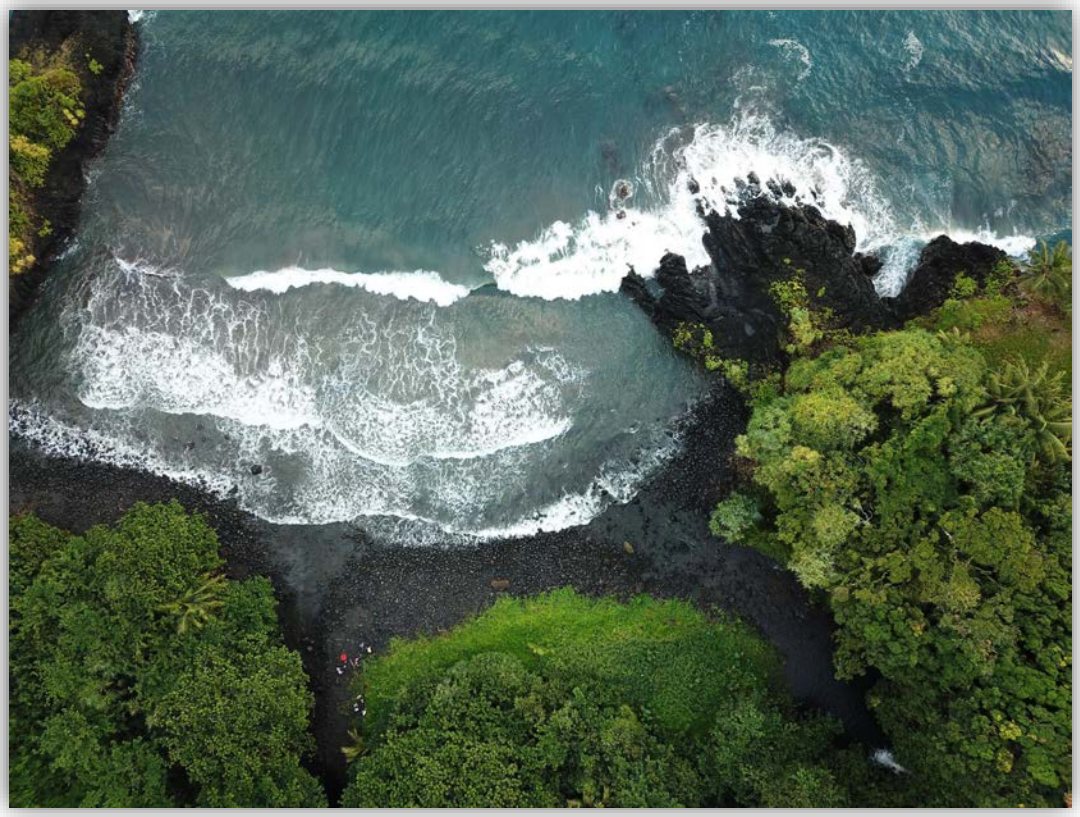
Division of Aquatic Resources

Aquatic species monitoring of East Maui streams and estuaries at 100% baseflow conditions

For the Commission on Water Resource Management



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Takeaways:

- CWRM and DAR expanded their collaboration of stream monitoring to study how stream biota responded to restoration of 100% baseflow conditions in East Maui at Waiohue, East Wailua iki and West Wailua iki streams. Additionally, two sites operating with permitted diversions were included for reference, Honomanū (East Maui) and Honokōhau streams (West Maui) as well as Keālia Lagoon (Central Maui).
- By leveraging USFWS Sportfish Restoration federal funding DAR expanded this East Maui study to measure how juvenile fish in estuaries responded to restoration of 100% baseflow in streams that provide freshwater to these essential fish habitats. Sites with permitted diversions were included in the estuary study also.
- Monitoring in both streams and estuaries yielded valuable data that will serve as baselines for comparison under future management practices in all six sites.
- This is the first study in Hawai‘i to include estuaries in determining best water management practices for streams.
- In estuaries this study demonstrated that juvenile fish are using this critical habitat in East Maui, an important result to improve sustainable fishing for many coastal species.
- At one estuary 65 fish species were recorded. This level of fish diversity in Hawaiian estuaries is comparable to levels found on Hawaiian coral reefs and underscores the importance of continued monitoring of Hawaiian estuaries, an understudied ecosystem.
- In streams monitoring documented both recruitment and upstream migration of native stream biota under conditions of continuous stream flow.
- DAR broadened our biological monitoring to include traditional monitoring practices in streams and estuaries as well as introducing a new method to measure biological diversity using environmental DNA (eDNA). This is a significant advancement for monitoring because Hawai‘i ranks high among global biodiversity hotspots.
- eDNA sampling also detected the presence of mammals, such as big horn sheep, pigs, cows, and rabbits, in watersheds. These results show that eDNA from aquatic habitats can be applied broadly to better gauge overall ecosystem health in entire watersheds.
- This study is contributing to our long overdue recognition in management that productive streams feed estuaries, and healthy estuaries are needed for sustainable fishing.

