AIA KE KUMU WAIWAI MA MAKALAWENA: ANALYSIS OF THE NATURAL RESOURCES WITHIN THE MAKALAWENA LOKO WAIʻŌPAE COMPLEX.

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ABSTRACT

Loko wai‘ōpae (anchialine pools) habitats are tidally influenced, land-locked aquatic habitats with subterranean connections to the sea that are home to unique assemblages of organisms, such as the endemic shrimp ‘ōpae‘ula (*Halocaridina rubra)*. However, the stability of anchialine environments are threatened by sea level rise, pollution due to groundwater contamination and runoff, as well as disturbance by invasive species. In the present study, I worked with community partners to characterize the Makalawena loko wai‘ōpae complex and investigate extrinsic drivers of ‘ōpae‘ula abundance. The Kona landscape is marked by ‘a‘ā and pāhoehoe lava flows, which has facilitated distinct organic and inorganic succession. I hypothesized that lava flows in Makalawena act as hydrological barriers to isolate loko wai‘ōpae from one another. I assigned ponds to regions based on surface lava flow topology: northern ponds dominated by ‘a‘ā-lava rock (North), central ponds amongst pāhoehoe-lava rock (Mid), and ponds surrounded by sandy, mixed-rock composition in the south (South). All but two ponds in the Mid region have ‘ōpae‘ula predators. Our data indicate that the Makalawena loko wai‘ōpae complex partitions into two types of water chemistry. North loko wai‘ōpae are characterized by higher salinity and lower nutrients, whereas Mid and South loko wai‘ōpae have lower salinity and higher nutrients. As high levels of Nitrogen and Phosphorus can be harmful to aquatic life, and ‘ōpae‘ula aren’t able to reproduce in high salinity environments, I hypothesized that Nitrogen, Phosphorus, and Salinity would have a negative correlation with ‘ōpae‘ula abundance. In the Mid and South regions, ‘ōpae‘ula abundance positively correlated with mean phosphate and total dissolved phosphorus concentrations, respectively. Mean nitrate/nitrite and total dissolved nitrogen concentrations negatively correlated with ‘ōpae‘ula abundance in the North and South regions, whereas, they were positively correlated in the Mid region. Salinity was not significant, however, silicate concentrations were positively correlated with abundance in the North and Mid regions. Since the Mid correlation plot showed no negative correlations, and those are the only two ponds without predators, we may be able to say that the negative correlations in the other pond regions are due to predator presence, which would have implications for ‘ōpae‘ula management across the complex. Decreases in ‘ōpae‘ula abundances are of critical concern to lineal descendants, customary practitioners and loko wai‘ōpae caretakers. Informed by practitioner knowledge, we investigated the relationship of ‘ōpae‘ula behavior/abundance and natural cycles (tides, moon phase, diel). ‘Ōpae‘ula are preyed upon by invasive fish species that are more active during the day, and as mentioned earlier, ‘ōpae‘ula are not able to reproduce in high salinity environments, yet oral reports have stated that they emerge to mate in the pools during certain moon phases. Therefore, I hypothesized ‘ōpae‘ula abundance would be highest at night, during low tides, and during full moons. Our data indicate that ‘ōpae‘ula prevalence is highest at night but are not affected by tidal phase or moon phases. Understanding the relationship of these factors to ‘ōpae‘ula abundance will be critical as Hawai‘i’s coastal zones experience increasing salt water intrusion and nutrient influences. Moreover, as these shrimp are highly valued as superior palu (chum/bait) to catch ‘ōpelu (mackerel scad, *Decapterus macarellus*), understanding ‘ōpae‘ula abundance is intimately linked to perpetuation of Native Hawaiian fishing practices along the North Kona coast. Our observations will inform development of ‘ōpae‘ula monitoring and harvesting approaches for ‘ōpelu fishing.

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MLW	Makalawena loko wai‘ōpae
ppt	parts per thousand
OAI	‘ōpae‘ula abundance index
CPUE	Catch per unit effort
SLR	sea level rise
NELHA	Natural Energy Laboratory of Hawai‘i Authority
HH	Highest high tide
HL	Higher low tide
LH	Lower high tide
LL	Lowest low tide

1. INTRODUCTION

1.1 Anchialine ecosystems: life at the extremes

Anchialine ecosystems are tidally influenced land-locked saline or brackish water bodies, found along coastal zones with subterranean connections to the sea. Formation of anchialine environments require a containment structure that forms either by dissolution of karst or creation of lava tubes through volcanism, which both allow persistence of the mixed salinity bodies of water (Mylroie & Mylroie, 2011). Thus, anchialine pools are limited to areas where limestone or fresh lava occur adjacent to the sea, across the Caribbean, Indo-Pacific, and Hawaiʻi. While these brackish aquatic ecosystems lack surface connections to the ocean, anchialine pool depth is dependent upon the water table or freshwater lens. Rhythmic tidal influences are assumed to result in large and dynamic fluctuations in physical and geochemical characteristics (Craft et al., 2008) that are idiosyncratic to specific anchialine pools.

Consequently, anchialine flora and fauna who inhabit these ponds exhibit high tolerance to environmental extremes (Iliffe & Kornicker, 2009) with high rates of endemism (Holthuis, 1973). Food webs in these rare habitats are primarily driven by detritus with algae and microbial mats providing nutrition for higher trophic levels. Anchialine fauna often display highly discrete biogeographic distributions. Current hypotheses suggest that faunal succession in anchialine environments derive through vicariance as a result of geological isolation or dispersal processes (Iliffe & Kornicker, 2009).

Over half of the world's anchialine pool habitats are located in the Hawaiian Islands - more than 600 pools on Hawaiʻi Island, Maui, Oʻahu (Brock, 1985; Chai, 1993). Anchialine pools are naturally occurring with a few notable exceptions: in 1965, a man-made anchialine habitat was formed as a result of testing military explosives on the island of Kahoʻolawe (Brock & Bailey-Brock, 1998) and Lake Kauhakō on Molokaʻi was once classified as an anchialine pool (Maciolek, 1982) , but has since lost its subterranean connection to the sea and is now considered a meromictic lake (Donachie et al., 2004). Although there are no surface connections to the sea, the salinity of Hawaiʻi's anchialine pools can range from about 2 ppt to 34 ppt (Bailey-Brock & Brock, 1993). While these naturally occurring ecosystems support several resources pertinent to

Hawaiian Island communities, including access to fresh groundwater, few have been systematically surveyed.

1.2 Aia i hea ka ʻai a Kona? ʻĀina momona in extreme environments

1.2.1 Kekaha Wai ʻOle: Hydrology and Geology of Kona, Hawaiʻi

While the loʻi (flooded agroecosystems) are often the iconic depiction of Native Hawaiian agriculture (Lincoln et al., 2018), our kūpuna (ancestors) demonstrated an astounding capacity to adapt to the diversity of ecotones and habitats in the natural environment, developing social-ecological land management systems to promote ʻāina momona (abundant lands) (Malo, 1903a). Koʻolau (windward) regions of the Hawaiian islands were based on stream-fed agriculture and kalo (taro, *Colacasia esculenta)* while kona (leeward) regions were reliant upon rain-fed agricultural systems (sweet potato, breadfruit) (Handy et al., 1972). ʻĀina maloʻo (drylands) of the kona regions held equivalent significance to subsistence of the Hawaiian people and were also ʻāina momona.

These agricultural innovations supported robust population growth through the 16th - 18th century (Kirch, 1997) that motivated the development of novel governance and resource management practices including the apportionment of six moku-o-loko (district) by the aliʻi Umi-a-Liloa on Hawaiʻi Island: Puna, Hilo, Hāmākua, Kohala, Kona and Kaʻū (Malo, 1903b). The Kona moku extends from the shore across the entire volcanic mountain of Hualālai, and continues to the summit of Maunaloa (Fornander, 1878). Rift zones are typical of shield volcanoes, occurring as a linear zone of fissures and low permeability rocks along the flanks of the volcano, from where lava erupts (Walker, 1999). Studies show freshwater in the Kona area is derived mainly from several meteoric sources including rain, snow and intercepted cloud vapor that precipitates (Fackrell et al., 2020; Okuhata et al., 2022; Tachera et al., 2021; Tillman et al., 2014). These elements of precipitation either evaporate, are transpired by vegetation, run off, or recharge the ground-water systems (Oki, 1999). While the northernmost portion of North Kona was called "Kekaha-wai-ʻole o nā Kona" (Waterless Kekaha of the Kona districts), historical studies show though the Kona district is limited in surface water abundance, it is rich in groundwater (Maly, 1998) and rich in its fisheries resources (Maly & Maly, 2004), such as loko waiʻōpae.

1.2.2 Mea Ku'ono'ono: Hawaiian Anchialine Ponds

In this study, I employ the phrase loko wai'ōpae (loko wai - freshwater pond; 'ōpae - shrimp) to describe anchialine ponds inhabited by 'ōpae'ula (6 species of red-coloured shrimp, *Halocaridina rubra, Metabetaeus lohena, Antecaridina lauensis, Periclimenes pholeter*, *Procaris hawaiana,* and *Calliasmata pholidota).* Anchialine ponds are also known as kaheka (Maly & Maly, 2004). Historically, Hawaiians considered the people of an ahupua'a (land division) that held loko wai'ōpae as ku'ono'ono (wealthy/well-off), especially in the arid region of Kona. They were a significant source of pride, were highly valued, and well maintained. They were especially prized as habitat for providing 'ōpae'ula. Early studies identified *H. rubra* as the most common endemic Atyidae, a family of shrimp commonly found in freshwater, species in the lava pools, with reports of *H. rubra* being extremely abundant around Hawai'i (Holthuis, 1973), and thus most commonly associated with the Hawaiian name 'ōpae'ula. Here, we use the term 'ōpae'ula to refer to all six aforementioned species. These hypogeal shrimp utilize both the subterranean rock interstices connected to the water table as well as the surface pond habitats. 'Ōpae'ula are about 1 - 2 cm in maximum length and have been characterized as omnivorous, typically feeding on algae, plankton, bacteria, and diatoms. These shrimp have been documented to reach 20 years and are most commonly red in color, hence the meaning "red shrimp", but can also range in color from clear to pink to red (Holthuis, 1973). The relatively young age of the Kona lava flows, a majority of which are less than 3,000 years old (Moore et al., 1987), together with the arid climate of the Kona coast, provide an opportunity to investigate the influence of microbial ecological succession on the maintenance of 'ōpae'ula communities (Sterling et al., 2022).

1.2.3 'Ōpae'ula: Keystone species in the North Kona 'ōpelu fishery

'Ōpae'ula and loko wai'ōpae have an important social-ecological role in the food systems of the Kona community (Titcomb, 1972), such as the North Kona 'ōpelu fishery, have been difficult to perpetuate. 'Ōpelu (*Decapterus macarellus*) have always been an important food fish and baitfish to Native Hawaiians (Titcomb, 1972) whose significance is further affirmed by its mention in the Kumulipo, the Hawaiian origin chant. Though 'ōpelu are found on the ko'olau and kona sides of the Hawaiian Islands, the calm deep waters off of West Hawai'i support both a daytime net fishery and a nocturnal hook and line fishery (McNaughton, 2008), which amounts to the second largest nearshore fishery in the state. Hoop-netting is the customary technique for catching ʻōpelu and involves attracting and concentrating a school of ʻōpelu over a net using palu (chum). Netting grounds are located at koʻa (customary fish aggregation areas). Traditionally, ʻōpaeʻula were considered to be superior palu used to catch ʻōpelu. Even during months when ʻōpelu fishing was kapu (forbidden, set aside), January through July, ʻōpaeʻula were gathered and used together with lepo (bottom sediment) to sustain the koʻa and train the ʻōpelu to aggregate in these areas. The ʻōpelu fishery in turn supported healthy aku (skipjack tuna) and ahi (big eye and yellowfin tuna) populations. Over time, hoop-nets have been replaced by easier, more-efficient, yet environmentally costly methods of fishing and subsequently, the sustainable practice of using ʻōpaeʻula as bait fell out of practice. As the frequency of this practice has decreased, loko waiʻōpae have become neglected ecosystems which are no longer actively managed for ʻōpaeʻula abundance. Thus, characterizing loko waiʻōpae and ʻōpaeʻula will provide information about the biogeochemical and ecological characteristics of anchialine ponds, and how to manage them for the future.

1.3 Increasing threats to anchialine environments

Anchialine ecosystems are idiosyncratic and thus face equally distinctive challenges. In many parts of the world, tourism development, limestone quarries, and groundwater pollution are either destroying or contaminating habitats, resulting in large-scale habitat loss. Disposal of sewage and other wastewater into cesspools or by pumping down boreholes contaminates groundwater with nitrates, detergents, toxic metals and pharmaceuticals, which depletes dissolved oxygen (Iliffe & Kornicker, 2009). In addition to these changes in water quality, loko waiʻōpae are subject to predation by invasive species. Moreover, the severity of each of these stressors could be exacerbated by sea level rise.

1.3.1 Sea level rise impacts

Coastal habitats are directly tied to sea level position and shoreline migration, responding vertically and horizontally to shifts in sea level (Mylroie & Mylroie, 2011). Studies in Hawaiʻi's urbanized low-lying coastal areas show a significant increase in sea-level rise causing flooding, and groundwater and cesspool inundation (Habel et al., 2017; McKenzie et al., 2021).

Sea level change also result in direct and immediate change in the position of the freshwater lens which supply loko waiʻōpae. Thus, anchialine fauna that depend on groundwater are vulnerable to habitat changes caused by 21st century sea-level rise (SLR) (Marrack, 2016). It is anticipated that SLR will impact biogeography and evolutionary processes within anchialine habitats, altering opportunities for gene flow forcing bottleneck events (van Hengstum et al., 2019). It is predicted that up to 80% of current anchialine pools will be lost by 2080 along 40 km of coastlines (Marrack et al., 2021). Paradoxically, due to climate change and an increase in groundwater flooding forecasts total pool counts will rise from 509 in 2018 to 1,000 by 2080. Similarly, SLR is quickly becoming a major concern for coastal ecosystems. Flooding is projected to cause surface connections to the sea and may destroy up to 80% of current loko waiʻōpae (Marrack, et al., 2021). Though inundation will create new loko waiʻōpae inland, it will also facilitate increased dispersal of non-native fishes into these new pools.

These shocks and stressors pose formidable challenges for management, communities, and native flora and fauna dependent on these habitats. Anchialine habitats risk additional SLR-related degradation from invasion of non-native species, groundwater pollution, and increased contact with human infrastructure (Marrack et al., 2021). Importantly, anchialine environments have not been included in climate change risk assessments, therefore there is a limited understanding of how SLR will impact these ecosystems globally.

1.3.2 Groundwater and runoff contamination from development

Shoreline development is a major threat to loko waiʻōpae. Along the Kona coast, many loko waiʻōpae have been filled in as part of coastal development projects related to expanding tourism infrastructure and residential areas. Lava tubes that can constrain loko waiʻōpae are high-efficiency flow paths within the basalt aquifer. In some cases, runoff from nearby development has led to groundwater contamination from pesticides, fertilizers and sedimentation (Knee et al., 2008). The mechanism by which anthropogenic changes in nutrient inventories mediate changes in epithilon composition and biomass and trophic level interactions have been observed but are poorly understood (Sakihara et al., 2015). In addition, sedimentation can cause serious degradation by reducing circulation, increasing eutrophication, and reducing the physical space available for the native biota in the pools (Marrack, 2015).

