) which was a 95.8% match. BLAST similarity searches of the sequences generated by Sherwood et al. (2020) were conducted with *rbc*L, COI, and SSU and did not reveal any matches or near-matches (>99%) to *C. tumulosa* with highest pairwise identities of 89.8%, 94.5%, and 97.2%, respectively.

In total, 113 species of *Chondria* are either currently accepted taxonomically, of unresolved taxonomic status, or have been transferred to other genera within the family Rhodomelaceae (Guiry and Guiry 2024). Of these, 25 species exhibit range overlap with potential source regions identified through the modeling analysis with 18 in Japan, 3 in the Line Islands including Palmyra, 2 in Johnston, 2 in Clipperton, and 2 in the Galápagos Islands (Table 1; Table S2). DNA barcodes are not available on NCBI GenBank for 12 of these 25 species.

## Discussion

Oceanographic modeling indicated the most likely source for the arrival of *C. tumulosa* in PMNM is Japan, the central Pacific, encompassing Johnston and the Line Islands including Palmyra Atoll, or the ETP islands of Clipperton and the Galápagos Archipelago. Despite signs that the species may be introduced into the region (Sherwood et al. 2020; Lopes et al. 2023; Fumo et al. 2024; Williams et al. 2024), its introduction pathway remains unknown. The establishment of a narrowed set of search regions for environmental detections increases the likelihood of identifying the *C. tumulosa* source which would allow for essential research into the origin, evolutionary history, ecological traits, and potential for control of *C. tumulosa*, aiding in safeguarding the protected and culturally significant PMNM (Wiener and Wagner 2013; Gaymer et al. 2014; Kikiloi et al. 2017).

Although PMNM is not as strongly influenced as the MHI by anthropogenic factors such as overfishing, eutrophication, and sedimentation, PMNM still faces global challenges (Selkoe et al. 2009). Climate change impacts, such as ocean warming (Sarmiento et al. 2004), contribute to marine heat waves (Frölicher, Fischer, and Gruber 2018) leading to mass coral bleaching (Couch et al. 2017). Additionally, climate change may cause shifts in hurricane intensity and storm paths (Mendelsohn et al. 2012; Wehner, Zarzycki, and Patricola 2019). These events not only cause widespread damage (Pascoe et al. 2021) but also have the potential to further distribute marine debris (e.g., Hu et al. 2023) and non-native species (e.g., Steiner et al. 2010), including *C. tumulosa* (Lopes et al. 2023). Other global challenges facing PMNM are sea level rise impacts (Shope, Storlazzi, and Hoeke 2017), as well as the accumulation of marine debris (Royer et al. 2023; Benandon et al. 2024) which is increasingly recognized as a vector for invasive species introductions (Carlton et al. 2018; Chong et al. 2023; Haram et al. 2023; Carlton and Schwindt 2024). Further, *C. tumulosa* continues to spread in PMNM (Lopes et al. 2023), likely aided by marine debris transport (Fumo et al. 2024). The introduction of the species to the MHI is a major concern because the archipelago has a history of economically and ecologically taxing marine algal invasions (Smith, Hunter, and Smith 2002; Conklin and Smith 2005; Veazey et al. 2019), including further spread aided by asexual reproduction (Thornton et al. 2024).

The oceanographic modeling explicitly targeted marine debris as a dispersal vector across the expanse of the Pacific Ocean and included the incorporation of real-world GPS information from tagged flotsam to optimize the model diffusivity parameter. *Chondria tumulosa* may be suited to passive rafting via entanglement, as it has been observed drifting while attached to flotsam or as detached fragments carried by currents (Lopes et al. 2023). Upon arrival in a new site or atoll, the ability to fragment and grow likely facilitates rapid spread and accumulation– as evidenced by the genetic signatures of asexual reproduction described at Manawai (Williams et al. 2024). Rafting may not only transport the species within the northern PMNM, but also may be responsible for its initial introduction (Fumo et al. 2024). Although Hawai‘i is one of the most isolated archipelagos in the world, there are many routes for *C. tumulosa* to enter the region. A factor that has generated increasing concern in recent decades is that Hawai‘i in general, and PMNM in particular, are near the Great Pacific Garbage Patch. This is perhaps the world’s largest collection of drifting flotsam, including debris from across the Pacific (Maximenko, Hafner, and Niiler 2012), and hosts living communities of coastal species in pelagic environments (Haram et al. 2023). Particles landing at Manawai in the CMS output were derived from numerous locations across the expanse of the Pacific Ocean, demonstrating this reality. Nevertheless, our model results narrow the potential drifting routes and source regions for particles coming from their respective source regions and thus aid in the broader search for *C. tumulosa*.