Summary. After more than a century of freshwater diversion, designated streams in East Maui were returned to natural flow conditions. This encouraging change in natural resource management restores habitat for the nine endemic species found in Hawaiian streams. The Commission on Water Resource Management asked the Division of Aquatic Resources to conduct a baseline study of stream biota under 100% baseflow conditions at Waiohue, East Wailua iki and West Wailua iki streams (Fig. 1). These baseline data are necessary to determine how aquatic species and ecosystems function at 100% baseflow conditions and to provide a means to compare how these species and ecosystems may respond under future water management practices that are planned for East Maui streams.

With this project DAR is applying several novel approaches to advance adaptive natural resource management. Monitoring in streams was extended to include juvenile sportfish monitoring in downstream estuaries. Estuaries are also freshwater-dependent ecosystems and serve as juvenile

habitat for many coastal species. Importantly, this is the first effort to introduce estuaries into discussions about water flow restoration in streams in Hawai‘i. In both freshwater-dependent ecosystems DAR is using traditional fish monitoring methods concurrently with biodiversity monitoring using environmental DNA (eDNA). Applying eDNA monitoring expands the scope of the project to include both vertebrate and invertebrate species. This is especially significant because many of these species are rarely detected by traditional monitoring methods. Additionally, two streams, Honokōhau and Honomanū, and a lagoonal estuary, Keālia Lagoon, currently operating with permitted diversions, are included for comparison (Fig. 1). This project highlights the efforts of DLNR to improve adaptive management of these important freshwater-dependent ecosystems as well as to enhance collaboration between DAR and CWRM.

Results to date indicate that by expanding the scope of biological monitoring, DAR is improving our understanding of the roles of freshwater inflow in streams and estuaries. Both visual monitoring (traditional) and eDNA biodiversity monitoring in streams recorded the same native species present. However, eDNA sampling in streams detected introduced species that were not found with visual monitoring. In estuaries, cast net monitoring (traditional) demonstrated clear evidence that juvenile fishes are using these sites. Results from eDNA biodiversity sampling were equally encouraging (Table 1). For example, at one site 65 fish species were recorded. Importantly, this is the highest level of fish biodiversity reported in a Hawaiian estuary to date and is comparable to fish diversity on Hawaiian coral reefs. Invertebrate biodiversity in streams reached 140 species at Honokōhau Stream. In estuaries invertebrate diversity was highest at Waiohue estuary with 104 species. Genetic markers used to detect fish species with eDNA sampling are broadly applicable to other vertebrate lineages, such as mammals. Biodiversity monitoring detected a broad list of introduced vertebrate species, such as sheep and cows, associated with streams and estuaries of Maui. By documenting the presence of large mammals in a watershed, managers can follow-up with this information to inspect for potential negative impacts by these introduced species, such as stream bank erosion.

Introduction. Freshwater-dependent aquatic ecosystems of Hawai‘i are largely shaped by precipitation. Rainfall at high elevations moves downslope to the coast, as both surface water and groundwater, forming complex hydrological processes that regulate, in part, biotic communities in streams, anchialine ponds, and estuaries. Aquatic life in freshwater-dependent ecosystems is managed by DAR. While surface water and groundwater resources, essential to the existence of these aquatic ecosystems, are regulated by CWRM.

