Kū Hou Kuapā: Cultural restoration improves water budget and water quality dynamics in Heʻeia Fishpond

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Abstract: In Hawaiʻi, the transition from customary subsistence flooded taro agroecosystems, which regulate stream discharge rate trapping sediment and nutrients, to a plantation-style economy (c. the 1840s) led to nearshore sediment deposition – smothering coral reefs and destroying adjacent coastal fisheries and customary fishpond mariculture. To mitigate sediment transport, Rhizophora mangle was introduced in estuaries across Hawaiʻi (c. 1902) further altering fishpond ecosystems. Here, we examine the impact of cultural restoration between 2012-2018 at Heʻeia Fishpond, a 600-800-year-old walled fishpond. Fishpond water quality was assessed by calculating water exchange rates, residence times, salinity distribution, and abundance of microbial indicators prior to and after restoration. We hypothesized that R. mangle removal and concomitant reconstruction of sluice gates would increase mixing and decrease bacterial indicator abundance in the fishpond. We find that Heʻeia Fishpond’s physical environment is primarily tidally driven; wind forcing and river flux are secondary drivers. Post-restoration, two gates in the northeastern region account for >80% of relative flux in the fishpond. Increase in exchange rates during spring and neap tide and shorter minimum water residence time corresponded with the reconstruction of a partially obstructed 56 m gap together with the installation of an additional sluice gate in the fishpond wall. Lower mean salinities post-restoration suggests increased freshwater influx due to R. mangle removal. Spatial distribution of microbial bio-indicator species inversely correlated with salinity. Average abundance of Enterococcus and Bacteroidales did not significantly change after restoration efforts, however, average abundance of a biomarker specific to birds nesting in the mangroves decreased significantly after restoration. This study demonstrates the positive impact of biocultural restoration regimes on water flushing and water quality parameters, encouraging the prospect of revitalizing this and other culturally and economically significant sites for sustainable aquaculture in the future.

Keywords: mariculture; aquaculture; community restoration; conservation ecology; Native Hawaiian fishpond; microbes; microbial source tracking
1.0 Introduction

1.1 Native Hawaiian fishpond mariculture and food security

As the catch rate of our global fisheries levels off due to degradation of the environment and even collapse of specific fish populations, the demand for aquaculture production of fish is projected to increase markedly [1]. Concerns over sustainable food production have brought indigenous models of resource management to the fore. Hawai‘i currently imports about half of our seafood [2] and local aquaculture is estimated to supply only ~20,000 lbs annually [3], but this was not always the case. For centuries, Native Hawaiians developed marine aquaculture that utilized natural enrichments via freshwater from surface and submarine groundwater discharge in managed estuaries, fishponds (loko i‘a) [4]. These walled fishponds (loko i‘a kuapā) were intentionally built in natural embayments at the interface of freshwater streams and the ocean where nutrients from streams promoted the growth of primary producers in constrained brackish ecosystems. The kuapā regulates freshwater inflow to size slotted sluice gates (mākāhā), creates a low wave energy environment within the fishpond, impedes water flux into and out of the fishpond and ensures that a minimum volume of water is retained in the fishpond at all times, especially at extremely low tides. In this system, unicellular photosynthetic microbes form the base of a complex food web that yielded energetically efficient protein production of crustaceans and herbivorous fish species. Fishpond stewards (kia‘i i loko i‘a) practiced stock enhancement, leveraging knowledge of juvenile fish migration to trap target species behind mākāhā until reaching maturity and preventing entry of large predators. In addition, kia‘i i loko regulate flux or harvest fish by blocking mākāhā. It is estimated that fishponds in Hawai‘i could have yielded approximately 2 million pounds of fish per year total historically [5,6].

1.2 The legacy of land use change and invasive species on loko i‘a

Physical changes (sedimentation, storm damage, development, disuse) and biological invasions have dramatically altered many loko i‘a. Beginning in the 1800s, a shift from subsistence to plantation economy led to erosion and siltation of the nearshore environment. In an attempt to mitigate and stabilize these impacts, mangroves were introduced to Hawai‘i in 1902 [7]. Mangroves are highly appreciated in their native habitats for the ecosystem services they provide throughout the tropics - shoreline protection and sediment stabilization [8], litterfall subsidy [9] and provision of nursery grounds [8]. Thus, by modifying their environment, mangroves have cascading effects for resident biota, acting as important ecosystem engineers.

However, in Hawai‘i, mangroves have caused a variety of negative ecological and economic impacts that motivate their removal [10]. Mangrove’s preference for halotopic ecotones favor their growth in estuaries with their root systems obstructing mākāhā, decreasing water velocity, flushing, circulation and residence time of loko i‘a and the streams that feed them [11–13]. Instead of sandy habitats, mangrove vegetated areas have high sedimentation rates and anoxic sediments due to bacterial decomposition of mangrove leaf detritus [11,14]. Moreover, mangrove draws down nitrogen and phosphate and decrease dissolved oxygen from overlying waters, potentially inhibiting primary production rates in loko i‘a [13]. Importantly, the absence of mangrove feeding specialists in Hawai‘i have resulted in poor assimilation of mangrove-derived nutrients from introduced stands [15] because detritivores native to Hawai‘i are not adapted to utilizing mangrove detritus, which tends to be tannin-rich and nitrogen-poor [16].

Post-World War II, a combination of urbanization, the introduction of invasive species, as well as stochastic events (e.g., storms, floods, tsunamis and lava flows) resulted in widespread deterioration of Hawaiian fishponds across the state [6]. By 1977, only 28 loko i‘a were still in production and by 1985, merely 7 loko i‘a were in commercial or subsistence use [6]. The loss of actively maintained loko i‘a exacerbated the spread of invasive mangrove in coastal estuaries [17].

1.3 Biocultural restoration of loko i‘a: He‘eia Fishpond as a model

Driven by a desire to re-establish customary practices, provide economic opportunities to local communities and improve production of crustaceans and herbivorous fish, a grassroots movement...
of loko i’a restoration has gained momentum since the early 2000s [18–20]. Hui Malama Loko I’a is a statewide network of indigenous kia’i loko dedicated to restoring loko i’a for food production [21]. Loko i’a restoration generally entails mangrove removal and dry stacking of basalt with coral/rubble internally. Typical mangrove clearing practices in Hawai’i include the removal of the above-sediment mangrove biomass, leaving intact the prop roots and the root-fiber mat within the sediment. Despite the increase in loko i’a restoration across the state, we know of no published data on the effects of mangrove removal and loko i’a infrastructure repair on water circulation dynamics and water quality.

Located on the windward side of O’ahu Island, Hawai’i (Figure 1A), He’eia Fishpond (also known as Pihi Loko I’a) is a loko i’a kuapā estimated to have been built 600-800 years ago atop the Malauka’a fringing reef [22] and has been at the forefront of fishpond restoration in Hawai’i. Rhizophora mangle was introduced to the He’eia estuary in 1922 to control runoff from upstream agriculture and stabilize sediments [11,15]. The circulation and flux patterns within He’eia Fishpond were compromised during the Keapuka Flood. On May 2, 1965, the highest discharge rate on record from Ha’ikū and Ioleka’a streams occurred during the Keapuka Flood [23]. Flood waters first broke the kuapā in the northwestern sector adjacent to He’eia Stream, creating a 183 m opening in the loko i’a wall. Historical tidal data [24] indicate that the flood likely occurred during a perigean spring tide (a.k.a. King Tide), thus the build-up of internal pressure within the loko i’a coupled with an extremely low tide outside the loko i’a, likely caused a 56 m break in the kuapā on eastern seaward side as well (Figure 1B, “Ocean Break”).

Figure 1. Study site: He’eia ahupua’a and fishpond. (A) The He’eia ahupua’a (social-political governance unit, usually organized along watershed boundaries) is located on the northeast/windward side of O’ahu Island, HI. He’eia ahupua’a is outlined in yellow, He’eia Stream (blue line) originates as Ha’ikū Stream near the ridgeline of the Ko’olau Mountains and converges with Ioleka’a Stream before entering Hoi wetlands and flowing into and past He’eia Fishpond (shaded red) into Kāne‘ohe Bay. Weather stations on Moku o Lo’e and Luluku (HI15) rain gauge are indicated by white dots. (map downloaded from USGS National Map Viewer) (B) Bio-cultural restoration over the course of this study. Freshwater and marine inputs into He’eia fishpond via mākahā (sluice gate) locations and names, yellow: community stewards Paepae o He’eia, white: He’eia Coastal Ocean Observing System; time period of this study (black line) in the context of the
chronosequence of mangrove removal and wall rebuilding. From 1965 - 2015 a 100 m break in the kuapā (C) altered flow patterns in the fishpond. From 2014-2015, Paepae o He’ eia (POH) and community volunteers repaired the kuapā and built a mākāhā (Kaho’ okele) (D), (E) From 2014-2017, POH removed invasive R. mangle and repaired kuapā and mākāhā infrastructure on the north quadrant of the fishpond bordering He’ eia Stream (photo courtesy of Sam Kapoi).