1.3.3 Predators and invasive species

Loko wai‘ōpae serve as protected habitats for herbivore grazers such as ‘ōpae‘ula (Brock, 1992) and other native biota. Moreover, the geographic distribution of this class of fauna was not predator-driven. Instead, it is hypothesized that these fauna originate due to dispersal processes influenced by tidal forcing and interactions with ocean currents that transport ‘ōpae‘ula larvae between islands and underground fissures between connected anchialine ponds.

Human introductions of non-native species such as tilapia (*Oreochromis sp./Sarotherodon sp.*), mosquitofish (*Gambusia affinis*), guppies (*Poecilia reticulata)*, and the omnivorous Tahitian prawn (*Macrobrachium lar*) prey on ‘ōpae‘ula and have severely impacted their populations in Hawaiian anchialine systems (Brock et al., 1987; Carey et al., 2011). Previous studies assessing the impact of predator pressure on ‘ōpae‘ula abundance on Hawai‘i Island suggest that *H. rubra* exhibit predator-dependent diel migration from underground to the open areas of loko wai‘ōpae (Carey et al., 2011). Methods to assess predator-prey interactions involving invasive fish and ‘ōpae‘ula have utilized a Catch Per Unit Effort (CPUE) method to determine ‘ōpae‘ula abundance (Capps et al., 2009), which we have also attempted as a method for determining ‘ōpae‘ula abundance in this study.

1.4 Motivation for research

Though western research on loko wai‘ōpae spans back to the 1900’s, ‘ike kupuna (Indigenous knowledge and practices) are documented extensively in oral tradition. The loko wai‘ōpae complex at Makalawena, Kona represents the opportunity to characterize and investigate the nature of anchialine environments and the extrinsic drivers that shape ‘ōpae‘ula abundance. While loko wai‘ōpae managers have monitored habitats within the Makalawena loko wai‘ōpae (MLW) complex, a comprehensive study of Makalawena has not been undertaken. This study endeavors to begin to address management needs relating to restoration and maintenance of these threatened systems.

As a means to ensure the enduring preservation of loko waiʻōpae and the culturally significant and endemic species of ʻōpaeʻula, this project bridges the gaps in existing knowledge by investigating abiotic factors within the Makalawena loko waiʻōpae complex, characterizing the types of distinct habitats and determine which factors most strongly correlate with ʻōpaeʻula abundance. Managers of loko waiʻōpae characterize a pristine system as having low turbidity, cyanobacterial crusts, shallow biogenic sediments, and fauna dominated by ʻōpaeʻula (*H. rubra* and other hypogeal crustaceans) (Kamehameha Schools, personal communication) whose constant grazing prevent epiphytic macrophytes from dominating and overgrowing the pools.

1.5 Hypotheses

1.5.1. Considerations for measuring ʻōpaeʻula abundance

Since ʻōpaeʻula are able to live underground, the abundance measured was not total population abundance, but was instead an observable abundance. The observable abundance is the abundance of visible ʻōpaeʻula in each pond at the time of surveying. Observable abundance can be influenced by many factors, such as, salinity fluctuations, predator presence, and mating. ʻŌpaeʻula are also potentially able to travel between ponds via underground connections. Because of this, population size may be larger or smaller than observable abundance. To minimize the effects of counting migrating individuals, multiple teams of surveyors were deployed at the same time for separate ponds.

1.5.2 Hypothesis 1: Abiotic parameter differences in water column across Makalawena loko waiʻōpae (MLW)

Rationale: The Makalawena loko waiʻōpae (MLW) complex is composed of both ʻaʻā and pāhoehoe lava flows of different ages, which has caused geological and biological disjunction in organic and inorganic succession across regions. Also, the northern region of Makalawena is adjacent to a rift zone, which may affect underground water flow paths. We predict that physicochemical properties will likely vary across MLW.

> **H1.1**: Physicochemical properties vary significantly across Makalawena loko waiʻōpae regions due to vicariance caused by different lava flows.
>
> > **Null**: Physicochemical properties will not vary across MLW regions.

H1.2: The heterogeneity of Makalawena loko wai‘ōpae physicochemical properties will affect ‘ōpae‘ula observable abundances.

Null: ‘Ōpae‘ula observable abundance will not vary significantly across MLW as a function of the physicochemical differences between ponds.

1.5.3 Hypothesis 2: Natural environmental cycles' correlation with ‘ōpae‘ula observable abundances within the MLW complex

Rationale: ‘Ōpae‘ula are unable to reproduce or survive for long periods of time in high salinity environments. I hypothesize that the observable abundance will be higher during lower tides, when freshwater influence is greater (i.e., the ‘ōpae‘ula will retreat underground, where there is more freshwater, when saltwater influence is greater). In addition, previous research has demonstrated that the most common species of ‘ōpae‘ula, *H. rubra*, have an active nocturnal preference with higher observable abundances during the night, having a tidally-linked migration showing patterns of emergence and submergence with incoming and ebbing tides (Brock, 1985; Chai, 1993). Many aquatic organisms may exhibit visible mating and/or spawning behaviors during full moons that require aggregation in the open, therefore, the observable ‘ōpae‘ula abundance will be greater in the ponds during full moons. Many of the ponds in this study have predators that are diurnal, therefore we predict that ‘ōpae‘ula populations to be more active and abundant at night.

H2.1: ‘Ōpae‘ula observable abundance will be negatively correlated with tidal height (e.g., higher ‘ōpae‘ula observable abundance at low tide).

Null: ‘Ōpae‘ula observable abundance will not vary significantly between high and low tides.

H2.2: ‘Ōpae‘ula observable abundance will be positively correlated with full moon phases (e.g., higher ‘ōpae‘ula observable abundance at full moon when compared to new moon).

Null: ‘Ōpae‘ula observable abundance will not vary significantly between new and full moon phases.

H2.3: ‘Ōpae‘ula observable abundance will be higher at night than during the day.

Null: ‘Ōpae‘ula observable abundance will not vary significantly between day and night.

In this study, we assessed physical and chemical parameters of 13 ponds at Makalawena. The assessment of physical and chemical parameters allowed us to identify possible geological barriers created by the most recent lava flow in 1801 and its effects on loko wai‘ōpae and ‘ōpae‘ula abundance. Furthermore, this assessment allowed characterization and clustering of ponds by physical parameters into three unique regions, North, Mid and South. We further investigated the influence of tidal, lunar, and diel cycles on ‘ōpae‘ula abundance to gain insight into understudied behavioral patterns in each region at Makalawena.

2. RESEARCH DESIGN AND METHODS

2.1 Place-based Methodology

2.1.1 Establishing pilina (relationship) and kuleana (accountability) to the Kekaha Kai community

As a Native Hawaiian, I practice science through the lens of my cultural beliefs using western scientific techniques with the intention of applying this perspective to the co-development of knowledge with communities to steward Hawaiʻi's natural resources. Because of a strong personal desire to help restore, protect, and maintain loko waiʻōpae, I began laying the groundwork for my thesis project in 2017, two years before entering the Marine Biology graduate program. Relationship building was necessary in order to ask and receive permission to access to ʻāina kulaiwi (ancestral lands) as well as vital information about the sites and cultural practices of the area and organisms. To ensure the research for this project was done ethically, I reached out to communities to understand their needs, setting intentions, creating partnerships and practicing open communication, as well as using historical knowledge and implementing traditional cultural practices when appropriate.

My community partners in this project include the Kaʻūpūlehu Marine Life Advisory Committee, the Queen Liliʻuokalani Trust, the Natural Energy Laboratory of Hawaiʻi Authority, the State of Hawaiʻi Department of Land and Natural Resources, and the Kamehameha Schools. The long-term goal of our collective work is to support ʻōpelu fishers, resource managers and community members in perpetuating customary ʻōpelu fishing practices which depend on restoring, protecting, and maintaining loko waiʻōpae. By volunteering at community workdays and through my role as a research technician with a previous University of Hawaiʻi project, my intention to work in a respectful and reciprocal relationship were clearly communicated. While working in these communities, I learned of a desire amongst the community to increase ʻōpaeʻula abundance and protect ʻōpaeʻula habitats. Through interactive dialog with cultural practitioners, community members, and resource managers, we co-identified the science needs associated with loko waiʻōpae resilience and adaptation to impacts and changes to the living resources within the loko waiʻōpae and the potential of using these resources to return to traditional ʻōpelu fishing practices. Throughout this project, I have shared my findings with community partners and stakeholders in Kona to ensure a mutually beneficial relationship.

2.1.2 Establishing pilina (relationship) and kuleana (accountability) to Makalawena

A central value that guides science practices in the Alegado lab is that any member of our laboratory conducting research within Indigenous spaces and with Indigenous communities should be prepared to participate in appropriate cultural protocols before, during, and after fieldwork has been performed. Makalawena is a wahi pana (legendary place) with numerous Native Hawaiian cultural sites including iwi kupuna (ancestral remains), therefore researchers take on additional kuleana (responsibility) to conduct themselves in a pono (proper, with integrity) manner. Makalawena is owned by the Kamehameha Schools, a private landowner whose land base was established through inheritance of ancestral lands to Ke Aliʻi Bernice Pauahi Bishop, a high ranking aliʻi (chief). Access to Makalawena was predicated on transparency around the research procedures undertaken and acquiring permission to access the property was given only after I was introduced to the ʻāina by Kamehameha Schools resource managers and a cultural practitioner of lineal descent from Makalawena, Aunty Kuʻulei Keakealani. After establishing a connection to the ʻāina of Makalawena through protocol, I took part in a formal introduction session where I was taught the history and usage of the area, was shown the loko waiʻōpae, and taught about the significance of each loko.

In accepting our kuleana to Makalawena, we determined that each member of the field team also needed to assume obligations to place, as well as over the course of the entire project. Therefore, field team members were required to: 1) carry themselves with the right intention, and 2) be of Indigenous ancestry, and/or have a lineal connection to place. Prior to the beginning of sample collection, cultural protocols for cleansing and protecting the campsite and its occupants were performed. Appropriate cultural protocols were conducted each day to honor kūpuna (elders, both in this realm and the ancestral realm) and akua (natural phenomena) of Makalawena and team members. Team members were invited to participate or connect to the ceremonies by opening their naʻau (mind and heart) and setting intentions internally. Once field work was completed, we broke camp and completed a closing protocol to re-establish noa (state free of obligations) for the field team and the ʻāina of Makalawena. After on-island sample processing was complete, our field team performed a final cultural protocol with hoʻokupu (offerings) at the piko of Hawaiʻi Island, Maunakea.

2.2 ‘Āina kulāiwi: Makalawena, Kona, Hawai‘i

The moku of Kona is divided into three ‘okana (subdivisions): Kekaha Wai‘ole, (Kona) Kai‘ōpua, and Kapalilua. Kekaha Wai‘ole is the ‘okana to the north that encompasses the Makalawena ahupua‘a. Makalawena extends mauka (toward the uplands) up to the land division of Mahai‘ula on the northside, down to the sea at the kuanalu (surfbreak), and toward the land division of Awake‘e to the south (Maly, 1998). Makalawena lies in Kekaha, in the district of Kona ‘Akau (North Kona). Makalawena was historically rich in natural resources and was favored and maintained by families who held residence into the later 19th century to the 20th century (Maly, 1998).

One of the main and abundantly prized natural resources within Makalawena were loko wai‘ōpae. The loko wai‘ōpae at Makalawena are an approximately 310,000 m^2 complex of anchialine pools (Google Earth: Version 9.167.0.0, 2022) located in the district of Kona in the ahupua‘a of Makalawena on Hawai‘i Island (Figure 1). Oral tradition describes at least 70 known loko wai‘ōpae with Makalawena. The 1800-1801 Hu‘ehu‘e lava flow and subsequent coastal changes have left about 50 loko wai and fewer loko wai‘ōpae. The Kīholo aquifer supports all the loko wai‘ōpae across the entire ahupua‘a of Makalawena (Maly, 1998) and are recharged by underground, rain-fed springs and underground tidal flow from the adjacent ocean. With an annual rainfall range of 204 - 750 mm (Frazier et al., 2016; Maly, 1998), there are no streams or other surface water connections to the ponds at Makalawena. Within the Makalawena complex, Kapo‘ikai (‘Ōpae‘ula pond) is designated as the Makalawena Marsh National Natural Landmark and support a resident population of the endangered non-migratory ae‘o (Hawaiian stilt) as well as being the principal nesting site for the ‘alae kea (Hawaiian coot) and the only known breeding area on the Hawai‘i Island for the auku‘u (black-crowned night heron). All fieldwork was conducted with the permission of the private landowner, Kamehameha Schools (Natalie Kurashima, Integrated Resource Manager and Namaka Whitehead, Senior Natural Resource Manager, Kamehameha Schools Natural and Cultural Ecosystems Division).

2.3 Sampling regime

Of Makalawena's 50 plus ponds, we completed sampling and surveying of 13 loko waiʻōpae within the Makalawena complex (Figure 1-3) over four field seasons: July 2020, November 2020, February 2021, and May 2021. Loko waiʻōpae were selected to span the Makalawena complex and had been previously observed to have ʻōpaeʻula (Kamehameha Schools, personal communication). Some pools also have fish that prey on ʻōpaeʻula, such as *Gambusia affinis, Poecilia reticulata,* and other members of the Pociliidae family (herein referred to as predatory fish unless specified). No sampling or surveying was conducted within Kapoʻikai as it is a designated bird sanctuary. The 13 pools were divided into three regions based on location: North, Mid, and South. The four North pools (Square Roots, Lukiki, Moʻo, CVS) are north of ʻŌpaeʻula Pond and are surrounded by an ʻaʻā flow (Figure 2). The four middle (Mid) pools (Pots, Golden Pots, Harry, and Golden Lepo) are south of ʻŌpaeʻula Pond and are surrounded by a pāhoehoe flow. The five South pools (Northwind, 3 Keiki, Mint Waters, Aholehole, Toads) are located between the edge of an ʻaʻā flow and sandy substrate (Figure 2). Sampling and surveying was completed during Hoʻoilo (wet season, November - April) and Kau (dry/hot season, May - October) at both new moon and full moon phases (Table 1).

During each of the four field seasons, sampling and surveys were split into two data collection periods, which I explain in further detail below in the "2.4 Discrete sampling methods" and "2.5 48-hour surveying methods" sections. On the first day of each field season, each team member was trained in kilo (observational) practices specific to this project, which included 1) moon phase, 2) weather, 3) predator estimation and behavior, 4) habitat description, 5) hiding spot estimation, 6) algae or bacterial crust estimation, and 7) general observations about surrounding area. An example of the data sheet used to record kilo is in Appendix I. The kilo data was used to characterize each loko waiʻōpae. Pond measurements of the length, width, and depth were taken at the highest high tide and lowest low tide, once during kau and once during hoʻoilo. ʻŌpaeʻula average length was calculated in each pond during the final field season by measuring 10 randomly chosen ʻōpaeʻula in each pond. To measure the ʻōpaeʻula, a GoPro recorded underwater footage of the surveyors placing a ruler next to different ʻōpaeʻula, then the video footage was reviewed and the lengths from the tip of the rostrum to the end of the uropod were recorded, then averaged for each pond. The 48-hour surveying data collection period began in the afternoon on day 1, through day 2, and into the morning of day 3, then after an 18-hour waiting period, the discrete sampling was conducted in the early morning period on day 4 (Figure 4).