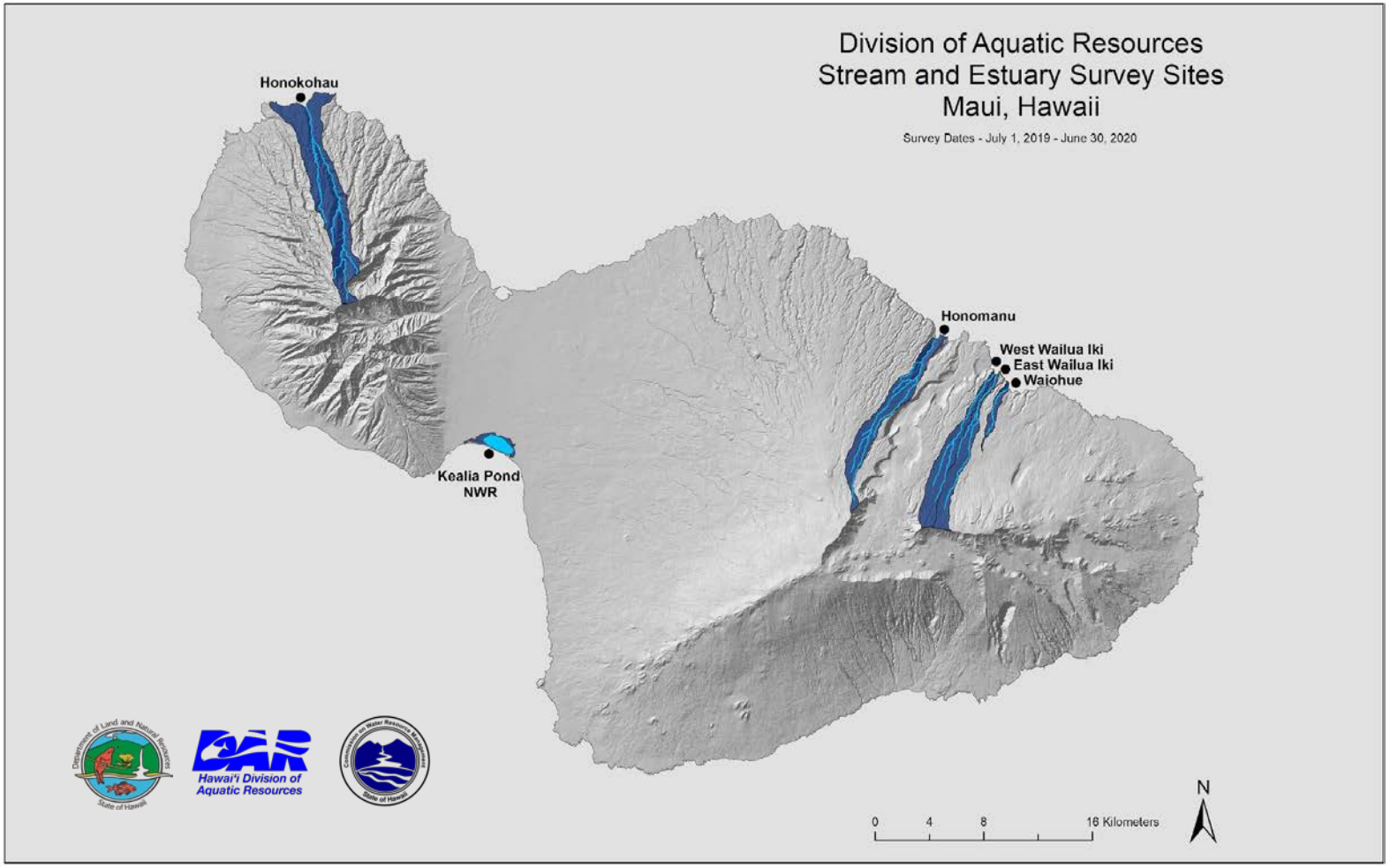


Fig. 1. The location of six Maui study streams and their watershed boundaries.

Table 1. Biodiversity detected from eDNA sampling in Quarter 1 (August 2020); Quarter 2 (October/November 2020; Quarter 2 (January/February 2021). Bold indicates total number of species detected in each watershed.

	Summer Quarter 1				Fall Quarter 2				Winter Quarter 3			
Site	**Vertebrate species**	**Fish species**	**Invertebrate species**	**All Species**	**Vertebrate species**	**Fish species**	**Invertebrate species**	**All Species**	**Vertebrate species**	**Fish species**	**Invertebrate species**	**All Species**
East Wailua iki Stream	–	–	–	–	8	5	30	38	13	9	82	95
West Wailua iki Stream	–	–	–	–	9	6	40	49	13	8	78	91
East Wailua Estuary	–	–	–	–	14	12	57	71	12	10	59	71
West Wailua Estuary	–	–	–	–	27	25	73	100	11	10	44	55
Wailua watershed	–	–	–	–	**31**	**25**	**97**	**128**	**24**	**18**	**109**	**133**
Paakea Stream/Estuary	–	–	–	–	9	6	44	53	7	5	61	68
Waiohue Stream	–	–	–	–	6	4	46	52	8	4	60	68
Waiohue Estuary	–	–	–	–	21	17	67	88	24	19	104	128
Waiohue watershed	–	–	–	–	**22**	**17**	**81**	**103**	**26**	**20**	**115**	**141**
Honokohau Stream	20	10	66	86	8	6	46	54	5	5	140	145
Honokohau Estuary	25	16	62	87	56	52	89	145	51	47	64	115
Honokohau watershed	**27**	**16**	**80**	**107**	**57**	**52**	**100**	**157**	**51**	**47**	**147**	**198**
Honomanu Stream	18	6	79	97	7	3	28	35	18	9	97	115
Honomanu Estuary	36	26	92	128	47	42	78	125	75	65	36	111
Honomanu watershed	**41**	**26**	**107**	**148**	**48**	**41**	**85**	**133**	**75**	**65**	**105**	**180**
Maalaea Bay	22	19	22	44	22	20	23	45	21	20	6	27
Kealia Lagoon	13	9	2	15	16	14	2	18	14	13	33	47
Kealia	**35**	**28**	**24**	**59**	**36**	**32**	**25**	**61**	**22**	**21**	**38**	**60**

Table 2. Table of surveys by type completed in streams and estuaries for each quarter.