As a result of the shift from a constrained to a radically unconstrained system, the fundamental functioning of the loko i’ a changed: the volume became strongly tidally dominated and fish production using customary mariculture techniques could no longer be practiced. A dense mangrove forest around the mouth of He’ eia stream expanded into the loko i’ a, growing along and eventually obscuring the kuapā and effectively decreasing the amount of surface freshwater delivered to the loko i’ a. Sediment loading from He’ eia Stream, agriculture and urbanization overwhelmed the original mechanisms by which material was flushed out of the loko i’ a [25]. Progressive accumulation of terrigenous particulates on the coral benthos, accelerated by a dense mangrove root mass decreased the average fishpond depth to ~1 m [26]. These conditions - increased salinity, organic matter, and turbidity likely facilitated a shift in the biological diversity and composition of the loko i’ a away from desirable aquaculture species and toward invasive macroalgae.

Though limited restoration in the past 25 years enabled conventional net pen aquaculture in the loko i’ a, the ecosystem became steadily more eutrophic. In 1988, Mark Brooks leased the fishpond, installing a 0.9 m retaining wall of cement cinder blocks that reduced the tidal influence and prevented water exchange except at spring tides (Figure 1C). In addition, a previous flood in 1927 deposited a portion of the kuapā into the interior of the loko i’ a creating a mangrove stand where a 2,000-3,000 introduced cattle egrets (Bubulcus ibis) established a rookery (Fig 1B). The potential for human and animal health impacts from microbial contamination is a central concern in maintaining an ecologically balanced and productive loko i’ a [27,28]. Limited circulation within He’ eia exacerbates this issue, particularly from given the rich source of guano and nutrients produced by the egret colony.

In the present study, we partnered with the non-profit organization Paepae o He’ eia, kia’ i loko of He’ eia Fishpond, to assess the impacts of restoration from 2012-2018. Since 2001, Paepae o He’ eia has sought to foster cultural sustainability and restore and maintain a thriving fishpond for the local community by linking traditional knowledge and contemporary management practices. With the help of over 50,000 community volunteers, Paepae o He’ eia has resurrected the kuapā along its historical footprint and progressively removed over 2 km of invasive R. mangle (Figure 1B-1E). In this contribution, we addressed the following questions: 1) How does kuapā infrastructure repair including mangrove clearance around the fishpond periphery affect circulation dynamics such as water exchange rates, residence time? 2) How does the potential for increased freshwater and marine inputs alter the overall salinity distribution of the loko i’ a? and 3) How do these changes in the physical characteristics of water in the loko i’ a alter microbial bioindicators for fecal contamination?

2. Methods and Materials

2.1 Study site

He’ eia Fishpond (21°26’10.74” N, 157°48’28.05”W) is a 0.356 km² embayment located on the windward side of O’ahu Island, Hawai’i (Fig 1A). The loko i’ a is completely enclosed by 2.5 km of kuapā and is bordered by Kāne’ ohe Bay to the south and east, He’ eia Stream to the north, and a remnant irrigation ditch (auwai) running longitudinally along its entire west bank. The Ha’i’ikū Stream near the ridgeline of the Ko’olau Mountains converges with the ‘Ioleka’a Stream and becomes He’ eia Stream before entering the Hoi wetland. Within the Hoi wetlands, a portion of He’ eia Stream is diverted through a network of auwai, irrigating taro patches. At the terminus of the watershed, He’ eia Stream historically splits, either flowing south in the auwai that parallels He’ eia Fishpond or east toward Kāne’ ohe Bay. A forest of R. mangle occupies the northwest and western periphery of He’ eia Fishpond.
Mākāhā are interspersed along the kuapā, connecting the fishpond to exterior water sources and regulating surface and seawater exchange with the fishpond (Figure 1B, Table 1). Hereafter, names of mākāhā follow the convention used by Paepae o He‘eia. Designations from previous studies [29,30] are also given. For the past 50 years, mākāhā channels in He‘eia Fishpond have concrete floors with vertical walls composed of basalt and coral rubble with either a semi-permeable barrier fence or grid constructed from wood or plastic (Figure 2). With the exception of Kaho‘okele, the floor of the mākāhā are slightly higher than the natural bottom of the loko i‘a. All fieldwork was conducted with the permission of Paepae o He‘eia and the private landowner, Kamehameha Schools (Joey Char, Land Asset Manager, Kamehameha Schools Community Engagement and Resources Division).

**Table 1.** Mākāhā locations, heading, and dimensions.

<table>
<thead>
<tr>
<th>Mākāhā</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Heading</th>
<th>Width (m)</th>
<th>Height (m)</th>
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<td>80°/260°</td>
<td>3.05</td>
<td>1.75</td>
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<td>48°/228°</td>
<td>1.88</td>
<td>1.19</td>
</tr>
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</table>

**Figure 2.** Post-restoration rating curves at each mākāhā over various tidal stages. Water volume flux (m$^3$ s$^{-1}$) relative to the water level (m) is shown for all 6 mākāhā, *best fit line* in red, 9% confidence intervals, dashed pink line. Positive values indicate flux and negative values indicate flux out of the fishpond.
To evaluate the current direction (\(\phi\)), water level (m) and water velocity (m s\(^{-1}\)) into and out of the fishpond, Sontek Argonaut Shallow Water (SW) Profilers (SonTek, San Diego, CA) were deployed over the course of 7 days in each mākāhā (Fig 2, Table S1). The Argonaut SW Profiler and battery housing was oriented facing into the channel and mounted to 0.7 x 0.7 m metal mooring with ~25 kg weights and placed at the bottom of each mākāhā channel. Several flood and ebb tide tidal cycles were recorded to capture one full neap and spring tide using a high-frequency measurement interval of 20 s with an averaging interval of 10 s. The blanking distance was set to the minimal amount of 0.07 m, as the mean water column was < 0.50 m.

Precipitation, tidal state, wind direction, and speed were used as criteria for selecting pre- and post-restoration dates (Table S2). Daily (cm 24 hrs\(^{-1}\)) and cumulative precipitation over 4 days (cm 96 hrs\(^{-1}\)) obtained from the National Oceanic and Atmospheric Administration Luluku (HI15) rain gauge station (http://www.prh.noaa.gov/hnl/hydro/hydronet/hydronet-data.php) consistent with previous studies [30,31]. Mean stream streamflow (mean m\(^3\) s\(^{-1}\)24 hrs\(^{-1}\)) calculated using data from US Geological Survey discharge station (Haʻikū Station #16275000) accessed from http://waterdata.usgs.gov. Wind direction and magnitude was determined from automatic weather station Moku o Loʻe (21.4339° N, 157.7881° W), 1.5 km from Heʻeia Fishpond (http://www.pacioos.hawaii.edu/weather/obs-mokuoloe/). The station is positioned ~5 m above sea level and is outfitted with an Eppley 295-385 nm ultraviolet (UV) radiometer, a LiCor 200SZ Pyranometer, and a LiCor Quantameter (400-700 nm). A sea level gauge with a water temperature probe, located ~10 m offshore of the weather station at a depth of ~1 m, was used for tidal data (http://tides.mobilegeographics.com/locations/3854.html).

Flux data and water velocity measurements (m s\(^{-1}\)) acquired from the Sontek Argonaut SW Profiler were used to generate rating curves for each mākāhā at each tidal state (spring flood tide, SF; spring ebb tide, SE; neap flood tide, NF; and neap ebb tide, NE) using the following equation:

\[
\phi = wd v, \tag{1}
\]

where \(w\) is the respective mākāhā width (m), \(d\) is the water level vector (m) changing over time with the tide, and \(v\) is the water velocity (m s\(^{-1}\)) through the mākāhā channel [29,30]. To account for bidirectional water flow in the mākāhā due to tidal forcing, water volume flux was determined for an entire tidal cycle at the following tidal stages: SF, SE, NF, and NE. Full tidal amplitude was used when splitting the data set based on tidal stage. Rating curves were fitted using a poly-fit function with a best-fit line and 95% confidence intervals in Matlab (The MathWorks Inc., Natick, MA). Here, flux values for Kahoalāhui/Triple Mākāhā were calculated by taking flow measurements at the northernmost mākāhā channel (Kealohi) and multiplying by three.

Based on water volume flux, mean and maximum flow through each mākāhā were calculated for four tidal cycles (SF, SE, NF, NE). To account for varying tidal cycle length caused by mixed semidiurnal tides in Kāneʻohe Bay, individual mākāhā flow rates were normalized by calculating the total volume of water (m) moving through a mākāhā channel at a given tidal cycle and the hourly flux rate.

Fishpond volume was calculated using 728 bathymetric depth measurements normalized to mean low low water from a reference HOBO® water level logger (Onset, Bourne, MA) deployed at an interior site within the fishpond (21.43466° N, W 157.80699° W) that recorded tidal fluctuations during bathymetry mapping [26], Timmerman et al., unpublished. In 2018, we deployed a HOBO® water level logger at the same location to recollect reference water level data over a 10-day period. Reference pressure data was corrected for atmospheric pressure fluctuations using a second HOBO® logger situated on land to record atmospheric pressure fluctuations.

