Final Report for the Hawai'i Invasive Species Council FY2023

How do harbors jumpstart algal invasions?

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Summary of activities:

Motivated by the probability that our most potent invasive seaweed, *Chondria tumulosa*, will arrive from Papahānaumouākea Marine National Monument and be introduced in an urban Honolulu harbor, we began an exercise to characterize the parameters in harbors where other invasive algae already exist to see if tissue analysis of those invasive algae gave insight into the presence of elevated nutrients or wastewater in three harbors. This information could help guide protocols for where returning vessels dock in Oʻahu waters, and provides a low-cost framework for monitoring health of Oʻahu harbors in the future.

Background:

A comprehensive state-wide study of wastewater contamination in nearshore marine areas was conducted in an earlier study (Smith et al. 2022) and provided data to assist the State of Hawai'i in prioritizing areas for cesspool upgrades by measuring wastewater pollution analyzing field collected tissues of invasive algae and modeling methods for coastal waters of four major Hawaiian Islands: Kaua'i, O'ahu, Maui, and Hawai'i Island. This study coupled algal bioassays with the knowledge of watershed pollution from EPA summaries (How's my waterway:

<u>https://mywaterway.epa.gov/community</u>). In so doing, we began to see patterns where EPA identified impaired waterways likely drained into coastal and harbor regions, setting up conditions that could also favor invasive algae such as *Acanthophora spicifera* and it's closely relative, *Chondria tumulosa*. Thus we proposed that slower circulating, protected regions such as harbors could retain inflowing impaired fluids and create nutrient enriched settings ideal for invasive algae. We proposed to test this idea by contrasting two harbor types – one with abundant input from impaired waterways and a second with no overland flow of any stream. This is the first time where algal bioassay approach has been applied to assess the health of harbors in tropical regions in the context of favoring invasive species. If you allow that vessels coming to Hawaii is a proxy for the pressure of new marine introductions (Figure 1), then we need new tools to characterize the potential success of these threats.

Past research that provides new tools:

The potential for residential "onsite sewage disposal system" or OSDS wastewater to impact human health and coastal ecosystems is a cause of increasing concern throughout the State of Hawai'i (Abaya et al., 2018; Amato et al., 2016; Amato et al., 2020; Dailer et al., 2010). In 2016-2017, these concerns and federal EPA guidelines prompted the State of Hawai'i Department of Health to ban the installation of new cesspools and the Hawai'i State Legislature to provide financial assistance for upgrades of existing cesspools in sensitive areas (Act 120) and require the upgrade of the more than

88,000 cesspools in the state by 2050 with wastewater disposal systems that provide a higher level of wastewater treatment (Act 125). Field studies of the relationship between wastewater discharge to the groundwater and the resulting nutrient and contaminant loading in the coral reef communities has been studied on the local scale in Kahalu'u and Puakō (Abaya et al., 2018), Waialua and Waimanalo (Amato et al., 2020), at the island wide scale for Maui (Dailer et al., 2010) and O'ahu (Amato et al., 2020). Previous groundwater modelling studies have used computer simulations to estimate impact of residential OSDS wastewater on the coastline on a state-wide scale (Whittier and El-Kadi 2009; Whittier and El-Kadi 2014). With the funding provided though Act 132 (2018), the University of Hawai'i collaborated with the State of Hawai'i, Department of Health to comprehensive state-wide study of wastewater contamination in nearshore marine areas (Smith et al. 2022) and provide data to assist the State of Hawai'i in prioritizing areas for cesspool upgrades by measuring wastewater pollution using both field (tissue analysis of invasive seaweeds) and modeling methods in the coastal waters of four major Hawaiian Islands: Kaua'i, O'ahu, Maui, and Hawai'i Island.

Tissue Analysis of Invasive Seaweeds Provide Useful Indicator of Nitrogen.