2.4 Discrete sampling methods

At each site (Figure 2), the pond water's physical parameters were measured, such as Temperature (ºC), Dissolved Oxygen (DO %), Salinity (ppt), and pH. The parameters were measured using a multi-parameter sonde (YSI Pro Plus; YSI Incorporated, Yellow Springs, OH). The YSI multi-parameter sonde was calibrated in the field before sampling. Calibration included calibrating according to manufacturer instructions for dissolved oxygen: DO % in water saturated air (1-point calibration), salinity: specific conductance or conductivity (brackish water calibration value of 10,000 µS/cm), and pH: pH (3-point calibration). The sonde was held in place for approximately 3 minutes before reading. During this time, samples were collected for measurement of dissolved macronutrients: Silicate (SiO_4), Phosphate (PO_4^{3-}), Nitrate+Nitrite ($NO_3^- + NO_2^-$), Ammonia+Ammonium ($NH_3 + NH_4^+$), Total Dissolved Phosphorus (TDP), and Total Dissolved Nitrogen (TDN). At each site, 1 L of water was collected from surface waters for macronutrient analyses in acid-washed and autoclaved Nalgene bottles after being triple rinsed with ambient surface water. All samples were stored on ice until further processing for microbes and macronutrients at the lab. Once at the lab, each 1 L water sample was filtered through a 47 mm diameter, 0.45-µm pore size Millipore membrane filter (MF-Millipore, Merck KGaA, Darmstadt, Germany), then filters were stored for further downstream processing of microbes. Filtrate (250 mL) was collected in sterile Nalgene bottles and processed at NELHA (Kona, HI) to measure macronutrients. ʻŌpaeʻula observational abundance and predator presence was also recorded during this time. The methods for measuring observational abundance is in section 2.6. For the purposes of this study, predator presence was recorded as present or not present.

2.5 48-hour surveying methods

According to oral interviews with knowledgeable loko waiʻōpae caretakers, ʻōpaeʻula are often seen mating during full moon periods. For this reason, surveys were conducted during full and new moon periods to see if there would be an increase in observable abundance during the full moon period. Since ʻōpaeʻula can live much of their lives unseen and underground, observable abundance refers to the amount of visible ʻōpaeʻula in the ponds, available to count and/or harvest. The kaulana mahina (Hawaiian moon calendar) consists of 30 moon phases divided into 3 anahulu (10 moons): hoʻonui (waxing), piha (full moons), hōʻemi (waning) with new moon and full moon phases occurring over a 3–4-night period. Since Hawaiʻi has a mixed tide, meaning there are two high tides and two low tides of unequal heights within a 24-hour period, surveys were conducted, over a 48-hr period, at the flood and ebb of each major tidal change (i.e., 8 times within the 48-hr period, Figure 4). To survey each pond as close to tidal extreme as possible, the team was split into smaller groups to cover sites simultaneously. Team members would start surveys in the same order each time, 15 minutes prior to the reported peak tide according to the mobile tide app, Tides (7th Gear, LLC; Version 2.43, 2022). All surveys would be completed within 30-45 minutes of the peak of the tide. Surveys included measuring 1) each pond's physical parameters with a YSI multi-parameter sonde, as described in section 2.4, 2) observational abundance of ʻōpaeʻula, as described in section 2.6, and 3) predator presence (i.e., present or not present).

2.6 Measuring observational abundance of ʻōpaeʻula

Initially (July 2020), we adopted a modified catch per unit effort (CPUE) method as previously described (Capps et al., 2009). Briefly, active ʻōpaeʻula (e.g., animals that were not in cracks or crevices; either resting on or slowly swimming) were counted. Ponds were sampled using a 30 cm wide net for large ponds or a 12 cm wide net for smaller ponds. However, because the ponds did not have large areas with flat substrate and instead had large crevices, data were highly inconsistent. Also, in July 2020, we used a semi-quantitative method by D. Chai (personal communication) to estimate ʻōpaeʻula abundance. To minimize the effects of small observation errors and for efficiency, ʻōpaeʻula abundance was discretely binned into the categories to generate ʻōpaeʻula abundance indexes (OAI) scores: Bin 0 = 0 ʻōpaeʻula, Bin 1 = 1 - 99 ʻōpaeʻula, Bin 2 = 100 - 249 ʻōpaeʻula, Bin 3 = 250 - 399 ʻōpaeʻula, Bin 4 = 400 - 499 ʻōpaeʻula, Bin 5 = 500 - 999 ʻōpaeʻula, and Bin 6 = 1000+ ʻōpaeʻula. Total observational abundance was estimated for each pond at each timepoint. We continued to monitor observational abundance at Golden Lepo, Harry, and Aholehole, however, since the OAI score was always 0, they were removed from the dataset. In subsequent field seasons, each pond, except Golden Lepo, Harry, and Aholehole, was measured at the highest tide for length and width. The size of the pond determined how many quadrats we chose. We counted all ‘ōpae‘ula within a randomly chosen 36 cm x 36 cm quadrat and scaled up the total pond abundance by pond size. If the pond was larger than 1.5 m x 1.5 m, we repeated the quadrat counts for another randomly chosen area. If the pond was larger than 6.0 m x 6.0 m, we repeated the quadrat counts 4 times. In the third and fourth field season (February 2021, May 2021), we divided larger ponds into smaller sections for ‘ōpae‘ula counts with more sections in larger ponds and less in smaller ponds. Each section was equal to approximately the same length and width.

2.7 Statistical analysis for discrete sampling data set

All analyses were performed in R (Version 2022.07.0+548 "Spotted Wakerobin", R, PBC, 2022). For the discrete sampling data set, ten physicochemical parameters were measured in each loko wai‘ōpae; Temperature, DO, Salinity, pH, SiO_4, PO_4^{3-}, $NO_3^- + NO_2^-$, $NH_3 + NH_4^+$, TDP, and TDN. Means and standard deviations for each parameter across all samples per pond were calculated (Table 2). To summarize how the ten physicochemical parameters covary across the ponds, a principal components analysis (PCA) was performed using the R function *princomp* and was visualized using the *ggbiplot* package (Figure 5). To determine which physicochemical parameters varied significantly by site (site = individual ponds; Figure 2), Kruskal Wallis Rank Sum Tests were run for each variable measured using the function *kruskal.test()*. The same test was also performed by region (region = North, Mid, South; Figure 2). For the variables that varied significantly by site and region ($p \leq 0.05$), pairwise differences between sites were tested using the post-hoc Dunn’s Pairwise Test with the Bonferroni p-value adjustment. To explore the correlation between the ten physicochemical parameters and the ‘ōpae‘ula abundance index, we utilized the *corrplot* package in R to create a correlation plot for each region. Each region’s correlation plot includes data from all four field seasons for the ponds that had ‘ōpae‘ula present, where the North plot ponds are CVS, Lukiki, Mo‘o, and Square Roots, the Mid plot ponds are Pots and Golden Pots, and the South ponds are Northwinds, 3 Keiki, Mint Waters, and Toads (Figure 2). Abundance indexes for each site and each method of data collection (e.g., discrete vs. 48-hour) were visualized using bubble plots by corresponding latitude and longitude.

2.8 Statistical analysis for 48-hour surveying data set

For the 48-hour surveying data set, linear mixed models were used, in R, to determine if ‘ōpae‘ula abundance varied significantly with the natural environmental cycles (e.g. tide, moon, time of day) and site using the *lme4* package in R. Region is a fixed explanatory factor for all models because distinctions between regions were observed in the analyses for H1. To explore trends in ‘ōpae‘ula abundance the models defined in (1) - (4) were fitted:

$$\mathrm{OAI} = \beta_0 + \mathrm{Site}_{0s} + \mathrm{Region}\beta_r + \varepsilon \tag{1}$$

$$\mathrm{OAI} = \beta_0 + \mathrm{Site}_{0s} + \mathrm{Region}\beta_r + \mathrm{Tide}\beta_{td} + \varepsilon \tag{2}$$

$$\mathrm{OAI} = \beta_0 + \mathrm{Site}_{0s} + \mathrm{Region}\beta_r + \mathrm{Time}\beta_t + \varepsilon \tag{3}$$

$$\mathrm{OAI} = \beta_0 + \mathrm{Site}_{0s} + \mathrm{Region}\beta_r + \mathrm{Moon}\beta_m + \varepsilon \tag{4}$$

$$\mathrm{OAI} = \beta_0 + \mathrm{Site}_{0s} + \mathrm{Region}\beta_r + \mathrm{Tide}\beta_{td} + \mathrm{Time}\beta_t + \varepsilon \tag{5}$$

Where OAI is the ‘ōpae‘ula abundance index, $\mathrm{Region}\beta_r$ is the magnitude of the linear effect of region (north, mid, and south), $\mathrm{Tide}\beta_{td}$ is the magnitude of the linear effect of the period of the tide (High high, High low, Low high, Low low), $\mathrm{Time}\beta_t$ is the magnitude of the linear effect of the time of sampling (day vs. night), Site_{0s} is the sample site included as a random effect such that $\alpha \sim N(0,\tau^2)$, $Moon\beta_m$ is the magnitude of the linear effect of moon phase, and ε such that $\epsilon \sim N(0,\sigma^2)$. Model (1) is the null model. To determine which model was significant, an ANOVA was performed with models (2) - (5) against model (1). The best fit model was selected based on the Aikake information criterion (AIC) using the function *model.sel* from the *MuMIn* package and visualized using the *ggplot2* package. Since we were curious about how the environmental cycles together may affect ‘ōpae‘ula observational abundances, model (5) was determined to investigate the influence of tide, time, region, and site as a random effect on ‘ōpae‘ula abundance. Model (5) was the best fit model when compared against six (excluding model 1, the null model) other models (see Appendix II) and selected based on the AIC.

3. RESULTS

3.1 A description of the MLW ecosystem, flora, and fauna

3.1.1 North region

The Square Roots loko wai‘ōpae (Figure 3A) is a large (22.05 m wide x 27.40 m long, and 78 cm deep) pond that is greatly influenced by tides, pool size increasing and decreasing dramatically with tides (maybe add figure with image at high and low tide). It is filled with predatory fish such as *Gambusia affinis, Poecilia reticulata,* and other members of the Pociliidae family that prey on ‘ōpae‘ula. However, during extreme lows, Square Roots pond becomes three smaller ponds. ‘Ōpae‘ula abundance in the Square Roots pond has exceeded 5,000 individuals during a night time sampling.

The Lukiki pond (Figure 3A) is 8.00 m x 6.95 m and 18.00 cm deep with an aquatic plant, *Ruppia* sp., in the center. During the low tides, the water level recedes to the edges of the *Ruppia* sp., and allows ‘ōpae‘ula to hide from predators.

The Mo‘o loko wai‘ōpae (Figure 3A) is 9.00 m x 7.10 m, and 58.00 cm deep. It has a mound of sediment in the center with some akulikuli (*Sesuvium portulacastrum*) growth. At high tides, the mound is submerged and the ‘ōpae‘ula use the akulikuli to hide from predators.

The CVS pond (Figure 3A) is large (No length recorded, 2.65 m wide and 1.50 m deep) and deeper than the rest of the North ponds in this study. It houses several reef fishes such as, *Kuhlia sandvicensis, Abudefduf abdominalis, Kyphosus bigibbus* as well as smaller predator fish (e.g., *Gambusia affinis, Poecilia reticulata*). The ‘ōpae‘ula in this pond are on the smaller side (i.e., < 1 cm in length) and rarely seen. The CVS loko wai‘ōpae has the least amount of ‘ōpae‘ula at night compared to the other North ponds.

3.1.2 Mid region

The Pots loko wai‘ōpae (Figure 3B) is a narrow (1.37 m x 1.52 m, and 90.00 cm deep) pond without any predators. Salinity in the pond is low (e.g., 5.88 - 6.61 ppt), and algae growth is low for the amount of ‘ōpae‘ula present and size of the pond. ‘Ōpae‘ula are smaller in this pond than other nearby ponds (i.e., ~ 1 cm in length). At night, the ‘ōpae‘ula population decreases (Table 4).

The Golden Pots (Figure 3B) pond is located in a deep hole in pāhoehoe and is 6.07 m long x 4.36 m wide, and 79.00 cm deep. The pond is rocky and has a very fine sediment bottom. There is a large abundance of ‘ōpae‘ula (commonly over 1,000 ‘ōpae‘ula, Table 4) and no predatory fish.

The Harry loko wai‘ōpae is a large pond (measurements not taken) that has been overtaken by an invasive wetland plant (*Bacopa monnieri*). The entirety of the pond is not visible because of the wetland plant and ‘ōpae‘ula have not been observed during the surveys. After the first field season, this pond was no longer sampled.

The Golden Lepo loko wai‘ōpae (Figure 3B) is a very large pond (measurements not taken, but visually largest in this study) filled with a thick layer of silty sediment. A few areas of the pond are clear of sediment, but no ‘ōpae‘ula were observed here during this study. This pond has only been sampled once because it did not have enough water (< 3 cm deep) to take measurements using a YSI or collect water for nutrient measurements. There are many predator fish, toads, and tadpoles observed in this pond.

3.1.3 South region

The Northwind loko wai‘ōpae (Figure 3C) is a large pond (15.95 m x 5.21 m, and 1.20 m deep) where many predator fish were observed. *Ruppia* sp. and round algae masses are also present in this pond. ‘Ōpae‘ula were consistently observed in this pond and are more abundant at night.

The loko wai‘ōpae, 3 Keiki (Figure 3C), is, at the Higher tide (HH), a wide-reaching pond (23.01 m x 23.62 m, and 3.90 m deep) that separates into 3 or 4 ponds at both lower tides (LH, HL, LL). This pond is an exceedingly rocky pond with predator fish present. The ‘ōpae‘ula in this pond are smaller than the ‘ōpae‘ula in nearby ponds (i.e., ~ 1 cm in length). There is no visible algae growing in these ponds at any time and aside from a small patch of pale yellow sediment (which is only accessible during the higher tide), there is no visible bacterial growth either.

The Mint Waters loko wai‘ōpae (Figure 3C) is 17.83 m long x 4.72 m wide, and 24.38 cm deep. During the day, ‘ōpae‘ula are extremely rare. This pond is dominated by sandy substrate with algae or bacterial mat growth in some areas.

The Aholehole loko wai‘ōpae (Figure 3C) is a large pond (measurements not taken) with cyanobacterial mats covering the rocks in the pool. This pond is named for the large school of āholehole that is found here. During the high tide this pond connects with the Mint Waters pond. Because the two ponds merged so frequently, after the first field expedition, samples were only taken at the designated Mint Waters sampling site.

The Toads loko wai‘ōpae (Figure 3C) is a large and deep pond (18.23 m x 12.74 m, and 2.00 m deep) that contains predator fish. *Ruppia* sp. is found along the sides of the pool and occupies about 75% of the pool. ‘Ōpae‘ula are abundant (ranges from over 500 to 1000+, Table 4) at night and individuals are observed on each other forming piles.

3.2 Physicochemical properties of MLW complex

To visualize the associations between physicochemical properties in the pools, a principal component analysis (PCA) was performed at each site. The horizontal axis, PC1, explains about 57.8% of variability, whereas the vertical axis, PC2 explains about 13.6% of variability (Figure 5). PC1 is positively correlated with Salinity, and negatively correlated with TDP, TDN, pH, DO, SiO_4, PO_4^{3-}, NO_3+NO_2. PC2 is positively correlated with temperature, and negatively correlated with DO, pH, and NH_3 + NH_4^+. The North ponds form a distinct group with distinct water chemistry that is characterized by higher salinity and lower nutrient concentrations. To investigate this further, Kruskal Wallis tests were performed by site (e.g., individual ponds) and region (e.g., North, Mid, South).