Rafting particles transiting from the ETP largely pass through the central Pacific and are subsequently connected with Hawai‘i via the North Equatorial Current, Hawai‘i Lee Counter

Current, North Hawai‘i Ridge Current, and Subtropical Counter Current (Maximenko, Hafner, and Niiler 2012). Evidence of this pathway's potential to transport C. tumulosa into the central Pacific comes from pumice originating from the eastern Pacific's Revillagigedo archipelago which was found in the Line Islands (Kiritimati Island) and also in Hawai‘i, through in lower abundance than at Kiritimati (Jokiel and Cox 2003). Many of the particles backtracked to the ETP from Manawai generally passed through the central Pacific during their transit, narrowly missing the Line Islands and neighboring archipelagoes. Thus, particles departing the ETP may settle in the central Pacific prior to their transit northward into Hawai‘i. Though the physical and oceanographic distances from the ETP to the central Pacific are great, ongoing connectivity continues to exist between the two regions in reef fishes (Roberton, Grove, and McCosker 2004; Lessios and Robertson 2006) and coral larvae (Wood et al. 2016; Romero-Torres et al. 2018) lending feasibility to the ETP as a source region in the CMS run, though we do note larval durations in some fishes and corals may not be indicative of algal dispersal. The satellite derived SST measurements recorded from Darwin and Wolf Islands at the Northwestern corner of the Galápagos archipelago are warmer overall than those of Manawai yet share a maximum value of 28.7 ºC. Coral reefs are rare in Galápagos, but most of these reefs occur in the warmer waters surrounding the remote islands of Darwin and Wolf (Glynn et al. 2018). The combination of slightly higher yet largely similar thermal exposure in the northern part of the archipelago and the presence of topographically complex reefs, which *C. tumulosa* typically grows on, suggests that these islands could be a suitable environment for the species.

Alternatively, connectivity of PMNM with Japan and Indo-Polynesia is well-documented, because the species makeup of macroalgae in PMNM consists mainly of a mix of species with affinities to these regions (McDermid and Abbott 2006; Randall 2007; Kawai et al. 2023; Kittle et al. 2024). Algal connectivity with Japan was further evidenced during the earthquake of 2011 in Tōhoku– a region of Japan with high landings in the model output– which generated marine debris that transited across the North Pacific via the Subtropical Gyre into Hawai‘i (Carlton et al. 2017). The Tōhoku earthquake transported numerous algae out of Japan including a previously undescribed species recovered from drift material (West et al. 2016), species which exhibited close phylogenetic relationships with those of Tōhoku (Hanyuda, Hansen, and Kawai 2018), and many with high invasion potential (Hansen, Hanyuda, and Kawai 2018). Japan's Cape Inubo and Izu Islands exhibit cooler SSTs compared to Manawai. Although Cape Inubo was the highest-ranked settlement hexagon in the backtracking model, and we do not yet know the physiological tolerances for *C. tumulosa*, this region is substantially cooler than the conditions currently associated with abundant growth of the species at Manawai (Sherwood et al. 2020). Moreover, Inubo lies beyond the northern limit of coral reefs in Japan (Yamano et al. 2001; Wepfer et al. 2022). However, species can shift their niche space during invasion, as seen in the red alga *G. vermiculophylla*, which occurs in warmer waters in its non-native range compared to its cooler source populations along the coastlines of northern Honshu (including Tōhoku) and Hokkaidō (Sotka et al. 2018). This asymmetry in niches (*sensu* Hutchinson 1957) limits the value of

640 species distribution modeling analyses for *C. tumulosa*, where the abiotic limits of the species are
641 unknown and the current distribution in PMNM exists in a narrow range of environmental
642 parameters. The Izu Islands, despite being relatively close to Cape Inubo, are warmed by the
643 Kuroshio Current, which carries tropical waters northward from Southeast Asia, transporting
644 subtropical fauna along with it (Yamano et al. 2001; Nakano et al. 2008). Although the Izu
645 Islands support a few coral species, these occupy little reef space (Iwasaki 2017). The Ogasawara
646 Islands, to the south of the Izu Islands, are composed of a mixture of fauna and flora derived
647 from the Izu Islands, Mariana Islands, and Ryuku Islands supporting coral reefs composed of a
648 mixture of these species with a high level of endemism (Nakano et al. 2008; Wepfer et al. 2022;
649 Açıkbaş et al. 2024). Additionally, Cape Inubo marks a biogeographic break point at 35 ºN,
650 contributing to strong phylogeographic structure in red algae (Krueger-Hadfield et al. 2017;
651 Krueger-Hadfield et al. 2021). Thus, the Eastern coast of Japan spanning all the way from the
652 Ogasawara Islands to Tōhoku (north of Cape Inubo) represents a potential source region
653 spanning a range of SST and settlement likelihood gradients.
654
655 The Micronesian region showed a lower level of landings in comparison to the Central Pacific
656 and Japan. We note however that particles originating from Indo-Polynesia, including
657 Micronesia, typically enter Hawai‘i through the use of intermediary islands including, among
658 others, Johnston Atoll, Kingman Reef, and the Line Islands (Kobayashi 2006; Skillings, Bird,
659 and Toonen 2011). Johnston Atoll in the central Pacific is often considered an outpost of PMNM
660 (Skillings, Bird, and Toonen 2011) being primarily connected to the central islands near Lalo
661 (French Frigate Shoals) (Grigg 1981; Kobayashi 2006). However, if Johnston Atoll is considered
662 part of the Hawaiian biogeographic region (Crandall et al. 2019), then identifying it as a source
663 does not fully resolve the species origins outside of Hawai‘i.
664
665 With a lack of *C. tumulosa* eDNA detections from Johnston Atoll and from Kiritimati Atoll in
666 the Line Islands, should the species have arrived at Manawai from Micronesia, it may have done
667 so by transiting via the North Equatorial Counter Current, Subtropical Counter Current, or North
668 Pacific Subtropical Gyre and circumventing intermediary islands (Hu et al. 2015; Wang and Wu
669 2018). However, the North Equatorial Counter Current flows at very low latitudes, making it
670 likely that particles following this route would encounter multiple archipelagos en route to
671 Hawai‘i, thereby reducing the likelihood of successful transit without contacting another
672 settlement polygon, and thus decreasing the modeled observed landings there. The Subtropical
673 Counter Current, which runs in an easterly direction at roughly the latitude of the Micronesian
674 region, strengthened in the decades preceding the discovery of *C. tumulosa* in the PMNM (Wang
675 and Wu 2018) suggesting an increased potential to transport marine debris.
676
677 Alongside these dynamics are the ENSO-derived variations in the currents of the North Pacific
678 (Hu et al. 2015). Despite the strong role of ENSO in the ETP (Wang and Fiedler 2006), the
679 phenomenon did not impact the likelihood of particle landings from this region nor did it impact