	Summer Quarter 1				Fall Quarter 2				Winter Quarter 3				Spring Q4
Site	**Stream visual**	**Cast net visual**	**Cast net POE**	**eDNA**	**Stream visual**	**Cast net visual**	**Cast net POE**	**eDNA**	**Stream visual**	**Cast net visual**	**Cast net POE**	**eDNA**	**Stream visual**
East Wailua iki Stream								✓	✓			✓	
West Wailua iki Stream					✓			✓	✓			✓	
East Wailua Estuary						✓	✓	✓		✓	✓	✓	
West Wailua Estuary						✓	✓	✓		✓	✓	✓	
Paakea Stream/Estuary								✓				✓	
Waiohue Stream (Lower)					✓			✓	✓			✓	
Waiohue Stream (Upper)									✓				✓
Waiohue Estuary						✓	✓	✓		✓	✓	✓	
Honokohau Stream				✓	✓			✓				✓	✓
Honokohau Estuary		✓	✓	✓		✓	✓	✓		✓	✓	✓	
Honomanu Stream				✓	✓			✓	✓			✓	✓
Honomanu Estuary		✓	✓	✓		✓	✓	✓		✓	✓	✓	
Kealia Lagoon		✓	✓	✓		✓	✓	✓		✓	✓	✓	

For more than a century streams have been diverted for commercial agriculture and other human uses, a historically predominant management practice for this natural resource throughout Hawai‘i. The thinking at the time was: why waste freshwater by allowing it to flow into the ocean. For freshwater-dependent aquatic ecosystems the practice of diversion meant that fundamental hydrological and biological processes were severely altered resulting in persistently degraded habitats. For example, freshwater species require different ecosystems to complete their life cycle. Streams are habitat for adults and the ocean is where their larvae develop and disperse. The concrete and metal structures constructed to divert water in streams act as physical barriers to the natural movement among streams, estuaries, and the ocean that endemic freshwater species require. Present day management of water and aquatic resources is centered on finding balance for water demands among human uses and the diverse aquatic ecosystems that are dependent on freshwater inflow and connectivity.

This report summarizes the first year of a research and monitoring collaboration between CWRM and DAR. Findings will be applied to improve natural resource management using integrated and adaptive approaches for both DAR and CWRM. The study focuses on three streams in East Maui where water extraction practices ceased in 2016. Two additional streams and a lagoonal estuary with water diversions in place serve as sites for comparison. DAR has been collecting baseline data since August 2019 to improve our understanding of how aquatic species respond to the return of 100% baseflow as defined by CWRM. These baseline data are necessary to compare to data that can be collected in the future under water management practices that are planned for East Maui streams by CWRM. For example, certain streams are slated to be managed at 64% baseflow (H90) in the future. By collecting aquatic species data under differing baseflows, DAR and CWRM can better adapt our collective management strategies for freshwater-dependent ecosystems.

This study is notable because DAR is collecting baseline data that are aligned with our mission, which is to work with the people of Hawaii to manage, conserve and restore the state’s unique aquatic resources and ecosystems. Previously, to understand how biota respond to stream flow restoration, most of the work has centered on defining minimum flow standards, in selected streams, required for endemic stream fishes to complete their life cycle. While this approach remains important, with this study it was critical for DAR to take a different approach by broadening monitoring efforts to include species found in diverse aquatic ecosystems dependent on freshwater. In short, DAR retooled our monitoring approach with two key improvements. Specifically, because Hawai‘i is a biodiversity hotspot it is important for DAR to incorporate biodiversity monitoring for aquatic species. To achieve this, DAR is using environmental DNA (eDNA) to measure how flow restoration impacts both vertebrate and invertebrate species diversity. This is pioneering work because it applies eDNA sampling to compare biodiversity responses both spatially, among different sites and ecosystems, and temporally, among different seasons.

