AN ASSESSMENT OF ENVIRONMENTAL CONDITIONS THAT DEFINE SUITABLE HABITAT FOR THE UPSIDE-DOWN JELLYFISH (*CASSIOPEA* SPP.) ON O‘AHU

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ABSTRACT

The upside-down jellyfish, *Cassiopea*, is a genus of scyphozoans that in the past few decades, has become widely invasive across the subtropics and tropics, including the island of Oʻahu. Their biology, including a mechanism that allows them to pump out nutrient-rich porewater as well as a symbiosis with photosynthetic zooxanthellae, give *Cassiopea* spp. high tolerance to stressors and may increase their invasibility as the effects of climate change intensify. This study investigated what factors influence seasonal presence of the jellyfish, what constitutes suitable habitat, and how the jellyfish potentially impact nutrient cycling in their nonnative environments. The first part of this study focused on a temporal survey over ten months at the interior end of the Ala Wai Canal in Waikīkī, during which water and sediment nutrients were analyzed and used alongside water quality measurements to construct a logistic regression to determine which were most important for jellyfish presence. Only a few jellyfish were found at two of 18 sampling intervals. In the second part of the study, I conducted a spatial survey of ten sites across Oʻahu that currently or previously hosted invasive populations of *Cassiopea* spp. Water quality measurements and nutrient concentrations in porewater and the water column were used to construct several generalized linear models in order to determine the most influential factors for jellyfish presence, density, and size. Water column turbidity and nitrate concentration both had negative relationships with jellyfish presence and density, reinforcing the importance of the symbionts as well as posing new questions about nitrate tolerance and whether elevated nitrate could disrupt physiological processes. Salinity and sediment organic matter were also found to be positively related to jellyfish presence, which reinforces current understanding of preferred habitat conditions for the genus, sources of nutrition, and how they may benefit from anthropogenic impacts. Jellyfish tended to be larger at sites with higher pH, suggesting that the

health of these populations may begin to suffer in the future as ocean acidification intensifies. These findings suggest that several environmental factors may be key in crafting a multifaceted management solution for preventing and eliminating invasions of *Cassiopea* spp.

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by

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This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Marine Science at Hawaiʻi Pacific University. We the undersigned have examined this document and have found that it is complete and satisfactory in all respects, and all revisions required by the final examining committee have been made.

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Chapter 1: Literature Review

Introduction and Study Objectives

Scyphozoa is a class of marine invertebrates that has a history of coastal biological invasions, where it can aggregate in huge blooms that disrupt local water chemistry, thus altering the abiotic and biotic factors in their ecosystems (Guy-Haim et al. 2020). These jellyfish consume large amounts of organic carbon, nitrogen, and phosphorus as their population booms, and then experience sudden and massive die-offs, resulting in a large influx of biomass to the bottom of coastal bodies of water (Lebrato & Jones 2011; Pitt et al. 2009). The decaying biological matter supports abundant microbial communities that deplete oxygen and cause localized acidification (Sweetman et al. 2016).

One such genus of scyphozoans, *Cassiopea* spp., can also alter environments to which it is not native through physical and chemical changes. The genus *Cassiopea* spp. comprises a global tropical and subtropical species complex with 12 species (Collins et al. 2025). Their common name, upside-down jellyfish, derives from their tendency to settle with the dorsal bell surface down on soft benthos, exposing their ventral surface and oral arms to maximize access to sunlight in clear, shallow, protected waters. Sun exposure is necessary for their endosymbionts to drive photosynthesis (Lampert 2016). A comprehensive understanding of the ecology and evolution of the *Cassiopea* species complex is hindered by the difficulty to identify medusae at the species level due to their morphological similarities and plasticity (Enrique-Navarro et al. 2022; Holland et al. 2004; Maloney et al. 2024). *C. andromeda*, like many other species of jellyfish, tolerate a wide range of salinities—30 ppt to 50 ppt—and temperatures—26° C to 34° C—creating greater opportunity for invasion (Aljbour & Agusti 2025; Mahapatro et al. 2023; Toullec et al. 2024). With increasing sea surface temperature, *Cassiopea* spp. has expanded their range throughout the tropics (Rowe et al. 2022; Schembri et al. 2011; Tran Manh et al. 2020), where they play a variety of roles in their new habitats.

When invasive, *Cassiopea* spp. likely have both positive and negative effects on their environment. For instance, blooms of *C. andromeda* can have detrimental effects such as disruption of shrimp aquaculture in Brazil (de Araújo et al. 2020). In Hawaiʻi, blooms of *Cassiopea* spp. also impact operations at outdoor aquaculture ponds (Gerald Crow, personal communication) and loko iʻa, traditional Hawaiian fishponds (Ingrid Lassleben, personal communication; Keliʻi Kotubetey), which are culturally important and provide food for local communities. Despite this, they have also been shown to be important ecosystem engineers through a bell-pumping mechanism (Jantzen et al. 2010). After settling on soft sediment, they pulse their bells to pump nutrients out of the substrate, increasing nutrient flux (Jantzen et al. 2010). While these effects have so far only been observed in the lab, it remains unclear which, if any, ecosystem effects are occurring in Hawaiʻi's natural environments. Interestingly, this pulsation rate slows in warmer water, which could alter the role *C. andromeda* plays in nutrient cycling as sea surface temperature continues to rise (Béziat & Kunzmann 2022).

In addition to their ecological importance, *Cassiopea* spp. are model organisms due to their symbiosis. *Cassiopea* spp. have a symbiotic relationship with specialized dinoflagellates called zooxanthellae, which perform photosynthesis with chlorophyll a and c from where they reside in the oral arms of the jellyfish (Arossa et al. 2021; Enrique-Navarro et al. 2022). When the jellyfish are settled with their oral arms directed upwards, the symbionts receive enough sunlight to perform photosynthesis, providing their host with carbohydrates, amino acids, fatty acids, and oxygen in exchange for a safe environment and inorganic nutrient wastes (Arossa et al. 2021; Mortillaro et al. 2009). Study of the symbiotic relationship between *Cassiopea* spp. and their photosynthetic zooxanthellae can provide additional knowledge about similar cnidarian symbiotic relationships that can potentially be applied to coral reefs, which are highly threatened marine ecosystems that are negatively impacted by rising ocean temperatures and falling pH (Toullec et al. 2024).

Cassiopea spp. are also potential bioindicator species for environmental factors which are difficult to measure, such as inorganic phosphates or contamination by heavy metals, and could be useful because of their worldwide distribution (Ames et al. 2020; Béziat & Kunzmann 2022; Todd et al. 2006). Crucially, *Cassiopea* spp. appear to have taken advantage of changing climate to invade and become abundant in new environments (Keable & Ahyong 2016; Nabipoura et al. 2015; Schembri et al. 2011), making research on the genus more timely than ever. *Cassiopea* spp. are predicted to thrive under these changing conditions because their medusae have high thermal tolerances and are limited by cold temperatures rather than warm (Aljbour et al. 2018). Jellyfish can benefit from anthropogenic activity when their predator population is reduced by heavy fishing pressure (Liu 2023) or when eutrophication increases jellyfish blooms by providing nutrients or creating new habitat (Arai 2001). Understanding the biology and ecology of *Cassiopea* spp. is necessary to better understand and evaluate their invasive impact and ability to harness climatic changes as an advantage.

This study will focus on the environmental conditions that constitute a suitable habitat for the establishment and persistence of the invasive upside-down jelly in Hawaiʻi, hereafter referred to as *Cassiopea* spp. Specifically, I utilize two approaches: 1) a temporal survey of the Ala Wai Canal, where jellyfish were previously recorded, and 2) a snapshot spatial survey of ten sites across Oʻahu. During the temporal survey, my goal is to link changing water quality parameters to *Cassiopea* spp. presence. For the spatial survey, my goal is to link water quality parameters and nutrient concentrations to *Cassiopea* spp. density. I will also measure water quality parameters and habitat characteristics (e.g., water depth, turbidity, and mangrove cover). In addition to exploring what environmental conditions are most important for *Cassiopea* spp. invasion, I will also analyze nutrient concentrations in the water column and in the sediment at each site.

Background

Invasive Species

Invasive species are either purposefully or unintentionally, introduced to a geographic location outside of their natural range, where they are able to establish new populations that cause undesirable ecological, economic, and/or human health impacts (Ricciardi 2013). These species did not evolve in these areas in which they are novel, and as such, can disrupt native ecosystems (Ricciardi 2013). Two potential impacts of invasive species are predation and competition with native species, sometimes to the point of extinction; biological invasions have been found to be the main cause of recent extinctions across several major taxa (Bellard et al. 2016), and are one of the primary drivers of biodiversity loss in the Hawaiian Islands. Predation and competition can also lead to decreases in biodiversity and trophic cascades, causing changes in the physical environment because of altered or lost ecosystem services (Mack et al. 2000; Walsh et al. 2016). Due to the potential negative impacts of invasive species becoming established in an environment, management of invasive species, including preventative measures against invasion as well as controlling and eradicating populations of invasive species, is a crucial step to mitigating these damages (Ricciardi 2013). However, invasive species in general, and marine species in particular, can be extremely difficult to detect and eradicate.

Invasive marine species are generally able to disperse over larger distances than terrestrial species through the highly connected marine environment, either on their own or as a consequence of human activity, such as transport by ship ballast or by introduction of species for open water aquaculture (Giakoumi et al. 2019; Holland 2000; Molnar et al. 2008). The mobility of marine species renders many of the management techniques for terrestrial species less effective against them. Other management strategies, such as the use of genetic analysis to evaluate the risk of establishment and potential impacts of marine invasive species, hold great promise but are underutilized or still being developed (Holland 2000). The impacts of marine invasive species can also be dramatic and widespread, ranging from concentrated effects on one or few species, including keystone species. These effects can ripple out into trophic cascades and invasional meltdown, as is the case with introduced oyster species *Crassostrea gigas*, which form reefs on the coast and alter nutrient paradigms through filter-feeding (Molnar et al. 2008).

The Effects of Climate Change on the Ocean

Since the 1770s, when the onset of the Industrial Revolution began to introduce anthropogenic CO_2 into the atmosphere, ocean surface warming, acidification, eutrophication, and other changes caused by climate change in marine environments have been increasing at a growing rate (Garcia-Soto et al. 2021). Climate change has caused a rapid overall increase in ocean heat content and sea surface temperature, having increased by as much as 1.1° C since 1910 (Calvin et al. 2023; Garcia-Soto et al. 2021). Changing environmental temperature is known to drive distribution and range shifts in the ocean, with species moving out of their native geographic ranges or vertically in the water column to avoid thermal stress (Dahms & Killen 2023), and to follow changing distributions of food or prey organisms (Thorne & Nye 2021).

Increased anthropogenic input of CO_2, which spiked to almost 45 gigatons in 2019 (Calvin et al. 2023), results in a more acidic ocean, negatively affecting marine organisms, especially those with calcareous body structures (Shi & Li 2023). Eutrophication, the influx of excessive nutrients into a body of water, causes blooms of organisms, including jellyfish, and hypoxic conditions which are further exacerbated by climate change (Arai 2001). Climate change has been shown to decrease precipitation in the tropics and subtropics, which change hydrological dynamics, frequency of fire, and changing nutrient cycling through temperature effects, which lead to high nutrient loads (Meerhoff et al. 2022).

The Holobiont of Cassiopea *spp.*

Much like scleractinian corals (Knowlton & Rohwer 2003), *Cassiopea* spp. are part of a holobiont, an assemblage of a host organism and the microbial communities contained within it (Röthig et al. 2021). *Cassiopea* spp. host one or more species of the photosynthetic dinoflagellate *Symbiodinium* (Ohdera et al. 2018). These symbionts provide each medusa with energy through photosynthesis when sunlight is available at both high and low irradiance, which allows the host to access carbon fixed by symbionts and survive in conditions where there may not be a sufficient food supply to exist solely on a heterotrophic diet (Jantzen et al. 2010; Röthig et al. 2021). In addition to providing nutrition, symbionts can also support the regenerative abilities of the genus, which allow them to recover from serious injury and loss of tissue (Mammone et al. 2023; Ostendarp et al. 2022). *Cassiopea* medusae experience bleaching, characterized by a visually detectable loss of pigment in the host tissue (Hankins & Pomory 2008). Bleaching is the process where symbionts are expelled from the host under stressful conditions, typically elevated sea surface temperature or increased exposure to UV radiation (Glynn 1993). However, compared to corals, *Cassiopea* spp. may be more tolerant to bleaching, as they have been shown to retain their symbionts at the same temperatures (around 33° C) when corals begin to bleach under heat stress conditions, the density of symbionts (relative to the wet weight of the jellyfish) remains constant for a period of time (Jones et al. 1998; Lampert 2016; Toullec et al. 2024). This response may be due to their utilization of both high and low irradiance by zooxanthellae, making them more physiologically flexible and able to continue performing photosynthesis over a range of light conditions (Mammone et al. 2021). Increased exposure to high temperatures and UV levels have also been shown to result in blue appendages in *Cassiopea* spp. and are associated with increased survival in situations where the jellyfish experience heat stress (Maloney et al. 2024). Low pH, a condition associated with climate change that strongly affects corals, has not been shown to impact symbiont density in *Cassiopea* spp. (Weeks et al. 2019). Medusae can still lose symbionts under certain stressors, such as persistent dark conditions or in temperatures exceeding 34° C, but show no visual signs of bleaching at first, leading to the term, "invisible bleaching" (Toullec et al. 2024). The bleaching of *Cassiopea* spp. is unlike corals, which begin to lose pigmentation rapidly when losing symbionts (Glynn 1993), and although the thermal threshold for coral bleaching varies, bleaching can occur as low as 32–33° C (Glynn 1993). Understanding why *Cassiopea* spp. has more resilient relationships with its symbionts could be key to understanding and preventing coral bleaching, which has massive detrimental impacts on coral reef habitats and tropical coastal ecosystems (Puntin et al. 2022).