Kruskal Wallis comparisons of DO, salinity, SiO_4, NO_3 + NO_2, NH_3 + NH_4^+, PO_4^{3-}, TDN, and TDP varied significantly by site ($p \leq 0.05$) and DO, salinity, pH, SiO_4, NO_3 + NO_2, PO_4^{3-}, TDN, and TDP were shown to be significantly variable by region ($p \leq 0.05$). The only parameter not significant in either comparison was temperature (Kruskal-Wallis = 0.29 by site and 0.06 by region). Pairwise differences varied for each parameter, but overall, the North ponds showed significant differences from the Mid and South ponds (Table 6).

3.2.1 Physicochemical effects on ʻōpaeʻula abundance

Correlations between the physicochemical parameters and ʻōpaeʻula abundance were visualized using correlation plots with significant values indicated by asterisks ($p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$) (Figure 6). OAI is the farthest right column for all correlation plots. The North ponds' OAI are significantly positively correlated with SiO_4, and significantly negatively correlated with DO, $NO_3 + NO_2$, and pH. The Mid ponds' OAI are significantly positively correlated with DO, SiO_4, PO_4^{3-}, $NO_3 + NO_2$, and TDN**.** The South ponds' OAI are significantly positively correlated with TDP, and significantly negatively correlated with pH, $NO_2 + NO_3$, and TDN.

3.3 Natural environmental cycles' effects on ʻōpaeʻula abundances

3.3.1 Moon phases vs. ʻōpaeʻula abundance

When comparing the null hypothesis (model 1) to model 4 to investigate the effect of moon phases, there was a slight negative effect of the new moon phases (i.e. -0.37). I could not reject the null, so my hypothesis was not supported ($p = 0.15$). The boxplots in figure 8 display the OAI changes with each moon phase at each site. For this and the following models, Region was a fixed effect and Site was used as a random effect. Region, alone, was included in the null model.

3.3.2 Tidal periods vs. ʻōpaeʻula abundance

When comparing the null hypothesis (model 1) to model 2 to investigate the effect of tidal periods, there was a slight negative effect of the HL and LH tides (i.e., -0.33 and -0.07, respectively), and a slight positive effect of the LL on OAI (i.e. 0.25). I could not reject the null, so my hypothesis was not supported ($p = 0.45$). The figure 9 boxplots display the OAI changes with each tidal period at each site.

3.3.3 Time vs. ʻōpaeʻula abundance

When comparing the null hypothesis (model 1) to model 3 to investigate the effect of time, Time, specifically Night, had a significant positive effect (i.e., 2.04), thus supporting our hypothesis (p

= 2.2e-16). The figure 10 boxplots display the OAI changes with day and night times at each site. For clarity, day is defined as all times after sunrise and before sunset. Night is defined as all times after sunset and before sunrise.

4. DISCUSSION

4.1 Water chemistry and ‘ōpae‘ula abundance is influenced differentially

The observable abundance of ‘ōpae‘ula populations within the MLW complex is significantly impacted by geomorphology, nutrient concentrations, and predator presence. Correlations between pond water nutrient concentrations and OAI differed amongst locations, where the Mid ponds (ponds without predators) had no negative effects on ‘ōpae‘ula abundance (Figure 6).

4.1.1 North ponds are geomorphologically separate from Middle and South ponds

As stated earlier, the North ponds are surrounded by an ‘a‘ā-flow, whereas the Mid and South ponds are within pāhoehoe and a mixture of ‘a‘ā and sandy substrate, respectively. According to Peterson and Tilling (1980), and my geological observations of the separate flows, the 3,000-5,000 yr old flow has potentially lifted an older flow near the Mid ponds, which may have caused the newer flow to overlap the existing older flow, inhibiting our sites. The evidence for this is the dull appearance of the pāhoehoe flow surrounding the Mid ponds as well as the significantly different physicochemical properties of the ponds in the separate locations.

Overall, the varying geochemistry of the North ponds are driven by their higher salinity values relative to the middle and south ponds (Figure 4). The North ponds also have significantly lower SiO_4, NO_2+NO_3, TDN, PO_4^{3-}, and TDP than the Mid and South ponds (Table 2, Table 4). Although the North ponds seem to have much higher salinity, the highest recorded value is only 20.56 ppt, where the mean for the North ponds is 16.08 ppt (Table 2), which is still a much fresher environment than the nearby ocean. The higher SiO_4, NO_2+NO_3, TDN, PO_4^{3-}, and TDP concentrations in the Mid and South ponds could be due to greater freshwater influence ($\mu = 6.10$ for Mid; $\mu = 5.74$ for South) and that freshwater could be carrying more nitrogen and phosphorus.

4.1.2. Predation across the Makalawena complex

For the Mid ponds, only data from ponds with ‘ōpae‘ula (Pots and Golden Pots) were included. ‘Ōpae‘ula abundance from ponds in the Mid region had no predators and were not negatively correlated with any measured parameters. Since there were no negative correlations in the ponds without predators, we surmise that negative correlations between ‘ōpae‘ula abundance and measured parameters in the other pond regions may be attributable to predator presence. In the North region, ‘ōpae‘ula abundance positively correlates with SiO_4, whereas in the South ponds, abundance positively correlates with TDP. There are also differences in each location’s positive correlations. In these ponds, where predators are common, ‘ōpae‘ula abundance does not follow a pattern. With that said, the parameters that were negatively correlated in the north were slightly different from the negatively correlated parameters in the south, so these negative correlations may be due to something more than predator presence, such as microbial differences, which may also be due to a hydrological barrier between the North region and the other two regions. The North ponds are the closest to the rift zone near the Makalawena Ahupua‘a border (Figure 1), so it can be possible that the rift zone extends into Makalawena. This may be evidence that there is a hydrological barrier between the North ponds and the rest of the ponds and that the North ponds are separated geomorphologically.

4.1.3 Differences in ‘ōpae‘ula abundance survey methodologies

Prior to this study, ‘ōpae‘ula abundances at the MLW complex and other North Kona areas were collected using either a catch per unit effort (CPUE) method or loose overall estimations (e.g., none, few, some, many), or were only taken during a “snapshot” of an entire day (discrete sampling), or both (Capps et al., 2009; Carey et al., 2009; Chai et al., 1993). Using a CPUE method in these rocky, crevice filled ponds, where the “catch” are only a few centimeters in size and have been conditioned to escaping or hiding from predators proved to have many limitations. The “loose overall estimations” does not give a usable abundance understanding for potential harvesting needs. This study has adapted to these limitations and created a new, more accurate method of collecting abundances. Although the loose overall estimation can be used to understand successes or failures of future restoration efforts, the CPUE method is unadvisable and should not be used in future ‘ōpae‘ula abundance studies. Due to the nature of this study (two separate data sets and methods), we were able to record abundances using the “snapshot”, discrete sampling method, as well as a more continuous, 48-hour survey method of recording abundances. To visualize the difference in abundance estimations, bubble plots were made using mean OAI for each method (Figure 7). When the discrete sampling method was compared to the 48-hour surveying method, I saw some very obvious differences in observed abundance, where the 48-hour surveying method had a greater abundance since it also included night abundance counts. This could have major impacts for management. Ponds that may seem to be a lost cause or may seem to be too much restoration work for too few ʻōpaeʻula might actually have more ʻōpaeʻula than we thought, and we just arenʻt looking at the right times.

4.2 Establishing correlations for abundance using natural environmental cycles

Traditionally, Hawaiʻi was plentiful with kilo practitioners, who would have been able to know how certain natural environmental cycles, such as tidal periods, moon phases, or day vs. night may affect certain behaviors of their places and their organisms. However, kilo practitioners are not as prevalent as they once were, so that knowledge and way of knowing has been lost for many places and many organisms. Understanding general trends for how environmental cycles affect important resources, like ʻōpaeʻula, is vital for managers to know in order to maintain their ʻōpaeʻula populations.

4.2.1 Moon phases

The effect of the new moon in model 4 was slightly more negative on abundance (Figure 8), so my hypothesis was not supported. However, since we only conducted surveys during specific lunar cycles (i.e. full and new), I cannot confidently say that moon phases do not affect abundance. Therefore, it is still necessary to observe if the steady abundances are consistent during other lunar cycles, such as the kū, ʻole, lāʻau, or kāloa moons, which are the waxing, half, and waning moon phases.

4.2.2 Tidal periods

The effect of the HL and LH tides was slightly more negative than the HH tide on OAI. The effect of the LL tide was slightly more positive than the HH tide on OAI in model 2 (Figure 9). However, these effects were not significant, therefore, we can confidently say that tides alone (i.e., without added factors) do not affect ʻōpaeʻula abundances. However, during field observations at some ponds, I did notice differences in abundance that aligned with specific tides at night. Therefore, I fitted and ran model 5 that included Tide and Time as fixed effects. Model 5 was compared to models (1) - (4) and was selected as the best fit model based on the AIC, which means that observed abundances at the majority of the ponds increase significantly during certain tides at certain times of the day. More data is needed to understand which tide and what time of day abundance is greatest, but this model does provide insight into complexities of ʻōpaeʻula behaviors.

4.2.3 Time

The effect of night in model 3 was significantly more positive (Figure 10), therefore, we can confidently say that time does affect ʻōpaeʻula abundances. The hypothesis H2.3 is supported by model 3 because of a significant positive effect on observed abundance at 80% of the ponds (Harry, Golden Lepo, and Aholehole were not used in the models) during the night. Predators are much more active during the early morning hours and the early evening hours, so this may be a reason why ʻōpaeʻula are more abundant at night in the ponds with predators. According to some studies, ʻōpaeʻula in ponds without predators do not exhibit diel migration (Capps et al., 2009; Carey et al., 2009). However, Pots and Golden Pots do not have predators, yet ʻōpaeʻula abundances were greater during the day and decreased at night suggesting they follow a migration pattern opposite of the ponds with predators. Since the populations in Pots and Golden Pots have never been exposed to predators, we can assume that this higher abundance during the day and lower at night is the result of the natural behaviors of ʻōpaeʻula migration at MLW.

4.3 Recommendations

4.3.1 Pilina with the community

It is important to note that although this study had sampling dates that spanned just one year, the duration of this project exceeded four years and is still ongoing. Conducting research on ʻāina kulāiwi (native homelands) requires the researcher to connect with the community and peoples of the area of study. As a kanaka ʻōiwi, study sites always have kinship connections to people, even if I am not yet aware of them. Indigenous land-based research also requires the researcher to meet the standards of reciprocity to not only the caretakers of these sites, but also the native peoples of the land and ʻāina itself. As conservation researchers, we agree to conduct our research for the purposes of protecting waters, lands, and beings. All research done on ʻāina kulāiwi is conservation research. All research done in Hawaiʻi is done on ʻāina kulāiwi and must be conducted with a sense of kuleana (responsibility) at all times.

4.3.2 Pond specific recommendations

The Makalawena loko waiʻōpae differ slightly in temperature and pH, but are highly variable in salinity and dissolved oxygen with the North sites being higher in salinity than the Mid and South sites (Table 2). All loko waiʻōpae have extremely high silicate concentrations relative to sea water. This is most likely due to the chemical composition of the surrounding lava rock, which is composed of high amounts of silica. Generally, the North ponds are lower in silicate, phosphate, and N+N. The lower silicate concentration is due to the higher salinity since silicate is used quickly in saltwater environments. These data suggest that the North region is more affected by tidal influence and oceanic inputs than the Mid and South regions. The lower phosphate and N+N in the North region may be due to the lack of surrounding vegetation of the north ponds, which is not the case for the Mid and South region. Also, generally, the Mid region has steady and constant ʻōpaeʻula abunances, whereas the North and South regions' abundances fluctuate with time of day.

North region

Square Roots loko waiʻōpae (Figure 3A):

At low tides, the fish are abundant and can be easily caught with a net during this time because of the decrease in pond area. The dry season low tides would be an ideal time to remove predatory fish due to the extreme low tides and the low ʻōpaeʻula abundance during the day (Table 4). The process to remove predatory fish would take multiple attempts and removal should coincide with any pond that may connect with the Square Roots pond during a high tide. If biotic crust is maintained and predator fish controlled, the Square Roots pond has the potential to be a harvesting pond because of the high abundance observed at night.

The Lukiki loko waiʻōpae (Figure 3A):

Although there are predator fish in this pond, the ʻōpaeʻula are able to hide in the *Ruppia sp.* since the fish tend to remain on the outer parts of the *Ruppia*, near the edges of the pond. The removal of predator fish is possible, but will likely take several attempts since some of the smaller predator fish can hide in the *Ruppia.*

The Moʻo loko waiʻōpae (Figure 3A):

Based on visual surveys, this pond connects with the Kapo‘ikai pond at high tides. This will make the removal of predatory fish difficult because the fish can evade capture by swimming to other ends of the pond and hide in smaller crevices of the rockwall.

The CVS loko wai‘ōpae (Figure 3A):
It would be very difficult to remove fish from this pond because of the large size, elongated shape with single access point for managers and numerous crevices for smaller predator fish to hide. This pond is also frequented for recreational purposes by the general public.

Mid region
The Pots loko wai‘ōpae (Figure 3B):
Oral interviews suggest that this pond was once used as a drinking well, however, the salinity levels have increased since last reported in 2019 (1.08 ppt in 2019 to an average of 6.11 ppt in 2020-2021). Currently, no predators, but ‘ōpae‘ula are smaller than the nearby ponds, which may be due to lack of sufficient food since there is no visible algae or bacterial growth in this pool. The ‘ōpae‘ula observed abundance is steady, but averages less than 500 observed. Furthermore, due to the small size of the shrimps, this is not an ideal harvesting pool.

The Golden Pots loko wai‘ōpae (Figure 3B):
Due to the lack of predators and the large size of the pond, this an ideal habitat for ‘ōpae‘ula. Although these ‘ōpae‘ula tend to feed on the algae in the pond, ‘ōpae‘ula were observed feeding on animal carcasses that have fallen in the hole. This pond has the potential to be a harvesting pond because of the high abundance seen during day times. Removal of the surrounding vegetation may aid in decreasing some of the nitrogen concentrations in the water and help with some of the sedimentation on the bottom of the pond.

South region
The Northwind loko wai‘ōpae (Figure 3C):
The predator fish will be difficult to remove from this pond, but if enough *Ruppia* remains, the ‘ōpae‘ula population should remain abundant as they often hide in the *Ruppia*.

The 3 Keiki loko waiʻōpae (Figure 3C):
The ʻōpaeʻula are smaller than the nearby ponds, which may be due to lack of sufficient food since there is no visible algae or bacterial growth in this pool. It would be extremely difficult to remove predator fish from this pond due to the abundance of nooks, and crevices for them to escape and hide in.

The Mint Waters loko waiʻōpae (Figure 3C):
At high tide, the ʻōpaeʻula hide in the grass and pōhuehue (*Ipomoea pes-caprae*) that grow along the sides of the pond. The ʻōpaeʻula in this pond are often white or very light red in color. Predator fish are present in this pond, but during low tide, could be easily removed. It may take a couple attempts for removal due to the amount of predator fish present and the size of the pond.

The Toads loko waiʻōpae (Figure 3C):
This pond is a site frequently used for recreation by the general public and as a "bathing" pool where recreationalist would rinse their bodies after swimming in the ocean. The ʻōpaeʻula vary in coloration ranging from white to light red in color and occasionally are observed to be bright red. During the wet season, large abundances of tadpoles are found in these ponds. The fish would be difficult to remove because they have learned to hide from humans since many humans frequent the pool.