Additionally, DAR is expanding the scope of monitoring to include how estuaries, as juvenile fish nurseries, respond to flow restoration in their contiguous streams. This is the first-time in Hawai‘i that estuaries and their streams have been studied concurrently. Many estuaries are intimately linked to streams because streams are a chief source of freshwater inflow to estuaries. Estuaries with adequate freshwater inflow are thought to be larger and more productive compared to sites found downstream of highly altered and diverted streams. Also, stream species are strongly associated with estuaries during two stages in their life history. Initially, when larvae hatch in a stream they move as plankton through an estuary on their way to develop and grow in coastal currents. When the planktonic stage is nearing

completion and larvae are ready to return to streams to settle out as post-larval fish, they pass through estuaries for a second time. In healthy streams tens of thousands of freshwater larvae and post-larvae can pulse into or out of a single stream, far too many for any one stream to support. This productivity is ecologically important for estuarine food webs because freshwater larvae and post-larvae provide pulses of food for juvenile marine fishes that use estuaries as nursery habitats. *Productive streams feed estuaries, and healthy estuaries are needed for sustainable fishing*.

Methodology. From a list of streams in East Maui supplied by CWRM, DAR selected three streams/estuaries to monitor (Table 2) with 100% baseflow restored: East Wailua iki Stream, West Wailua iki Stream and Waiohue Stream (Table 3). Three additional sites currently operating with permitted diversions were selected by DAR, Honomanū Stream in East Maui, Keālia Lagoon in Central Maui, and Honokōhau Stream in West Maui. These diverted sites have estuaries that are currently monitored quarterly by DAR through its USFWS DJ Sportfish Restoration funded estuary project. For the estuary monitoring portion of this East Maui study DAR is leveraging federal funds to enhance and expand this state-funded project.

Table 3. Baseflow conditions at streams and estuaries monitored by DAR.

Site	Natural flow conditions	Current % Baseflow	Habitat	% Baseflow in future lease conditions
West Wailua iki	not diverted	100%	natural	100%
East Wailua iki	not diverted	100%	H90	64%
Waiohue	not diverted	100%	natural	100%
Honomanu	diverted	64%	H90	64%
Honokohau	diverted	<100%		<100%
Kealia	groundwater			

In both streams and estuaries two types of sampling were performed, traditional monitoring and eDNA biodiversity monitoring. An additional stream/estuary, Pa'akea Gulch, was sampled only with eDNA. Traditional monitoring in streams followed DAR-developed protocols for visual surveys to record species present, counts and size estimates of native freshwater species. For estuaries two independent DAR-developed protocols, visual cast net sampling and Probability of Encounter (POE) cast net sampling, were each used consecutively to report species present, counts, fork length, and to estimate weight (mass).

Environmental DNA (eDNA) is particles of organismal DNA that can be found suspended in the water column. eDNA originates from cellular material shed by organisms, such as mucus, skin, or excrement, in environments that can be sampled and detected using molecular methods (metabarcoding). To monitor biodiversity DAR collected and filtered water samples in both streams and estuaries. Laboratory work (metabarcoding) for eDNA samples was contracted to Hawaii Pacific University/Oceanic Institute (the contractor) and universal primers for vertebrates and invertebrates were used. Results, as partially processed data, for eDNA samples were provided by the contractor to DAR. Then DAR performed QA/QC protocols and analyzed eDNA results for this report. (This report has been updated from an earlier one that had eDNA results from only the first two quarters.)

This project is designed to be a time series study to reflect ecological conditions inherent to both Hawaiian streams and estuaries during wet and dry seasons. Streams are characterized as flashy. Heavy rainfall in the mountains can flood streams as water rushes to the coast. It is common for these freshets to be large enough to displace boulders, uproot vegetation along stream banks and scour out stream channels. These physical and ecological resets are natural and Hawaiian species are well-adapted to recover over time after these disturbances.

Estuaries in Hawai'i also have their own innate temporal variability. Unlike well-studied estuaries on the Mainland, sub-tropical estuaries in Hawai'i support settlement of juvenile fish species year-round, reflecting the wide range of reproductive strategies that are found in Hawaiian species. There are species that reproduce year-round. As a result, their larvae settle out of the plankton and into estuaries throughout the year. Other species are seasonal spawners, consequently juvenile settlement into estuaries also follows seasonal patterns. Therefore, monitoring strategies in both ecosystems must account for these underlying temporal shifts.

Each quarter is three months long and begins in July, following the state's fiscal year. Stream study sites were established beginning in the first quarter. In streams eDNA sampling began in quarter one at Honomanū and Honokōhau, while quarterly visual surveys started in quarter two (Oct-Dec 2019). In quarter two for the lower reach site of East Wailua Iki eDNA samples were collected and a study site was established however, due to fieldwork time restraints the site was not surveyed using visual methods until quarter three. In quarter three (Jan-Mar) project staff were unable to survey the lower reach of Honokōhau stream due to bad weather conditions. In quarter four (Apr-Jun), due to Covid-19 restrictions, project staff were unable to survey the lower reaches of Waiohue, West Wailua Iki, and East Wailua Iki streams. Estuary sampling began in August 2019 (quarter 1) and three sites (Honomanū, Honokōhau and Keālia estuaries) were surveyed for eDNA and cast net sampling using both visual and POE methods. For quarters 2 and 3 all six estuary sites sampling proceeded as planned however, public health restrictions affected the 4th quarter and no fieldwork was permitted.