The bell tissue of the medusae, which partially shields its symbionts from the negative effects of ultraviolet radiation, may be one reason why the *Cassiopea* spp. holobiont can tolerate stress conditions better than stony corals (Enrique-Navarro et al. 2022). Another factor that may allow for higher stress tolerance is the ability for zooxanthellae to be degraded by amoebocytes, immune cells of the host, a behavior which has not been observed in other similar holobionts. In an experiment by Toullec et al. (2024), *Cassiopea* hosts in heat stress conditions switch to increased catabolism and break down their own tissues for nutrition due to a reduced contribution from their symbionts. However, host immune cells also surround and phagocytose damaged symbiont cells, a process which was only discovered in 2023 and about which many questions linger, particularly about its function—whether it provides the host with nutrients, if it is a more efficient way of expelling symbionts that may be a burden on stressed hosts, and if it makes *Cassiopea* spp. more tolerant to heat stress. Future studies may aim to better understand the mechanisms that control degradation and answer these questions.

Diet and Feeding Behavior

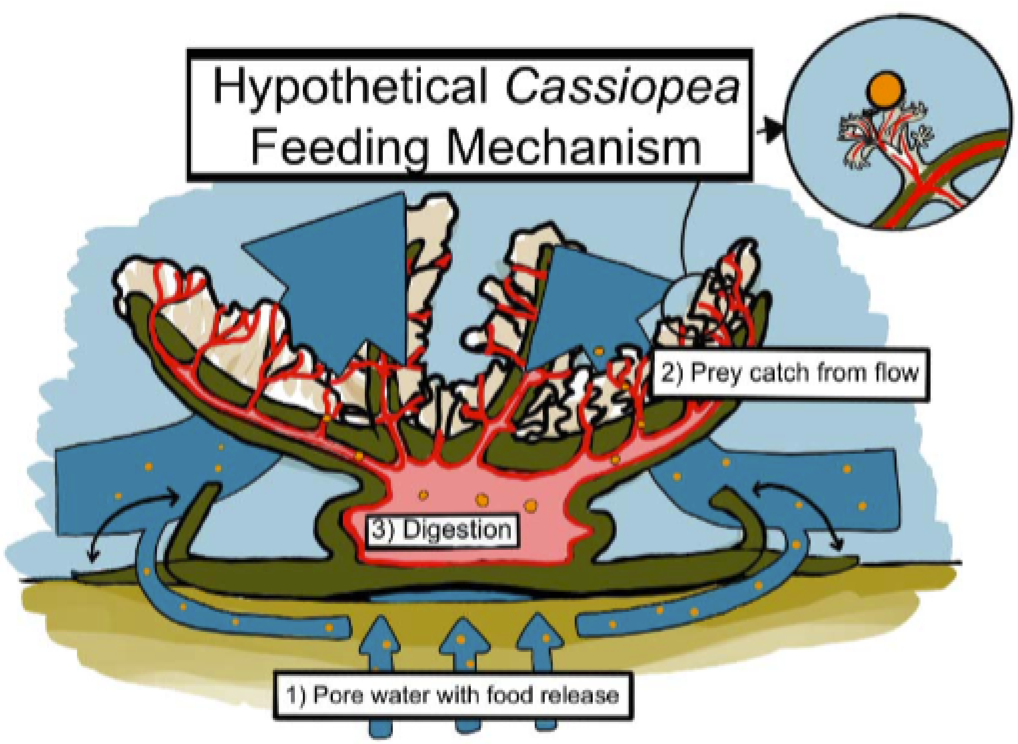

Figure 1: A diagram of a *Cassiopea* spp. feeding mechanism. Cassiosomes, clusters of nematocysts and symbionts that move actively using cilia, sting prey in the water column. The pumping action of the bell draws nutrients out of sediment, as well as bringing these prey and nutrients to the oral cavity and stomach. Figure from Muffett et al. 2025.

Cassiopea spp. have a degree of trophic plasticity due to their symbionts and their ability to feed by preying on zooplankton (Toullec et al. 2024). Prey items consumed by *Cassiopea* spp. include whole prey, such as a variety of small arthropods—isopods, amphipods, copepods, ostracods, and tanaids, among other things—nematodes, insects, and foraminifera (Muffett et al. 2025). They also consume disparate pieces of prey, such as gastropod pleiopods and crustacean parts, as well as egg masses, detritus, and microplastics (Muffett et al. 2025). The genus is functionally similar to photosynthetic organisms when in light conditions strong enough to support their zooxanthellae, and the level of energy contributed depends on the intensity of photosynthetically active radiation. However, the zooxanthellae of *Cassiopea* spp. are resilient and can provide a large input of energy to their hosts even in low light conditions (Mammone et al. 2021). In addition, the medusae can pulse their bells to pump nutrients from the substrate, increasing the capture of particulate organic matter (Jantzen et al. 2010). *Cassiopea* spp. medusae also release cassiosomes, clusters of nematocysts and symbionts that move actively using cilia. These cassiosomes kill prey very effectively, such as harpacticoid copepods or foraminifera (Ames et al. 2020; Muffett et al. 2025), and the jellyfish bell-pumping activity brings in food to the oral cavity (Figure 1). Because of these multiple sources of nutrition, *Cassiopea* spp. medusae change their feeding mode under different conditions. Heterotrophy alone is not enough to support adults (Gadomski & Sweet 2019). However, in high dissolved organic carbon conditions, *Cassiopea* spp. switches to heterotrophy and relies more heavily on this feeding mode (Tilstra et al. 2022). In several cases, after dredging or construction has caused an influx of organic carbon, cnidarians, including *Cassiopea* spp. medusae, have increased in number, though whether it is strictly due to the sudden availability of organic carbon for uptake or also due to the increase in suitable habitat created by dredging is unclear (Arai 2001). These blooms of *Cassiopea* spp. have implications for the feeding strategies of the jellyfish under eutrophic conditions and could lead to a change in local carbon cycling when whole populations are shifting to heterotrophy (Arai 2001).

Reproduction

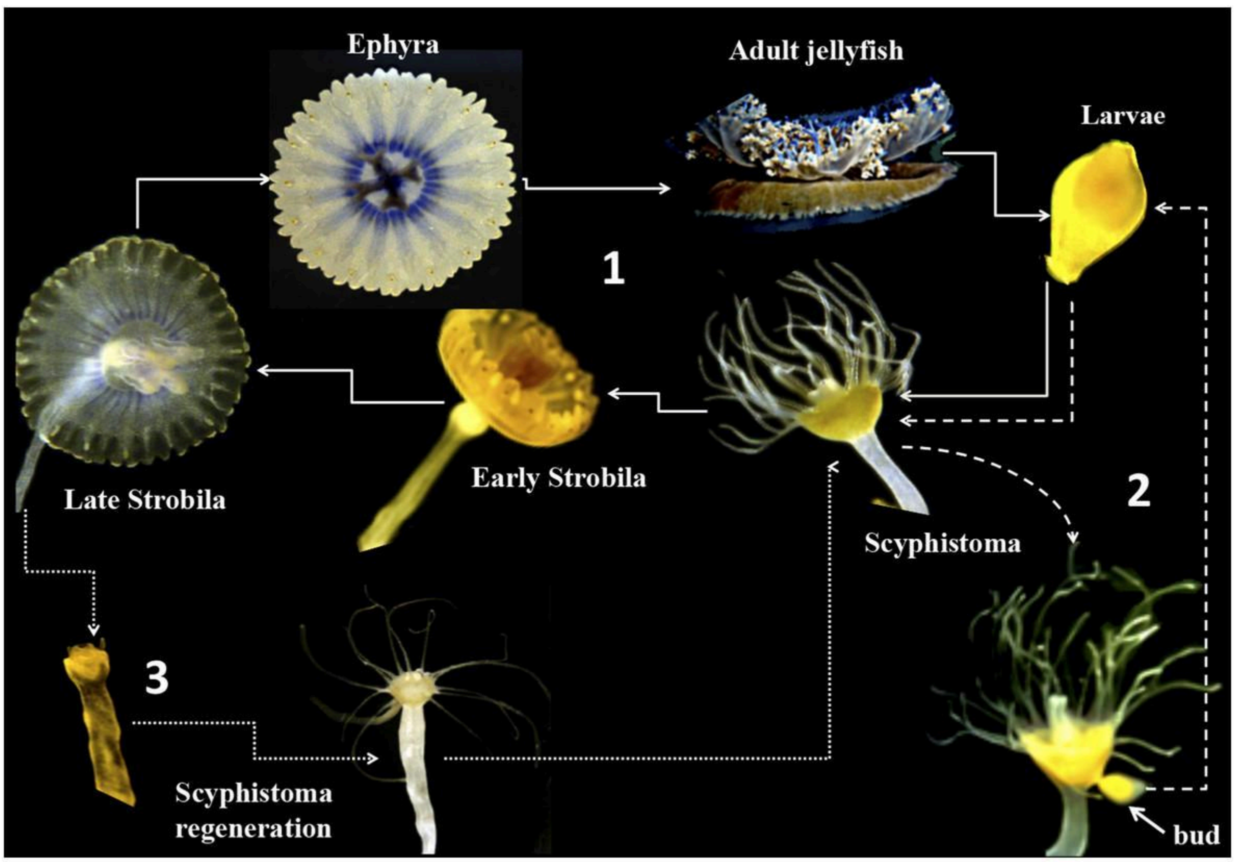

Figure 2: A diagram showing the life cycle of *Cassiopea* spp. Solid lines indicate the sexual reproduction stage of the life cycle, while dashed lines indicate the asexual reproduction stage of the life cycle, and dotted lines indicate where strobila can revert and return to the asexual part of the cycle (from Cabrales-Arellano et al. 2017).

Like many other schyphozoans, *Cassiopea* spp. have an alternating, two-stage life cycle (Figure 2), which differ from other jellyfish in some ways due to their unique habitat preferences (Hofmann et al. 1996). Male adult medusae release sperm into the water column, where they fertilize eggs which are then protected by females on sex-specific tentacles near the oral disk (Fitt & Costley 1998; Hofmann et al. 1996). Females protect the fertilized eggs until they hatch out into planula larvae, which eventually settle onto suitable substrate and metamorphose into a benthic polyp stage (Hofmann et al. 1996). Once polyps are settled and their oral openings have formed, they may acquire the necessary symbionts, forming the *Cassiopea* spp. holobiont (Hofmann et al. 1996). Once symbionts are acquired, the tentacled end of the polyp can reproduce by segmenting, a process known as strobilation, into ephyra, which mature into medusae (Hofmann et al. 1996). Strobilation can happen multiple times for the same polyp (Hofmann et al. 1996).

Though sexual reproduction is more common, *Cassiopea* spp. are also able to reproduce asexually and do so in the wild when entire populations are male (Hofmann et al. 1996; Holland et al. 2004). Instead of strobilation, polyps form buds, a life stage which is similar to larvae and that also must find and settle on suitable substrate before strobilating (Hofmann et al. 1996).

There are several controls in the life cycle of *Cassiopea* spp. which affect their reproductive success. In order to strobilate, polyps must have acquired symbionts; polyps without symbionts were found to never strobilate in the lab (Hofmann et al. 1996). The reason why symbionts are required for successful strobilation remains unclear, but photosynthesis is hypothesized to be a factor, as polyps with symbionts exposed to light are associated with a higher rate of strobilation; however, this is not the case with buds, which can lack symbionts and still strobilate (Hofmann et al. 1996). Another control for strobilation is temperature, as the environment needs to be above 20° C for the process to occur (Fitt & Costley 1998). Settlement and metamorphosis into the polyp stage is controlled by chemical cues, including oligopeptides, phorbol esters, and indoles, which interact with receptors in the larva or bud (Cabrales-Arellano et al. 2017; Deng et al. 2022; Hofmann et al. 1996). Due to the natural patterns of polyp settlement in nature onto surfaces such as algae and decaying mangrove leaves, and the failure of larvae and buds to settle onto clean surfaces or in water containing antibiotics, it is theorized that the bacterial communities that are present in the wild create these chemical cues (Fleck & Fitt 1999; Hofmann et al. 1996).

Phylogeography and Cassiopea spp. in Hawaiʻi

Cassiopea spp. are difficult to identify at species level for several reasons, including morphological plasticity and their evolutionary history. Each species shows a high degree of morphological variation, making it difficult to identify medusae, the adult form of many jellyfish species, based on gross morphology such as color or shape alone (Holland et al. 2004). Bell coloration is one such highly variable character; blue pigmentation is associated with individuals that are subjected to high levels of solar radiation but cannot be used for species identification (Anthony et al. 2022), especially in the field. Similarly, the color of a jellyfish is uninformative about the clade of its symbiont (Lampert et al. 2012). *Cassiopea* spp., such as the populations in the Florida Keys, often have sympatric evolutions, meaning that they evolved from a common ancestor but continue to live in the same range as that ancestor, resulting in a spatial overlap of many species. This can make genetic analysis of the species more difficult because of a relatively low level of genetic difference (Muffett & Miglietta 2023). DNA extraction and sequencing of the mtDNA cytochrome *c* oxidase I (COI) gene has been proven to show important evolutionary relationships for this genus that make it important for identification (Holland et al. 2004). Advances are being made in methods for species identification, but as it stands, DNA sequencing is crucial for *Cassiopea* spp. phylogenetics.

Around 20 years ago, genetic sequencing demonstrated the existence of cryptic species in *Cassiopea* spp. populations in Oʻahu due to high levels of genetic divergence, which suggest multiple anthropogenic introductions of the species to the island (Holland et al. 2004). The genetic differentiation between the windward (*C.* sp. 3) and leeward populations (*C. andromeda*) on Oʻahu exceeds the benchmark typically used to denote species, but new species have not yet been officially designated because of limited geographic sampling and the use of a single mtDNA marker. However, this finding highlights the need for a global reassessment of *Cassiopea* taxonomy using more than one genetic marker and more detailed morphological analysis, especially with the development of more sophisticated morphological identification. Despite evidence supporting the existence of cryptic species (Holland et al. 2004), many species may currently be unidentified due to insufficient data.

In this paper, populations of *Cassiopea* jellyfish are referred to as *Cassiopea* spp. so as to be as accurate as possible. No genetic analysis was performed as a part of this study, and not all species of *Cassiopea* can be reliably distinguished from one another by morphology alone, so the jellyfish in each observed population were not identified to the species level. In order to account for the potential of multiple species of *Cassiopea* present at each site and the possibility of hybridization between species, I use the plural *Cassiopea* spp. instead of sp.