4.4 Limitations, Implications, and Future Directions

Understanding abundances is important to know the limitations to harvesting ʻōpaeʻula, so being able to predict when abundances are highest is imperative to managers and caretakers of these environments. A key limitation of this study was the sampling periods. Due to the remote location, restricted access, and limited funding, we had to be strategic about the sampling periods. For this reason, we chose only 2 full moon periods and 2 new moon periods per season (Kau and Hoʻoilo). As stated earlier, a more accurate account of abundance distributions during moon phases would include other phases.

As implied earlier, some of the ponds' water chemistry parameters did not follow a correlation pattern when it came to ʻōpaeʻula abundance differences. Being that ʻōpaeʻula are only about 1-2 cm in size, populations could be affected by more than water chemistry. The microbial assemblages in the water column could be playing a role in abundance. ʻŌpaeʻula are the main grazers in loko waiʻōpae, so microbial assemblages could be affecting food availability or diversity. Microbes may also be contributing to differing nutrient concentrations in the water. During this study, microbial samples were taken. However, due to funding resources, those samples were not processed in time to be included in this paper.

During this study, we also noticed behaviors that were unique to the ʻōpaeʻula in each location (North, Mid, South). For instance, the North ponds would explode with ʻōpaeʻula abundance during the early morning hours (between 2 am - 4 am). During these hours, abundance often exceeded 5,000 individuals in Square Roots (the larger shallow North pond) where they were often observed feeding in large clusters. Whereas, in the South ponds, seeing large populations was often unpredictable, even at night. When large populations were noticed, they seemed to be piled on top of each other, forming stacks of ʻōpaeʻula clusters. It is unknown why they exhibited this behavior in the South ponds, but further studies will be done to understand these and other unique behaviors.

4.5 Conclusions

This study focuses on environmental impacts to anchialine pool ecosystems will help ecologists, conservationists, and environmental policy makers to determine an optimal anchialine pool ecosystem for ʻōpaeʻula population growth. Assessments and data results have aided in developing early and proper management of these ecosystems and their living resources. With climate change causing sea level rise in Hawaiʻi, anchialine pools and their unique organisms will be directly impacted. Therefore, implementing proper management practices for these ponds now will be imperative to combat the negative effects of sea level rise for these coastal resources. The results outlined in this study will inform managers and cultural practitioners on how to utilize the living resources within the anchialine pools for the ʻōpelu fisheries and assist in restoring the value and knowledge on a macroll scale to the almost forgotten loko waiʻōpae and ʻōpaeʻula. Results of the ʻōpaeʻula population growth study will support harvesting practices of the living resources, while paving the way for a more sustainable way of opelu fishing. It is also important to note that though the study focuses on the *H. rubra*, loko waiʻōpae are home to two other endangered shrimp species with uncertain population abundances. The methodologies outlined in this paper can be adapted and used in the assessment and the management of those species.

TABLES

Table 1. Field season dates for this study

	Kau (Dry Season)	Ho‘oilo (Wet Season)
New Moon*	July 2020	February 2021
Full Moon**	November 2020	May 2021

*Mauli, Muku, Hilo, Hoaka, and Kūkahi moons

**Akua, Hoku, Māhealani, Kulu, and Lā‘aukūkahi moons

Table 2. Physical characteristics of sample sites

Discrete physical samples' mean ± stdev, measured with YSI ProPlus multiparameter sonde.

Region	Name	Temperature (ºC)	Dissolved Oxygen (mg/L)	Salinity (ppt)	pH
North	CVS	22.40±*0.97*	4.91±*1.09*	15.65±*1.64*	8.17±*0.28*
	Lukiki	23.05±*0.79*	3.74±*2.24*	15.74±*1.63*	8.12±*0.26*
	Mo'o	23.20±*0.78*	2.19±*1.04*	15.74±*1.39*	8.12±*0.30*
	Square Roots	23.15±*1.00*	3.46±*2.52*	17.20±*2.48*	8.05±*0.27*
Mid	Harry*	25.10	3.59	6.79	8.09
	Golden Pots	23.20±*1.40*	5.46±*2.01*	5.93±*0.07*	8.40±*0.23*
	Pots	23.05±*1.14*	4.80±*0.62*	6.15±*0.06*	8.33±*0.27*
	Golden Lepo*	21.40	4.55	5.93	8.06
South	Northwind	22.68±*1.15*	5.09±*0.91*	5.85±*0.12*	8.20±*0.12*
	3 Keiki	21.83±*0.87*	6.52±*0.66*	5.57±*0.20*	8.37±*0.12*
	Mint Waters	21.93±*1.20*	4.82±*1.13*	6.11±*0.43*	8.39±*0.14*
	Aholehole*	22.60	7.40	5.29	8.40
	Toads	21.95±*1.11*	6.27±*1.50*	5.56±*0.14*	8.49±*0.18*

*only 1 data point

Table 3. Biogeochemical characteristics of sample sites

Discrete nutrient samples' mean ± stdev, measured with Astoria-Pacific A2 autoanalyzer.

Region	Name	Si (mg/L)	TDP (mg/L)	PO_4 (mg/L)	TDN (mg/L)	NO_2+NO_3 (mg/L)	NH_3+NH_4 (mg/L)
North	CVS	18750.80±*1576.65*	125.15±*28.84*	155.20±*10.06*	637.50±*98.75*	465.43±*70.05*	6.73±*2.40*
	Lukiki	18601.70±*853.17*	119.78±*20.46*	143.75±*14.53*	498.98±*64.24*	326.79±*152.10*	10.90±*3.47*
	Mo'o	18612.85±*997.06*	117.68±*25.96*	145.25±*3.87*	489.24±*79.96*	271.91±*184.02*	11.65±*6.88*
	Square Roots	17069.90±*2380.57*	122.68±*5.77*	137.00±*4.96*	502.54±*68.82*	289.40±*120.39*	12.13±*2.89*
Mid	Harry*	22816.00	146.00	148.30	487.90	143.30	0.10
	Golden Pots	27441.70±*2183.55*	204.75±*26.63*	252.45±*21.29*	1128.90±*135.71*	1005.06±*143.26*	16.55±*2.65*
	Pots	27044.85±*2406.95*	186.78±*17.43*	230.83±*7.90*	1220.30±*208.52*	1121.18±*179.90*	6.58±*3.22*
	Golden Lepo*	23199.60	185.20	209.60	665.20	620.50	50.20
South	Northwind	27589.25±*2863.62*	195.65±*25.43*	223.95±*1.23*	1121.70±*170.57*	921.15±*105.67*	14.15±*4.72*
	3 Keiki	28498.70±*4467.37*	197.80±*19.92*	236.95±*4.55*	1179.80±*115.42*	1164.48±*130.77*	7.30±*2.91*
	Mint Waters	25394.75±*2136.51*	188.65±*20.59*	222.43±*7.15*	1216.90±*128.34*	1062.68±*162.45*	17.98±*9.78*
	Aholehole*	25863.00	194.80	225.20	1196.40	1109.20	0.00
	Toads	25964.85±*5337.45*	186.65±*27.67*	217.18±*13.55*	1173.23±*82.57*	1062.93±*180.32*	8.78±*6.97*

*only 1 data point

Table 4. Biotic characteristics of study sites.

Biotic crust includes *Lyngbya* spp., *Schizothrix calcicola*, and other unidentified microbial mats/crusts. *Ruppia maritima* noted separately as they have the potential to provide protection from predators during the day, allowing for grazing. - = none, + = few, ++ = some, +++ = common, * = 1 data point.

Region	Site	ʻŌpaeʻula Abundance Index		ʻŌpaeʻula Predators	Algae	*Biotic crust*	*R. maritima*	*Other plant material*
		Day	Night					
North	CVS	0.94	3.07	*Poecilia reticulata* and other fish	++	+	-	-
	Lukiki	1.67	5.85	*Poecilia reticulata*	-	+++	+	-
	Moʻo	0.72	4.77	*Poecilia reticulata*	-	+++	-	*Sesuvium portulacastrum*
	Square Roots	0.83	5.77	*Poecilia reticulata*	-	+++	-	-
Mid	Harry*	0.00	0.00	Unknown	-	-	-	+++
	Golden Pots	6.00	5.17	None	+	-	-	+, sparse plant detritus
	Pots	4.28	3.25	None	-	-	-	-
	Golden Lepo*	0.00	0.00	*Poecilia reticulata*	-	+++	-	-
South	Northwind	4.88	5.31	*Poecilia reticulata*	++	++	+	-
	3 Keiki	0.88	4.39	*Poecilia reticulata*	-	+, accessible during HH	-	-
	Mint Waters	0.33	4.00	*Poecilia reticulata*	-	+	-	*Ipomea pes-caprae*
	Aholehole*	0.00	0.00	*Poecilia reticulata*and other fish	-	+++	-	-
	Toads	0.19	5.78	*Poecilia reticulata;* tadpoles	-	+	+	-

Table 5. Pairwise comparison of environmentals parameters by site (Posthoc Dunn's test)

Parameter	Region	Site	CVS	Lukiki	Square Roots	Moo	Harry	Golden Pots	Pots	Golden Lepo	North wind	3 Keiki	Mint Waters	Aholehole
Temperature (°C)	North	Lukiki	0.226	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.205	0.955	-	-	-	-	-	-	-	-	-	-
		Moo	0.181	0.899	0.944	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.079	0.322	0.340	0.363	-	-	-	-	-	-	-	-
		Golden Pots	0.310	0.844	0.800	0.746	0.265	-	-	-	-	-	-	-
		Pots	0.360	0.767	0.725	0.672	0.239	0.921	-	-	-	-	-	-
		Golden Lepo	0.397	0.107	0.099	0.090	**0.040**	0.137	0.154	-	-	-	-	-
	South	Northwind	0.592	0.499	0.464	0.422	0.156	0.632	0.704	0.236		-	-	-
		3 Keiki	0.592	0.081	0.071	0.061	**0.036**	0.121	0.147	0.611	0.284	-	-	-
		Mint Waters	0.800	0.143	0.128	0.111	0.055	0.205	0.242	0.493	0.430	0.778	-	-
		Aholehole	0.715	0.688	0.662	0.630	0.272	0.782	0.831	0.339	0.979	0.481	0.599	-
		Toads	0.693	0.108	0.096	0.083	**0.045**	0.159	0.190	0.550	0.352	0.888	0.888	0.539
Dissolved Oxygen (mg/L)	North	Lukiki	0.583	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.517	0.921	-	-	-	-	-	-	-	-	-	-
		Moo	0.094	0.260	0.304	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.476	0.715	0.762	0.728	-	-	-	-	-	-	-	-
		Golden Pots	0.398	0.163	0.136	**0.012**	0.213	-	-	-	-	-	-	-
		Pots	0.955	0.622	0.554	0.105	0.499	0.368	-	-	-	-	-	-
		Golden Lepo	0.776	0.950	0.901	0.439	0.735	0.413	0.803	-	-	-	-	-
	South	Northwind	0.735	0.375	0.324	**0.044**	0.354	0.612	0.693	0.618	-	-	-	-
		3 Keiki	0.102	**0.029**	**0.023**	**0.001**	0.081	0.430	0.091	0.188	0.195	-	-	-
		Mint Waters	0.855	0.714	0.642	0.136	0.551	0.304	0.899	0.866	0.602	0.069	-	-
		Aholehole	0.135	0.065	0.057	**0.011**	0.081	0.336	0.126	0.159	0.200	0.643	0.107	-
		Toads	0.190	0.063	**0.050**	**0.003**	0.123	0.642	0.172	0.266	0.331	0.746	0.136	0.504
Salinity (ppt)	North	Lukiki	0.944	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.612	0.662	-	-	-	-	-	-	-	-	-	-
		Moo	0.921	0.978	0.683	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.618	0.587	0.413	0.575	-	-	-	-	-	-	-	-

		Golden Pots	**0.047**	**0.040**	**0.013**	**0.037**	0.449	-	-	-	-	-	-	-
		Pots	0.205	0.181	0.076	0.172	0.762	0.473	-	-	-	-	-	-
		Golden Lepo	0.254	0.236	0.144	0.229	0.612	0.908	0.735	-	-	-	-	-
	South	Northwind	**0.025**	**0.021**	**0.006**	**0.019**	0.359	0.800	0.331	0.782	-	-	-	-
		3 Keiki	**0.002**	**0.002**	**0.000**	**0.001**	0.144	0.266	0.067	0.413	0.390	-	-	-
		Mint Waters	0.067	0.057	**0.019**	0.054	0.510	0.877	0.573	0.986	0.683	0.205	-	-
		Aholehole	**0.019**	**0.017**	**0.008**	**0.016**	0..143	0.273	0.121	0.338	0.350	0.695	0.233	-
		Toads	**0.001**	**0.001**	**0.000**	**0.001**	0.126	0.221	0.052	0.373	0.331	0.91	0.168	0.749
pH	North	Lukiki	0.683	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.508	0.800	-	-	-	-	-	-	-	-	-	-
		Moo	0.767	0.910	0.714	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.624	0.817	0.943	0.762	-	-	-	-	-	-	-	-
		Golden Pots	0.278	0.135	0.081	0.167	0.240	-	-	-	-	-	-	-
		Pots	0.473	0.260	0.167	0.310	0.345	0.714	-	-	-	-	-	-
		Golden Lepo	0.482	0.656	0.776	0.605	0.866	0.165	0.247	-	-	-	-	-
	South	Northwind	0.855	0.822	0.632	0.910	0.708	0.205	0.310	0.557	-	-	-	-
		3 Keiki	0.430	0.231	0.147	0.278	0.323	0.767	0.944	0.229	0.331	-	-	-
		Mint Waters	0.331	0.167	0.102	0.205	0.269	0.910	0.800	0.187	0.248	0.855	-	-
		Aholehole	0.314	0.206	0.154	0.233	0.237	0.748	0.581	0.176	0.262	0.612	0.695	-
		Toads	0.065	**0.024**	**0.012**	**0.032**	0.098	0.447	0.260	0.061	**0.043**	0.291	0.382	0.873
SIO (mg/L)	North	Lukiki	0.955	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.652	0.693	-	-	-	-	-	-	-	-	-	-
		Moo	0.978	0.978	0.672	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.510	0.487	0.345	0.499	-	-	-	-	-	-	-	-
		Golden Pots	**0.007**	**0.006**	**0.002**	**0.007**	0.302	-	-	-	-	-	-	-
		Pots	**0.014**	**0.012**	**0.004**	**0.013**	0.373	0.822	-	-	-	-	-	-
		Golden Lepo	0.465	0.444	0.310	0.454	0.955	0.336	0.413	-	-	-	-	-
	South	Northwind	**0.009**	**0.007**	**0.002**	**0.008**	0.319	0.955	0.866	0.254	-	-	-	-
		3 Keiki	**0.008**	**0.007**	**0.002**	**0.007**	0.310	0.978	0.844	0.345	0.978	-	-	-
		Mint Waters	0.052	**0.046**	**0.017**	**0.049**	0.569	0.464	0.612	0.618	0.499	0.481	-	-