the central Pacific landings. Landings from Japan were highest during neutral phase ENSO periods. This pattern is likely associated with the changes in circulation and marine debris concentration patterns in the North Pacific associated with ENSO (Howell et al. 2012). The incidence of marine debris in PMNM increases (decreases) during El Niño (La Niña) periods because of the southward (northward) migration of the Subtropical Convergence Zone (STCZ); however, the arrival of debris remains high regardless of ENSO phase (Morishige et al. 2007). During El Niño (La Niña), though, the Kuroshio Current weakens (strengthens) and may impact the exportation and retention of marine debris in the associated recirculation gyre (Howell et al. 2012). Elevated neutral period landings from Japan to Manawai are thus likely associated with the change in strength of the Kuroshio and movement of the STCZ to a favorable median condition.

Clearly, much variability exists in the pathways and source regions in the CMS output. The model primarily captured the drift routes and source regions with the highest probability of providing drift material to Manawai despite the existence of many other lesser highlighted routes. Though there is structuring of marine populations across the Indo-Pacific (e.g., Keith et al. 2013; Bowen et al. 2016), the region is generally well-connected (Crandall et al. 2019). Hence, a confirmed detection of *C. tumulosa* in the Indo-Pacific may suggest the species has a broader distribution and is not endemic to the detection site. Indeed, there is also the possibility that *C. tumulosa* has been exported from PMNM to other regions. Basin-scale searches for *C. tumulosa* will remain warranted even after initial detections outside of PMNM and should be followed by genetic analyses to determine structure of the population throughout its native range in order to identify the pathway of introduction to PMNM and provide a more complete picture of the species' introduction history.

The varying mean rafting durations of *C. tumulosa* to Manawai are long, at 421 days from Japan, 587 days from the central Pacific, and 1014 days from the eastern Pacific. Though the physiological limits (e.g., nutrient availability, temperature and salinity tolerance, light exposure) and therefore the potential rafting duration of *C. tumulosa* are unknown, there are indications that the species can transit for periods sufficient to survive these trips. In order to be an effective rafting species, *C. tumulosa* must be able to hold onto floating objects, establish and compete successfully in this environment, and persist during its voyage (Thiel and Gutlow 2005). Generally, organisms best suited to rafting are those which do not feed on their host raft, reproduce asexually, and are small (Thiel and Gutlow 2005). There is evidence that *C. tumulosa* exhibits these traits because it has been observed rafting in PMNM (Lopes et al. 2023) and can persist via thallus fragmentation (Williams et al. 2024). Additionally, it may occur cryptically (Nichols et al. 2025) and can dominate otherwise healthy reef systems (Sherwood et al. 2020), contributing to its impact. Further, for *C. tumulosa* to raft effectively, it must reattach to the substrate, a trait it likely possesses because it attaches via haptera (Sherwood et al. 2020). Thus, spore production may not be necessary for initial establishment, and the dominance of the

tetrasporophyte phase (Williams et al. 2024) suggests the life cycle could be reestablished in its new environment (Krueger-Hadfield 2020). Should the alga have arrived to PMNM via rafting, its dispersal duration may have been exceptionally long given the species remained within tolerance thresholds for light, temperature, and nutrients (Theil and Haye 2006; Carlton et al. 2017; Lopes et al. 2023; Benadon et al. 2024). In such cases, episodic events, though rare, could disperse *C. tumulosa* across biogeographic boundaries and this may have been facilitated by marine flotsam, such as natural pumice and wood, or marine debris (Jokiel and Cox 2003; Thiel and Haye 2006; Carlton et al. 2017). Debris from the 2011 Tōhoku earthquake began landing in North America and Hawai‘i ~300 days after the earthquake (Carlton et al. 2017), implying that transit times across these great distances can be rapid. Survival over long periods was also observed following this event, as a large mass of buoys and ropes derived from Tōhoku arrived in Hawai‘i >2000 days after the earthquake with living biota aboard (Carlton et al. 2017; 2018). Landings in Japan occur more rapidly and regularly than those passing through the formidable Eastern Pacific Barrier where corals, among other organisms, are generally isolated from their central and western Pacific counterparts (Baums et al. 2012). However, modeling of coral propagule dispersal assuming a 120-day competency period suggests corals successfully settle from the ETP to the central Pacific on a regular basis (Connolly and Baird 2010; Wood et al. 2016). Further, global modeling assessing the transit times of particles into the world's oceanic accumulation centers in subtropical gyres suggests that particles arriving into the North Pacific garbage patch have a minimum transit time from Japan, the ETP, and the central Pacific of <250 days (Maximenko, Hafner, and Niiler 2012). This minimum travel time may not represent a realistic transit in which particles must contact a habitat suitable to their establishment. Indeed, particles may nearly pass a settlement location for long periods of time without settling and many particles, even after landing successfully, are not competent in their newfound environment (Carlton et al. 2017; 2018). Nevertheless, the broad scale movement of living biota attached to marine debris across oceanic basins in short time spans can and does occur episodically. The drift times from the CMS run in this study vary greatly, suggesting the model captured a wide range of potential drifting pathways and potential lifespans with some relatively short transits occurring from all regions of interest falling within the realm of possibility, especially considering *C. tumulosa* may have been attached to marine debris and is likely to disperse via thallus fragments rather than spores that have a much shorter longevity.