To calculate post-restoration loko iʻa volume, the difference in reference tidal state from pre-restoration (2007) and post-restoration (2018) was applied to the bathymetry dataset at SF, SE, NF, NE tidal states with Station Moku o Loʻe as a reference. A rectangular grid with ~1 m spacing and a natural neighbor interpolation was adopted to estimate depths in between measured bathymetry points in Matlab. For each tidal state, a trapezoidal rule was used with no smoothing applied. The small mangrove island located in the northwest quadrant of the fishpond was excluded from our calculations.
To derive minimum residence time in He‘eia Fishpond, the amount of water exchanged during ebb flood transition was calculated for neap and spring tide using the following equations [30]:

$$\tau_{\text{HFS}} = \frac{\text{He‘eia Fishpond Volume Exchanged (spring high tide – spring low tide)}}{\text{He‘eia Fishpond Volume (spring high tide)}}$$  \hspace{1cm} (2)

$$\tau_{\text{HFN}} = \frac{\text{He‘eia Fishpond Volume Exchanged (neap high tide – neap low tide)}}{\text{He‘eia Fishpond Volume (neap high tide)}}$$  \hspace{1cm} (3)

To determine residence time, the following assumptions were made: fishpond water column is mixed uniformly, all flood and ebb tides are 6 hours long, and mākāhā are the only source of water exchange with the following equation,

$$\varphi = 0.01$$  \hspace{1cm} (4)

where $\varphi$ is the percentage of water remaining after 1 flushing cycle (12 hours) and $x$ is the residence time in flushing cycles.

2.3 Sampling regime

This study utilized on-going efforts by Nā Kilo Honua o He‘eia (http://nakilohonua.org), a He‘eia coastal ocean observing research collective at the University of Hawai‘i at Mānoa, which has carried out monthly sampling at He‘eia Fishpond since 2007 [29] to collect discrete samples. To minimize the variability of physical and chemical characteristics of the loko i‘a due to tidal exchange, samples were collected during neap tide over a period of 3–4 hr. The pre-restoration (2014) sampling grid was comprised of 10 stations within the fishpond (P1–P10) whereas the post-restoration (2017) sampling grid was comprised of 11 stations within the fishpond (L01-L10), each of the intact mākāhā (M01–M06), Tables S3 and S4 respectively. Reference endmembers for oceanic input were taken outside the kuapā at Kaho‘okele/Ocean Break (E01) whereas endmembers for surface freshwater were collected in He‘eia Stream between the Hoi wetland and He‘eia Fishpond outside the kuapā (E02). To minimize the disturbance of the water column and benthos prior to measurements, stations were approached against prevailing currents and winds. Salinity was measured using a YSI Professional Plus (ProPlus) multiparameter sonde (YSI Xylem Brand, Yellow Springs, OH). At each station, a measurement was taken ~ 5-10 cm below the water surface (“surface”) and 5-10 cm above the benthos (“bottom”) by allowing the instrument reading to stabilize for 2-3 minutes before recording values.

Eleven stations were selected for discrete sampling for microbes: Kaho‘okele/Ocean Break, Wai‘anae, and 9 stations in the fishpond interior. Pre-restoration (P01-P10, Ocean Break) and post-restoration (L01-L03, L06-L11, Kaho‘okele, Wai 2) locations differed slightly (Table S3, Figure 5A). At each station, 1L polycarbonate bottles were acid washed and rinsed with ambient surface water three times, before immersion at the surface to fill the bottle completely. Samples were stored at 4 °C and processed within 2 hr of collection. Seawater was filtered through a 47 mm diameter, 0.45 μm filter (MCE, Millipore, Sigma, Burlington, MA) and stored at -80 °C prior to DNA extraction.

2.4 Microbial source tracking

Total genomic DNA (gDNA) was extracted from filters using the PowerWater DNA Extraction kit (QIAGEN, Germantown, MD) following the manufacturer’s instructions. Quantitative PCR (qPCR) was used to determine the abundance of bacterial 16S rRNA genes from mammalian fecal indicator bacteria Enterococcus using assay Entero1a [32–34] and Bacteroidales using assay GenBac3 [35–37]. Quantification was performed with the KAPA PROBE FORCE qPCR system (Wilmington, MA) using KAPA PROBE FORCE qPCR Master Mix (20 μL reactions), 400 nM specific Taqman primers (Table 2) and template gDNA diluted 1:5. Standards were run in triplicate using an 8-point, 5-fold serial dilution. Cycling parameters for all assays were: 95 °C for 2 min, 45 cycles of 95 °C for 15 sec and annealing/extension at 60 °C for 30 sec. Ct values were converted to concentrations per 100 mL using the manufacturer’s software. The standards used for the Entero1a and GenBac3 assays were...
genomic DNA extracted from *Enterococcus faecalis* strain V583 (ATCC® 700802D-S™) and *Bacteroides thetaiotaomicron* strain VPI 5482 (ATCC® 29148™), respectively.

Primers previously shown to detect avian fecal contamination in water [38] were tested on *B. ibis* fecal DNA (Table 2). Briefly, fecal material was collected from birds present on the small mangrove island on the loko i’a interior. Total genomic DNA was extracted from avian feces using the DNeasy PowerSoil Kit (Qiagen, Germantown, MD) following the manufacturer’s instructions.

qPCR using GFC primers targeting the 16S rRNA gene from *Catellicoccus marimammalium* used the KAPA SYBR FAST qPCR system (20 μL reactions), 400 nM primers, and gDNA diluted 1:5. Cycling parameters were as follows: 95 °C for 3 min for enzyme activation, followed by 40 cycles of 95 °C for 3 sec and annealing/extension at 60 °C for 20 sec. Ct values were calculated as previously described with uncultured *Catellicoccus* sp. 16S rRNA gene, partial sequence (Genbank accession number JN084062) used as a standard.

### Table 2. 16S rDNA oligos used in this study.

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<td></td>
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</table>

### 2.5 Statistics

Statistical significance for pre- and post-restoration events was determined with a pairwise Welch’s t-test to account for differences in variance. Mean baseline events pre-restoration and mean baseline events post-restoration for salinity and log-transformed numbers of microbial biomarker abundance were compared with the t-test for statistical significance in R (R Foundation for Statistical Computing) with the p-value for statistical significance set to p < 0.05. In addition, correlation of GFC/GenBac3/Enterol1a distribution with salinity, date, and location was tested using a generalized additive mixed model (GAMM) in R (R Foundation for Statistical Computing). Mean baseline salinity and log-transformed numbers of microbial biomarker abundance pre- and post-restoration was plotted with a contour plot function in Matlab (The MathWorks Inc., Natick, MA).

### 3. Results

#### 3.1 Restoration from 2014-2018 shifted relative flux contributions of each mākāhā

Before the restoration, fish movement could not be regulated using the mākāhā system. Thus, Paepae o He’ eia centered their research, education, and aquaculture activities around quarter-acre fish pens. From 2006-2009 Paepae o He’ eia produced approximately 1.2 metric tons of Pacific threadfin. Two events occurred in 2009, in which there was massive fish mortality. These events prompted a re-evaluation of the use of conventional rearing techniques in He’ eia Fishpond. Repairing the kuapā would eliminate the need for net pen aquaculture, enabling fish stock to move throughout the entire fishpond toward cooler and/or more oxygenated areas in response to future environmental stress. Moreover, Paepae o He’ eia hypothesized that consistent freshwater input and nutrients, via functional mākāhā would increase primary productivity and subsequently increase the biomass of native herbivores in the loko i’a. Biocultural restoration from 2012 to 2018 targeted the gap in the seaward kuapā and the section bordering He’ eia Stream (Figure 1B).

The restoration phase involving repair of the 56 m kuapā gap spanned 2014-2015 and was known as *Pani ka puka* (Shut the door). Kia’i loke used traditional external materials (*pohaku pele*, basalt rock) and a mix of traditional and contemporary internal materials (*ko’a*, coral rubble, and remnant cinder blocks) to rebuild the north and south segments of broken kuapā simultaneously and meeting in the middle. Rather than rebuild a continuous kuapā spanning the entire seaward wall of the loko i’a, Paepae o He’ eia elected to install a new mākāhā (Kaho’okele) to increase fishpond circulation,
increase oxygenation of the water column, and promote recruitment of marine species (Fig 1 C-D).

Mākahā site selection was based on empirical kia‘i loko observations of areas with the highest abundance and diversity of marine life (ex. fish, oysters, macroalgae, sponges). Per Bernoulli’s principle, as the kuapā gap narrowed, the velocity of water exchange increased, scouring the bottom of the new mākahā to approximately 3 m, revealing the historic coral benthos. In support of their hypothesis, kia‘i loko noted an increase in fish aggregation around Kaho‘okele over the course of Pani ka Puka that has persisted.

Historically, the volume and location of surface water input into He‘eia Fishpond from the Hō‘oiki Stream and concomitant mangrove removal (Fig 1E) in order to alter the path of surface water into the loko i‘a. Over this period of this study, Paepae o He‘eia commenced kuapā restoration along He‘eia Stream and concomitant mangrove removal (Fig 1E) in order to alter the path of surface water into the loko i‘a. Kia‘i loko posited that restoring the wall and mākahā would increase the rate of water exchange and flow rate, which might improve fish passage into the estuary. R. mangle was initially removed from the remnant kuapā and nearby fishpond interior by clear-cutting and incineration on site. With the exception of 2014-2015, the mean rate of restoration was 154.84 ± 17.33 m yr⁻¹, totaling 619.35 m kuapā (Table S5).