Invasional Meltdown Hypothesis (IMH)

One important species interaction for *Cassiopea* spp. occurs with *Rhizophora mangle*, the invasive red mangrove tree. A paper by Hoffman et al. (1996) demonstrated that planula larvae and buds of the jellyfish detect chemical cues released from deteriorating mangrove leaves, which signal the larvae to settle on them and begin metamorphosis into polyps. The chemical cue's effectiveness is negatively impacted by antibiotic treatments in the water, which suggests that bacterial biofilms are involved in producing the chemical cue. The importance of mangroves for *Cassiopea* spp. development was also supported by findings that peptidic cues from *Vibrio alginolyticus* can induce settlement of *Cassiopea* spp. planulae (Fleck & Fitt 1999; Hofmann et al. 1996). The relationship between *R. mangle* and *Cassiopea* spp. suggests that, in order for medusae to settle new areas, they must already be colonized by mangroves.

Red mangrove was intentionally planted in the Hawaiian Islands in 1902 on Moloka‘i (Allen 1998), around half a century before *Cassiopea* spp. were recorded (Holland et al. 2004). Sites where *Cassiopea* spp. medusae were sampled on O‘ahu and Moloka‘i by Holland et al. (2004) almost exactly overlap with the map of mangrove stand distribution (updated by Allen in 1998), which supports the idea that mangroves are required before *Cassiopea* spp. can successfully invade an area and become established. The nature of the relationship between *R. mangle* and *Cassiopea* spp. is currently not well-characterized. For example, *Cassiopea* spp. appear to rely on *R. mangle* for planular settlement and development, but it is not clear if *R. mangle* benefits in a mutualistic sense from their presence in any way. While *Cassiopea* spp. jellyfish exhibit behaviors that make them ecosystem engineers, such as increasing nutrient flux, the degree to which these effects may benefit *R. mangle* has not been investigated. There may also be indirect effects caused by *R. mangle* that shape the way that *Cassiopea* spp. interact with other species.

Because of the reliance of *Cassiopea* spp. on *R. mangle*, the invasional meltdown hypothesis provides a useful framework for management by supporting regional predictions of the spread and establishment of the upside-down jellyfish (Fleck & Fitt 1999). The invasional meltdown hypothesis was proposed as an alternate theory to biotic resistance, which is the idea that invasive species will have to overcome species that are already established in order to spread to new areas, making it more difficult for nonnative species to colonize new areas as the number of species increases (Simberloff & Von Holle 1999). In contrast, the invasional meltdown hypothesis proposes the opposite pattern to what is expected with biotic resistance: nonnative species can facilitate the survival and settlement of other nonnative species, making the environment easier to invade as more invasive species settle. This hypothesis predicts that facilitative interactions make environments with an increasing number of nonnative species easier to invade by additional invasive species (Stachowicz 2001). Signs of an invasional meltdown include direct effects, like an increase in survival or population size in one species due to another and indirect effects, where one species alters the impact that another species has on a third species (Simberloff & Von Holle 1999). While the invasional meltdown hypothesis is broadly supported across a variety of ecosystems (Braga et al. 2018), alternate hypotheses propose that some nonnative species interact with many others in an ecosystem and broadly change its structure, which does not necessarily add to the potential for further invasion (Devanna et al. 2011).

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Chapter 2: Temporal Survey

Introduction

The scyphozoan genus *Cassiopea* comprises a global tropical and subtropical species complex with some interesting and unusual life history features and behaviors (Holland et al. 2004; Jantzen et al. 2010). Their common name, upside-down jellyfish, derives from the fact that they tend to favor clear, shallow, protected waters and soft substrates, where they settle with their bells facing the sediment, exposing their ventral surface, tentacles, lappets (a fleshy flap or lobe), and oral arms. This upside-down orientation maximizes exposure to sunlight, which is necessary for their endosymbionts to drive photosynthesis (Lampert 2016). The genus's ability to survive in a wide range of environmental conditions may be one reason that they are invading environments all around the subtropics.

Though *Cassiopea* spp. are highly tolerant to a wide range of environmental conditions, especially compared to other marine cnidarians, salinity and temperature impact their survival and development (Maloney et al. 2024). *C. andromeda* can tolerate temperatures as low as 26° C and as high as 34° C, at which point they begin to lose their symbionts (Toullec et al. 2024). In addition, temperatures of 18° C or lower disrupt the development of *C. xamachana*, with tentacle growth being stunted and impacting the ability of the animals to feed (Fitt & Costley 1998). Increased temperatures also cause increased bell pulsation rate in *C. xamachana*, which may indicate that their metabolic processes are sped in the presence of environmental stress (Maloney et al. 2024). *C. andromeda* can survive in salinities as low as 30 ppt and as high as 50 ppt, but lower or higher salinities produce physiological stress in them, including reduced body mass and a switch to anaerobic metabolism (Aljbour & Agusti 2025). Based on these temperature and salinity thresholds, we might expect that populations of *Cassiopea* spp. would display seasonal patterns in a tropical ecosystem, such as on the island of O‘ahu.

Weather on O‘ahu is characterized by two distinct seasons: kauwela, the hotter, drier season from May to October, when temperatures are higher and there is decrease in the amount of precipitation relative to ho‘oilo, the cooler, wet season from November to April. Based on this seasonality, *Cassiopea* spp. populations are more likely to bloom during kauwela, as they are more strongly limited by their lower temperature and salinity tolerance thresholds than their upper limits (Aljbour et al. 2018; Aljbour & Agusti 2025). The purpose of this study was to conduct a temporal survey, encompassing both kauwela and ho‘oilo, to understand the conditions that affect *Cassiopea* spp. and their seasonal presence at one location on O‘ahu, the Ala Wai Canal. This location was selected due to the environmental conditions at this site, with *Cassiopea* spp. expected to bloom in response to urban run-off and hypereutrophication (Laws et al. 1993). I measured *Cassiopea* spp. density together with water quality over a 10-month period and addressed two hypotheses: (1) *Cassiopea* spp. will be present on dates with comparatively higher water temperature, salinity, and lower dissolved oxygen (DO), and (2) the concentration of ammonium, nitrate, and phosphate will be elevated when *Cassiopea* spp. are present.

While *Cassiopea andromeda* were first documented in the Ala Wai Canal in 1950, it is not clear whether these are still the species that occupy the canal. For the sake of accuracy, this study refers to the jellyfish by the genus name *Cassiopea* spp., due to the fact that jellyfish DNA was not sequenced during the study (Holland et al. 2004). In order to account for the potential of multiple species of *Cassiopea* present at each site and the possibility of hybridization between species, I use the plural *Cassiopea* spp. instead of sp.

Methods

Study Site

Due to its location in downtown Waikīkī, an urban center on the island of Oʻahu, the canal is polluted by runoff from residential and industrial areas, leading to a great influx of organic carbon and hypereutrophication (Laws et al. 1993). The canal also experiences significant seasonal changes in sea surface temperature and salinity, which may dictate the seemingly-seasonal presence of *Cassiopea* spp. observed there in the past (Knor et al. 2024). These conditions might be expected to lead to blooms of *Cassiopea* spp., and I therefore measured *Cassiopea* density together with water quality over a 10-month period. My first hypothesis was that *Cassiopea* spp. will be present on dates with comparatively higher water temperature, salinity, and lower dissolved oxygen (DO). Additionally, *Cassiopea* spp. pulse their bells to pump nutrients out of the sediment, increasing nutrient flux (Jantzen et al. 2010). My second hypothesis is therefore that the concentration of ammonium, nitrate, and phosphate will be elevated when *Cassiopea* spp. are present compared to when they are absent.

To account for small-scale spatial variation, three subsites at the inland mauka end of the Ala Wai Canal were selected for sampling. These subsites were sampled regularly over a period of 10 months, from October 2024 to July 2025, for a total of 18 sampling dates.

Water Quality

Water quality parameters, in particular, temperature, salinity, pH, and dissolved oxygen (DO), were measured *in situ* at each subsite using a YSI meter. Samples from the upper layer of water, the topmost 0.1 m, were collected from each subsite for nutrient analysis starting in March 2025.

Cassiopea Density

While sampling at the Ala Wai, I visually surveyed the end of the canal for any medusae, whose light bodies are easy to see against the dark sediment of the canal, even from a distance. I was the only observer, and always conducted surveys in the morning or afternoon, with times ranging from 8:00 to 14:00, based on the low tide time of the day. Medusae were point-counted, as their densities were too low and the canal was too wide to justify use of a quadrat or transect. Using satellite imagery of the Ala Wai Canal, I calculated the area that I visually surveyed while sampling, 50m wide and 150 m long, an area of 7500 m^2. I then calculated the density by dividing the number of medusae counted on each date by the area.

Precipitation and Tidal Height

I used publicly-available data from the National Oceanic and Atmospheric Administration data: tidal height at the time of surveys and daily precipitation data (taken from https://tides.willyweather.com/hi/honolulu/ala-wai-boat-harbor.html) recorded at the Honolulu station (ID 1612340). I averaged daily precipitation data recorded by the National Oceanic and Atmospheric Administration at Beretania Pump Station 705 and US1HIHN0023 station, and then I used these average values to calculate 3-, 5-, and 7-day cumulative rainfall preceding my surveys.

Nutrient Analysis

Water samples were filtered through a Pall Corporation 1-µm glass fiber filter on the day of collection and then frozen for batch analysis of ammonium, nitrate, and phosphate at a later date. Analysis was conducted at Hawaiʻi Pacific University's Downtown Science Laboratory facilities.

The phenate method was used to determine the concentration of ammonium, using the protocol detailed in Standard Methods for the Examination of Water and Wastewater 4500-NH_3 Nitrogen (2017). To 5 mL of the filtered water sample, I added 0.2 mL phenol solution, 0.2 mL sodium nitroprusside solution, and 0.5 mL oxidizing solution. This solution was mixed well and allowed to develop in the dark for an hour before the absorbance was read at 640 nm with a VWR UV-1600PC spectrophotometer.

The concentration of nitrate in water samples was determined by the colorimetric brucine method as outlined by EPA (Environmental Protection Agency) Method 352.1 Nitrate (1971). The pH of samples was adjusted to approximately 7 with acetic acid or sodium hydroxide, and then 5 mL of standards and samples were pipetted into the sample tubes along with 1 mL of 30% sodium chloride solution, then swirled to mix, and placed in a cold water bath (0–10° C). Five mL of 80% sulfuric acid was pipetted into each tube and mixed by swirling, then allowed to cool before 0.25 mL brucine-sulfanilic acid reagent was added and mixed, then all tubes were placed in a 100°C water bath for 25 minutes. Tubes were then transferred to a cold water bath, and the absorbance of the sample was measured at 410 nm by a VWR UV-1600PC spectrophotometer.

The concentration of phosphorus was determined by the colorimetric ascorbic acid method as outlined by EPA Method 365.3 Phosphorus (1978). Five mL of the water sample was combined with 0.1 mL of the sulfuric acid (11N) before adding 0.4 mL of ammonium molybdate-antimony potassium tartrate reagent and finally 0.2 mL of ascorbic acid; samples were mixed by inversion after each reagent addition. The tube was allowed to sit and develop for approximately five minutes before being read at 650 nm with a VWR UV-1600PC spectrophotometer.

We used linear regressions based on gravimetric standards of known concentrations to determine the concentration of ammonium ($R^2 = 0.999$), nitrate ($R^2 = 0.998$), and phosphate ($R^2 = 0.999$), which allowed us to convert spectrophotometer readings to concentrations.

Logistic Regression

While I initially considered a linear mixed effect model to determine the effect of environmental factors on the density of *Cassiopea* spp. in the Ala Wai Canal, I determined this approach was inappropriate due to the very low densities and infrequent presence of *Cassiopea* spp. at each sub-site. Instead, I performed a logistic regression to examine the effects of the same factors on their occurrence (presence or absence). Logistic regressions fit a binary dependent variable to several independent variables using a maximum likelihood estimation (Walker & Duncan 1967). The seven factors examined were: temperature, salinity, DO, tidal height, precipitation, season (kauwela or ho‘oilo), and subsite (the same 3 subsites were sampled over the whole sampling period).

Two outliers, a DO value of 277% and a salinity value of 34.11 ppt (notably higher than the other measurements from that date), were excluded from the dataset after plotting them on a dot chart. On three of the sampling events, the DO at the first subsite was notably higher than the other two. DO is the parameter that takes the longest to settle, so these outliers were removed due to the suspicion that the YSI meter was not sufficiently warmed up and settled. By looking at a dot chart, two nitrate outliers were detected and removed, which were also one to two orders of magnitude above the average concentration for the sampling date. This left 18 data points for analysis.

Because logistic regression assumes that no factors are collinear, factors were then checked for using a correlation matrix, which lists and plots the correlation coefficient between each factor in the analysis. I defined collinearity as two factors with a Pearson correlation coefficient equal to or greater than 0.7. Dissolved oxygen content mg/L and percent saturation were collinear ($r = 0.99$, $P < 0.0001$), which was expected, but we chose to use percent saturation because we are comparing oxygen across dates that show a variety of salinities and temperatures. At this stage, we also chose five-day cumulative precipitation to represent precipitation in our model. I considered a variety of precipitation measurements including a daily average, a three-day cumulative of precipitation, a five-day cumulative, and a seven-day cumulative. The three-day and five-day cumulative precipitation were collinear ($r = 0.89$, $P < 0.0001$), as were the five-day and seven-day cumulative precipitation ($r = 0.71$, $P < 0.0001$). I ultimately chose to use the five-day cumulative value because of previous documentation of its use in another study done in this area (Nigro et al. 2022).

A logistic regression was performed to evaluate the relationship between *Cassiopea* spp. occurrence and the concentration of ammonium, nitrate, and phosphate in the Ala Wai. For this regression, we only considered the presence of *Cassiopea* spp. alongside the concentrations of nutrients. None of the nutrient concentrations were collinear with one another.

Results

Jellyfish were only observed twice during the sampling period, and both times, at low densities. On March 21, 2025, three medusae were spotted, at a density of 0.0007 medusae/m^2. On April 18, 2025, two medusae were observed, at a density of 0.0005 medusae/m^2.