Algal bioassays for tissue Nitrogen (N) has been used for over a decade to detect wastewater in coastal environments around the globe (Dailer et al., 2010 and references within). When N is abundant in nearshore waters, seaweed have the ability to store N in their tissues which causes their overall percent tissue nitrogen (%N) to increase. Because seaweed are continuously absorbing nutrients to grow, the composition of the nitrogen in the tissue reflects the average nitrogen conditions over several days to weeks (Dailer et al., 2012). In addition, the isotopic ratio of nitrogen in seaweed tissues can be used to determine the dominant nitrogen source because N from different sources tend to have different isotopic ratios. Isotopic nitrogen ratios of ¹⁵N:¹⁴N are expressed as the δ^{15} N signature or value, which is a measure of the abundance of the heavy Nitrogen-15 isotope (¹⁵N) in relation to the lighter nitrogen isotope ¹⁴N. Wastewater is generally enriched in ¹⁵N because bacteria preferentially uptake the lighter isotope of ¹⁴N leaving the wastewater enriched in the heavier isotope of ¹⁵N, consequently increasing its δ^{15} N value (Heaton 1986). Chemical fertilizer, naturally occurring and atmospheric nitrogen have nearly equal amounts of ¹⁵N and ¹⁴N, consequently resulting in low (near 0‰) δ^{15} N signatures. Evaluation of the seaweed δ^{15} N value in tandem with the percent of N in the sample (%N) gives insight into both the dominant source of that N (δ^{15} N) and relative amount of coastal N loading (%N) in coastal waters where collected (Amato et al., 2016, Amato et al., 2020; Dailer et al., 2012). Seaweed samples therefore can indicate the relative amount of N loading (%N) and the dominant source of that nitrogen (δ^{15} N) in coastal waters where collected. Naturally-derived nitrogen is characterized by low seaweed $\delta^{15}N$ and %N, agriculturally-derived nitrogen is characterized by low seaweed $\delta^{15}N$ and high %N, and wastewater-derived N is characterized by high seaweed δ^{15} N at 6 ppth or higher and moderate to elevated %N at 2 % N or above (Amato et al., 2016).

Methods:

This study used two approaches to assess nutrient pollution that favors invasive algae around three harbor area: Barber's Point, Ke'ehi and Kewalo coastal regions. The first approach queried the EPA How's My Waterway data for presence of impaired waterways in coastal and harbor regions to select three sites that are publicly accessible at low tides for algal collections on O'ahu.

The second approach collected invasive algae, specifically *Acanthophora spicifera* as well as occasional native species if *A. spicifera* was not available. Samples were processed following an established tissue analyses protocol, measured stable isotopes of nitrogen (N) as an indicator for sewage-derived wastewater because N is abundant in wastewater, negatively impacts aquatic ecosystems, is generally nonreactive in groundwater, and previous studies indicate that it is an

effective N-based wastewater tracer in Hawai'i (Abaya et al., 2018; Amato et al., 2016; Amato et al., 2020; Dailer et al., 2010; Hunt and Rosa 2009) and throughout the tropics (Fong et al., 2003; Garrison et al., 2007; Shuler et al., 2019).

Multi-person, expert teams were established and trained in Standard Operating Procedures, for this study. At each site, one or more of a selected set of seaweed species were collected in triplicate, spaced apart by 1 meter. Seaweed samples were returned to labs on each island, cleaned, dried, and analyzed for the amount of nitrogen in a seaweed as a percentage of mass (%N) and the isotopic value of that nitrogen ($\delta^{15}N$).

Results:

For each sampling, the average from two parameters (seaweed $\delta^{15}N$ values, seaweed %N values) were ranked to produce a comparative assessment. Sample areas were then designated into one of the three following categories of wastewater influence: 1) wastewater dominant (6 ppth or above for tissue $\delta^{15}N$), 2) wastewater influenced (4-6 ppth for tissue $\delta^{15}N$), and 3) little to no wastewater detected (0 to 4 ppth for tissue $\delta^{15}N$).

Tissue analysis of samples from invasive and native algae collected at Barber's Point (Figure 2) show little to no wastewater detected from all tissue analyses ($\delta^{15}N = 3.7 \text{ ppth} \pm 1.2$, SD, n= 37), values that are found at the bottom of the state-wide ranked comparison (Smith et al. 2022), a healthy habitat with little wastewater detected. This conclusion is also backed up by the %N data (%N = $1.2\% \pm 0.3$ SD, n= 37). These swaths generally have very good agreement between the results of seaweed parameters and the expectation for low pollution because no impaired waterways flow into Barber's Point Harbor.

While accessing Honolulu Harbor was not feasible (Figure 5), we did work in two alternative urban Honolulu Harbors that are on either side of Honolulu Harbor. The major source of nitrogen to one of these Harbors, Ke'ehi lagoon (Figure 3), was predicted and confirmed to be from wastewater sources ($\delta^{15}N = 6.5 \text{ ppth} \pm 0.9 \text{ SD}$, n= 33) with %N in tissues (%N = 1.2% $\pm 0.2 \text{ SD}$, n= 33). Kewalo. (Figure 4) had elevated levels in some spots but on average fell in the intermediate range for wastewater exposure ($\delta^{15}N = 4.8 \text{ ppth} \pm 0.5 \text{ SD}$, n= 15), but with the highest % N average value suggesting fertilizers or other N-sources are present (%N = 1.8 % $\pm 0.3 \text{ SD}$, n= 15). Among and between site comparisons show these patterns among sites are statistically distinct (p<0.001).