3.1.1 Characterizing mākahā flux post-restoration (2018)

Four mākahā along the eastern kuapā (Hīhīmanu, Kaho‘okele, Nui, Kahoalāhui, Figure 2) were assumed to have bi-directional flow mediated by the semi-diurnal tidal cycle in Kāne‘ohe Bay. Three mākahā in the north and northwest sectors of He‘eia Fishpond were documented since the early 1900s to provide conduits for surface water inputs into the loko i‘a (Figure 1B). Wai 1 and Wai 2 were restored over the course of this study. Wai 1 is located closest to the mouth of He‘eia Stream and allows the bidirectional exchange of fresh and oceanic water [30] whereas Wai 2, located 100 m upstream, has a unidirectional flow of surface water into the loko i‘a. The most upstream mākahā was destroyed during flood events in 1927 and 1965 and has not yet been restored and, measurements with current meters in this area was not possible.

Meteorological conditions during water volume flux sampling events both pre- and post-restoration have a small amount of variability (Table S2). While daily rainfall ranged from 0.05 cm to 1.32 cm in 2012 (pre-restoration) (mean 0.76 ± 0.6 cm), it ranged slightly higher from 0 cm to 2.29 cm (mean 1.23 ± 0.87) in 2018 (post-restoration). Similarly, Ha‘i kū Stream discharge ranged from 0.04 m³ s⁻¹ to 0.07 m³ s⁻¹ (mean 0.06 ± 0.013) in 2012 (pre-restoration), and from 0.06 m³ s⁻¹ to 0.11 m³ s⁻¹ (mean 0.085 ± 0.03) in 2018 (post-restoration). Wind direction ranged from E to NE (average wind direction ~50°) with magnitude ranging from 10 to 13 knots pre-restoration and from E to NE (average wind direction ~60°) with magnitudes of 3-13 knots post-restoration.

Flood tide onset and end were defined as low slack water (LSW, flux = 0 m³ s⁻¹) tide stage and high slack water (HSW, flux = 0 m³ s⁻¹), respectively. Conversely, ebb tide onset and end were defined as HSW and LSW, respectively. LSW levels range from 0.2 m at Kahoalāhui to 0.65 m at Kaho‘okele and Wai 1, whereas HSW levels range from ~0.5 m at Kahoalāhui to 1.1 m at Kaho‘okele. It is likely that the water level at Wai 1 is consistently high due to continuous baseline stream flow.

Peak flood occurs mid-way between slack tides, thus the water level to water volume flux relationship, the rating curve, typically resembles a “C” curve or vertical sine function. Rating curves were generated to understand the influence of 4 tidal stages (Spring Flood, SF; Spring Ebb, SE; Neap Flood, NF; and Neap Ebb, NE) on water volume flux (m³ s⁻¹) and water level (m) for each mākahā with positive values corresponding to water volume flux into and negative values corresponding to water volume flux out of the fishpond (Figure 2). We noted that Wai 2 exhibits an atypical rating curve due to a wooden board in the mākahā that restricts discharge into the loko i‘a only when the water level is higher than the board (Figure 2, Wai 2).

Mean and peak water volume flux was highest during flood tides at all mākahā. The fastest mean water volume flux (4.18 m³ s⁻¹ at SF and 2.26 m³ s⁻¹ at NF) and peak water volume flux (9.70 m³ s⁻¹ at SF and 5.41 m³ s⁻¹ at NF) were recorded at mākahā Nui, Table 3. In addition, flood tidal cycle duration was shorter than ebb at all mākahā at both Spring and Neap, mean tidal duration was 5.23 ± 1.20 hr and 8.00 ± 0.84 hr for SF and NF, respectively, whereas mean tidal duration was 6.09 ± 0.73
hr and 15.67 ± 1.38 hr for SE and NE respectively. Taken together, the shorter lag time at high water vs. low water, longer-duration dropping tides and stronger flood than ebb currents suggest that Heʻeia Fishpond is a flood-dominant system.


<table>
<thead>
<tr>
<th></th>
<th>Mean flux (m³ s⁻¹)</th>
<th>Peak flux (m³ s⁻¹)</th>
<th>Tidal cycle duration (hr)</th>
<th>Cum. flux per tidal cycle (m³)</th>
<th>Flux rate (m³/hr)</th>
<th>Volume exchanged per tidal cycle (m³)</th>
<th>Relative flux</th>
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<td><strong>Spring Flood</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wai 2</td>
<td>0.05</td>
<td>0.16</td>
<td>4.43</td>
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<td>0.93</td>
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<td>1569</td>
<td>7140</td>
<td>3.37%</td>
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<td>0.92</td>
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<td>5601</td>
<td>24420</td>
<td>12.74%</td>
</tr>
<tr>
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<td>9.70</td>
<td>6.29</td>
<td>97800</td>
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<td>97800</td>
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</tr>
<tr>
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</tr>
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<td>Nui</td>
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<td>-76320</td>
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<tr>
<td>Kahoʻokele</td>
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<td>-3.12</td>
<td>5.50</td>
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<td><strong>Neap Flood</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>Wai 2</td>
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<td>0.20</td>
<td>7.41</td>
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<td>1863</td>
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<td>Hīhīmanu</td>
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<td>0.24</td>
<td>8.20</td>
<td>1560</td>
<td>190</td>
<td>1560</td>
<td>1.10%</td>
</tr>
<tr>
<td><strong>Neap Ebb</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wai 2</td>
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<td>5640</td>
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<tr>
<td>Wail</td>
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<td>-0.57</td>
<td>15.50</td>
<td>-9880</td>
<td>-637</td>
<td>-9880</td>
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</tr>
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<td>Kahoolāhui</td>
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<td>-0.30</td>
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<td>-17100</td>
<td>-1103</td>
<td>-17100</td>
<td>10.69%</td>
</tr>
<tr>
<td>Nui</td>
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<td>-3.19</td>
<td>14.09</td>
<td>-81298</td>
<td>-5770</td>
<td>-81298</td>
<td>50.83%</td>
</tr>
<tr>
<td>Kahoʻokele</td>
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<td>-1.80</td>
<td>17.10</td>
<td>-53280</td>
<td>-3116</td>
<td>-53280</td>
<td>33.31%</td>
</tr>
<tr>
<td>Hīhīmanu</td>
<td>-0.08</td>
<td>-0.25</td>
<td>14.34</td>
<td>-4020</td>
<td>-8020</td>
<td>-4020</td>
<td>2.51%</td>
</tr>
</tbody>
</table>

3.1.2 Changes in relative water volume flux post-restoration

We evaluated the relative contribution of each mākāhā to loko i'a water exchange during SF, SE, NE, and NE in order to gain insight into how restoration altered circulation in Heʻeia Fishpond. Prior to restoration, Ocean Break, the 0.9 m elbow wall bridging the 56 m gap in the eastern kuapā was lower than the adjoining sections of wall, restricting water exchange to high tidal stages, when the water level exceeded the height of Ocean Break. The highest mean water volume flux at all tidal states was measured at Nui, which accounted for roughly half of the total water volume exchanged during one tidal cycle post-restoration (Table 3, Figure 3). The newly added mākāhā channel at Kahoʻokele accounted for the second largest volume of water flux post-restoration with roughly a quarter of influx and a third of outflux (Table 3, Figure 3). Kahoolāhui is comprised of three individual mākāhā post-restoration and together they account for the third largest water volume - roughly 10% of contribution to total water volume flux. The lowest water volume flux among the ocean-influenced mākāhā was measured at Hīhīmanu with considerably lower mean velocities and relative water flux contributions of < 5%. In 2018, flux rates measured at Wai 1 were similar to pre-restoration with a relative flux magnitude of 3-7% and low mean flow rates (Figure 3). Wai 2 displayed unidirectional flow into the fishpond only, regardless of tidal state with solely positive flow velocities and accounting for the lowest water volume flux measured.

When comparing site-specific volume flux rates pre-restoration (2012) to post-restoration (2018), it becomes evident that the relative magnitude of water volume flux specific to each mākāhā changed due to restoration practices: The total amount of water volume exchanged in a complete tidal cycle decreased from 241,413 m³ pre-restoration to 194,700 m³ post-restoration for flood tide and decreased from -241,685 m³ pre-restoration to -173,080 m³ post-restoration (Table 4). Pre-restoration, Ocean Break facilitated the largest amount of volume exchange contributing approximately ~80% to total water exchange at both flood and ebb tidal cycles (81.94% for flood, 79.76% for ebb) with mean water velocities of 11.53 m s⁻¹ and -13.55 m s⁻¹ [39]. Pre-restoration, Nui contributed the second largest
amount of volume exchange with 12.88% for flood and 11.12% for ebb tide and mean velocities of 1.75 m³ s⁻¹ and 0.5 m³ s⁻¹ [39]. While contributing only 10% to water exchange pre-restoration, post-restoration Nui is presently the site with largest water volume exchange. Post-restoration, Nui facilitated about half of the volume exchanged (50.24% at flood tide, 44.1% at ebb tide) with much higher mean water volume flux of 4.18 m³ s⁻¹ and -3.6 m³ s⁻¹ (Table 4) than pre-restoration. In contrast to pre-restoration, Kahoʻokele now accounts for the second largest volume exchanged (27.93% and 39.01% for flood and ebb tide respectively) with lower mean water volume flux of 2.02 m³ s⁻¹ and -1.1 m³ s⁻¹ compared to pre-restoration. The relative contribution in the magnitude of three channels constituting triple mākāhā increased about six-fold for flood tide and five-fold for ebb tide from pre-restoration to post-restoration (from 1.71% to 12.54% for flood tide and 2.41% to 11.68% for the ebb tide, Table 4). Hīhīmanu did not experience significant changes due to restoration: While accounting for 1.69% at flood and 2.03% for ebb pre-restoration, it now accounts for 3.61% and 2.76% at flood and ebb, respectively (Table 4). Mean water volume flux ranged from -0.12 m³ s⁻¹ to 0.28 m³ s⁻¹ pre-restoration and are now -0.17 m³ s⁻¹ to 0.39 m³ s⁻¹.