Variability

Some seasonal variability can be observed in all the factors, but the degree of variability is different for each (Fig. 1). Temperature, though it varies little (29 ± 1.6, $\bar{x}$ ± SD, CV = 5.6%), seems to be consistently lower from late November to late March, when it begins to rise. Salinity is more variable than temperature (30 ± 6.3, CV = 21%), which drops in early April and remains low into May, which may be explained by higher rainfall events in April and May. DO varies the most over the sampling period (54 ± 25, CV = 47%), while pH varies the least (7.9 ± 0.24, CV = 3.1%). Five-day cumulative precipitation reaches a peak at one of my sampling events in May, at the beginning of the dry season. Both the wet season and the dry season sampling events show high variation in five-day cumulative precipitation (6.9 ± 7.8, CV = 113%), and tidal height also varies quite a bit (0.13 ± 0.19, CV = 146%).

Water Quality Model Results

From the logistic regression analysis, no factors had a statistically significant effect on *Cassiopea* spp. presence ($\alpha = 0.05$, df = 51): five-day cumulative precipitation ($z = 1.6$, $P = 0.12$), temperature ($z = -1.6$, $P = 0.11$), wet season ($z = -0.09$, $P = 0.93$), salinity ($z = 1.5$, $P = 0.15$), DO ($z = 1.2$, $P = 0.22$), and tidal height ($z = -1.4$, $P = 0.17$).

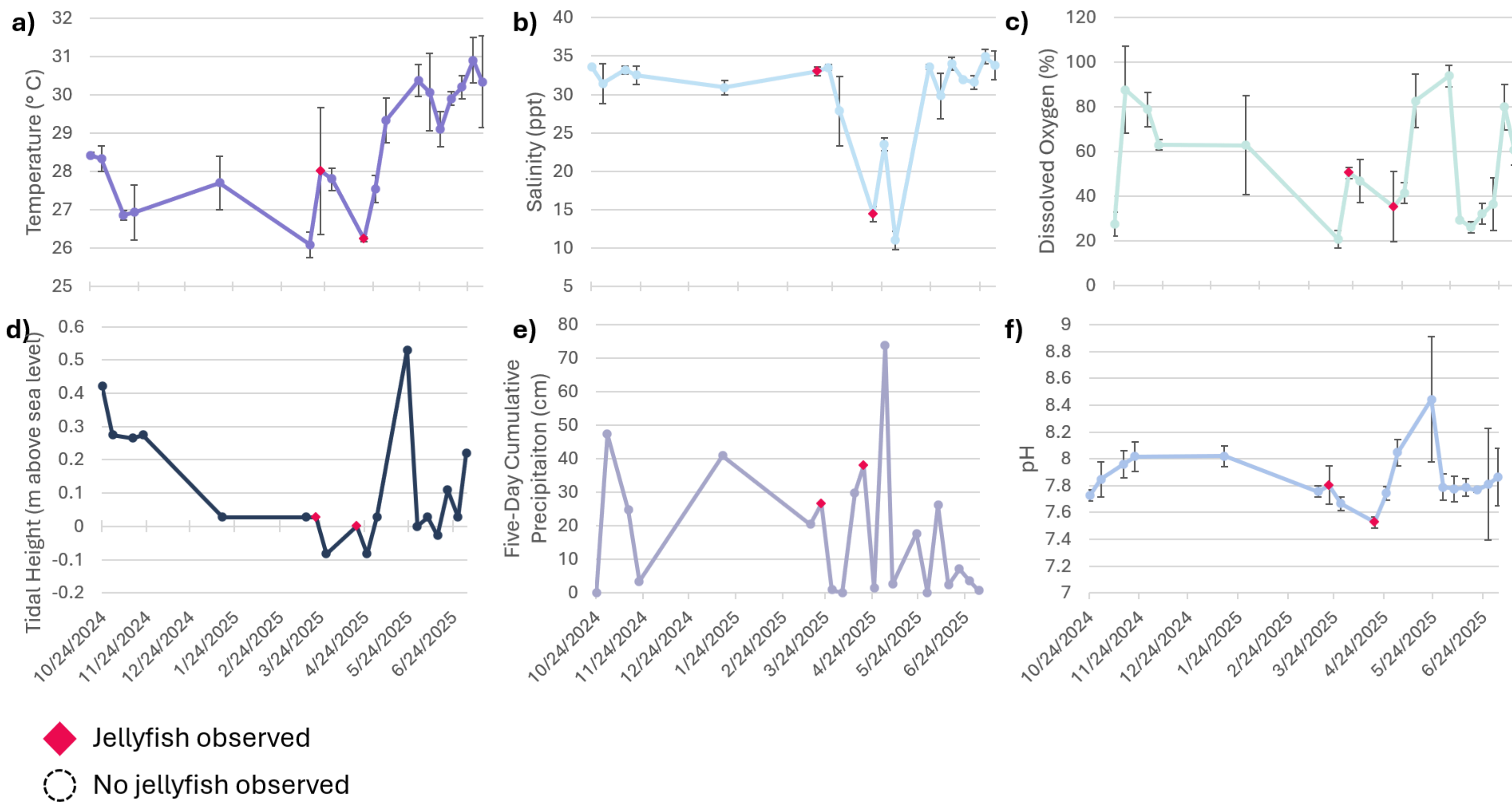

Figure 1: Time series of six water quality parameters, measured at the three subsites per sampling day: (a) Temperature (° C) (b) Salinity (ppt), (c) Dissolved oxygen (% saturation), (d) Tidal height (meters above sea level), (e) Five-day cumulative precipitation (cm), and (f) pH. Pink diamonds indicate sampling dates where jellyfish were observed, and colored points indicate sampling dates where none were observed. Error bars represent the standard deviation.

Nutrient Model Results

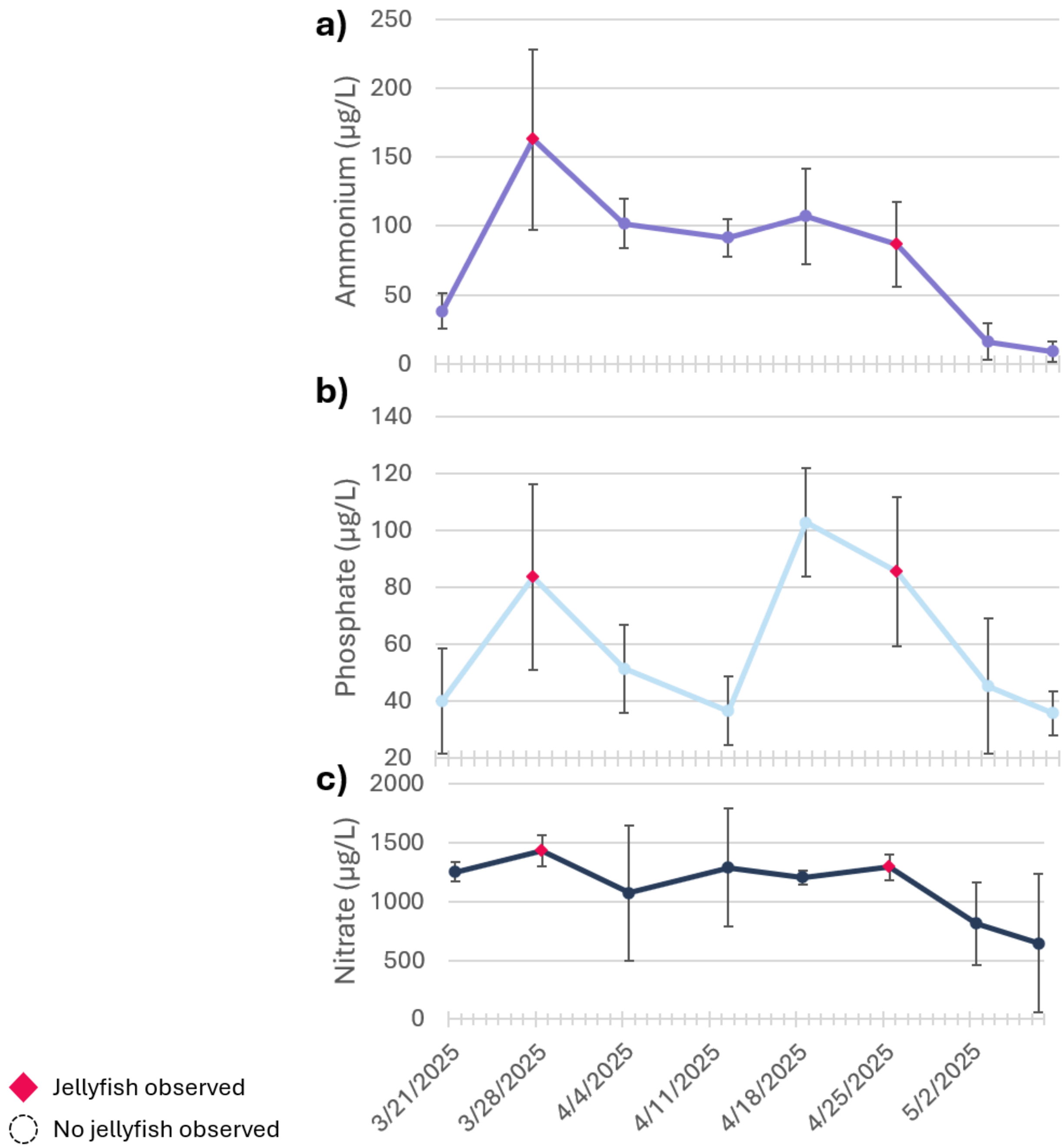

Figure 2: Time series of the concentration of nutrients in the water column, averaged across the three subsites: (a) Ammonium (µg/L), (b) Phosphate (µg/L), and (c) Nitrate (µg/L). Pink diamonds indicate sampling dates where jellyfish were observed, and colored points indicate sampling dates where none were observed. Error bars represent the standard deviation.

Compared to most water quality measurements, nutrient concentrations vary more (Fig. 2). Ammonium varies the most (91 ± 74, CV = 81%), followed by phosphate (57 ± 30, CV = 53%) and nitrate (1116 ± 400, CV = 36%). Concentrations of different nutrients do not appear to follow the same trend over time.

In the logistic regression, ammonium (df = 26, z = −0.93, P = 0.35) and nitrate (z = 0.46, P = 0.65) were not statistically significant (α = 0.05), but there was a weak positive relationship with phosphate (z = 1.6, P = 0.096).

Discussion

Cassiopea spp. were observed only twice during the 21 sampling events, and the densities were very low (i.e, << 1 jellyfish per m^2). No significant relationships were found between the factors I examined and the presence of *Cassiopea* spp. in the Ala Wai Canal. Contrary to my hypothesis, I observed *Cassiopea* spp. towards the end of the wet season on two days when 5-day cumulative precipitation was in the top third of all of my sampling events. I also observed a weak trend with *Cassiopea* spp. presence being associated with higher phosphate levels in the water column, but this was not statistically significant (P = 0.096). Because of the relatively low flow rate in the Ala Wai Canal, which should prevent strong mixing and dilution of any increases in nutrients, a measurable increase in nutrient concentrations was expected, but the effect may still be relatively localized to the areas surrounding individual jellyfish (Jantzen et al. 2010; McMurtry et al. 1995). Higher phosphate levels are possibly associated with runoff during rain events in the Ala Wai (Nigro et al. 2022), and with such low densities of *Cassiopea* spp., higher phosphate levels are unlikely to be associated with their bell pulsating mechanism.

The lack of any significant relationships is an interesting result from this study, which likely stems from the very low density and frequency of appearance of *Cassiopea* spp. medusae at the site. There are only two sampling dates where jellyfish were present, and there were only three and two medusae present, respectively. This lack of jellyfish made it difficult to quantify any potential differences in jellyfish-present and jellyfish-absent conditions, and did not allow me to investigate if there is a density-dependent relationship.

While there is little variation in jellyfish density, there is greater variability in site conditions, the most extreme examples being nutrients and dissolved oxygen. Such variation makes it difficult to identify patterns in water quality or nutrient concentrations when jellyfish are present or absent, especially when I only observed jellyfish at 2 of the 21 sampling events. These observations suggest that the Ala Wai is not the ideal site to study *Cassiopea* spp., as their densities appear to be low year-round, not adhering to a summer bloom population as was anticipated. It is possible that *Cassiopea* spp. no longer occur in the canal as they did historically, and that sites where their appearance is relatively recent or which possess different physical characteristics may be preferred habitat for the genus.

Climate change and anthropogenic impacts on the environment may increase the invasive potential of *Cassiopea* spp. through their alteration of marine environments. Jellyfish blooms are associated with a variety of human activities, such as aquaculture, eutrophication, and harvest of seafood (Purcell 2012). With increasing sea surface temperature, *Cassiopea* spp. have expanded their range geographically throughout the tropics (Rowe et al. 2022), and the holobiont's ability to perform photosynthesis at a high enough rate to meet and exceed carbon demands, even at low irradiation levels, could be another mechanism by which they are outcompeting native species and invading new habitats (Mammone et al. 2021; Welsh et al. 2009). While the Ala Wai Canal experiences several of these anthropogenic and climate change impacts, especially eutrophication, the fact that jellyfish occurrence here is low may indicate that there is some complexity with these impacts and how they affect *Cassiopea* spp. populations (Laws et al. 1993). The complexities of the impacts of climate change on *Cassiopea* spp. populations require further study.

Monitoring *Cassiopea* populations over time illuminates the impact of changing environmental conditions, both from smaller seasonal changes as well as pressing and potentially dramatic alterations from climate change. In order to better understand the impact that a variety of water quality conditions has on *Cassiopea* spp. populations with a range of densities, additional sites were added to create a spatial survey in Chapter 3.