		Aholehole	0.170	0.159	0.098	0.165	0.573	0.749	0.859	0.612	0.776	0.762	0.887	-
		Toads	0.052	**0.046**	**0.017**	**0.049**	0.569	0.464	0.612	0.618	0.499	0.481	0.998	0.887
NO2 + NO3 (mg/L)	North	Lukiki	0.652	-	-	-	-	-	-	-	-	-	-	-
		Square												
		Roots	0.464	0.778	-	-	-	-	-	-	-	-	-	-
		Moo	0.447	0.757	0.978	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.403	0.581	0.708	0.722	-	-	-	-	-	-	-	-
		Golden												
		Pots	0.097	**0.035**	**0.017**	**0.015**	0.059	-	-	-	-	-	-	-
		Pots	**0.024**	**0.007**	**0.003**	**0.003**	**0.024**	0.554	-	-	-	-	-	-
		Golden												
		Lepo	0.762	0.557	0.444	0.433	0.368	0.454	0.262	-	-	-	-	-
	South	Northwind	0.237	0.102	0.056	0.052	0.113	0.632	0.284	0.656	-	-	-	-
		3 Keiki	**0.011**	**0.003**	**0.001**	**0.001**	**0.015**	0.383	0.778	0.194	0.177	-	-	-
		Mint												
		Waters	**0.049**	**0.015**	**0.007**	**0.006**	**0.037**	0.757	0.778	0.345	0.430	0.573	-	-
		Aholehole	0.130	0.072	**0.048**	**0.046**	0.063	0.643	0.929	0.338	0.444	0.929	0.789	-
		Toads	0.052	**0.017**	**0.007**	**0.007**	**0.039**	0.778	0.757	0.354	0.447	0.554	0.978	0.776
NH3 + NH4 (mg/L)	North	Lukiki	0.205	-	-	-	-	-	-	-	-	-	-	-
		Square												
		Roots	0.125	0.789	-	-	-	-	-	-	-	-	-	-
		Moo	0.102	0.714	0.921	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.476	0.130	0.092	0.081	-	-	-	-	-	-	-	-
		Golden												
		Pots	**0.011**	0.200	0.311	0.360	**0.020**	-	-	-	-	-	-	-
		Pots	0.910	0.248	0.155	0.128	0.433	**0.015**	-	-	-	-	-	-
		Golden												
		Lepo	**0.027**	0.159	0.216	0.240	**0.021**	0.551	**0.033**	-	-	-	-	-
	South	Northwind	0.065	0.564	0.757	0.833	0.060	0.481	0.083	0.297	-	-	-	-
		3 Keiki	0.888	0.260	0.163	0.135	0.423	**0.016**	0.978	**0.034**	0.088	-	-	-
		Mint												
		Waters	**0.043**	0.447	0.622	0.693	**0.046**	0.602	0.055	0.354	0.855	0.059	-	-
		Aholehole	0.433	0.113	0.079	0.069	0.955	**0.017**	0.393	**0.018**	0.051	0.383	**0.039**	-
		Toads	0.473	0.583	0.414	0.360	0.243	0.067	0.545	0.079	0.260	0.564	0.190	0.216
PO4 (mg/L)	North	Lukiki	0.602	-	-	-	-	-	-	-	-	-	-	-
		Square												
		Roots	0.266	0.554	-	-	-	-	-	-	-	-	-	-
		Moo	0.673	0.921	0.490	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.922	0.817	0.545	0.866	-	-	-	-	-	-	-	-
		Golden												
		Pots	**0.002**	**0.000**	**0.000**	**0.000**	**0.039**	-	-	-	-	-	-	-

		Pots	**0.036**	**0.009**	**0.001**	**0.012**	0.154	0.311	-	-	-	-	-	-
		Golden Lepo	0.689	0.465	0.269	0.504	0.693	0.117	0.354	-	-	-	-	-
	South	Northwind	0.132	**0.043**	**0.009**	0.054	0.293	0.108	0.554	0.581	-	-	-	-
		3 Keiki	**0.008**	**0.001**	**0.000**	**0.002**	0.075	0.652	0.573	0.200	0.248	-	-	-
		Mint Waters	0.151	**0.050**	**0.011**	0.063	0.314	0.094	0.508	0.612	0.944	0.221	-	-
		Aholehole	0.297	0.170	0.081	0.191	0.368	0.354	0.776	0.612	0.929	0.521	0.893	-
		Toads	0.248	0.094	**0.023**	0.115	0.408	**0.050**	0.345	0.742	0.725	0.132	0.778	0.755
Total Dissolved Nitrogen (mg/L)	North	Lukiki	0.414	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.464	0.933	-	-	-	-	-	-	-	-	-	-
		Moo	0.353	0.910	0.844	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.533	0.915	0.873	0.972	-	-	-	-	-	-	-	-
		Golden Pots	0.125	**0.019**	**0.023**	**0.014**	0.111	-	-	-	-	-	-	-
		Pots	**0.032**	**0.003**	**0.004**	**0.002**	**0.048**	0.545	-	-	-	-	-	-
		Golden Lepo	0.873	0.499	0.533	0.454	0.536	0.418	0.233	-	-	-	-	-
	South	Northwind	0.139	**0.022**	**0.027**	**0.016**	0.119	0.955	0.508	0.439	-	-	-	-
		3 Keiki	0.059	**0.007**	**0.009**	**0.005**	0.069	0.725	0.800	0.302	0.683	-	-	-
		Mint Waters	**0.032**	**0.003**	**0.004**	**0.002**	**0.048**	0.545	1.000	0.233	0.508	0.800	-	-
		Aholehole	0.219	0.081	0.091	0.069	0.143	0.796	0.901	0.398	0.769	0.972	0.901	-
		Toads	0.076	**0.010**	**0.012**	**0.007**	0.081	0.811	0.714	0.336	0.767	0.910	0.714	0.915
Total Dissolved Phosphorus (mg/L)	North	Lukiki	0.778	-	-	-	-	-	-	-	-	-	-	-
		Square Roots	0.693	0.910	-	-	-	-	-	-	-	-	-	-
		Moo	0.693	0.910	1.000	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.776	0.643	0.593	0.593	-	-	-	-	-	-	-	-
		Golden Pots	**0.010**	**0.004**	**0.003**	**0.003**	0.182	-	-	-	-	-	-	-
		Pots	**0.049**	**0.024**	**0.018**	**0.018**	0.336	0.554	-	-	-	-	-	-
		Golden Lepo	0.200	0.144	0.126	0.126	0.430	0.735	0.972	-	-	-	-	-
	South	Northwind	**0.021**	**0.010**	**0.007**	**0.007**	0.240	0.800	0.735	0.859	-	-	-	-
		3 Keiki	**0.014**	**0.006**	**0.004**	**0.004**	0.206	0.910	0.632	0.789	0.888	-	-	-
		Mint Waters	0.052	**0.026**	**0.019**	**0.019**	0.345	0.536	0.978	0.957	0.714	0.612	-	-
		Aholehole	0.117	0.081	0.069	0.069	0.311	0.957	0.749	0.822	0.915	0.986	0.735	-
		Toads	0.056	**0.028**	**0.021**	**0.021**	0.354	0.517	0.955	0.943	0.693	0.593	0.978	0.722

‘Ōpae‘ula Abundance Index	North	Lukiki Square	1.000	-	-	-	-	-	-	-	-	-	-	-
		Roots	0.373	0.373	-	-	-	-	-	-	-	-	-	-
		Moo	0.630	0.630	0.683	-	-	-	-	-	-	-	-	-
	Mid	Harry	0.301	0.301	0.638	0.466	-	-	-	-	-	-	-	-
		Golden Pots	0.056	0.056	**0.005**	**0.017**	**0.025**	-	-	-	-	-	-	-
		Pots	0.300	0.300	0.054	0.129	0.091	0.381	-	-	-	-	-	-
		Golden Lepo	0.301	0.301	0.638	0.466	1.000	**0.025**	0.091	-	-	-	-	-
	South	Northwind	0.136	0.136	**0.017**	**0.049**	**0.048**	0.672	0.651	**0.048**	-	-	-	-
		3 Keiki	0.683	0.683	0.630	0.942	0.438	**0.020**	0.148	0.438	0.058	-	-	-
		Mint Waters	0.569	0.569	0.748	0.930	0.500	**0.013**	0.108	0.500	**0.040**	0.872	-	-
		Aholehole	0.301	0.301	0.638	0.466	1.000	**0.025**	0.091	1.000	**0.048**	0.438	0.500	-
		Toads	0.815	0.815	0.511	0.804	0.375	**0.032**	0.204	0.375	0.085	0.861	0.737	0.375

Table 6. Pairwise comparisons of environmental parameters by region.

Temperature salinity, dissolved oxygen, and pH values from YSI Sonde measurements. Macronutrient measurements from discrete sampling. Kruskal-Wallis comparison by region. North = 4 North ponds combined, Mid = 4 Mid ponds combined, South = 5 South ponds combined (Post-hoc Dunn's Test).

Parameter	Region	Mid	North
Temperature (°C)	North	1.000	-
	South	0.199	0.088
Dissolved Oxygen (mg/L)	North	0.373	-
	South	0.623	**0.004**
Salinity (ppt)	North	**0.007**	-
	South	0.153	**0.000**
pH	North	0.376	-
	South	1.000	**0.035**
Silica (mg/L)	North	**0.000**	-
	South	1.000	**0.000**
NO2 + NO3 (mg/L)	North	**0.002**	-
	South	1.000	**0.000**
NH3 + NH4 (mg/L)	North	1.000	-
	South	1.000	1.000
PO4 (mg/L)	North	**0.000**	-
	South	1.000	**0.000**
TDN (mg/L)	North	**0.001**	-
	South	1.000	**0.000**
TDP (mg/L)	North	**0.000**	-
	South	1.000	**0.000**
‘Ōpae‘ula Abundance Index	North	0.108	-
	South	0.351	1.000

FIGURES

Figure 1. Geology of North Kona

North Kona is marked by lava flows from Hualālai and span ahupuaʻa (marked in black). Recent flows (dark blue) are from the A.D. 1800 -1801 Huʻehuʻe flow; older flows (lighter blues) range from 1,500 to >11,000 years old. An estimation of a nearby rift zone is marked by a red dotted line. Makalawena ahupuaʻa is owned by Kamehameha Schools (outlined in yellow).

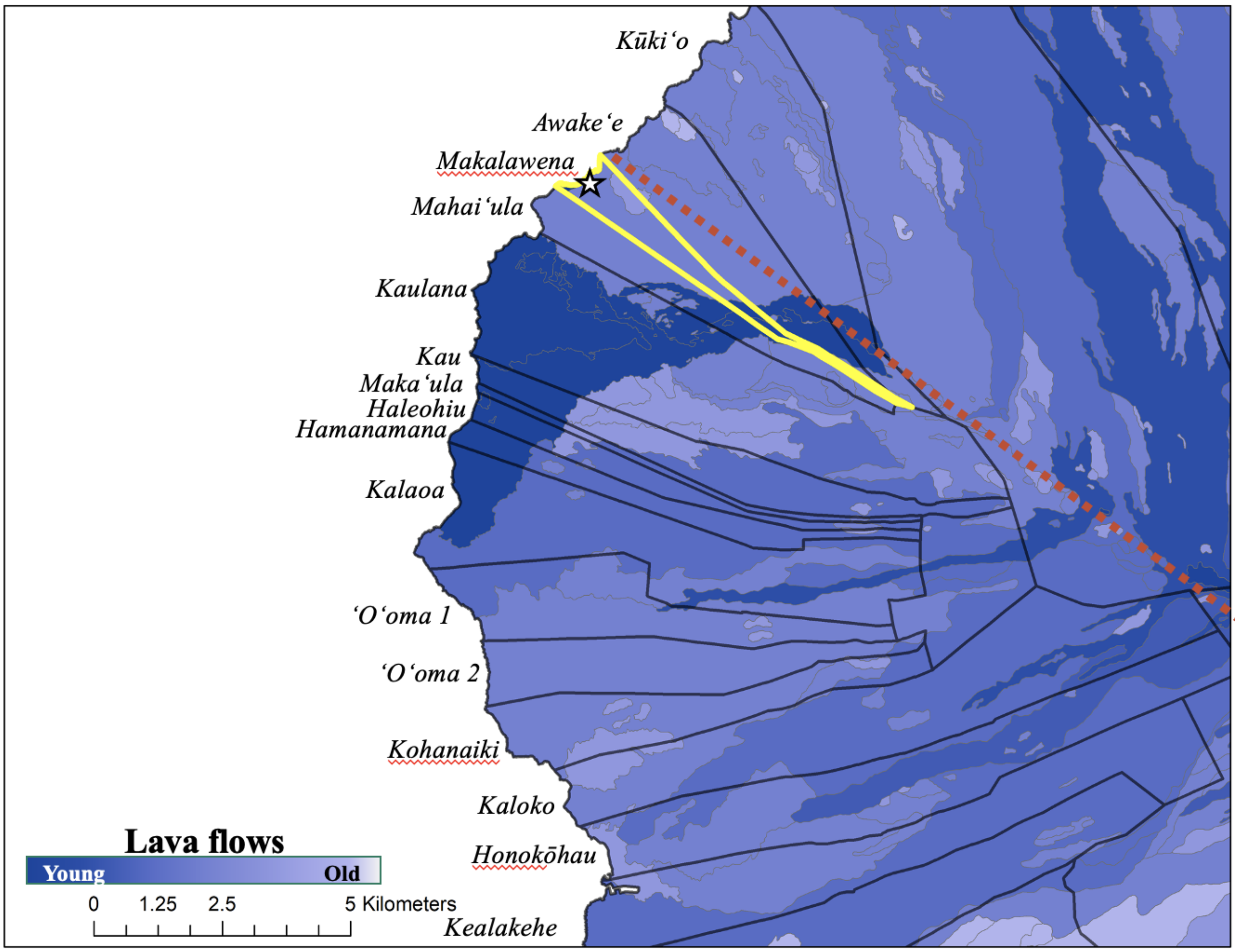


Figure 2. Map of sample sites at Makalawena in Kona, Hawai‘i.

Figure showing Hawai‘i island in top left with white box indicating general study area. Right image is a google earth image of sample sites. Filled circles = ponds with predators; Outlined circles = predator-less ponds. North region pond = blue circles ; Mid = green circles; South = red circles.