**Figure 3. Relative water flux post-restoration dominated by Mākāhā Nui, and Mākāhā Kahoʻokele.** Relative water flows through each mākāhā during spring flood tide; spring ebb tide; neap flood tide; neap ebb tide. Arrow lengths are visual representations of the relative magnitude of water flux at each mākāhā, normalized to the total flux for each respective cycle. Mākāhā location, filled red circles.

In terms of overall volume exchange, the river mākāhā continue to play minor roles in water exchange. Water volume flux at the two river mākāhā at Wai 1 overall has increased from pre-restoration to post-restoration: Water passing through Wai 1 increased from 0.93% pre-restoration to 5.1% post-restoration for flood tide, and 2.4% pre-restoration to 5.7% post-restoration for ebb tide. Water volume flux increased from 0.09 m³ s⁻¹ and 0.1 m³ s⁻¹ pre-restoration to 0.4 m³ s⁻¹ and 0.32 m³ s⁻¹ post-restoration. Pre-restoration Wai 2 accounted for 0.85% of water exchange during flood tide and...
accounts for a slightly decreased water exchange of 0.67% post-restoration for flood tide. For ebb tide, the water exchange reversed from 2.28% pre-restoration to -3.25% post-restoration.

Restoration resulted in a significant shift in water exchange in the seaward kuapā. Prior to restoration, Ocean Break accounted for the most water exchange during spring tide (~80%) whereas post-restoration, the relative contribution of Kaho‘okele was nearly one-third, at ~30% water exchange. Instead, Nui now exchanges the greatest water exchange, ~50%. Thus, the spatial pattern of flushing in He‘eia Fishpond remains dominated by the mākāhā in the northeast quadrant of the loko i‘a for all tidal stages. Nui, Kaho‘okele, and Kahoalāhu‘u together contributing for a water exchange of 92% of water volume at spring flood, 94% at spring ebb, 91% at neap flood and 95% at neap ebb tide whereas the southern and eastern edges of the fishpond experience relatively low flushing.

Table 4. Change in flux rates through mākāhā pre-restoration (2012) and post-restoration (2018).

<table>
<thead>
<tr>
<th>Mākāhā</th>
<th>Flood Tide</th>
<th>Ebb Tide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-restoration</td>
<td>Post-restoration</td>
</tr>
<tr>
<td></td>
<td>Volume exchange per tidal cycle (m$^3$)</td>
<td>Relative flux</td>
</tr>
<tr>
<td>Wai 2</td>
<td>2057</td>
<td>0.85%</td>
</tr>
<tr>
<td>Wai 1</td>
<td>2249</td>
<td>0.93%</td>
</tr>
<tr>
<td>Kahoolāhu‘u</td>
<td>4106</td>
<td>1.71%</td>
</tr>
<tr>
<td>Nui</td>
<td>31301</td>
<td>12.88%</td>
</tr>
<tr>
<td>Kaho‘okele/OB</td>
<td>197820</td>
<td>81.94%</td>
</tr>
<tr>
<td>Hīhi‘manu</td>
<td>4081</td>
<td>1.69%</td>
</tr>
<tr>
<td>Mākāhā Total</td>
<td>241,413</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

3.2 Decrease in loko i‘a volume and residence time post-restoration

The majority of the loko i‘a has relatively uniform and shallow bathymetry of ~0.9 m with the deeper portions around the mangrove island and Ocean Break [26]. Prior to restoration, water exchange along the eastern kuapā only occurred when the water depth exceeded the height of the elbow wall at Ocean Break. Pre-restoration, ~90% of loko i‘a water exchange occurred in the northeast corner of the fishpond via Ocean Break (~80%) and Nui (~10%), suggesting that the eastern half of the fishpond was better mixed and less stratified than the western side of the fishpond [30]. Timmerman et al., unpublished. Water volume exchange before restoration was also found to be largely tidally driven, with the greatest volume exchange at midtide: ~77% during spring tide and ~42% during neap tide.

Given changes in flux in He‘eia Fishpond due to restoration, we determined post-restoration loko i‘a volume and residence time for SF, SE, NF, NE. He‘eia Fishpond is deepest during SF tide (Figure 4A), averaging 0.89 ± 0.12 m with a minimum water depth of 0.63 m in the center of the loko i‘a and a maximum water depth of 1.46 m around the mangrove island in the northwestern corner of the fishpond. During SF, the maximal volume of the loko i‘a is 264,730 m$^3$ (Figure 4B). The minimal water volume occurs during SE tide when the loko i‘a is 48,060 m$^3$ or 20% of the SF volume (Figure 4B). The mean fishpond depth at spring ebb tide is 0.17 m ± 0.12 m and ranges from 0 m in the fishpond center to 0.74 m around the mangrove island in the northwestern corner of the fishpond. The NF tidal volume is 149,550 m$^3$, 56% of the SF tidal volume, with a mean depth of 0.50 m ± 0.12, ranging from 0.25 - 1.08 m. NE depth ranges from 0 - 0.79 m, averaging 0.22 ± 0.12 m. NE tidal volume is 63,160 m$^3$. Restoration regimes resulted in a considerable change of loko i‘a volume from pre- (2007) to post-restoration (2018): SE tide fishpond volume decreased 16,010 m$^3$, SF volume decreased 17,990 m$^3$, NE volume decreased 14,890 m$^3$ and NF volume increased 15,660 m$^3$ (Figure 4B). Thus, as a result of removing the elbow wall and installing a sixth mākāhā (Kaho‘okele), He‘eia Fishpond is shallower and has a lower volume at all tidal states except NF.

We calculated that post-restoration, approximately 82% of the fishpond water is exchanged during the ebb-flood transition at spring tide. During the neap tide ebb-flood transition, 58% of the fishpond water is exchanged. To be consistent with previous work by Young [30], we defined one
flushing cycle as the time that it takes to flush out 82% of fishpond water during spring ebb tide and to replenish that water again with new Kāneʻohe Bay water during spring flood tide or 12 hr. Based on the assumption that the incoming water would mix uniformly with the water remaining in the loko iʻa during the first flushing cycle (18%), about 3 flushing cycles are required to mix the initial 18% of water to a <1% dilution. Therefore, the post-restoration minimum residence time of Heʻeia Fishpond is ~32 hr or under 3 flushing cycles, and occurs during spring tide when water exchange is maximal. In contrast, when water exchange is minimal (e.g., neap tides), the maximum residence time is 64 hr. More than 5 flushing cycles or 64 hr are required to mix the 42% of water retained down to <1% dilution. Water exchange during ebb flood transition experienced a 4.51% increase (from 77.34% pre-restoration to 81.85% post-restoration, Table 4) at spring tide. During neap tide water exchange increased 16.06% (from 41.71% pre-restoration to 57.77% post-restoration, Table 4). As a result, minimum water residence time decreased from 38 hours at spring tide pre-restoration to 32 hours (~1.5 days) at spring tide post-restoration and maximal residence time during neap tides decreased from 102 hours (~8.5 days) at spring tide pre-restoration to 64 hours (~5.5 days) at spring tide post-restoration.

**Figure 4.** Comparison of Heʻeia Fishpond depth and volume pre- vs. post-restoration over various tidal stages. (A) Fishpond depth (m) for spring flood, spring ebb, neap flood, neap ebb pre-restoration (top row) vs. post-restoration (bottom row). (B) Fishpond volume (m³) for each tidal stage pre-restoration (grey) vs. post-restoration (black).

### 3.3 Spatial salinity distribution significantly altered due to restoration

The water column geochemistry of Heʻeia Fishpond is influenced by the mixing of distinct water masses: surface water from Heʻeia Stream, whose discharge depends on precipitation; submarine groundwater discharge, comprised of a mixture of fresh water from an underground aquifer and recirculated seawater [31]; and seawater from Kāneʻohe Bay that fluctuates with tidal pumping. Built at the interface of Heʻeia Stream and Kāneʻohe Bay, Heʻeia Fishpond exhibits a typical vertical salinity gradient - a less dense, freshwater lens atop a more dense, saltier water mass - although mixing of these water masses does occur with increased river flow, winds, and tides. A major motivation for the biocultural restoration of Heʻeia Fishpond was to increase the freshwater influence in the loko iʻa. Kiaʻi loko hypothesized that brackish conditions would drive primary production of diatoms - a major food source for juvenile mullet, which is a target species. Surface and bottom salinities were measured using a handheld YSI at several locations in Heʻeia Fishpond (Figure 5A). We selected two pre-restoration sampling events from 2014 and three post-restoration sampling events from 2017 with similar meteorological conditions (Table S3 and S4). Salinity measurements from pre and post-restoration work were analyzed as an indicator of fishpond circulation, mixing, and stratification.