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Chapter 3: Spatial Survey

Introduction

The genus *Cassiopea*, comprised of 12 species and commonly referred to as upside-down jellyfish, is present throughout the subtropics (Collins et al. 2025). They are native to the subtropics, including the Caribbean Islands and the Red Sea, but have also been introduced widely, including the coasts of Brazil, Vietnam, and Australia, the Persian Gulf, and the Mediterranean Sea (de Araújo et al. 2020; Forskål 1776; Keable & Ahyoung 2016; Nabipoura et al. 2015; Pallas 1774; Schembri et al. 2011; Tran Manh et al. 2020). *Cassiopea* spp. are considered invasive in places for the harmful human health, economic, and ecological impacts they cause (de Araújo et al. 2020). This genus feeds by releasing cassiosomes, bundles of nematocysts (stinging cells) and mucus that move freely through the water, either passively with the movement of the water or actively with cilia, and kill prey (Ames et al. 2020). In addition to killing prey items consumed by *Cassiopea* spp., however, the cassiosomes remain in the water and can cause relatively serious stings, posing a risk to human health (Muffett et al. 2021). The indiscriminate effects of the cassiosomes also harm other organisms in their ecosystems, both by depleting shared prey populations and by potentially stinging them, which poses a concern for both natural ecosystems and aquaculture operations alike (Keliʻi Kotubetey, personal communication).

Not only do their invasions cause a range of negative impacts, *Cassiopea* spp. are of great concern due to their resilience to a wide variety of environmental conditions. *C. andromeda* can tolerate salinity ranging from 30 to 50 ppt, and can even survive those as low as 20 ppt or as high as 60 ppt, though they do so with significant stress (Aljbour & Agusti 2025). *Cassiopea* spp. are also tolerant of a range of pH, surviving from 7.5 up to 8.2 without impacts to their health, though a pH of 7.0 causes stress, including reductions in bell diameter, pulsation rate, and deformation (Thayer et al. 2022; Weeks et al. 2019). *Cassiopea* spp. medusae can also tolerate temperatures as low as 20° C and as high as 34° C before they experience lethal stress (Aljbour et al. 2018; Toullec et al. 2024). *Cassiopea* sp. also pulse their bells against soft substrate to draw out and assimilate nutrients from porewater (Jantzen et al. 2010).

The resilience of *Cassiopea* spp. can be attributed, in part, to contributions from other species, both to their symbionts as well as species with which they may have a facilitative relationship. *Cassiopea* spp. possess photosynthetic endosymbionts, which can provide the medusae with the products of photosynthesis and support their metabolism, allowing the medusae to survive in areas with few opportunities for heterotrophic feeding (Ohdera et al. 2018; Röthig et al. 2021). The bacteria present in the *Cassiopea* spp. microbiome also allow them to survive in eutrophic areas (Röthig et al. 2021). The roots of red mangrove (*Rhizophora mangle*), whose presence has been posited as a prerequisite for *Cassiopea* spp. settlement, help with sediment retention along banks of bodies of water, which has the benefit of reducing turbidity in the water, which may help the symbionts of *Cassiopea* spp. harness sunlight for photosynthesis (Allen 1998; Hoffman et al. 1996). The ability of the genus to survive a broad range of environmental conditions has allowed them to widely invade the subtropics, including the Hawaiian Islands (Keable & Ahyong 2016; Nabipoura et al. 2015; Rowe et al. 2022; Schembri et al. 2011; Tran Manh et al. 2020).

Cassiopea spp. have been reported in the Hawaiian Islands since the 1940s, though phylogeographic analysis of the genus has suggested multiple anthropogenic introductions (Holland et al. 2004). Genetic sequencing has also revealed the presence of a cryptic species, *C. sp. 3*, on the island of Oʻahu, which has also been found in Australia, though no new species have yet been designated (Holland et al. 2004; Rowe et al. 2025). *C. sp. 3*, was found on the windward side of the island, and *C. andromeda* were found on the southern shore of the island (Holland et al. 2004). It is not clear whether these are still the species that occupy these areas, so in order to account for the potential of multiple species of *Cassiopea* present at each site and the possibility of hybridization between species, I use the plural *Cassiopea* spp. instead of sp.

In addition to the questions that remain about the taxonomy of *Cassiopea* spp. in the Hawaiian Islands, new and urgent problems have arisen in the past 20 years that have not been a focus of research. While *Cassiopea* spp. have invaded a variety of habitats across the island of Oʻahu, their invasion into traditional traditional Hawaiian fishponds, loko iʻa, has resulted in dense populations that disrupt regular operations of these culturally and economically important resources (Ingrid Lassleben, personal communication; Keliʻi Kotubetey, personal communication). These recent issues have motivated this third chapter, where I aim to describe what constitutes suitable habitat for *Cassiopea* spp.

Previous observations, discussions with local resource managers, and my literature review suggest that upside-down jellyfish exhibit seasonal blooms, increasing in density during the summer months, when their habitats are warmer and more saline, and decreasing in density during the winter, when the opposite occurs. In order to examine this pattern, a temporal survey was conducted wherein the Ala Wai was repeatedly sampled over a period of ten months. However, the variation in population density that was expected was not observed, and it is possible that location may play a bigger role in *Cassiopea* spp. population density than time or season. In order to test this idea, the number of sites was expanded, and these sites were sampled once to capture a variety of conditions over space instead of time. I conducted a spatial survey across ten sites on Oʻahu to capture a wide range of environmental factors. My first objective was to determine what physical and chemical factors define suitable habitat for *Cassiopea* spp. medusae. Second, I wanted to determine which environmental factors influence their density. My third objective was to determine what factors were associated with healthy *Cassiopea* spp. populations, using bell diameter of medusae as a proxy for health.

Methods

Study Sites

Ten field sites on the island of O‘ahu, where upside-down jellyfish populations have been reported in the past few decades in published literature, by stewards of the land, or on iNaturalist were sampled once (Figure 1) between August 21 2025 and September 29 2025 (Holland et al. 2004; Ingrid Lassleben, personal communication; Keli‘i Kotubetey, personal communication; Ty Spangler, personal communication).

Table 1: A table of all the sites examined during this study. Longitude, latitude, and depth averaged from all subsites at a site. Jellyfish presence in Holland et al. 2004 listed in the last column, with N/A meaning a site was not surveyed as part of that study.

	Number of Subsites	**Latitude (Decimal Degrees)**	**Longitude (Decimal Degrees)**	**Average Depth (m)**	**Jellyfish in Holland et al. 2004?**
Sand Island	3	21.30079933	-157.6038167	0.53	N/A
Magic Island Lagoon	3	21.28325567	-157.846909	0.52	N/A
James Campbell (JC) Green Pond	3	21.693453	-157.9534087	0.83	N/A
JC Intermediate Pond	3	21.69311867	-157.9532067	0.85	N/A
JC Jellyfish Pond	3	21.692964	-157.953481	0.65	N/A

Ala Wai Canal	3	21.41442233	-157.8631523	0.42	N/A
Mid-Pacific Country Club	3	21.39405933	-157.728421	0.31	*C. sp. 3*
Moli‘i Pond	9	21.51276789	-157.8448907	0.75	*C. sp. 3*
Ka‘elepulu	3	21.48065833	-157.8277317	1.21	N/A
He‘eia	15	21.436335	-157.8084146	0.92	N/A

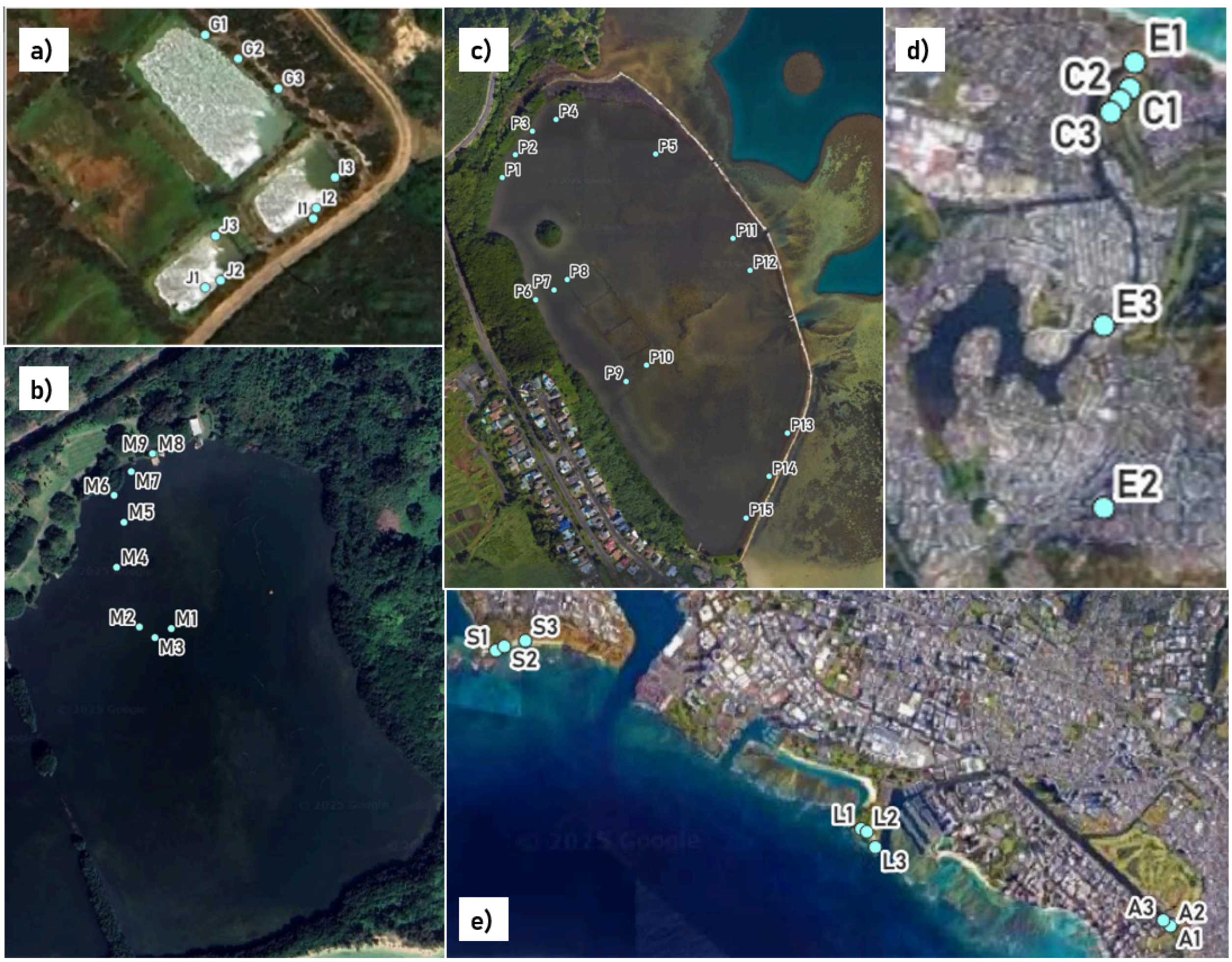

Figure 1: Map indicating site locations. (a) Sites at James Campbell National Wildlife Reserve in Kahuku. Points G1–G3 denote Green Pond, I1–I3 Intermediate Pond, and J1–J3 Jellyfish Pond. (b) Moli‘i Pond. (c) He‘eia Fishpond in He‘eia. (d) Kailua sites. C1–C3 are sites at the Mid-Pacific Country Club Golf Pond, and E1–E3 in Ka‘elepulu. (e) Downtown sites. S1–S3 are at Sand Island, L1–L3 at Magic Island Lagoon, and A1–A3 at the Ala Wai Canal. Satellite map from Google Maps, maps constructed in QGIS.

Moli‘i Pond at Kualoa Ranch and He‘eia Fishpond were both surveyed for this study. Both sites are active loko i‘a, traditional Hawaiian fishponds. *Cassiopea* spp. medusae have been observed at both sites in recent years and were also observed around a decade ago, before disappearing and then reappearing recently (Ingrid Lassleben, personal communication; Keli‘i Kotubetey, personal communication). At He‘eia, the jellyfish bloomed around 2015–2016, but hand removal was effective in keeping the population size low until the COVID-19 pandemic, when it ceased and the population subsequently bloomed (Keli‘i Kotubetey, personal communication; Dr. Rosie Alegado, personal communication).

The ponds at James Campbell National Wildlife Reserve also used to function as aquaculture ponds, but since being abandoned and purchased by the reserve, each pond has developed unique characteristics, with jellyfish present in two ponds but not the third, despite being located mere meters away from one another. There are notable visual differences between the ponds. The pond with the greenest, least clear water is referred to as Green Pond in this paper, while the other pond, which possesses some jellyfish and slightly green water, is referred to as Intermediate Pond.

Sand Island, Ka‘elepulu Pond, and the lagoon at Magic Island all possess characteristics that make them suitable for *Cassiopea* spp., and the jellyfish have been reported at all sites before, but are not currently present (Bob Bourke, personal communication; Dr. Brenden Holland, personal communication). The pond at Mid-Pacific Country Club was also surveyed for the Holland et al. 2004 paper but appears to no longer contain jellyfish. The final site to be surveyed is the Ala Wai Canal, an artificial waterway near Waikīkī (Laws et al. 1993).

Each of these sites has the fundamental physical characteristics of suitable habitat, including protected, relatively shallow clear salt water with soft sand or mud substrate. Suitable abiotic water parameters include salinity within the range of 30 and 50 ppt, as suggested by previous observations (Aljbour & Agusti 2025), and temperature between 26 and 34° C (Béziat & Kunzmann 2022) were observed at many sites—though, curiously, several subsites where medusae were found had values outside of this range.

Several of these sites were sampled 20 years ago as part of a phylogeographic assessment of the species on the island, but *Cassiopea* spp. medusae were only seen at a few sites when they were reassessed for this study (Holland et al. 2004). Conditions at sites sampled in the past may have changed in the intervening years so as to make the environment there unsuitable for *Cassiopea* spp., such as by removal of mangroves or cleanout of artificial water features, both of which occurred at the Hilton Lagoon in Waikīkī, a site which was sampled in 2004 but will not be included in this study (Dr. Brenden Holland, personal communication; Gerald Crow, personal communication). In order to account for small-scale spatial variation, at least three subsites were chosen per site; in larger ecosystems where *Cassiopea* spp. were present, I sampled more subsites (Table 1).