Uncovering the source of cryptogenic species is a global challenge (Carlton and Schwindt 2024). Lack of information on the native range of an organism obscures the ecological and evolutionary background of the species, complicating the prediction of its impacts and any evolutionary shifts that occurred during the introduction. For instance, there may be herbivores of *C. tumulosa* in its native range that do not exist in PMNM. Further population genetic studies can help uncover patterns of reproductive system variation and gene flow (e.g., see work in *G. vermiculophylla*, Krueger-Hadfield et al., 2016; 2017). Identifying *C. tumulosa* in the field should be supplemented by leveraging collections stored in global herbaria and generating DNA barcodes

(i.e., UPA, *rbc*L, COI) for these specimens. Identification of *C. tumulosa* through genetic methods may be especially important for the genus *Chondria* because it is exceptionally species-rich with 78 currently accepted species (Guiry and Guiry 2024). Further, several other genera in the family Rhodomelaceae superficially resemble *Chondria*, which has contributed to the confusion surrounding the taxonomy of this group. Morphologically distinguishing species of *Chondria* is challenging, particularly in the field. However, the morphological characteristics outlined by Sherwood et al. (2020) are critical to consider during the examination of collections stored in herbaria. Sampling should also be conducted on specimens falling outside of the currently recognized morphological description of *C. tumulosa* because the tribe Chondrieae remains poorly represented with DNA barcoding data. Of the currently accepted *Chondria* species (Guiry and Guiry 2024) with overlapping distributions with the modeled regions of interest, *C. acrorhizophora* Setchell & N.L.Gardner, *C. clarionensis* Setchell & N.L.Gardner, *C. econstricta* M.Tani & Masuda, *C. flexicaulis* W.R.Taylor, *C. lancifolia* Okamura, *C. minutula* Weber Bosse, *C. repens* Børgesen, *C. simpliciuscula* Weber Bosse, *C. stolonifera* Okamura, and *C. xishaensis* J.-F.Zhang & B.-M.Xia have no verified DNA sequences and should be targeted from global herbaria as a meaningful contribution in the search for matches to *C. tumulosa* (Holmes 1896; Setchell and Gardner 1930; Taylor 1945; Dawson 1963; Buggeln and Tsuda 1969; Segawa 1981; Yoshida, Nakajima, and Nakata 1990; Yoshida 1998; Tani and Masuda 2003; Titlyanov et al. 2006; Serviere-Zaragoza et al. 2007; Ruiz and Ziemmeck 2011; Tsuda, Fisher, and Vroom 2012; Titlyanov and Titlyanova 2012; Tsuda and Walsh 2013; Yoshida, Suzuki, and Yoshinaga 2015; Sutti et al. 2018; Titlyanov et al. 2019). One of these species, or another, may be a match to *C. tumulosa*, a finding that would expand our understanding of the species instantaneously. However, given the challenges of morphologically distinguishing *Chondria* species, especially from pressed material, some of the distribution records may have limitations in their reliability and require DNA clarification. Additionally, the morphology of *C. tumulosa* may differ in its native environment due to environmentally induced variation in morphology (e.g., Murren et al. 2022) and many algal species remain to be described (De Clerck et al. 2012). Finally, a phylogenetic reassessment of the genus *Chondria* and the tribe Chondrieae is warranted to clarify the systematics of this diverse group and enhance our understanding of the distribution of *C. tumulosa*. This framework can be extended to other cryptogenic red algae of unknown origin such as the putative Japanese *Pyropia* J.Agardh which appeared on the central coast of British Columbia, Canada, in 2015 (Lindstrom 2018).

Our recommended procedures for investigating model-guided source locations in the search for *C. tumulosa* are as follows: searches should continue by screening eDNA collected from regions of interest (e.g., Japan, Galápagos, Line Islands including Palmyra, Johnston Atolls) because this method increases sensitivity and minimizes survey costs (Jerde et al. 2011; Dejean et al. 2012; Smart et al. 2015; Nichols et al. 2025). Our screening of eDNA sample pools from the model-guided potential source regions did not detect *C. tumulosa*. However, further investigation into these regions may be warranted. The power of eDNA analysis lies in the ability to search for *C.*