Discussion and Conclusions

Wastewater discharge to coastlines was significant on all of the Main Hawaiian Islands included in this study earlier modelling work in Smith et al 2022 determined that cesspool nitrogen is the source of about 80% of the wastewater nitrogen discharging into coastal waters state-wide, and accounted for 83% of the wastewater nitrogen discharging into coastal waters on O'ahu. This additional N pollution above natural levels puts much of our O'ahu coastal regions at risk for invasion or continued expansion of invasive seaweeds.

In this study we provide the first set of data that characterize nutrient pollution in harbors that may be places where vessels carrying *Chondria tumulosa* hide. Strikingly, the outcomes of data correspond with simple early assessments of nutrient status based on EPA's web site that characterizes the health of a waterbody/ waterway. Significant differences were found among two paramters used to characterize harbor health based on algal bioassay: Barber's Point / Deep Draft Harbor appears to be the least likely site for an invasive seaweed to bloom because wastewater and algal tissue N are low $(\delta^{15}N = 3.7 \text{ ppth} \pm 1.2, \text{ SD}, n= 37; (%N = 1.2\% \pm 0.3 \text{ SD}, n= 37)$. In contrast, Ke'ehi Lagoon can be

characterized as a site where wastewater dominates as the main source of N. If Pearl or Honolulu Harbor are similarly high, other safeguards should be aggressively taken to prohibit *Chondria tumulosa* introductions to any of these three harbors from boats returning from Papahānaumokuākea Marine National Monument. This may require having those vessels tie up in at Barber's Point for a period of time to be determined.

Finally, fine scale sampling of seaweeds provides an integrated look at the available N in coastal waters at the resolution of meters of coastline and can be accomplished with relatively little cost. While these results do not provide a complete picture of coral reef health, these data suggest that there is a significant risk of invasive seaweeds invasion in the wastewater dominated harbors such as Ke'ehi but also likely in adjacent Honolulu and Pearl Harbors.

Deliverables:

Participant in *Chondria* Conference, May 10, 2024 and discussed the need to consider harbor health. Meetings with Ewa community and Honokai 'ohana on May 18, 2024 and December 7, 2024. USFWS funded *Chondria* research at Midway to begin characterizing the plant biology. Discussions with NOAA about comparable sampling for algal bioassay, in Pearl Harbor. **Figure 1.** The North Pacific is heavily used as maritime pathways connecting ports on the Easter and Western Pacific coasts. Traffic also moves North to South connecting these ocean regions that are usually bounded by equatorial currents. This image represents the positions of fishing boats, military vessels, tugs, tankers, vehicle and bulk carriers in the Central Pacific on 01/14/25, courtesy of marinevesseltraffic.com.



Figure 2. Barber's Point WATERSHED: Waimanalo Gulch (200600000501). SIZE: 7,538 acres / 30.50 km^{2.} No overland streams are shown on this image of the coastal region. The land mass is light blue while the coastal waters are light gray. This figure is courtesy of https://mywaterway.epa.gov/community/BarbersPoint,Hawaii/overview .



Figure 3. Keehi Beach Park Lagoon, WATERSHED: Moanalua Stream (200600000305) SIZE: 12,643 acres / 51.16 km². On this image of the coast, polluted overland streams are shown in red, green coastal regions are characterized as healthy, purple regions marks waters that remain uncharacterized. The land mass of interest is light blue while the coastal waters are light gray. This image is courtesy of EPA

https://mywaterway.epa.gov/community/KeehiBeachParkLagoon,UrbanHonolulu,HI,USA/overview



Figure 4. Kewalo Harbor. WATERSHED: Ala Wai Canal (20060000303) Honolulu, Hawaii SIZE: 12,075 acres / 48.86 km^{2.} On this image of the coast, polluted overland streams are shown in red, green coastal regions are characterized as healthy, purple regions marks waters that remain uncharacterized. The land mass of interest is light blue while the coastal waters are light gray. This image is courtesy of EPA

http://www.mywaterway.epa.gov/community/96822/overview



Figure 5. Sand Island State Beach Park WATERSHED: Waolani Stream (200600000304) SIZE: 12,610 acres / 51.03 km² On this image of the coast, polluted overland streams are shown in red, green coastal regions are characterized as healthy, purple regions marks waters that remain uncharacterized. The land mass of interest is light blue while the coastal waters are light gray. This image is courtesy of EPA

https://mywaterway.epa.gov/community/SandIslandStateBeachPark,UrbanHonolulu,HI,USA/ov erview