Surface salinity distribution pre- and post-restoration display a strong spatial gradient (Figure 5B, left panels). The highest salinities in both cases were measured along the ocean-ward kuapā near Nui and the Ocean Break/Kahoʻokele (station P10), while the lowest salinity was measured along...
He‘eia Stream near Wai 2 (station P3, L07). However, mean pre-restoration salinity was significantly higher than post-restoration salinity, 27.4 ± 4.86 ppt and 20.5 ± 10.41 ppt, respectively (p-value < 0.01).

With similar meteorological conditions, these data indicate a weaker freshwater influence and stronger salinity gradient pre-restoration. Before restoration, the freshwater wedge did not extend past the western edge of the mangrove island, where salinities ranged from 20 – 25 ppt (stations P2, P4, P5) and further west, salinities rose to 25 – 30 ppt (stations P1, P6, P7, P8, P9). Post-restoration however, salinity ranged from 0.10 – 32.59 ppt with the freshwater wedge from the river extended beyond the mangrove island, which ranged from 15 – 20 ppt (stations L06, L08, L09), with further west salinities rising to above 20 ppt (station L01 and L05) and 25 – 30 ppt (stations L02, L03, L04, L11, M03). The presence of strong spatial gradient throughout the restoration process suggests that freshwater from He‘eia Stream is more prevalent along the northwestern side of the loko i‘a, whereas tidal pumping from Kāne‘ohe Bay dominates the southeastern side of the loko i‘a.

As expected, bottom waters of the loko i‘a had a higher salinity than the surface, however, post-restoration salinity exhibited limited gradient structure post-restoration, whereas the loko i‘a bottom pre-restoration was entirely homogeneously mixed with no detectable freshwater influence (Figure 5B, right panels). Mean bottom salinities were significantly higher pre-restoration (31.99 ± 1.82 ppt) as compared to post-restoration (25.17 ± 8.12 ppt), p-value < 0.1. Post-restoration, the influence of freshwater from He‘eia Stream became more evident, with the majority of the loko i‘a salinity ranging from 20-25 ppt (Figure 5B, lower right panel). Similar to the surface salinity spatial distribution, highest measurements were taken near the Kaho‘okele and Nui and the lowest measured bottom salinities were taken at Wai 2.

**Figure 5.** Average salinity of He‘eia Fishpond surface and bottom waters decreased due to restoration. (A) Discrete sampling sites for microbial indicator species in the water column (blue circles) and/or salinity (pre-restoration, red fill, and post-restoration, orange fill). (B) Heat map of salinity as a proxy for the relative proportion of freshwater and ocean water in the fishpond. Gradient of higher salinity in the eastern sectors of the fishpond bordering Kāne‘ohe Bay and lowest salinity near the diffusive flow region closest to He‘eia Stream and the unrestored portion of kuapā is typical of an estuarine saltwater wedge.

3.4 Restoration-driven changes to circulation altered microbial biomarker spatial distribution

To understand the consequences of Paepae o He‘eia’s restoration regime on biological-physical interactions in the loko i‘a, we quantified the abundance of microbial biomarkers that have been used previously to track fecal contamination within bodies of water. We focused on 3 specific bacterial
groups: Enterococcus and Bacteroidales, indicators of contamination from mammals and C. marimanimmarium, an indicator for contamination from avian sources, to investigate how increasing freshwater inputs into the loko i’a potentially affect the biogeography of pathogens.

Discrete samples were collected from a network of stations across the loko i’a along a transect from Wai 2 to Kahoolawe to capture the salinity gradient observed previously (Figure 5A, L03, L06, L07, L09, L10). In addition, we sampled at a higher resolution around the mangrove island on the interior of the fishpond in order to consider the influence of the large B. ibis rookery housed in the R. mangle stand. Contrary to expectations, amplification of the 16S rDNA genes from the family Bacteroidales (GenBac3) and the genus Enterococcus (Enter 1a) from samples pre- and post-restoration showed no significant difference when averaged across all stations (Figure 6A). We hypothesized that grouping together data may have masked changes in biomarker spatial distribution that occurred due to restoration. We mapped the mean concentration (16S copies 100 mL⁻¹) onto the stations and used a rectangular grid with ~1 m spacing to determine whether the biogeography of Enterococcus and Bacteroidales changed from 2014 to 2017 (Figure 6B and 6C respectively). We found that prior to restoration, the mean concentration of Bacteroidales was higher than 10³ copies per 100 mL across the entire western side of the loko i’a. In contrast, post-restoration, Bacteroidales concentrations higher than 10³ copies per 100 mL were restricted to a geographically smaller area of the fishpond, adjacent to Wai 2 and the diffuse flow region and lower in the center of the loko i’a (Fig 6B and 6C, top row). Indeed, when grouped by salinity, freshwater stations showed a statistically significant decrease in Bacteroidales concentration post-restoration (Figure 6D, top row, white). GAMM analysis confirmed that concentration of Bacteroidales negatively correlates with salinity (Figure 6E, top row), with the highest concentrations found at stations with the lowest salinity.

**Figure 6. Spatial distribution and mean concentration of Bacteroidales, Enterococcus and Fusobacteria pre- and vs. post-restoration.** (A) Tukey box-plot diagrams showing concentration, log (16S copies/100 mL) of Bacteroidales (GenBac3), Enterococcus (Enter 1a), and Fusobacteria (GFC) for before (grey) and after (white) fishpond wall restoration from all sampling sites, outliers and 95% confidence intervals are indicated. Heat maps of the averaged abundance of pre-restoration (B) and post-restoration (C) date Bacteroidales (GenBac3), Enterococcus (Enter 1a), and Fusobacteria (GFC). Tukey box plot diagrams of Bacteroidales (GenBac3), Enterococcus (Enter 1a), and Fusobacteria (GFC) abundance binned by salinity (freshwater, brackish and marine) of sites pre- and post-restoration, 95% confidence intervals and outliers are indicated. (D) Tukey box-plot diagrams showing...
concentration, log (16S copies/100 mL) of Bacteroidales (GenBac3), Enterococcus (Entero1a), and Fusobacteria (GFC) for before (grey) and after (white) fishpond wall restoration binned by salinity. Outliers and 95% confidence intervals are indicated, *p < 0.05, ***p <0.001.

(E) Correlation between salinity and biomarker concentration using a generalized additive mixed model.

We also found that when values were grouped across all stations Enterococcus concentrations did not change significantly over the course of restoration (Figure 6A, middle row). However, unlike Bacteroidales, the spatial distribution of Enterococcus pre- and post-restoration were structured with highest concentrations along the western edge of the loko i'a (10^4 copies per 100 mL) and a decreasing gradient proceeding to the east down to 10^2 - 10^3 copies per 100 mL (Figure 6B and 6C, middle row).

The lack of difference in pre- vs post-restoration data was also supported by binning the stations along a salinity gradient (Figure 6D, middle row). As expected, the general additive mixed model (Figure 6E, middle row) confirmed that Enterococcus has increased survival in low salinity environments.

To assess B. ibis fecal contamination, we first developed microbial source tracking tools by adapting primers specific to the 16S rDNA gene of C. marimammalium (Table 2, GFCf and GFCr). These primer pairs had previously been used to detect fecal contamination from gulls, geese, ducks, and chickens [38]. GFC primers specifically amplified fecal DNA from B. ibis living at He'eia Fishpond (Figure S1) and were used to determine the extent of contamination from B. ibis fecal sources in the loko i'a. Pre-restoration, B. ibis fecal contamination was significantly higher across all stations (mean concentrations of 2.4 x 10^4 copies 100 mL^{-1}) as compared to 10^3 copies 100 mL^{-1} post-restoration (Fig 6A, bottom row), p < 0.01. In general, the spatial distribution of pre- and post-restoration B. ibis fecal indicator bacteria had weak correlations with co-registered salinity measurements (Figure 5). Pre-restoration concentrations of B. ibis fecal indicator bacteria were higher across all stations than both Bacteroidales and Enterococcus (Figure 6B), with greater than 10^3 copies per 100 mL detected at the oceanic stations. In contrast, post-restoration concentrations of C. marimammalium decreased by 2 orders of magnitude, and these differences were statistically significant at the fresh and brackish stations (Figure 6D, bottom row). GAMM indicate that while the negative correlation between B. ibis fecal indicator bacteria and salinity is not as strong as with Bacteroidales and Enterococcus, it does exist.

We note 2 interesting differences in microbial indicator concentrations and biogeography post-restoration. First, we note the appearance of a region where microbial indicator concentrations are low (Figure 6C). We note that differences in the station locations pre- vs post-restoration may have altered the interpolation of biomarker concentrations. Alternatively, this may suggest that post-restoration, circulation patterns in the center of the fishpond have resulted in a well-flushed zone.

Secondly, all three molecular markers exhibited high variability 16S copy concentration pre-restoration as compared to post-restoration at the marine stations (Figure 6D). We interpret this difference as an indication that pre-restoration, the loko i'a was less homogeneously mixed than post-restoration.