*tumulosa* in leveraged samples that were designed to study other organisms. In this manner, the search for *C. tumulosa* using eDNA can be optimized by opportunistically checking for detections using pooled samples from any collection region of interest, provided sample pools do not dilute target eDNA any lower than the present study; we suggest limiting pooled samples to <20 for reliable amplification of potential low-abundance sites. A positive eDNA detection of *C. tumulosa* may then be considered as a strong rationale to individually re-screen sites and initiate in-water surveys. The species can exhibit robust, cryptic, or epiphytic growth patterns, necessitating that phycologically trained in-water divers meticulously examine potential habitats and cryptic microenvironments. Specimens collected during in-water surveys should be cleaned of epiphytes and debris and stored in silica desiccant when in remote field sites or, if possible, flash frozen in liquid nitrogen. The remainder of the collected specimen should be examined for morphological similarity to *C. tumulosa* via microscopy and pressed to make an herbarium voucher. Samples matching *C. tumulosa* morphologically (or nearly so) should be extracted using a modified CTAB protocol (Doyle and Doyle 1989; Fumo and Sherwood 2023) and sequenced for the UPA barcode (Sherwood and Presting 2007) for comparison to published UPA sequences of *C. tumulosa* (Sherwood et al. 2020; Paiano et al. 2021). Any matches of high similarity should be sequenced additionally for the plastidial *rbc*L, mitochondrial COI, and nuclear SSU markers, and compared to published sequences using BLAST searches. Matches or very near-matches (>99%) for all three of these barcodes alongside an eDNA detection can be considered adequate evidence that *C. tumulosa* has been identified. However, full genomic sequencing and assembly of the plastidial genome with comparison to NCBI accession number MW309501 may also be performed for a more confident assessment (Paiano et al. 2021).

## Conclusions

The alga *C. tumulosa* is a putative non-native nuisance red alga in its only confirmed geographic location, PMNM, where its spread is likely aided by marine debris. Despite targeted sampling efforts from global herbaria and opportunistic eDNA surveys, there is no published record of the species' detection elsewhere, underscoring the challenge of searching across the vast Pacific Ocean. To address this, we suggest a model-guided search strategy to implement in the most probable source locations. These locations are: Japan's Izu Islands and Cape Inubo, the central Pacific islands of Johnston Atoll and the Line Islands including Palmyra, and in the ETP, Clipperton Atoll and the northern Galápagos archipelago. In short, this protocol begins with eDNA surveys followed by in-water surveys and DNA barcoding. The qPCR assay targeting *C. tumulosa* is sufficiently sensitive to uncover trace amounts of target eDNA from pooled samples. Through this process we identified a number of *Chondria* taxa that have yet to be sequenced for any molecular markers, with geographic ranges that include the potential source regions suggested by the oceanographic model. Implementing this systematic and explicit search for *C. tumulosa* is critical for locating and determining the native range of the species, which would also confirm its non-native status in Hawai‘i (raising it to invasive status). Identification of the native range of the species is ultimately necessary to resolve ecological parameters pertinent to

control mechanisms, thereby supporting conservation efforts in PMNM. We note that these methods can also be developed in other cryptogenic marine organisms to help fill in important ecological and evolutionary gaps that go beyond our example shown here in *C. tumulosa*.

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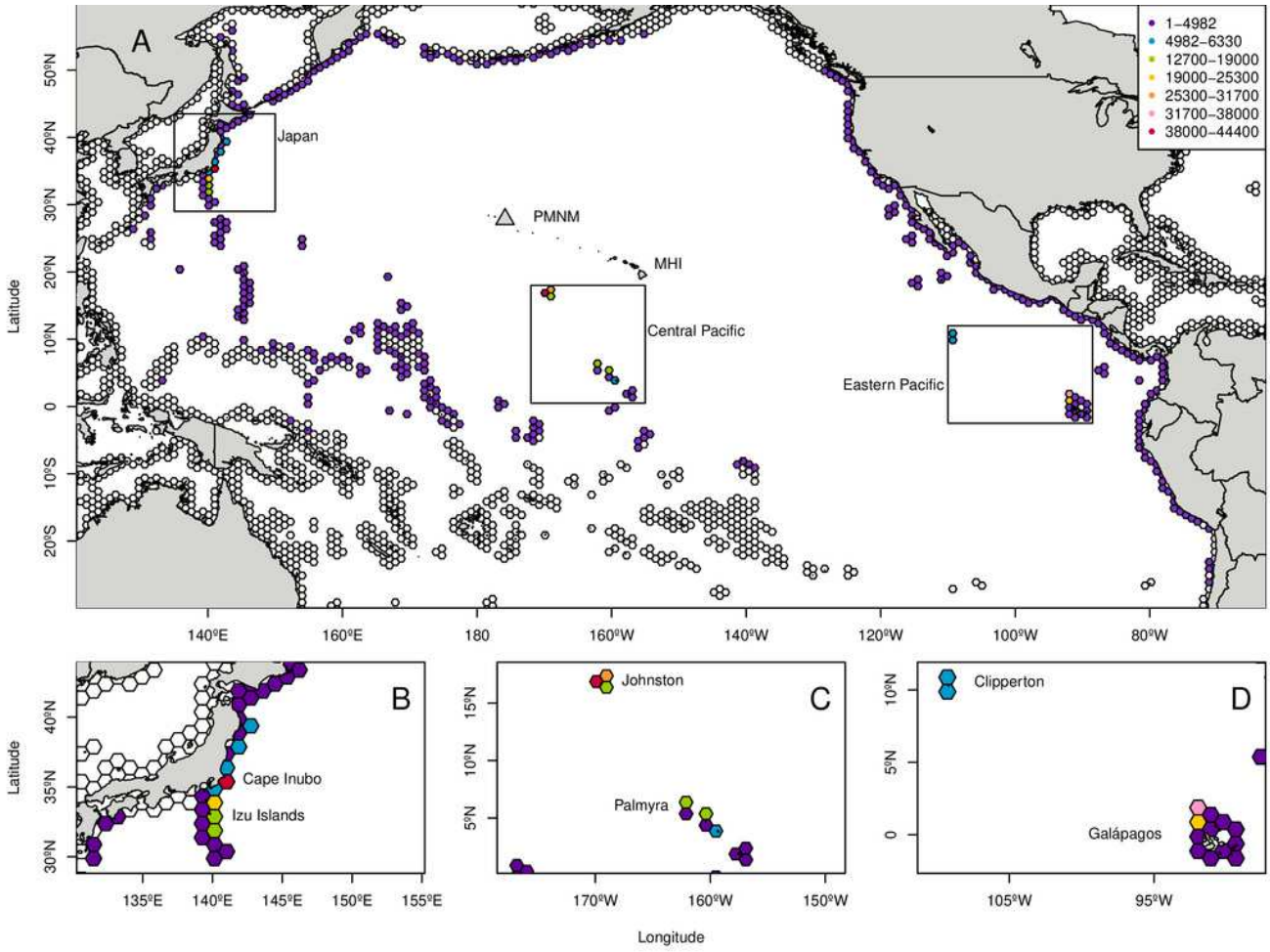
# Figure 1