Cassiopea *Density*

The density of *Cassiopea* at each subsite was measured using two methods to account for site-by-site characteristics, which may make one method less effective. The first method was a belt transect, whereby the number of jellyfish in a 0.25 m^2 area, defined by a quadrat, was counted. Counts were repeated along a transect of known length, flipping the quadrat over itself so that no medusae were double-counted. The second method was a net tow along a transect of known length, using a pool net with a head measuring 26.4 cm by 50 cm and an inflatable raft to avoid disturbing any medusae on the bottom. The net was periodically emptied into a bucket while going along the transect if it was full, in order to make room for other jellyfish along the transect. The first method was ideal in more shallow, clear waters, whereas the second accounted for any medusae that may not be seen with the naked eye, at sites with very small medusae as well as sites with more turbid water. Differences in the densities produced by each method can be compared and may be attributed to physical characteristics of each site. At one site, the Jellyfish Pond at James Campbell National Wildlife Refuge, the density of medusae collected during the net tow was too great to count, and visual estimates of the number left in the net were made after counting more than one hundred jellyfish less than 2 cm in diameter.

Bell Diameter

The bell diameter of *Cassiopea* spp. medusae were measured with a flat measuring board. Medusae collected during the net tow were deposited in a bucket, then immediately after capture, were placed on the boards, bell side-down. Bell diameter was measured to the nearest 0.5 cm, as marked on the boards. Up to 30 medusae were measured at each subsite. For a histogram of frequency of bell diameter size by site, see Appendix Figure 3.

Water Quality Measurements

Temperature, salinity, pH, and dissolved oxygen (DO), were measured *in situ* at each subsite using a YSI Pro Quatro Meter. Water samples from the surface of the water were also collected for analysis by an Apera TN420 portable turbidity analyzer.

One water sample (from the surface of the water) and one sediment sample (from the top 3 cm of sediment) was collected from each subsite. Water samples were filtered through a 1-µm glass fiber filter on the day of collection and then frozen for batch analysis at a later date.

Sediment was decanted into tubes for storage; one subsample was processed for sediment organic matter and the other was frozen at −20° C until it could be centrifuged to collect porewater.

Porewater Processing

Porewater processing was based on Vizza et al. 2017. Sediment samples were allowed to thaw for two hours at room temperature 72 hours before centrifuging. Several samples which were entirely sand and had very little or no water content had ultrapure water added to the 50 mL line before centrifuging. Tubes were centrifuged at 4000 RCF for 45 minutes at 4° C. Porewater was poured into a clean centrifuge tube, then filtered through a 1-μm glass fiber filter. Ultrapure water was added to samples with less than 35 mL, in 5 mL increments until the final volume was at least 35 mL.

Nutrient Analysis

All water column (WC) and porewater (PW) samples were analyzed for ammonium, nitrate, and phosphate using a VWR UV-1600PC spectrophotometer. Ammonium was measured using the phenolhypochlorite method (Solórzano 1969), nitrate was measured using the brucine method (Jenkins & Medsken 1964), and phosphate was measured using the ascorbic acid method (Murphy & Riley 1962). I used linear regressions based on standards of known concentrations to determine the concentration of ammonium ($R^2 = 0.997$), nitrate ($R^2 = 0.991$), and phosphate ($R^2 = 0.995$), which allowed us to convert spectrophotometer readings to concentrations. Dilution factors were used to convert the samples diluted with ultrapure water to the correct concentrations. For more details about the procedures of the nutrient analyses I conducted, please see Chapter 2.

Sediment Organic Matter

To determine the SOM, sediment samples were dried for 48 hours at 60° C and weighed. The dried samples were then placed in a muffle furnace and ashed for four hours at 500° C, which burned off the organic matter. The samples were then wet with ultrapure water and dried again for 48 hours at 60° C. The ash-free-dry mass is equal to the amount of organic matter present in the sediment, which was then scaled by the sediment dry mass to get organic matter (OM) %:

OM % = (sediment dry mass – sediment ashed mass)/sediment dry mass * 100.

Statistical Analysis

My first two hypotheses were that *Cassiopea* spp. would be present and at higher densities at sites with comparatively higher water temperature, salinity, sediment organic matter (SOM), nutrient concentrations in the water column and porewater, pH, and lower dissolved oxygen (DO) and turbidity (Table 2). Using bell diameter as a proxy for *Cassiopea* spp. health and life stage, my third hypothesis was that bell diameter would be larger at sites with comparatively higher pH, temperature, nutrient concentrations in porewater, and lower jellyfish density and turbidity (Table 3).

Before analysis of these three hypotheses, outliers were first detected using dot charts and removed if there was reasonable suspicion that they were recorded incorrectly. Two data points were removed this way: 1) A salinity value where pH was likely recorded as salinity and clearly did not match the other salinity values from the site, and 2) A WC phosphate value that was likely mixed up with a PW sample as it was one to two orders of magnitude higher than any other WC phosphate value. I also used a correlation matrix to examine all the predictor variables for collinearity and to ensure that no collinear variables were used in model selection. Response variables—jellyfish density by quadrat, jellyfish density by net tow, and bell diameter—were checked for normality and transformed using the bestNormalize package in R. The arcsin transformation was chosen for jellyfish density by quadrat, while net tow jellyfish density and medusa bell diameter were both transformed using the ordered quantile normalization transformations (ORQ).

Initially, I intended to use mixed effect model selection after Zuur et al. (2009), but this approach often led to the overfitting of models as the number of predictor variables of interest was high, but the subsites where jellyfish were found and all variables of interest were measured was relatively low (14 out of 48 total subsites). Additionally, during mixed effect model selection, I found that adding site as a random factor never improved the model. Therefore, I proceeded with generalized linear models (GLM) and Akaike Information Criterion (AIC) based model selection that penalizes for a larger number of parameters and corrects for small sample sizes (AICc; Burnham & Anderson 2002). The most likely model is the one with the lowest AICc value, and all remaining models are compared relative to this model using delta AICc (Δi). Models with a Δi less than or equal to 2 are considered to have substantial support, while models having a Δi greater than 7 have little support (Burnham & Anderson 2002). I used the MuMIn package in R for model selection, where I was also able to constrain the number of parameters allowed in any one candidate model based on the sample size. My intention was then to use the MuMIn package to average any models with substantial support ($\Delta i > 2$), but in all three analyses, there was only one model with substantial support. I also calculated a deviance-based R^2 for each of the best GLMs, which is based on the proportion of total deviance of a model explained by the predictors variables versus the null model (intercept only). Finally, I confirmed that the model with substantial support did not strongly violate assumptions of linear regression.

To determine which factors influence whether *Cassiopea* jellyfish were present (Table 1), I used a logistic GLM where the global, or most complex, model was:

Jellyfish Presence ~ Temperature + Salinity + DO + pH + Turbidity + SOM + WC Nitrate + WC Phosphate

Because a total of 43 subsites were used in this analysis, I constrained the total number of parameters that could be included in any candidate model to four, to ensure a sample to parameter ratio >10 (Concato et al. 1995; Harrell et al. 1985).

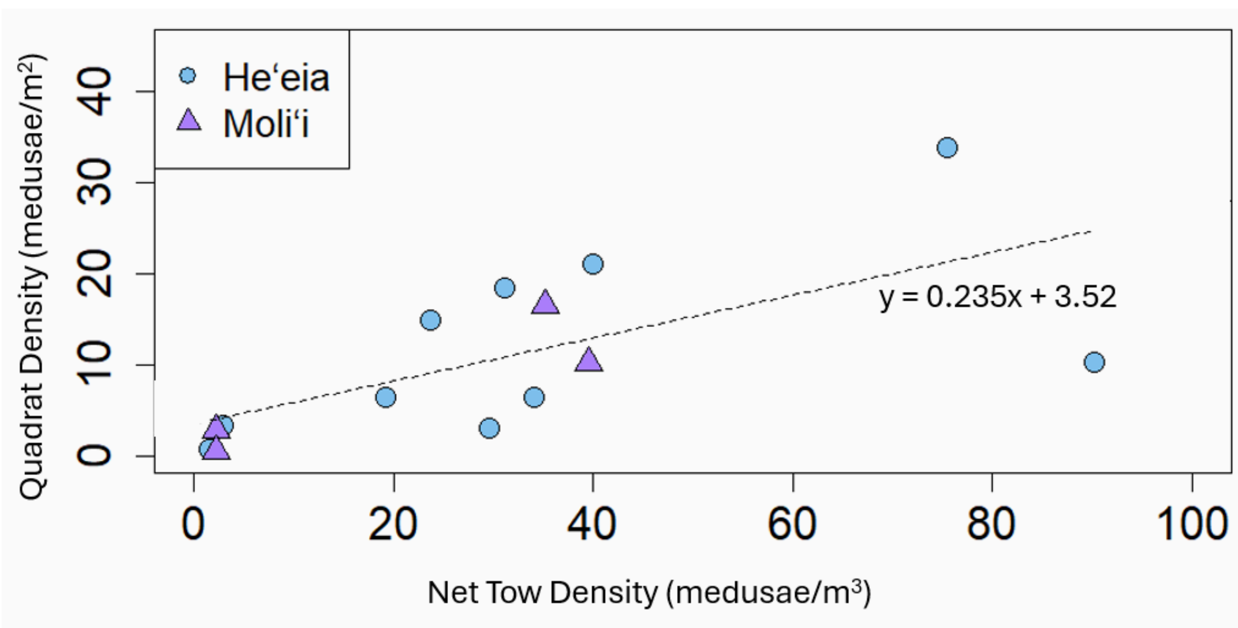

Figure 2: Linear regression of net tow density against quadrat density of *Cassiopea* spp. The dashed line represents the regression equation. To see the 95% confidence interval, see Appendix Figure 1.

The net tow density of jellyfish was correlated with the quadrat density (r = 0.66, P = 0.010) and was collinear after transformation (r = 0.80, P = 0.00066) (Figure 3). As such, quadrat density was chosen to represent jellyfish density.

Because a total of 14 subsites were used in this analysis, I constrained the total number of parameters that could be included in any candidate model to two. To determine which factors influence *Cassiopea* spp. Density (Table 2), I used a GLM where the global, or most complex, model was:

Quadrat Density ~ Temperature + Salinity + DO + pH + Turbidity + SOM + WC N + WC P + PW A + PW N + PW P

Table 2: Table showing the hypothesized relationships between jellyfish presence or density and a suite of physical and chemical factors.

Predictor variable	Hypothesized effect	Rationale	References
Temperature	+	*Cassiopea* spp. are negatively affected by colder temperatures.	Aljbour et al. 2018
Salinity	+	*C. andromeda* have a relatively high salinity tolerance.	Aljbour & Agusti 2025
DO	−	*C. xamachana* and other *C.* spp. are associated with mangrove habitats, which contain low DO conditions.	Allen 1998; Fleck & Fitt 1999; Hofmann et al. 1996; Mattone & Sheaves 2017
pH	+	*Cassiopea* spp. are stressed when pH is 7.0, but can withstand at least up to 8.2 without issue.	Thayer et al. 2022; Weeks et al. 2019
Turbidity	−	*Cassiopea* sp. endosymbionts need access to sunlight in order to	Mammone et al. 2021

		perform photosynthesis.	
SOM	+	*Cassiopea* spp. use particulate organic matter and organic carbon as a food source, feeding blooms.	Arai 2001; Jantzen et al. 2010
Nutrients	+	*Cassiopea* sp. pump nutrient-rich porewater out of substrate, assimilating it as well as driving mixing with the water column.	Jantzen et al. 2010

To determine which factors influence *Cassiopea* spp. bell diameter, I used a GLM where the global, or most complex, model was:

Bell Diameter ~ Temperature + Turbidity + pH + PW A + PW N + PW P + Net Density

Because a total of 19 subsites were used in this analysis, I constrained the total number of parameters that could be included in any candidate model to two.

Table 3: Table showing the hypothesized relationships between bell diameter and a suite of physical and chemical factors.

Predictor variable	Hypothesized effect	Rationale	References
Temperature	+	Cold-treated *Cassiopea* spp. experience decreases in bell diameter.	Aljbour et al. 2018
pH	+	pH of 7.0 causes *Cassiopea* sp. bell diameter to decrease.	Thayer et al. 2022
Nutrients	+	*Cassiopea* sp. need nutrients for themselves as well as their symbionts.	Jantzen et al. 2010
Net Density	−	Density-dependent competition could decrease the size of individuals.	Stoner et al. 2022

Results

Habitat Suitability

Cassiopea spp. jellyfish were only found at He‘eia Fishpond, Moli‘i Pond, and the Intermediate and Jellyfish Ponds at James Campbell National Wildlife Reserve. Salinity, turbidity, SOM, and nitrate in the water column were the leading environmental factors determining whether jellyfish were present at a site, as evidenced by the best-fit model (Table 4). Generally, *Cassiopea* spp. were present at sites with higher sediment organic matter and salinity, but lower nitrate and turbidity in the water column (Table 4). Interestingly, salinity did not seem to have a noticeable effect alone without the other variables being controlled for in the model (Figure 4). The next best model had a $\Delta i = 7.36$, and therefore there was no other model with substantial support in explaining jellyfish presence.

Table 4: Summary of the only GLM with substantial support ($\Delta i = < 2$) for determining jellyfish presence. The AICc is 28 and the deviance-based R^2 is 0.54 and the RMSE (root mean square error) is 9.3.