Statements of Procedure (SOP) can be found in the appendix.

Results and discussion.

Site summary.

Wailua. Wailua is in the remote northeastern shoreline of Maui and includes East Wailua iki and West Wailua iki streams (Fig. 1). Both streams flow into Wailua Bay. These estuaries and lower stream reaches are accessed by helicopter.

East Wailua Iki Stream is perennial and has a total length of 9.6 miles (15.4 km). It has a stream order of 2. The watershed is made up of 100% conservation land. This project monitors two 100-meter reach sites on East Wailua Iki stream. The lower reach site is at an elevation of about 50 ft. The substrate composition in this lower reach consists of boulder, cobble, gravel, and some sand. The upper reach site is at an elevation of about 1200 ft. The substrate composition in this upper reach consists of boulder, cobble, and some bedrock and gravel.

West Wailua Iki Stream is perennial and has a total length of 9.4 miles (15.1 km). It has a stream order of 2. The watershed is made up of 99.7% conservation land, and 0.3% agricultural land. This project monitors one 100-meter reach site on the lower reach of West Wailua Iki. This lower reach site is at an

elevation of about 80 ft. The substrate composition in this lower reach consists of boulder, cobble, gravel, and some sand.

Three sub-estuaries make up Wailua Estuary: East Wailua iki and West Wailua iki riverine estuaries and Wailua Bay estuary. East Wailua Iki riverine estuary, sampled as an estuary site for this report, forms at the mouth of East Wailua Iki stream. The freshwater inflow at this site is not enough to keep the stream mouth open. As a result, a cobble berm is present most of the time. East Wailua iki riverine estuary ponds behind the cobble berm and opens only during flash flooding. Substrate composition of the estuary consists of mostly cobble and a few boulders. Water along the eastern shoreline remains turbid from detritus and suspended sediments due to water current and wind patterns within Wailua Bay. East Wailua estuary includes East Wailua Iki riverine estuary and eastern part of Wailua Bay.

East Wailua iki and West Wailua iki were sampled for eDNA during October 2019 (quarter 2) and January 2020 (quarter 3) (Table 2). Results for eDNA biodiversity sampling demonstrated similar results for both streams (Table 1). East Wailua iki had 5 fish species, 30 invertebrate species, and a total of 38 species for quarter 2. East Wailua iki in quarter 3 had 9 fish species, 82 invertebrate species, and a total of 95 species. Similarly, West Wailua iki recorded 6 fish species, 40 invertebrate species, and a total of 49 species in quarter 2. West Wailua iki in quarter 3 had 8 fish species, 78 invertebrate species, and a total of 91 species. For both streams' biodiversity was lower in quarter 2 compared to quarter 3 with temporal change in invertebrate biodiversity accounting for this difference.

West Wailua Iki at the lower reach was surveyed during October 2019 (quarter 2) and January 2020 (quarter 3). Hihiwai (*Neritina granosa*) and post-larvae fish recruitment occurred during these months and surveys documented presence of ʻOʼopu nopili (*Sicyopterus stimpsoni*), ʻOʼopu nakea (*Awaous stamineus*), and ʻOʼopu akupa (*Eleotris sandwicensis*).

East Wailua Iki at the lower reach was surveyed during January 2020 (quarter 3). Surveys documented *N. granosa* and post-larvae fish recruitment and a presence of *N. granosa*, ʻOʼpae ʻoehaʼa (*Macrobrachium grandimanus*), *A. stamineus*, *E. sandwicensis*, and *S. stimpsoni*.

West Wailua Iki riverine estuary, the second estuary site sampled, is located about 150 meters (500 feet) to the west of East Wailua Iki stream mouth. In contrast to East Wailua Iki, freshwater inflow is adequate to maintain continuous connection between West Wailua Iki stream and Wailua Bay. Previous surveys, before flow restoration, detailed a shallow stream mouth that was calf-deep and easy to cross. Surveys done for this report found dramatic changes to the geomorphology. Specifically, the banks of the stream have been eroded and the stream channel fronting the stream mouth is now greater than two meters deep. Benthic habitat consisted mostly of cobble and boulder. On the western portion of the bay water was consistently clear of sediment and detritus, in contrast to the eastern section of Wailua Bay. West Wailua estuary includes West Wailua Iki riverine estuary and western part of Wailua Bay.