4. Discussion

Embedded between land and sea, He'eia Fishpond is a powerful natural laboratory. We have been provided the unique opportunity to examine how historical land use change has altered the functions of coastal habitats and how biocultural restoration maintains and improves the integrity of these coastal ocean ecosystems in the face of rapid global change. In the current study, we utilized a comprehensive time series dataset of in situ deployments, discrete sampling, and empirical observations to draw a link between restoration efforts and changing fishpond circulation, as well as water quality dynamics. Specifically, we examined the impact of invasive mangrove removal around the northern fishpond periphery from 2014-2017 and Pani ka Puka, repair of the Ocean Break in 2015, presenting a comparison of pre- vs. post-restoration ecosystem dynamics along multiple parameters.
4.1 Ho’oniho ka niho: Water volume flux changes due to kuapā repair

Generally, understanding the physical environment of He’eia Fishpond will advance our knowledge of the dynamic biochemical and physical interactions in Hawaiian estuarine ecosystems. In repairing the physical infrastructure of He’eia Fishpond, Paepae o He’eia have set the stage for the ecology of the loko i’a to return to the original conditions engineered by kūpuna (elders, ancestors) of He’eia: a brackish body of water with a consistent volume, maintained by regulated mixing of fresh and marine inputs to facilitate phytoplankton growth. Our study confirms that during baseline conditions, coastal loko i’a circulation patterns are primarily driven by a combination of either tidal pumping or stream velocity, depending on the location of the mākahā [30,41]. Flux rates during SF and SE tides from mākahā bordering Kāne’ohe Bay (Hīhīmanu, Kaho’okele, Nui, Kahoalāhui), suggest that the fishpond is more influenced by oceanic inputs (> 95% total mean flux) than freshwater inputs (< 5% total mean flux) from He’eia Stream during baseline conditions at both pre- and post-restoration (Tables 3 and 4, Figure 3).

Prior to Pani ka Puka, He’eia Fishpond acted largely as an unconfined system during spring tides, when the spring flood tide exceeded the height of Ocean Break. In essence, because the Ocean Break was lower in height than the surrounding kuapā, the entire 56 m wide section of Ocean Break functioned like a mākahā when tidal pumping in Kāne’ohe Bay was higher than the provision elbow wall. Pre-restoration, we observed enormous water volume flux at spring tides, ~80% exchange almost exclusively from Ocean Break (Table 4). However, during neap tides, the loko i’a was more confined with less exchange and circulation in the southeastern portion of the fishpond. In 2015, this expansive section of the wall was repaired and Kaho’okele was built, shifting relative mākahā exchange rates at Kaho’okele to ~30% post-restoration. This dynamic is also reflected in mean water volume flux rates: Pre-restoration, Ocean Break had the highest mean water volume flux rates of ~12-14 m²s⁻¹ (Table 4), while the Kaho’okele flux rates post-restoration are dramatically lower (now ~1 m²s⁻¹, Tables 3 and 4). Mean water volume flux rates at other mākahā generally increased from pre-restoration to post-restoration, an indication that nearby mākahā somewhat compensate for the difference in flux between Ocean Break and Kaho’okele. However, the general “C” shape of rating curves remained similar (Figure 3). In its current state, the addition of Kaho’okele renders He’eia Fishpond a confined system at all tidal states with adequate water exchange in the southeastern region. These findings are supported by Ertekin et al. [42] who modeled circulation patterns at two different Ali‘i fishponds in Moloka‘i, which concluded that the number of mākahā plays a significant role in improving tidal circulation. They concluded that mākahā distance and location in relation to the physical forces at work (tidal activity, wind, fishpond bathymetry, stream location) affected circulation inside the fishpond.

Our results suggest that oceanic mākahā water volume flux is also dependent upon wind forcing, in particular for mākahā aligned with the trade winds (~70°). Nui and Kaho’okele account for ~50% and ~30% of total flux respectively, Figure 3. These mākahā also have the largest cross-sectional areas (Nui: 6.48 m; Kaho’okele: 3.05 m, Table 1), and are positioned most in-line with the predominant trade wind direction, Nui has a bearing of 63° and Kaho’okele has a bearing of 80° (Table 1). Wind blowing from the northeast across Kāne’ohe Bay, can accelerate (if the wind aids) or dampen (if the wind opposes) water flow through Nui and somewhat Kaho’okele, which is aligned with the predominant wind direction of 70°. We also noted that the channel floor of Kaho’okele is deeper than the adjacent benthos of both the loko i’a interior and Kāne’ohe Bay. Thus, the mākahā floor depth may allow slightly higher flux through Kaho’okele due to lower resistance to water volume flux. In contrast, Kahoalāhui and Hīhīmanu have considerably smaller relative flux (together accounting for ~13%, Figure 3) as the individual channels of Kahoalāhui have small cross-sectional areas and Hīhīmanu has the smallest cross-sectional area (2 m, Table 1), in addition to being positioned at 48° and 111°, respectively. The notion that wind can influence the rate of water flow through mākahā is supported by a study by Yang [43] who suggested that the rate of water flow through the mākahā may be altered by wind accelerating or dampening flow when the body of water was large enough. Kāne’ohe Bay and He’eia Fishpond are both large enough, and shallow enough to be affected by wind stress in such a way as to act as a secondary driver of flux in this system.

1 Interlock the stones [40]
We found that the river mākāhā have significantly lower relative flux rates during base flow conditions pre- and post-restoration (Wai 1 and Wai 2 together ~5%). Flux through Wai 1, the most seaward mākāhā along the Heʻeia Stream, is affected by tidal activity due to its proximity to Kāneʻohe Bay, making it the only freshwater mākāhā that allows bi-directional flow. Under baseline conditions, the relative flux of water passing through Wai 1 during flood tide is balanced by the amount of water flowing back out during ebb tide (Table 3). At flood tides, flowing out of Wai 1 is dampened by Heʻeia Stream, flowing in the opposite direction into the loko i‘a, while Heʻeia Stream flow is additive during ebb tides. Due to a dam-like structure in the mākāhā (Figure 2), Wai 2 has little to no detectable tidal signal and exhibit exclusive unidirectional flow from Heʻeia Stream into the loko i‘a that is largely dependent stream discharge and precipitation in the Heʻeia watershed [30,41,43]. During episodic storm events, strong freshwater influx can have pronounced effects on the fishpond system [30], yet our flux measurements were all conducted at baseline/low flow conditions. We anticipate that the relative contribution of river mākāhā vs. ocean mākāhā, as well as the balance between ebb vs. flood exchange, is likely to change if Heʻeia Stream discharge increases during storm events. Research comparing baseline to storm conditions to quantify how higher stream velocities affect loko i‘a flushing is currently underway and will be the subject of a subsequent contribution.

Assuming the Heʻeia Fishpond water balance is in steady state, the influx rates should be equivalent to outflux rates. However, we found the difference between spring and neap tidal cycle flow, the sum of flow (m³) for all mākāhā, to be -16,760 m³ or ~8% of total flow between SF and SE tide and 18,554 m³ or ~13% of total flow between NF and NE (Table 4). Post-restoration, this imbalance is most evident in 2 mākāhā: Kahoʻo okele, which accounts for 28% of influx, and 39% of outflux during spring tide, and Nui, which accounts for 40% of influx and 44% of outflux during spring tide. This pattern is evident at both spring and neap tidal cycles. We posit that trade winds accelerate flow into the fishpond at Nui during flood tide, which as previously discussed is aligned with the prevailing wind direction during sampling (63°, Table 1). However, during ebb tide, the wind force opposes outflow at Nui, and a small proportion of water flux is redistributed to other mākāhā channels thereby compensating for the reduced outflow at Nui (Table 3, Figure 4). However, these site-specific differences do not account for all the discrepancy observed pre- and post-restoration. We attribute discrepancies in flux balances to a number of factors. First, the influence of submarine groundwater discharge (SGD) into Heʻeia Fishpond is not accounted for in this study. Previous work quantifying SGD at Heʻeia Fishpond using radon isotope measurements found that the amount of water flux from SGD was equal to that of Heʻeia Stream discharge [31,44]. Second, the flux in the diffuse flow region (Figure 1B), as well as gains or losses of water through small holes in the kuapā, was not able to be quantified and has not been accounted for in our water budget. In addition, though every effort was made to choose tidal cycles similar in length and amplitude for rating curves, rating curves were calculated using in situ data from sequential rather than simultaneous deployments due to the limitation of instruments (Table S1). Some degree of variability in tidal length and amplitude among sites likely exists. Finally, the mixed semidiurnal tides cause large variations in tidal length (Table 3), giving rise to some uncertainty in the final water volume flux rates calculated.

4.2 Paepae ke alo: Volume, residence time, and salinity

Pani ka Puka affected loko i‘a volumes and residence times considerably (Figure 4). The addition of a sixth mākāhā (Kahoʻo okele) led to increased and faster outflux during both NE and SE tides, corresponding to lower volumes post-restoration. Conversely, whereas no water exchange occurred at Ocean Break during neap tides prior to restoration, Kahoʻo okele allows more influx during NF tide compared to before, resulting in a larger loko i‘a volume post-restoration during this tidal stage (Figure 4). These increased water masses cannot be compensated entirely with the flow (1 - 2 m³/s1) through Kahoʻo okele, which has a smaller cross-diameter, 3.05 m as compared to Ocean Break, 56 m (Figure 1C and 1D). We predicted a decrease in residence time post-restoration, particularly during neap tides Kahoʻo okele enables water exchange at all tidal states. We found that total exchange rates increased 5% during spring tides between pre- and post-restoration, and

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2 Raise the face of the wall [40]
minimum residence time during this tidal stage decreased 12% from 38 hr to 32 hr. For neap tides, water exchange increased 16% and maximum residence time decreased 37% from 102 hr pre-
restoration to 64 hr post-restoration. These data are tempered the following assumptions made in order to calculate residence time: (1) uniform mixing of the loko i’a water column, (2) all flood and ebb tides are 6 hours long, (3) mākāhā present the only source of water exchange. However, from salinity measurements at surface vs. bottom, it is evident that the water column is sometimes mildly stratified and not homogeneously mixed. Furthermore, upon analyzing the variability in length of tidal cycles, it becomes clear that there is a large range in variability (from 4.43 - 17.46 hr for tidal cycle duration, Table 3). Lastly, it is likely that there are other indirect sources of water exchange as SGD and the diffuse flow region. The difference in minimum and maximum residence times emphasizes the importance of differentiating between tidal states when looking at the effects of restoration on the physical environment of the loko i’a.