	Estimate	Standard Error	Z-score	P-value
Salinity	+0.45	0.17	+2.6	0.0082
Turbidity	−0.68	0.34	−2.0	0.047
SOM	+1.3	0.50	+2.6	0.0099
WC Nitrate	−25	8.9	−2.8	0.0047
Intercept	−11	4.6	−2.4	0.017

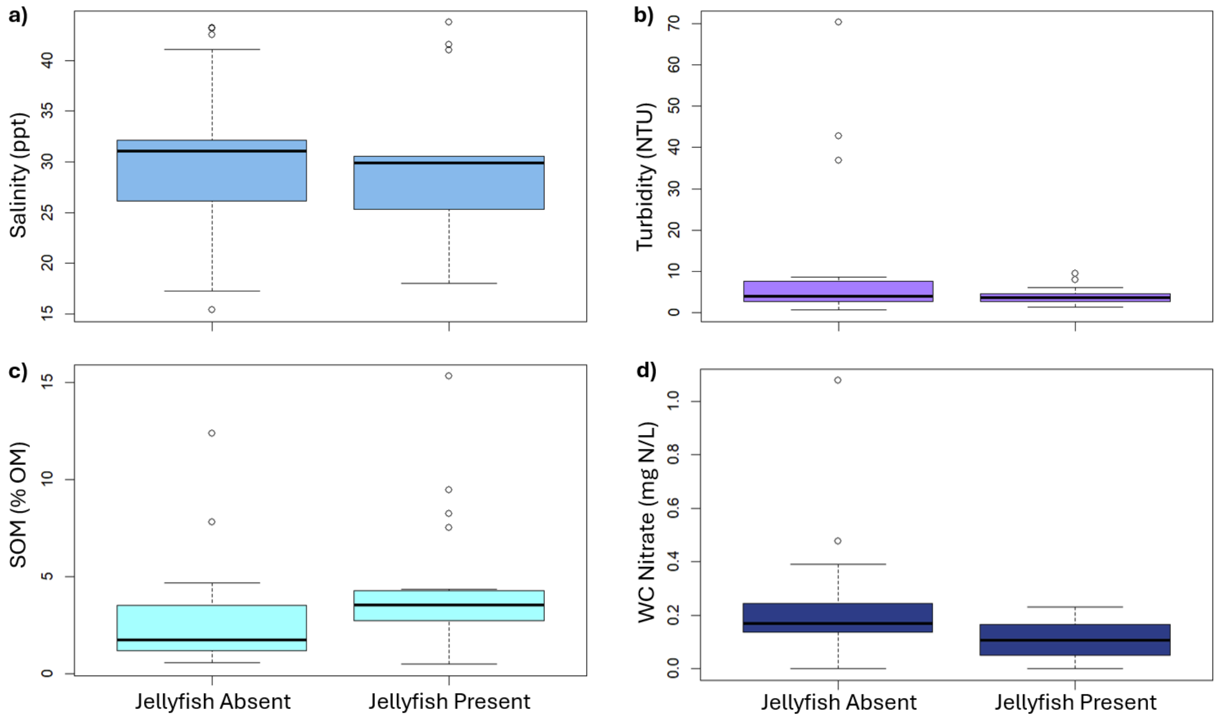

Figure 3: Box plots depicting significant factors from the logistic regression at sites with and without jellyfish. (a) Salinity (ppt). (b) Turbidity (NTU). (c) Sediment organic matter or SOM (%). (d) Water Column Nitrate (mg N/L water)

Quadrat Density

Only one model had substantial support for explaining quadrat density of *Cassiopea* spp. (Table 5), while the next best model had Δi = 7.62. Both turbidity and water column nitrate levels were significant predictors of *Cassiopea* density (Figure 5). However, this model selection only included data from Moli‘i Pond and He‘eia (Figure 5). The James Campbell sites with *Cassiopea* spp. were excluded due to quadrat counts of zero, but it should be noted that the Jellyfish Pond had two subsites with extremely high tow densities of 250 and 1400 medusae/m^3.

Table 5: Summary of the only GLM with substantial support (Δi = < 2) for determining quadrat density. The deviance-based R^2 is 0.81 and the RMSE is 0.42.

	Estimate	Standard Error	t-value	P-value
Turbidity	–0.56	0.10	–5.6	0.00017
WC Nitrate	–7.6	2.0	–3.8	0.0030
Intercept	2.4	0.37	6.4	< 0.0001

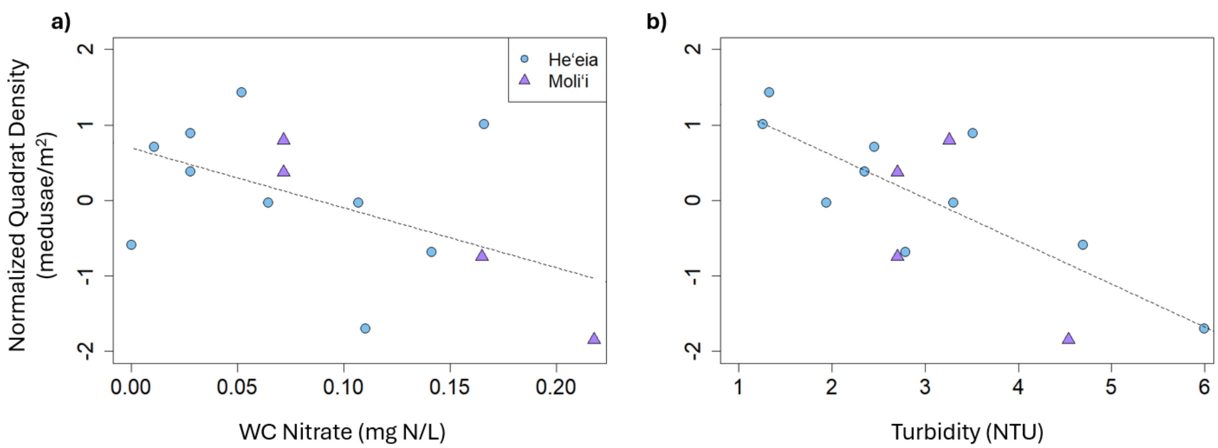

Figure 4: Linear regressions of normalized (arcsin) quadrat density of *Cassiopea* spp. plotted against the two predictor variables from the GLM with substantial support: (a) Water column nitrate (mg N/L water) and (b) Turbidity (NTU). Dotted lines represent the regression equations for each factor.

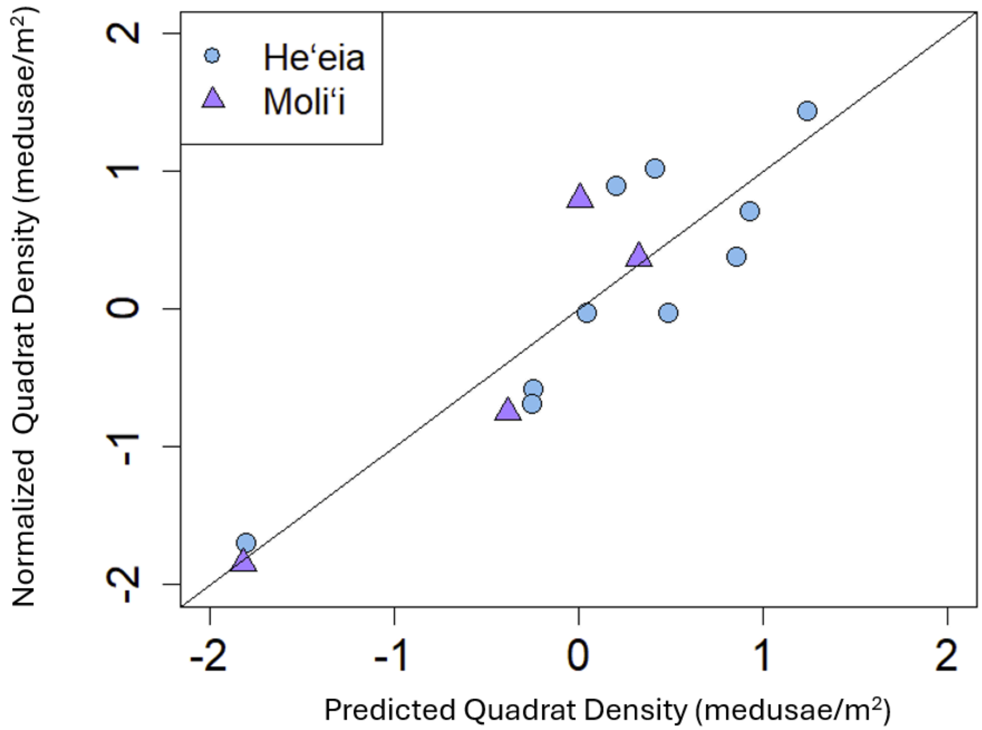

Figure 5: Scatterplot of predicted quadrat density against observed quadrat density of *Cassiopea* spp., both normalized with an arcsin transformation. Predictions come from the model in Table 5 that includes turbidity and water column nitrate as predictors, and the solid line is a 1:1 line. The deviance-based R^2 is 0.81 and the RMSE is 0.42.

Bell Diameter

In comparison to the other sites where *Cassiopea* spp. were present, the mean bell diameter at the James Campbell subsites was very small (< 4 cm), while the medusae at He‘eia (9 ± 4 cm; mean ± SD) and Moli‘i were larger (14 ± 7 cm). There was only one model with substantial support (Table 6), while the next closest model had a Δi of 2.22. All seven models within a Δi of 4 had pH as a positive predictor (Figure 6), but none of the other predictors variables other than pH were used more than once.

Table 6: Summary of the only GLM with substantial support ($\Delta i < 2$) for determining bell diameter. The AICc is 48.4 and the deviance-based R^2 is 0.47 and the RMSE is 0.71.

	Estimate	Standard Error	Z-score	P-value
pH	+2.5	0.66	+3.8	0.001
Intercept	–20	5.2	–3.8	0.001

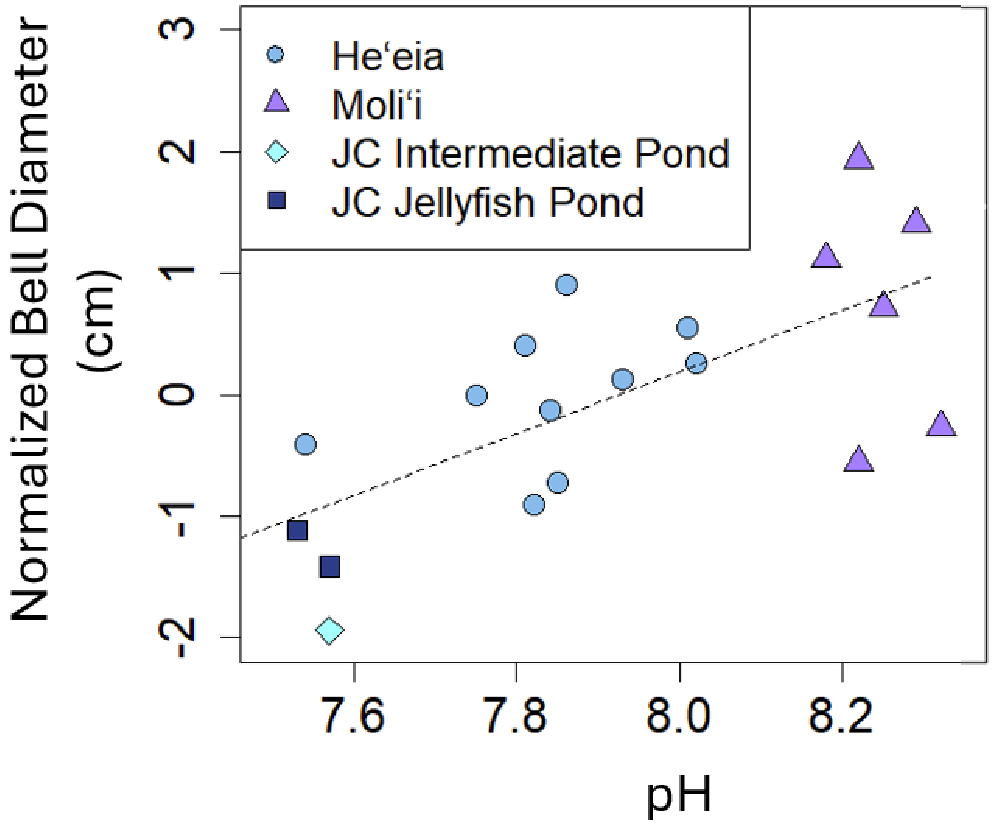

Figure 6: Scatterplot of pH against the ORQ-transformed bell diameter. The dotted line represents the regression equation from the model in Table 6. To see the 95% confidence interval, see Appendix Figure 2.

Discussion

Turbidity and nitrate were the best predictors of whether or not *Cassiopea* spp. was found at a site, but also how large of a population will be found there. Low turbidity is necessary for enough sunlight to pass through the water to the zooxanthellae located in the oral arms of *Cassiopea* spp. medusae (Enrique-Navarro et al. 2022). Without sufficient light levels, zooxanthellae cannot photosynthesize. Products of photosynthesis provide most, if not all of the nutrition and organic carbon that *Cassiopea* spp. need, and several studies have shown how heterotrophy alone is insufficient to sustain the medusae, so disruption to light levels has a huge negative impact on their fitness (Cates 1975; Röthig et al. 2021; Verde & McCloskey 1998).

The negative relationship with nitrate was contrary to my hypothesis. In laboratory environments, *Cassiopea* spp. bell pumping has been shown to increase the mixing of porewater with the water column and the nutrient concentrations in the water at sites where they are present (Jantzen et al. 2010). The fact that the increase in porewater nutrients was not observed in the wild in this study may point to additional factors, such as dilution of nutrients at the ecosystem scale or even mixing in these shallower loko iʻa ecosystems. A negative relationship between nitrate in the water column and the presence of *Cassiopea* spp. at high densities could suggest potential uptake of nutrients at high enough rates to deplete them in the water column, but this pattern would be more strongly supported by historically higher nitrate data that plummets following blooms of jellyfish. To my knowledge, stewards of Heʻeia and Moliʻi have not observed such a pattern.

A negative relationship may also reflect nitrate levels at these sites that are high enough to be harmful. Other marine holobionts that are similar to *Cassiopea* spp., such as stony corals or sea anemones and endosymbiotic *Symbiodinium*, are documented to be sensitive to eutrophication, especially enrichment by nitrate (Röthig et al. 2021). Nitrogen is usually limited in the environments of these species, and introducing an excess amount can alter the N:P ratio within the holobiont, causing dysbiosis, a sudden and disruptive change in the microbial community, or bleaching (Röthig et al. 2021). The *Cassiopea* spp. holobiont in particular has been noted as resistant to nitrate enrichment due to their ability to survive the loss of their symbionts (dysbiosis) and reacquire them easily, as well as their ability to strictly control how much dissolved inorganic nitrogen is given to symbionts (Röthig et al. 2021). Higher levels of nitrate could also possibly reduce the diversity of bacteria in the *Cassiopea* spp. microbiome through dysbiosis, which has been found to be key in managing nitrate in the host as well as the symbionts (Röthig et al. 2021). It is possible that high nitrate levels could disrupt the ability of *Cassiopea* juveniles to acquire symbionts, as well, which would prevent them from successfully completing their life cycle (Hofmann 1996). However, none of the nitrate concentrations at any of the sites exceeded the minimum threshold to pose as a stressor to a variety of aquatic invertebrates and fish, suggesting that they would not have exceeded the harmful threshold for *Cassiopea* spp. either (Banerjee et al. 2023). Another possibility is that nitrate responds to variation in land use, hydrology, habitat, or some other process the more directly influences the presence and density of *Cassiopea* spp.