Total modeled landings at source locations for Chondria tumulosa backtracked from Manawai (Pearl and Hermes Atoll), Hawai‘i

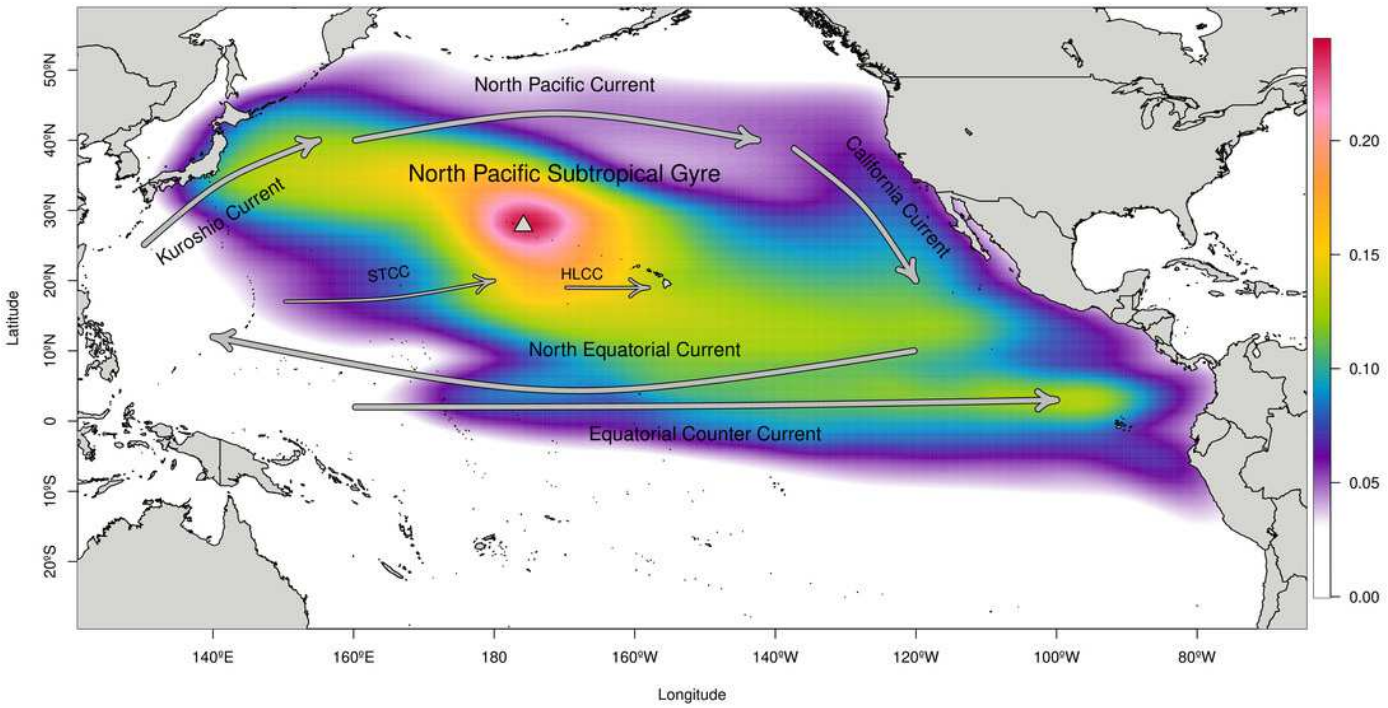
From December 30, 2015 to January 1, 2000, 100 particles per day (584,000 total) originating at the backward trajectory start point, Manawai, were traced until landing in a potential source location. Hexagonal polygons were the regions designated as landing areas in the Connectivity Modeling System. Each hexagon is colored by the number of landings in that settlement polygon throughout the model duration with colorless hexagons receiving zero landings throughout the modeled period. All landings throughout the modeled region are shown in panel A. The regions of highest landings were Japan (B), the central Pacific islands of Johnston Atoll, and the Line Islands including Palmyra (C), and the eastern Pacific islands of Clipperton and the Galápagos archipelago (D). The legend in panel A, indicating landings, applies to all panels. Manawai is indicated by a gray triangle, the Main Hawaiian Islands (MHI) and Papahānaumokuākea Marine National Monument (PMNM) are indicated.