East Wailua estuary and West Wailua estuary were sampled during October 2019 (quarter 2) and January 2020 (quarter 3) for both eDNA and cast net surveys. For East Wailua estuary in Quarter 2 a total of 71 species were detected using eDNA, including 12 fish species and 57 invertebrate species. Also, other vertebrates including the wild boar *Sus scrofa* was found. Similar diversity was sampled in Quarter 3 with 71 species, including 10 fish species and 59 invertebrates. Other vertebrate species sampled included the rat *Rattus rattus* and *S. scrofa.* The presence of land mammal eDNA in the waters

of an estuary, where rapid dilution of stream water is expected, may indicate the large populations of these mammals in close association to East Wailua iki Stream.

Diversity was higher in West Wailua estuary compared to East Wailua estuary in Quarter 2. There were 100 species detected in West Wailua estuary with 27 vertebrate species of which 25 were fish species. Like the vertebrate diversity, invertebrates were also more speciose in West Wailua estuary compared to the other site because the continuously flowing stream results in a more productive estuary. Quarter 3 results were significantly lower compared to quarter 2. These results are not considered ecologically significant considering that the contracted lab informed DAR that mistakes in sample processing had occurred in the lab. DAR requested the return of all unused eDNA samples from this lab. There is potential to rerun these samples in the future with another lab.

Results from cast net sampling support this conclusion. The total catch using both sampling methods at East Wailua estuary was nominal compared to results from West Wailua estuary. Four fish were sampled using visual method and two by POE and all were native species. Species richness was low with four different species documented by both methods. Three sport-fish species were documented by visual sampling methods and totaled four individuals: Two reticulated flagtail *Kuhlia sandvicensis*, one bluefin trevally *Caranx melampygus*, and one Hawaiian silverside *Atherinomorus insulalrum*. All were juveniles as indicated by their small sizes. POE sampling found 2 sport-fish: one juvenile *C. melampygus* and one adult sharpnose mullet *Neomyxus leuciscus*.

West Wailua Iki, in contrast, documented a total of N=119 specimens using both sampling methods. Visual methods accounted for 63 native fish and 4 introduced fish, while POE methods had 16 native and 36 introduced fish. Species richness was high with ten species collected by both methods. Visual methods recorded seven native and one introduced species and POE methods documented five native and one introduced species. The most abundant species sampled by visual sampling methods was *Kyphosus sandwicensis* Gray chub comprising 43% of the relative abundance. A total of 29 Gray chubs were caught, ranging from juveniles to adults (139 to 304 mm FL). This popular sport-fish usually appears grey in color however, there are different variations from yellow to white or multicolored. The yellow color variation was considered the “queen” of the school in old Hawaii. We observed a queen Gray chub in striking yellow color morph twice from the shoreline feeding with the school in the stream mouth. We did get the cast net over it once however, it managed to escape. It was a large adult. Hawaiian flagtail *Kuhlia xenura* was the second most abundant species caught at West Wailua Iki using visual methods with N=20 accounting for 30% relative abundance. Small juveniles (53mm FL) thru large adults (205mm FL) were documented. Five convict tang *Acanthurus triostegus* were collected and they ranged from juveniles to adults (78 - 159mm FL). Other sport-fish documented were striped mullet *Mugil cephalus,* bigeye trevally *Caranx sexfasciatus,* blackspot sergeant *Abudefduf sordidus*, and *Kyphosus hawaiiensis* Bicolor chub. There were four introduced kanda mullet *Moolgarda engeli* juveniles documented.

POE methods documented N=52 specimens from six species with the introduced *M. engeli* as the most abundant species (N=36; 69% relative abundance). *M. engeli* ranged from juveniles to sub-adults (112 to 173mm FL). The most abundant native species was *K. xenura* (21%) and all were juveniles (median 76mm FL). Other sport-fish documented were all juveniles of *C. sexfasciatus*, *M. cephalus* and *K. sandvicensis*.

Two sub-estuaries, less than 150 meters apart from one another, demonstrate the significance of freshwater inflow to estuaries. Results from both cast net sampling using visual and POE methods as well as eDNA sampling all clearly demonstrate that West Wailua estuary with its open stream mouth is measurably more productive for both juvenile and adult fishes than East Wailua estuary. There is both higher species richness as well as abundance at the site with continuous freshwater inflow to the ocean when compared to a site with lower inflow. Allowing more freshwater to enter Hawaiian estuaries is critical to the ecological restoration of this essential fish habitat.

Waiohue. Waiohue, is also along the remote northeastern shoreline of Maui. Two streams, Waiohue and Paakea, flow into Waiohue Bay. The estuary and lower stream reaches were accessed by helicopter for sampling. Waiohue's upper reach monitoring site is accessed by hiking in from the Hana Highway.