While the positive relationship between presence and salinity supported my hypothesis, this nuanced relationship relied on controlling for other factors (Figure 3a; Table 4). At sites where jellyfish were observed, salinity ranged from 18 to 44 ppt, which exceeds the lower limit that the jellyfish prefer, 30 ppt, but does not go past their preferred upper limit of 50 ppt; as such, increased salinity would decrease the stress on the medusae (Aljbour & Agusti 2025). These jellyfish appear to be surviving without bodily deformation even at salinities under 20 ppt, so their tolerable range could be greater than originally thought, or some other site factors may be mitigating the negative impact of low salinity on the jellyfish.

The observed positive relationship between SOM and jellyfish presence aligns with my hypothesis, based on the fact that *Cassiopea* spp. utilize organic matter in sediment for nutrition by actively filter feeding using a bell-pumping mechanism (Jantzen et al. 2010). Jellyfish, including *Cassiopea* spp., have been recorded to increase in population size after anthropogenic activity, such as dredging, increases available organic matter, but this effect may be conflated with other impacts of such activity, such as creating additional habitat (Arai 2001). Because *Cassiopea* spp. pump nutrients out of the sediment and utilize them, it is possible that SOM depletes over time in especially dense invasions of jellyfish, but SOM data would need to be recorded regularly over the course of an invasion to ascertain whether or not this is true (Jantzen et al. 2010).

The only significant factor predicting bell diameter was water column pH. This relationship was positive, as expected, because *Cassiopea* spp. appear to be more sensitive to lower pH than higher pH (Thayer et al. 2022; Weeks et al. 2019). Although we did not record a pH below 7.5 at any of our sites, a pH beneath which *Cassiopea* spp. and their zooxanthellae may begin to experience stress, a higher pH may still be preferred (Thayer et al. 2022). Low pH can cause bell deformation and disrupted swimming ability, but as they are resistant to the end-of-century estimate for ocean acidification (pH =7.6; Thayer et al. 2021), *Cassiopea* spp. will likely survive these effects, though they may begin to suffer decreases in bell diameter as the stress of acidification increases.

While *R. mangle* are important in the development of *Cassiopea* spp., their presence was not considered in any of our models (Fleck & Fitt 1999; Hofmann et al. 1996). This is in part due to the fact that an assessment of the current area cover of mangrove at these sites does not reflect their history, which is thought to be more influential than the current presence of mangrove.

Several of our sites—Moli‘i Pond, Ka‘elepulu, the Golf Pond at Mid-Pacific Country Club, the Ala Wai Canal, and He‘eia Fishpond—had *R. mangle* at the time of sampling, though Paepae o He‘eia has since finished its removal of all mangrove bordering the loko i‘a. However, despite removal or no current presence, the impact of mangrove, particularly on the sediment, can outlast the presence of the trees themselves (Allen 1998). As such, an assessment of current mangrove presence is not accurate in reflecting this. Another reason it was not included in the analysis was that it does not appear to be a prerequisite for *Cassiopea* spp. populations to colonize areas. James Campbell National Wildlife Reserve, which contained three of our sites, has no *R. mangle* within miles of the old aquaculture ponds, where *Cassiopea* spp. are currently located. Despite this, our highest net tow densities were observed there, indicating a large population of smaller jellyfish. This pond also had one of the smallest average bell diameters, many jellyfish being less than 1 cm in diameter, which possibly indicates young medusae and successful reproduction. Records and observations at these sites are not clear about whether or not *R. mangle* used to grow near the ponds, but if *Cassiopea* spp. are flexible enough to utilize other aquatic plants for reproduction, then their potential invasibility increases considerably, as removal of *R. mangle* may not be enough to disrupt reproduction. Future study may illuminate which plants are capable of supporting *Cassiopea* spp. strobilation, which is important for understanding and preventing further invasion.

Managing invasions of *Cassiopea* spp. is crucial for maintaining native ecosystems and protecting human health. Not only do stings directly harm people, the jellyfish disrupt aquaculture operations, especially the culturally significant loko i‘a of Hawai‘i (de Araújo et al. 2020; Keli‘i Kotubetey, personal communication). Current methods of management, including hand removal and tarping, are time- and labor-intensive, so finding ways to passively eliminate

Cassiopea populations through habitat alteration is crucial to management (Anderson et al. 2005; Keliʻi Kotubetey, personal communication). From our models, several factors have been identified that may contribute to our understanding of *Cassiopea* spp. invasion, and should be a focus of further study and trials in order to determine what may be effective in removing them. Salinity can be lowered by freshwater addition, but particularly in Hawaiʻi, the amount of water that can be added may be strongly limited (Keliʻi Kotubetey, personal communication). Nitrate can be added to bodies of water, but as *Cassiopea* spp. are considered to have a high tolerance to eutrophication, including nitrate enrichment, this approach may harm other fish or invertebrates before *Cassiopea* spp. are affected at all (Röthig et al. 2021). Turbidity is similar, being relatively easy to increase in a body of water and also often resulting from eutrophication and human activity, but carrying negative impacts for a variety of species, not just *Cassiopea* spp. (Higham et al. 2015; Ortega et al. 2020). SOM is a difficult factor to alter, but may be slowly altered by the removal of *R. mangle* (Allen 1998). Changes to pH in large bodies of water may be difficult to manage, as technology or methods that work in aquaria may not scale up, and also carry negative consequences for non-*Cassiopea* species (Mariu et al. 2023; Thayer et al. 2022). The relationships with environmental factors could be manipulated in microcosms or mesocosms to examine these mechanisms and whether directly altering these factors has any promise for stewardship actions aimed at fighting *Cassiopea* invasions. While this study focused on a variety of water quality and physical factors, additional factors may impact *Cassiopea* spp. more strongly. Though there is no single solution for eliminating *Cassiopea* spp., understanding the conditions which influence habitat suitability is important for preventing future invasions, and a combination of approaches will be key for controlling them.

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Appendix

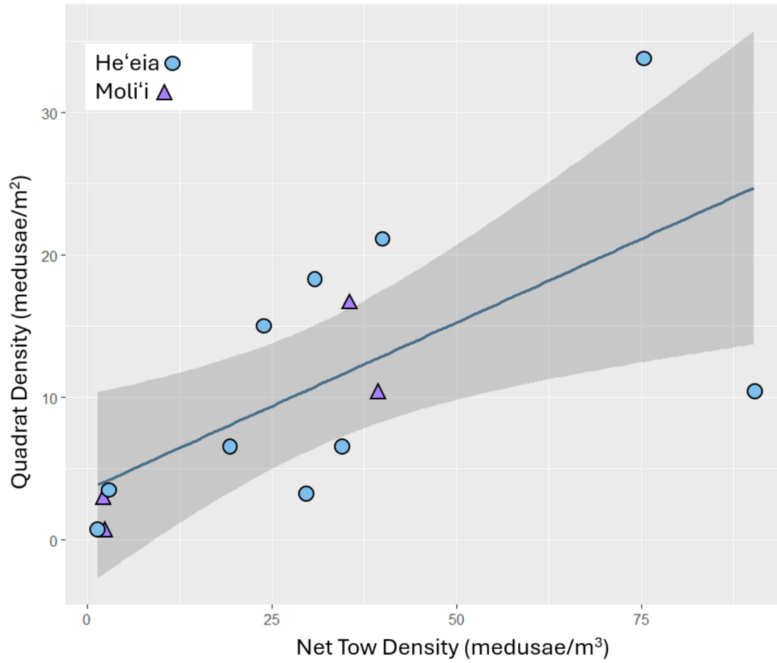

Figure 1: Linear regression of net tow density against quadrat density of *Cassiopea* spp. The error envelope shows the 95% confidence interval. The regression line is $y = 0.23x + 3.5$.

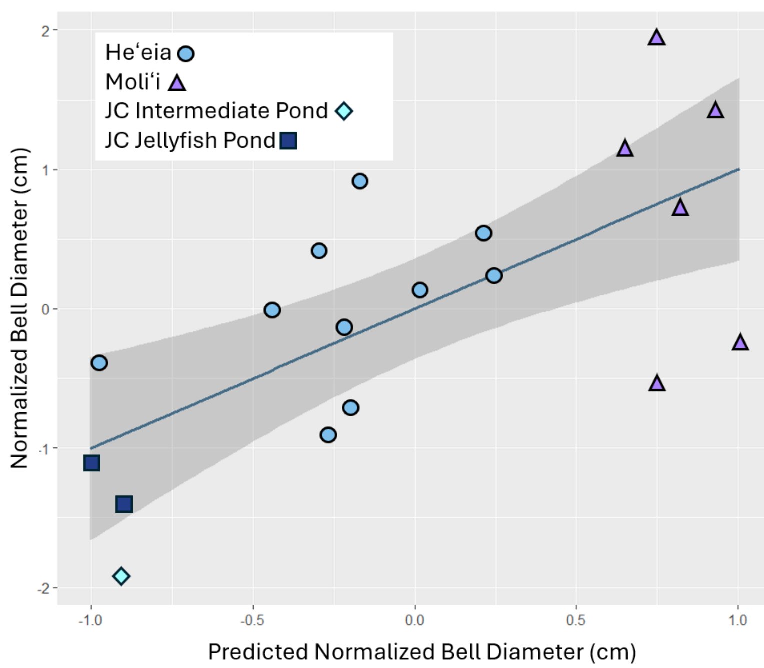

Figure 2: Linear regression of pH against the ORQ-transformed bell diameter. The error envelope shows the 95% confidence interval. The regression line is $y = x$.

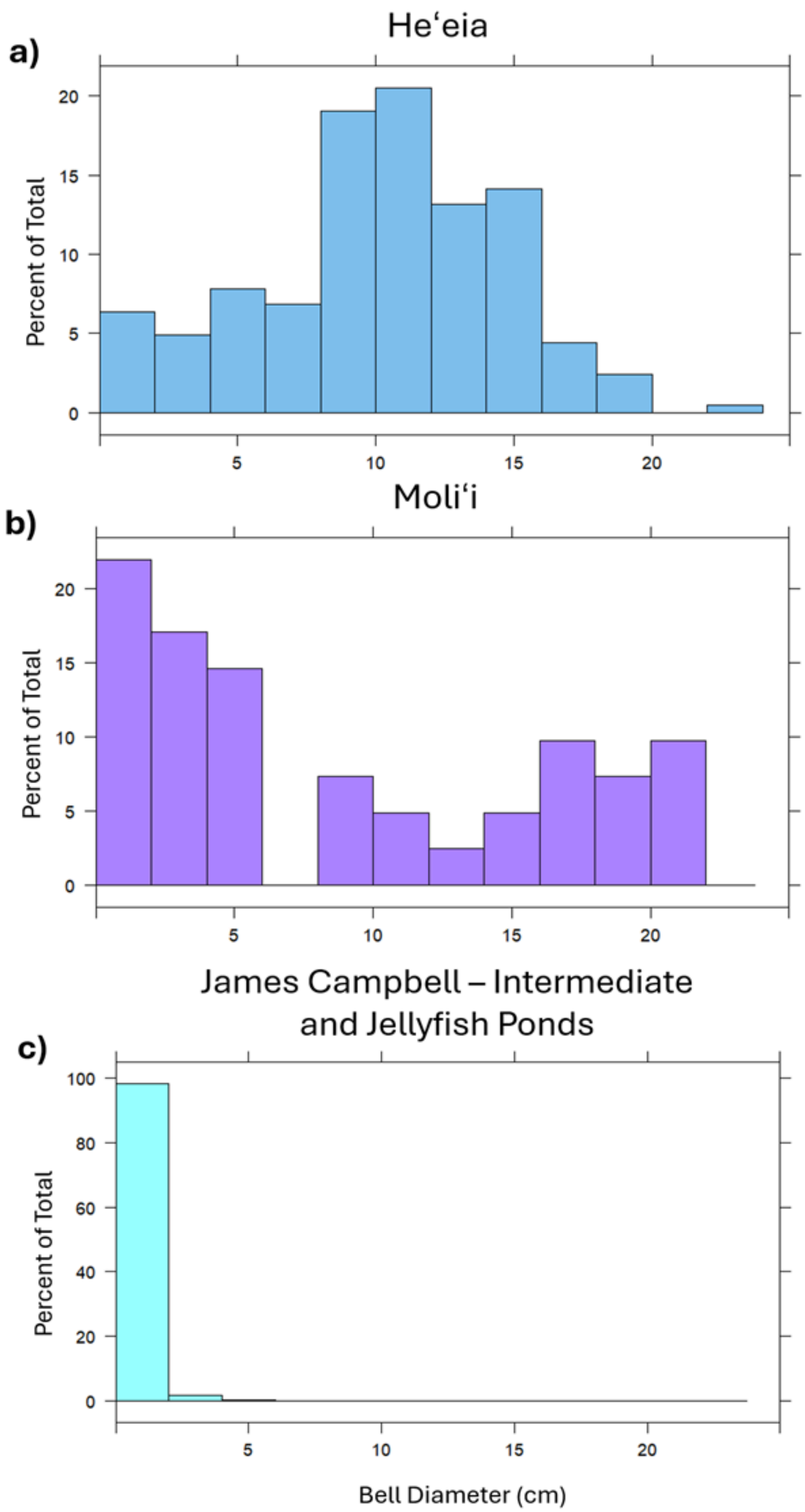

Figure 3: Histogram showing relative frequency of bell diameter at three sites. a) He‘eia (b) Moli‘i Pond and (c) James Campbell (combined Intermediate and Jellyfish Ponds).