A
B
C
D
1–4982
4982–6330
12700–19000
19000–25300
25300–31700
31700–38000
38000–44400
Japan
PMNM
MHI
Central Pacific
Eastern Pacific
Cape Inubo
Izu Islands
Johnston
Palmyra
Clipperton
Galápagos
Latitude
Longitude
50°N
40°N
30°N
20°N
10°N
0
10°S
20°S
140°E
160°E
180
160°W
140°W
120°W
100°W
80°W
35°N
135°E
140°E
145°E
150°E
155°E
15°N
5°N
170°W
160°W
150°W
105°W
95°W

# Figure 2

Density cloud of particle locations throughout the modeled backtracking period from Manawai (Pearl and Hermes) Atoll, Hawai‘i

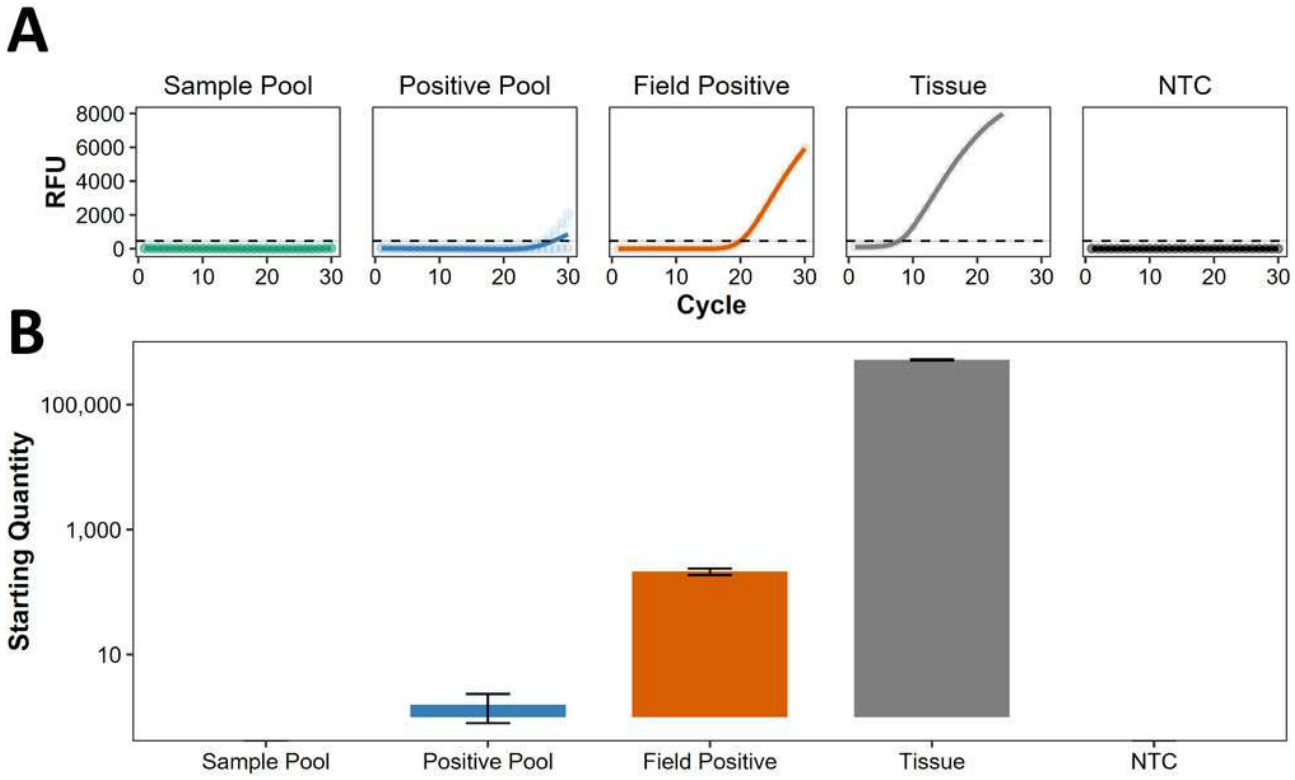
Colors indicate the proportion of particles located in each pixel throughout the study period, January 1, 2000 to December 30, 2015 using the Connectivity Modeling System. Particles originating at the backward trajectory start point, Manawai (indicated by a gray triangle), generally tracked towards the northwest or southeast until landing in a potential source region. Warmer colors represent regions with a higher concentration of particles, while cooler colors indicate areas with fewer particles during the modeled duration. The high concentration of particles surrounding Manawai reflects the start point of the model run. Currents influencing the dispersal of particles are shown in gray arrows and named. The North Pacific Subtropical Gyre is composed of the Kuroshio Current, North Pacific Current, California Current, and North Equatorial Current, which runs north of the eastward flowing Equatorial Counter Current. The Hawai‘i Lee Counter Current (HLCC) and Subtropical Counter Current (STCC) occur within the gyre, moving more weakly from west to east and are represented by thinner arrows.


North Pacific Current
North Pacific Subtropical Gyre
Kuroshio Current
California Current
STCC
HLCC
North Equatorial Current
Equatorial Counter Current
Latitude
Longitude
50°N
40°N
30°N
20°N
10°N
0
10°S
20°S
140°E
160°E
180
160°W
140°W
120°W
100°W
80°W
0.20
0.15
0.10
0.05
0.00

# Figure 3

Screening of pooled eDNA samples from potential source regions.