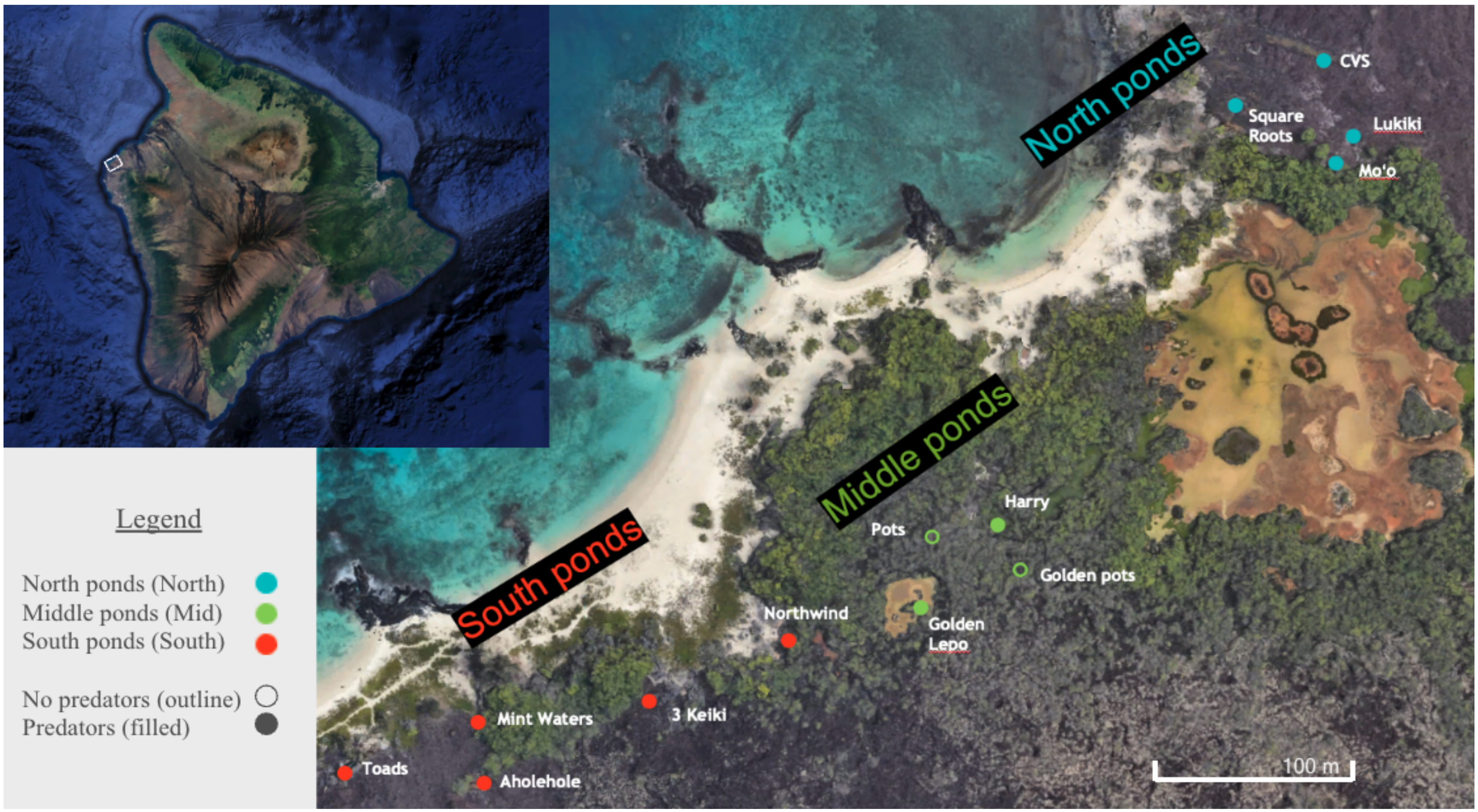


Figure 3. Makalawena Loko wai‘ōpae sample sites for this study.

North ponds from left to right (A): CVS, Lukiki, Mo‘o, and Square Roots. Mid ponds from left to right (B): Golden Pots, Pots, Golden Lepo, and Harry (not pictured). South ponds from left to right (C): Northwind, 3 Keiki, Mint Waters, Aholehole, and Toads.

Figure 4. Sampling regime for this study

Diagram showing an example of Hawai‘i’s mixed tidal cycle, which is characterized by two high tides and two low tides of unequal heights within a single day. The 48-hr surveys and samples (orange diamonds) were conducted during each major tidal change over a 48-hr period. The discrete samples (yellow diamond) were conducted just before and during daybreak on the next day after the 48-hour surveys were completed.

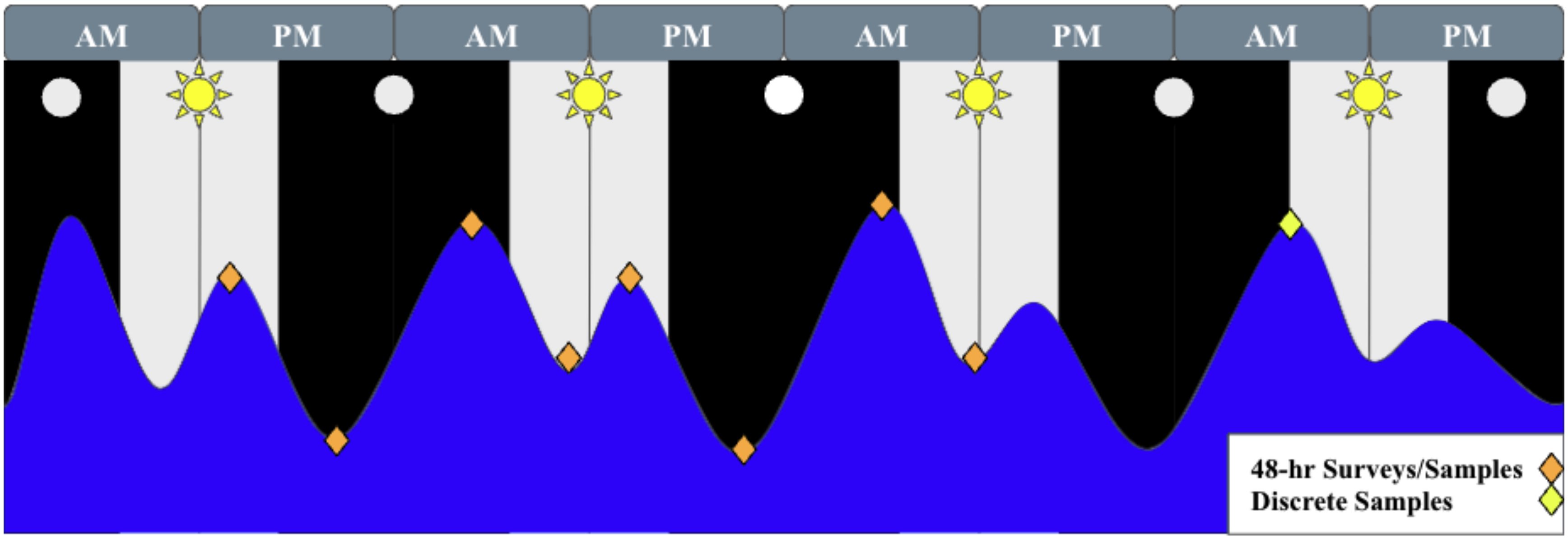


Figure 5. Principal component analysis of discrete samples' physicochemical parameters.

PCA plot showing clusters of samples based on their similarities. Each point represents a sample. The point colors represent what region the sample originates (North = blue, Mid = green, South = red). The circles represent the distribution of the samples within a region. The vector lines show how strongly each physicochemical parameter influences each sample.

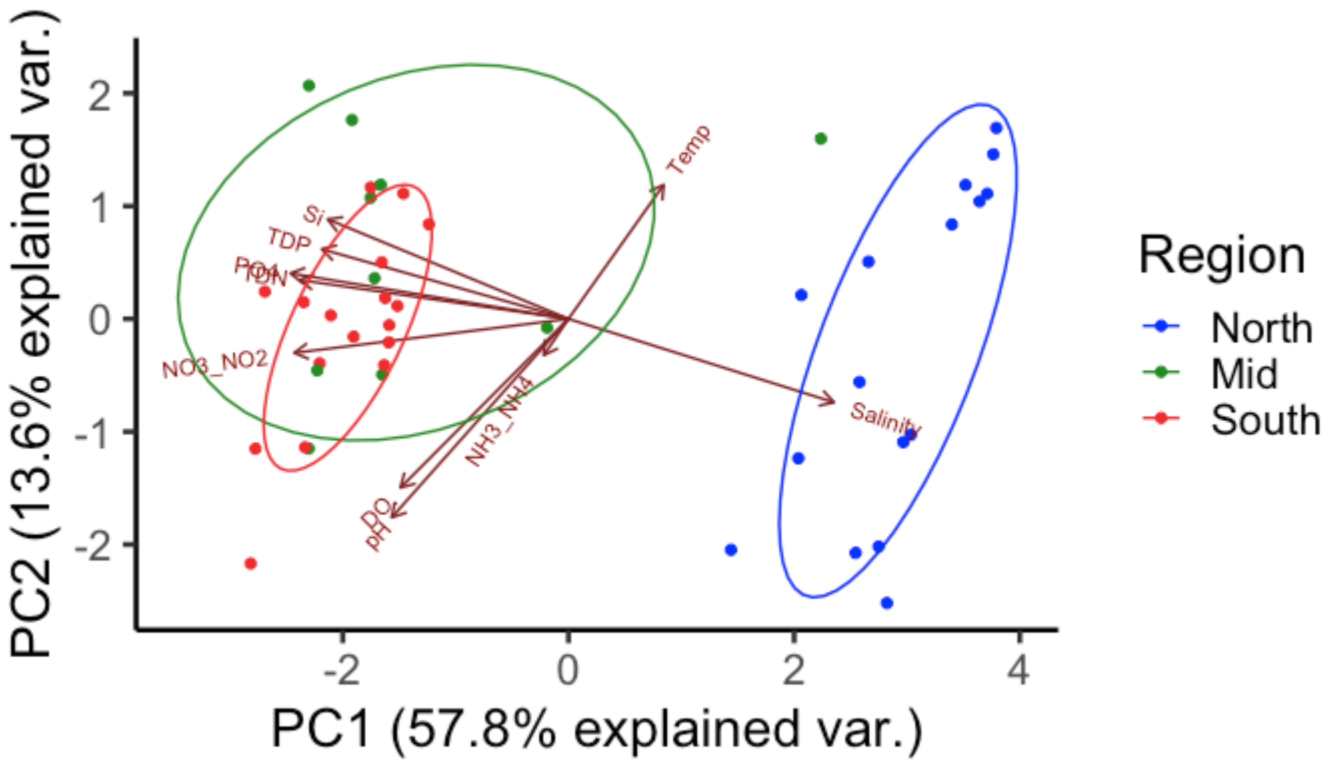
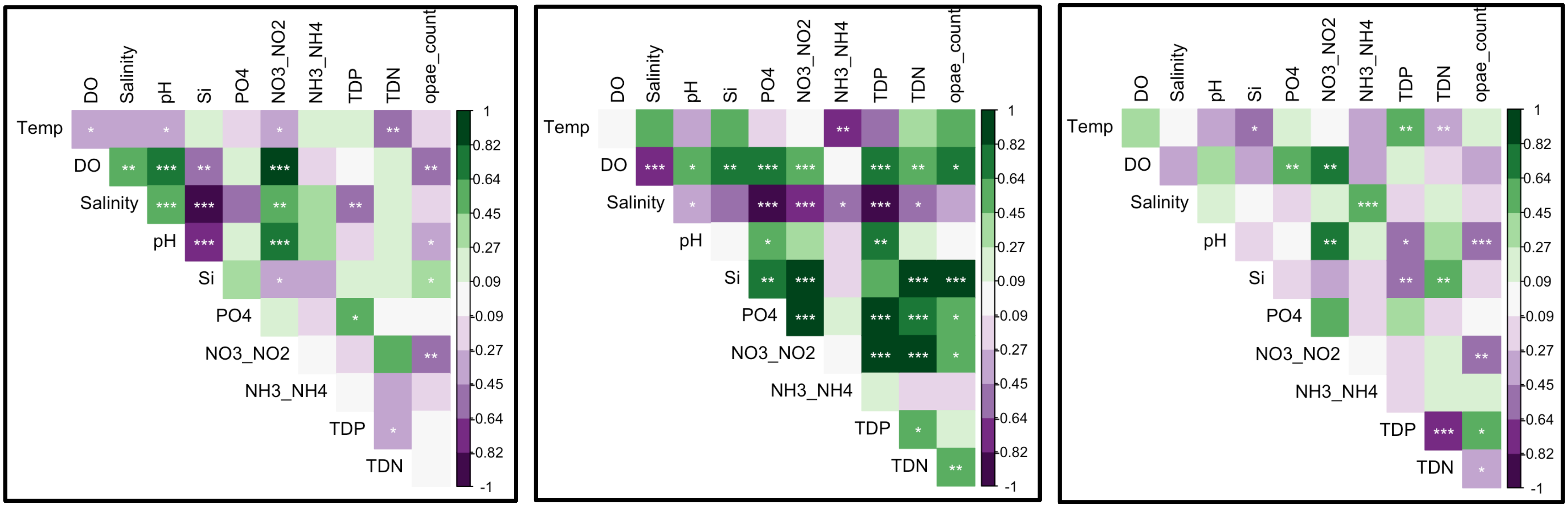


Figure 6. ‘Ōpae‘ula abundance correlation plots for each region.

North ponds’ (left plot) abundance is negatively correlated with dissolved oxygen, pH, and nitrate+nitrite; is positively correlated with silicate. Mid ponds’ (center plot) abundance is positively correlated with dissolved oxygen, silicate, phosphate, nitrate+nitrite, and total dissolved nitrogen. South ponds’ (right plot) abundance is negatively correlated with pH, nitrate+nitrite, and total dissolved nitrogen; is positively correlated with total dissolved phosphorus



North **Mid** **South**

Figure 7. Comparison of ‘ōpae‘ula abundance indexes by pond region

Discrete sampling results (left) show a lower mean value than the 48-hour tidal sampling results (right). All discrete samples were taken during a timescale that spanned from just before sunrise to just after sunrise, whereas the 48-hour tidal samples were taken throughout a 48-hr period. North region = blue circles; Mid = green circles; South = red circles.

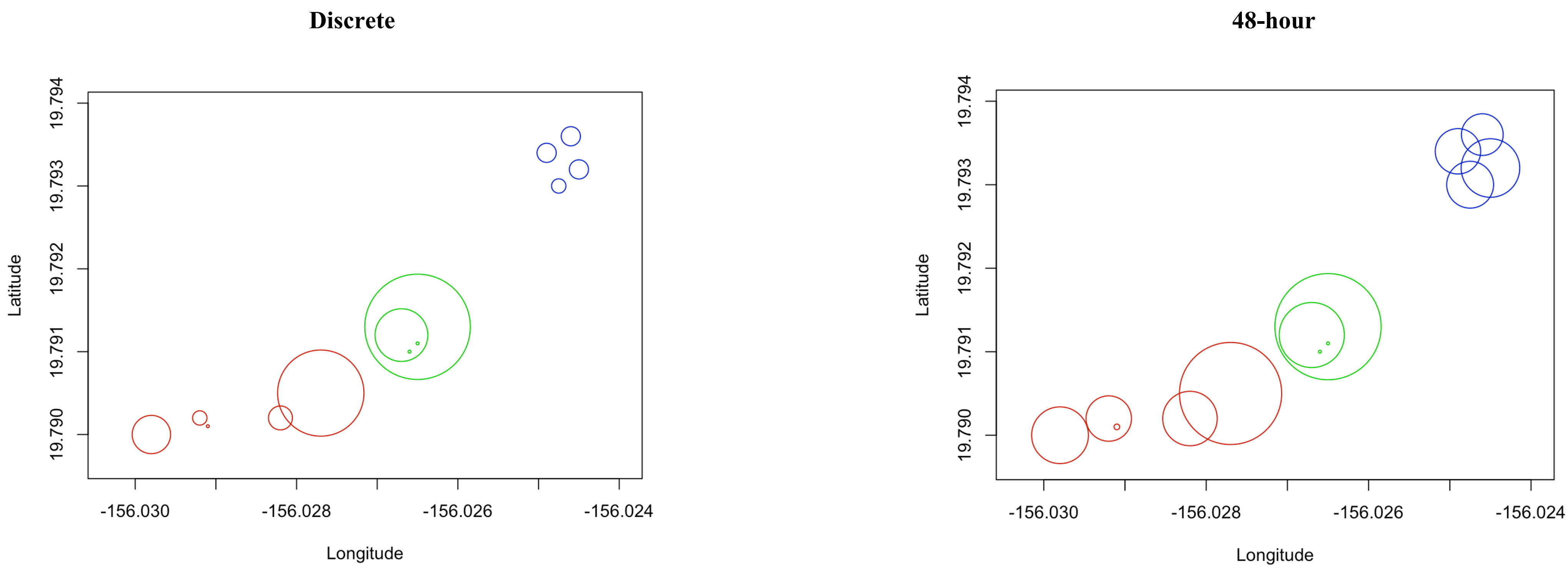


Figure 8. ‘Ōpae‘ula abundance index by moon phase

Boxplots of ‘ōpae‘ula abundance index during the new and full moon phases for each site. In general, Golden Pots and Northwind have the highest mean values, however, there is no statistical difference between the new and full moon phases for any site. The model plot (right) shows that as the OAI value approaches the neutral line (red vertical line), which indicates no effect. Red values = decreasing abundance, blue values = increasing abundance.