Concomitant mangrove removal around the stream mouth corresponded with an increase in water volume flowing through Wai 1 from ~1–2% pre-restoration to ~5% post-restoration (Figure 1E and Table 4) and a freshening of the loko i’a post-restoration. At the end of the period of this study, Wai 2 was not fully clear of R. mangle and also showed little change in discharge between pre- and post-restoration. We conclude that mangrove removal positively correlates with increased water flow and subsequently improved loko i’a circulation. Increased freshwater flux is also reflected in the salinity distribution, which shows a much stronger freshwater signal around the river mākāhā in post-restoration compared to pre-restoration (Figure 5B). We expect that continued removal of mangrove along the loko i’a periphery will increase stream velocity and the mass of freshwater entering He’e‘ia Fishpond. Moreover, the temperature of the surface water is often much lower than marine inputs and given concerns about fish stress linked to sea surface warming trends [29], mixing of cooler water may be beneficial to fish survival. In addition, increased freshwater and nutrient input may be beneficial for native macroalgae and phytoplankton to thrive, which is the primary food source for the herbivorous target fish species. While we can only speculate as to the historical biogeochemistry of He’e‘ia Fishpond, the abundance of evidence suggests that increasing freshwater input is necessary for proper management of native marine species. As this is the first study we are aware of that reveals a correlation between mangrove removal and improved loko i’a circulation, we recommend long-term monitoring of fish and phytoplankton diversity and biomass, particularly near the stream so that the connection between mangrove removal, stream flow, and nearshore fishery health can be fully understood.

4.3 Pani hakahaka³: Microbial indicators as markers of watershed connectivity

To assess water quality and associated human health risk, we used two broad-spectrum microbial bioindicators used by the US Environmental Protection Agency [45,46]. We used primers that targeted the Bacteroidales family (GenBac3) and the Enterococcus genus (Enterol1a), bacteria that are common in the feces of mammals, Table 2. These non-pathogenic microbes are easy to quantify and have decay rates similar to those of the pathogens of interest [47], hence, they can be strongly associated with the presence of pathogenic microorganisms derived from upstream in the watershed. By performing co-registered sampling of salinity and microbes, we were able to directly correlate fecal indicator concentrations with salinity, an abiotic factor that strongly influences survival rates [45,48,49]. We hypothesized that shorter residence time and increased water volume flux would lower the concentration of Bacteroidales and Enterococcus in He’e‘ia Fishpond. Instead, we found no significant difference in surface mammalian fecal indicator bacteria before and after restoration (Figure 6A). We found coherence between spatial distribution of mammalian fecal indicators with surface salinity (Figure 6B); post-restoration, lower salinity (e.g. more fresh water) in the northwestern sector of the loko i’a corresponded with even higher concentrations of surface bioindicators as compared to pre-restoration whereas higher salinity in the oceanic-dominated areas of the fishpond had even less fecal contamination than pre-restoration. As mammalian fecal contaminants are introduced via terrigenous freshwater runoff, the increase in river flushing detected post-restoration, we attribute the increase in mammalian fecal bacteria in the northwest area of the

³ Close gaps/vacancies [40]
We also evaluated an internal source of fecal pollution deriving from a large colony of *B. ibis* residing on the mangrove island on the loko `i`a interior. In order to quantify *B. ibis* fecal contamination, we optimized primers to *C. marinamammalium* (GFC, Table 2), an uncharacterized Gram-negative facultative anaerobe in the order of Lactobacillales (Fusobacterium) [50] originally developed to detect fecal contamination from gulls in coastal environments [38,51–53] for cattle egret fecal material (Figure S1). Unlike Bacteroidales and *Enterococcus*, we found a significant decrease in egret fecal bacteria post-restoration, suggesting that increased flushing and decreased residence times had a positive impact on water quality. The pattern of decreasing *C. marinamammalium* and consistent abundance of Bacteroidales and *Enterococcus* between the pre- and post-repair periods is intriguing and may be related to differential environmental reservoirs of the two clades targeted by the assays.

We also recognized the necessity of fixing *O. ioleka* to increased stream input. It is possible that mammalian indicator bacteria may accumulate in the freshwater lens at the surface of the fishpond, bacterial concentrations in bottom waters need to be considered. Because the expansion of freshwater niches are generally more favorable for these microbes to survive [49], these results emphasize the need for enhanced pollution reduction management upstream.

As Hawaiian watersheds are highly interconnected, loko `i`a provide snapshots of ecosystem health for the entire ahupua`a. Fecal contamination in our study site confirms the presence of leaking cesspools and/or septic tanks in the Ha`i`ikū and `Ioleka`a watersheds. This kind of pollution endangers plans for seafood production as well the public, who participate in numerous educational and cultural activities.

### 4.4 Pōhaku ka papale: Future Implications of revitalizing customary fishpond infrastructure

Since initiating wall restoration in 2005, Paepae o He`eia recognized the necessity of fixing Ocean Break in order to have a functioning fishpond. While aquaculture was possible using net pens, conventional methods were labor-intensive, expensive, and did not allow the entire 88 acres of the loko `i`a to be utilized for rearing fish. Large-scale fish mortality events in 2009, wherein only fish in the net pens died, served to further support the organization’s desire to return to traditional cultivation methods. The process to obtain the necessary federal and state permits to begin reconstruction took over three years. Importantly, the kia`i loko of He`eia Fishpond made the decision that restoring the Ocean Break would be an undertaking for the entire community, not just the organization. This approach to restoration not only revitalized the physical structure of the loko `i`a but revitalized the traditional Hawaiian practice of coming together as a community to maintain these cultural sites. Serendipitously, completion of the kuapā and Kaho`okele coincided with the 50th anniversary of the Keapuka flood, further affirming the appropriateness of taking a biocultural approach toward stewardship of this loko `i`a.

The design of the new kuapā with additional mākahā represents an innovation of the contemporary kia`i loko to mitigate future flooding risk. While deviating from historical photographs from the 1920s, it is likely that over the course of the 800-year existence of He`eia Fishpond kuapā infrastructure has been altered in response to hydrological and oceanic conditions. Kelly noted archeological evidence that the kuapā adjacent to He`eia Stream has been moved multiple times, potentially due to catastrophic floods [22], suggesting that placement and number of mākahā were dynamically managed. Paepae o He`eia revealed more contemporary evidence of this during the restoration of Nui, when concrete slotted mākahā, likely built in the 1900s, was found buried in the kuapā interior. Because kia`i loko were concerned about future floods and the integrity of a 3 m wall, they reasoned that having a mākahā would facilitate the release of water pressure during high flow events. The exact location of the mākahā was based on practitioner knowledge of

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4 Place the capstone on the top [40]
the circulation and biological diversity of the area. Thus, re-establishment of customary practices encompassed adaptation for increased resilience, as well as future fish recruitment.

A key dimension to restoring Heʻeia Fishpond has been the removal of invasive R. mangle, whose roots grow into the kuapā, separating the rock and coral. Furthermore, mangrove roots hold sediment transported from upstream and its leaf litter directly contribute to the organic matter in the pond, changing the chemistry of the benthos and water column. Mangrove canopies acted as a wind block, impeding circulation and oxygenation, creating heterogenous micro-niches within the loko iʻa. Moreover, kiaʻi loko observed that this non-native species also corresponded with the presence of non-native fish, and they speculated that mangrove removal would enable native aquaculture species to compete more effectively in this habitat, potentially by increasing fish passage into the estuary. Examining the rates of sediment transport from the loko iʻa out to Kāneʻohe Bay is needed, as well as a more comprehensive understanding of how this introduced species functions in non-native vs native landscapes.

Overall, this study clearly demonstrates the positive impact restoration regimes had on various physical and microbiological components of the fishpond ecosystem. Our results are encouraging and indicate that there is a significant potential for community-based restoration to revitalize this, and other, culturally and economically significant sites for sustainable aquaculture in the future. More recently, in part because of the ongoing concerted efforts of community organizations like Paepae o Heʻeia, the coastal area of Heʻeia was designated as National Estuarine Research Reserve (NERR) in January 2017 to advance research and protection of the Heʻeia ahupuaʻa by integrating the traditional Hawaiian ecosystem management approach with contemporary estuarine management practices.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Table S1: Heʻeia Fishpond in situ sampling regime, Table S2: Meteorological conditions pre- and post-restoration flux calculations, Table S3: Discrete sampling station pre- and post-restoration in Heʻeia Fishpond, Table S4: YSI and discrete sampling meteorological conditions pre- and post-restoration.


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