(A) Amplification curves (in relative fluorescence units, RFU) and (B) eDNA concentrations ("starting quantity" ± SE) using the species-specific qPCR assay for Chondria tumulosa against standards with known copy numbers. Samples from Kiritimati, Line Islands (n=22), Johnston Atoll (n=40), and Okinawa, Japan (n=20) were pooled by region ("sample pool") and amplified in triplicate. A second set of pooled samples per region ("positive pool") were each spiked with eDNA from a confirmed positive site ("field positive", n=2) in Papahānaumokuākea National Marine Sanctuary, where C. tumulosa was observed at 1% relative abundance. DNA extracted directly from a preserved C. tumulosa specimen ("tissue", n=1) and no-template controls ("NTC", n=4) are also shown. Critical thresholds of fluorescence (above which detections can be discriminated from background noise) are plotted with a dashed line.


A
Sample Pool
Positive Pool
Field Positive
Tissue
NTC
RFU
8000
6000
4000
2000
0
0
10
20
30
Cycle
B
Starting Quantity
100,000
1,000
10
Sample Pool
Positive Pool
Field Positive
Tissue
NTC

## Table 1(on next page)

Range overlap of Chondria species with regions of interest identified by the Connectivity Modeling System output as potential source locations for Chondria tumulosa in the Papahānaumokuākea Marine National Monument, Hawai‘i.

The species column includes those that are either currently accepted taxonomically, have unresolved taxonomic status, or have been transferred to other genera within the family Rhodomelaceae. Availability of DNA barcode sequences for these species from GenBank NCBI is indicated.

| Species | Japan | Line Islands including Palmyra | Johnston Atoll | Clipperton | Galápagos | Available NCBI GenBank sequences |
|---|---|---|---|---|---|---|
| Acanthophora muscoides | X | | | | | None |
| Acanthophora spicifera | X | | | | | 18S, 28S, COI, LSU, petB, psbA, rbcL, SSU, UPA |
| Chondria acrorhizophora | | | | | X | None |
| Chondria armata | X | | | | | COI, rbcL, SSU |
| Chondria capillaris | X | | | | | rbcL, SSU |
| Chondria clarionensis | | | | X | | None |
| Chondria crassicaulis | X | | | | | 18S, COI, LSU, SSU, rbcL |
| Chondria dasyphylla | X | | | | | 18S, 28S, COI, LSU, petB, psbA, rbcL, SSU, UPA |
| Chondria econstricta | X | | | | | None |
| Chondria expansa | X | | | | | 18S, COI, SSU, rbcL |
| Chondria flexicaulis | | | | | X | None |
| Chondria intertexta | X | | | | | rbcL |
| Chondria lancifolia | X | | | | | None |
| Chondria mageshimensis | X | | | | | rbcL |
| Chondria minitula | | X | | | | None |
| Chondria polyrhiza | X | | X | | | rbcL |
| Chondria repens | X | X | | | | None |
| Chondria ryukuensis | X | | | | | COI, SSU, rbcL |
| Chondria simpliciuscula | | X | X | | | None |
| Chondria stolonifera | X | | | | | None |
| Chondria xishaensis | X | | | | | None |
| Laurencia filiformis | | | | | | 18S, SSU, rbcL |
| Laurencia pinnata | X | | | | | None |
| Palisada perforata | X | | | X | | COI, psbA, rbcL |

## Table 2 (on next page)

Summary of Sea Surface Temperature (SST) for each location from the Optimum Interpolation Sea Surface Temperature (OISST) dataset

For each location, the minimum, median, mean, and maximum SST values (ºC) from OISST data covering the period from January 1, 2000, to December 30, 2015 (n=193 for each location) are presented along with the difference in SST between each location and Manawai, Hawai‘i, the site of the initial detection of Chondria tumulosa in the Papahānaumokuākea Marine National Monument. The latitude and longitude of the OISST grid cell used is presented alongside the location name.

| Location (latitude, longitude) | Manawai, Hawai‘i (27.875 ºN, 175.875 ºW) | Cape Inubo, Japan (35.625 ºN, 140.875 ºE) | Izu Islands, Japan (33.125 ºN, 139.875 ºE) | Johnston Atoll (16.875 ºN, 169.625 ºW) | Palmyra Atoll (5.875 ºN, 162.125 ºW) | Clipperton Atoll (10.375 ºN, 109.125 ºW) | Darwin & Wolf Islands, Galápagos (1.625 ºN, 91.875 ºW) |
|---|---|---|---|---|---|---|---|
| Minimum | 18.7 | 11.9 (-6.8) | 16.2 (-2.5) | 24.7 (+6) | 26.3 (+7.6) | 26.6 (+7.9) | 23.1 (+4.4) |
| Median | 23.9 | 19.3 (-4.6) | 22.5 (-1.4) | 26.6 (+2.7) | 28.3 (+4.4) | 28.1 (+4.2) | 25.9 (+2.0) |
| Mean | 24.0 | 19.6 (-4.4) | 22.7 (-1.3) | 26.6 (+2.6) | 28.2 (+4.2) | 28.1 (+4.1) | 25.9 (+1.9) |
| Maximum | 28.7 | 26.0 (-2.7) | 28.7 (0) | 29.3 (+0.6) | 29.7 (+1.0) | 29.8 (+1.1) | 28.7 (0) |