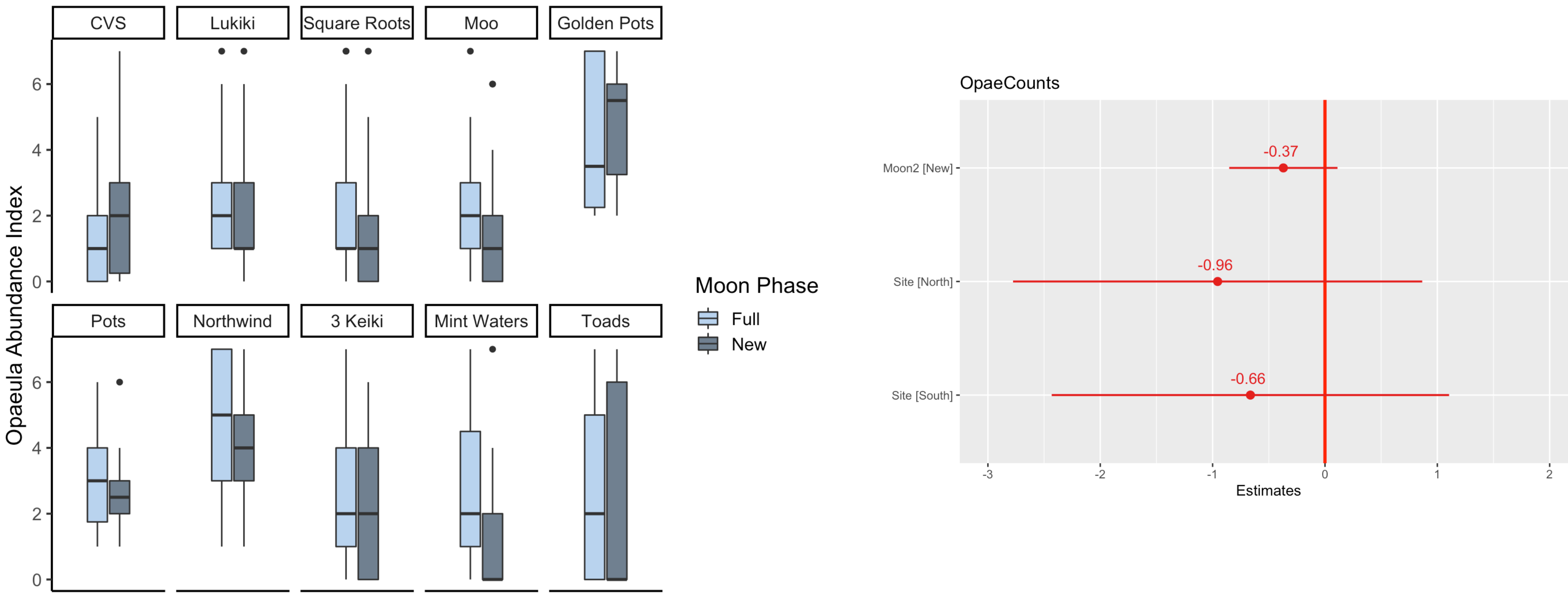


Figure 9. ‘Ōpae‘ula abundance index by tidal period

Boxplots of ‘ōpae‘ula abundance index during the mixed tidal cycles for each site. In general, Golden Pots and Northwind have the highest mean values, however, there is no statistical difference between the different tidal periods for any site. The model plot (right plot) shows that as the OAI value approaches the neutral line (red vertical line), it indicates no effect. Red values = decreasing abundance, blue values = increasing abundance.

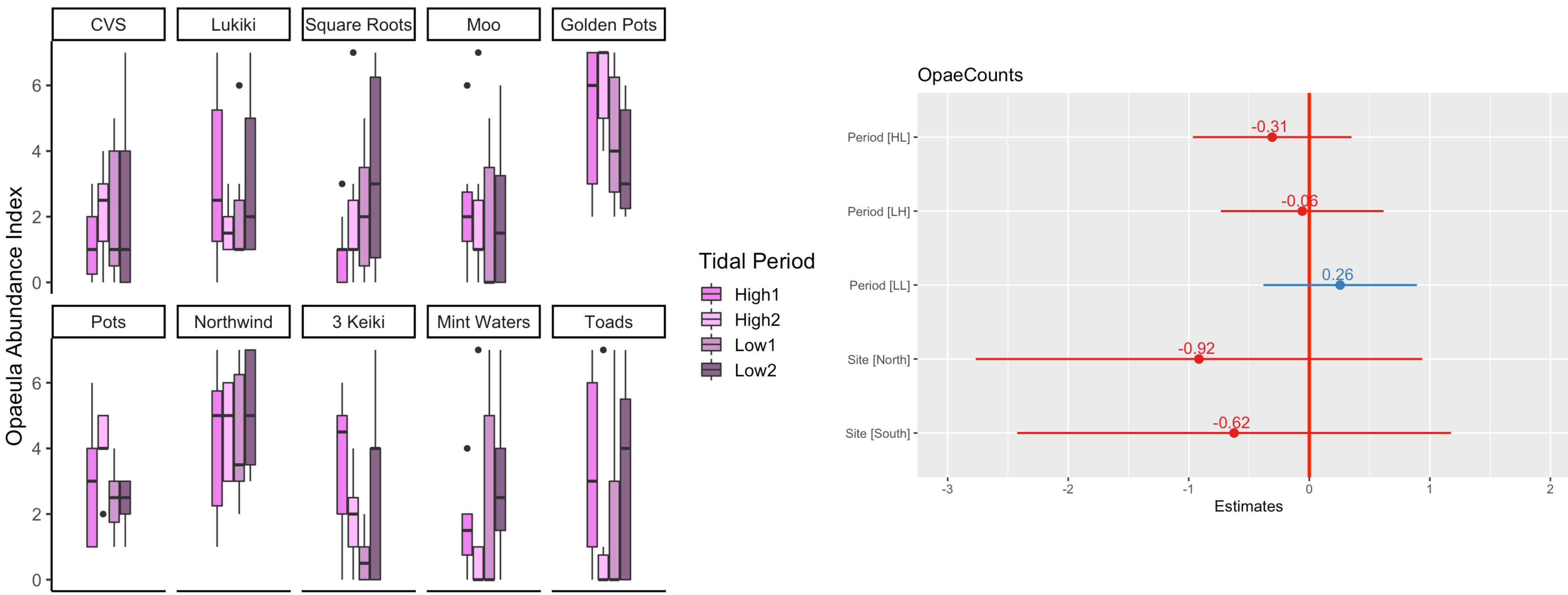


Figure 10. ʻŌpaeʻula abundance index by time

Boxplots of ʻōpaeʻula abundance index during day and night for each site. Aside from Golden Pots and Pots, all sites showed an increase in OAI during the night. The model plot (right plot) shows that as the OAI value approaches the neutral line (red vertical line), it indicates no effect. Red values = decreasing abundance, blue values = increasing abundance.

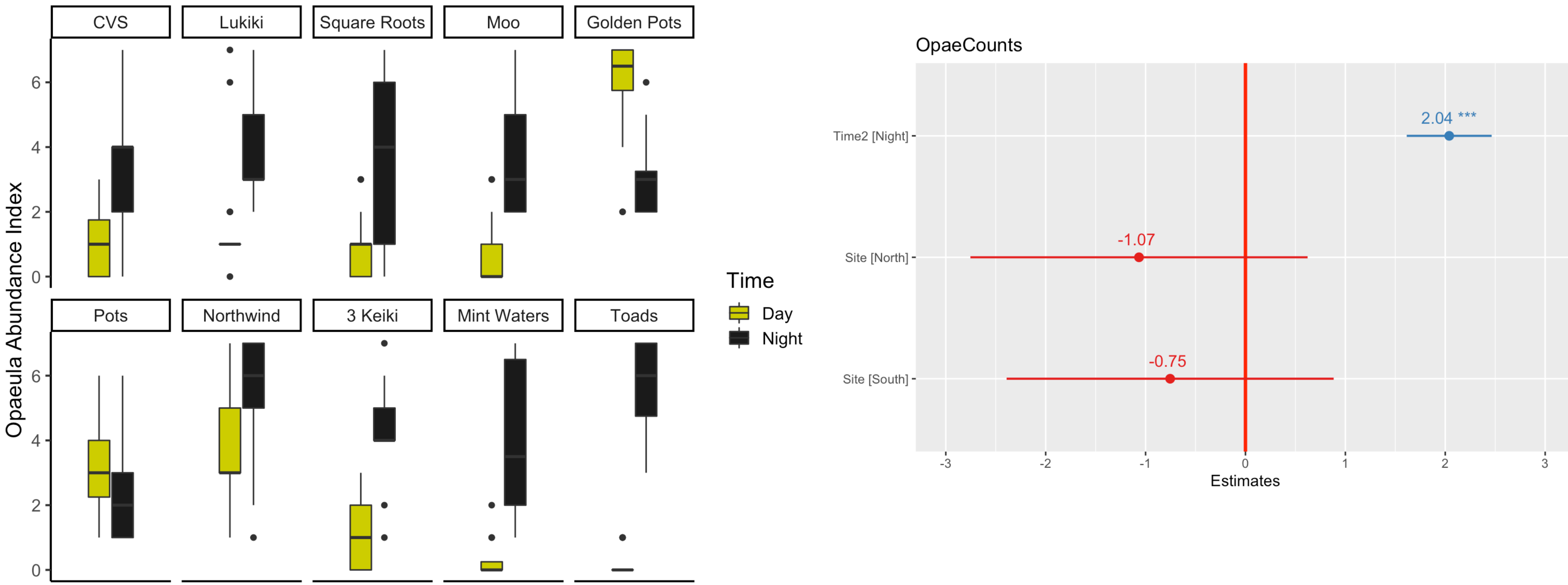
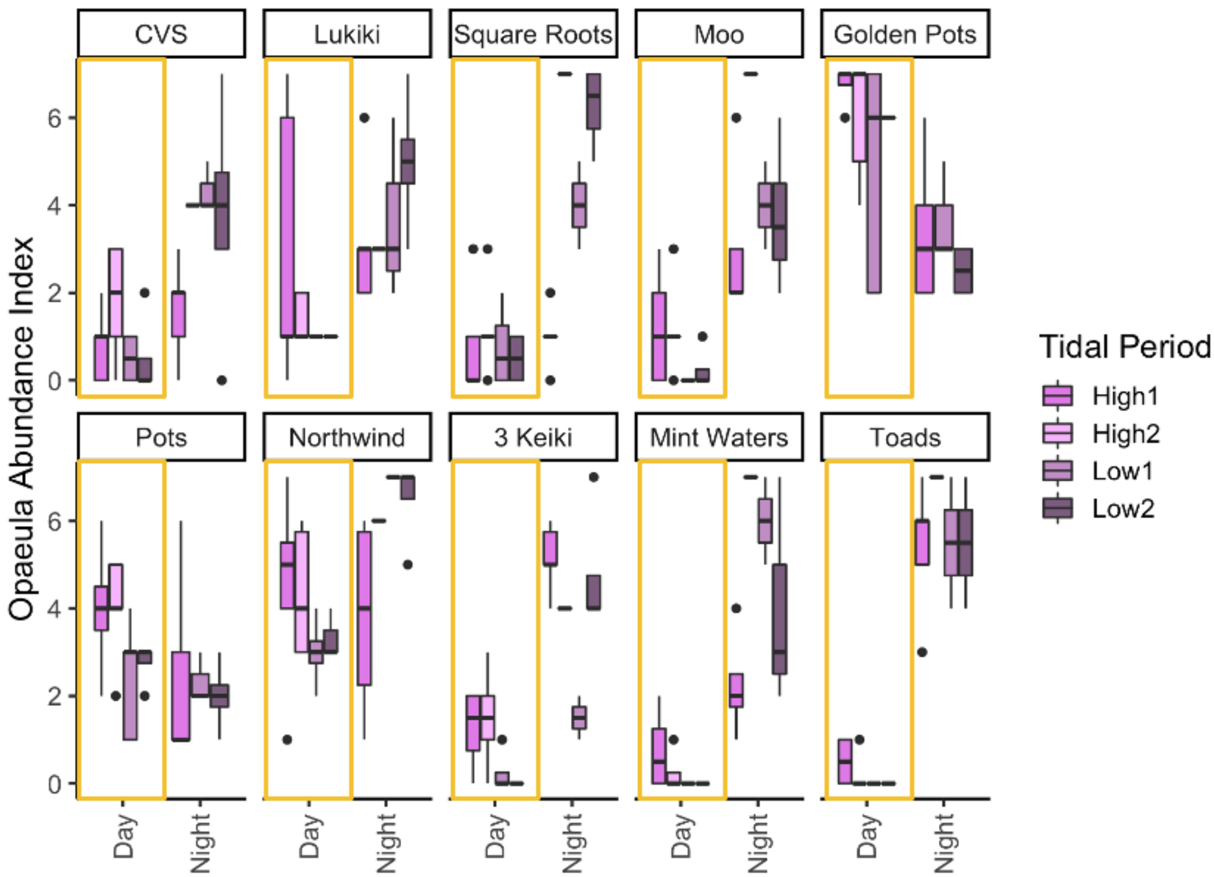


Figure 11. ‘Ōpae‘ula abundance index by tidal period and time.

Boxplots of ‘ōpae‘ula abundance index during the different tidal periods and time for each site. Day time tidal periods are indicated with a yellow box. When tidal periods were paired with time, the mean differences between the variables were greater than the differences when only comparing each variable, separately.



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APPENDIX I

Sampling report used to record kilo and YSI data.

ʻŌPAEʻULA FIELD SAMPLING REPORT **MOON:**

Observer/Sampler Name: ____________________ Contact Person: ____________________

Sample ID: ______________________________ Tide: ____________________

Date: _______________ Time: ______________ Last Sampled: (________________)

Location: ______________

FIELD TESTS:

Temp °C : ______________
DO% : ______________
DO (mg/L) : ______________
Sal (ppt) : ______________
Spec. Cond (μs/cm) : ______________
pH : ______________
Color : ______________
Odor : ______________

Weather:

Gen. obs. about area:

Count per Quadrant:

Predator Estimation and behavior (i.e. hunting, sleeping, chilling):

Habitat description (i.e. grass, rocks, algae):

Hiding spot estimation:

Algae/Bacteria Crust estimation:

APPENDIX II

Models used in determining which factors significantly affected ‘ōpae‘ula observable abundance. Model (1) is the null model that was used. Models (3) was the only significant single variable model. Model (5) was the best overall model according the AIC ranking.

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + \varepsilon \quad (1)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Tide\beta_{td} + \varepsilon \quad (2)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Time\beta_t + \varepsilon \quad (3)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Moon\beta_m + \varepsilon \quad (4)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Tide\beta_{td} + Time\beta_t + \varepsilon \quad (5)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Tide\beta_{td} + Moon\beta_t + \varepsilon \quad (6)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Time\beta_{td} + Moon\beta_t + \varepsilon \quad (7)$$

$$OAI = \beta_0 + Site_{0s} + Region\beta_r + Time\beta_{td} + Moon\beta_t + Tide\beta_{td} + \varepsilon \quad (8)$$