Waiohue Stream is perennial and has a total length of 2.6 miles (4.3 km). It has a stream order of 1. The watershed is made up of 99% conservation land, and 1% agricultural land. This project monitors two sites on Waiohue Stream; a lower 75-meter reach and an upper 100-meter reach. The lower reach site is at an elevation of about 15 ft. The substrate composition in this lower reach consists of boulder, cobble, gravel, and some bedrock and sand. The upper reach site is at an elevation of about 1300 ft. The substrate composition in this upper reach consists of bedrock, boulder, cobble, and some gravel.

Pa'akea Gulch is a perennial stream and has a total length of 2.1 miles (3.4 km). It has a stream order of 2. The watershed is made up of 69.6% conservation land, and 30.4% agricultural land.

Biodiversity in Waiohue Stream using eDNA reported 4 fish species, 46 invertebrate species, and a total of 52 species in quarter 2. In the following quarter 4 fish species, 60 invertebrate species, and a total of 68 species were detected.

Waiohue Stream at the lower reach was visually surveyed during October 2019 (quarter 2) and January 2020 (quarter 3). *N. granosa* and post larvae recruitment occurred in both quarters. Observations document the presence of *S. stimpsoni*, *A. stamineus*, and *E. sandwicensis*.

Waiohue Stream at the upper reach was surveyed during February 2020 (quarter 3) and June 2020 (quarter 4). Observations documented an abundance of Tahitian prawn *Macrobrachium lar* and small numbers of 'O'pae kala'ole *Atyoida bisulcata*.

Waiohue Estuary, a typical east Maui estuary with high surge, white water, and a narrow boulder beach, is composed of three sub-estuaries: Waiohue riverine estuary, Pa'akea riverine estuary, and Waiohue Bay. Both streams continually flow into the bay. One of the smaller sub-estuaries on Maui, Pa'akea riverine estuary begins just below a terminal waterfall in a coastal plunge pool that flows across a boulder stream for about 10 meters (30 feet) before emptying into the bay. This site was surveyed for eDNA only. Waiohue riverine estuary is located about 40 meters (130 feet) to the west of Pa'akea.

Pa'akea plunge pool had 53 and 68 species reported from eDNA sampling. In quarter 2 there were 6 fish species and 44 invertebrate species, while the next sampling detected 5 fish species and 61 invertebrate species. The only mammal detected was the rat *R. rattus*, unfortunately found during both sampling dates.

Waiohue Estuary had 88 species detected using eDNA in quarter 2 compared to 128 species in quarter 3. Fish biodiversity was similar in both sampling periods with 17 species and 19 species, respectively.

Differences in invertebrate biodiversity between the quarters explained the higher biodiversity reported for quarter 3 compared to the earlier date. Like Pa'akea plunge pool, the rat *R. rattus* was detected in the Waiohue estuary. Finding land mammal eDNA in estuaries, where water is diluted, indicates a large population of this introduced species. The wild boar *S. scrofa* was detected in Waiohue Stream.

Both sampling methods combined detected N=146 with no introduced species documented. Species richness was low with three species each documented by visual and POE sampling methods. The most abundant species was *K. xenura* (96% overall), ranging from juveniles to large adults (63 - 228mm FL). Median size was 150mm FL, indicating that many were large adults well over the legal size for take (127mm FL). It was exceptional to catch this many large *K. xenura* and this is likely due to the remoteness of Waiohue estuary as the fish are to a degree protected from the reach of human activities. The overall abundance of large *K. xenura* was remarkable; many were seen feeding in Waiohue riverine estuary, at its stream mouth, and along the shallow bay in the white water. The other species documented were two Hawaiian chub *Kyphosus hawaiiensis* juveniles and two *N. leuciscus* adults. An interesting observation was a distinct bronze colored shark seen swimming in the waves 20 meters offshore for an extended period. The presence of apex predators is an indication of ecosystem productivity.

POE methods also documented *K. xenura* as the most abundant species sampled (96%). Size ranged juveniles to adults (55 to 234mm FL). POE sampling confirmed the abundance of *K. xenura* throughout Waiohue estuary. Two other sport-fish species were also caught and were both adults: *Kyphosus cinerascens* High-fin chub and *Kyphosus vaigiensis* Brassy chub.

Honomanū. Located in east Maui, all sites in Honomanū are accessible by road.

Honomanū Stream is perennial and has a total length of 18.4 miles (29.7 km). It has a stream order of 2. The watershed is made up of 100% conservation land. This project monitors three 100-meter reach sites on Honomanu Stream. The lower reach site is at an elevation of about 80 ft. The substrate composition in this lower reach consists of boulder, cobble, gravel, and some sand. The middle reach site is at an elevation of about 60 ft. The substrate composition in this middle reach consists of boulder, cobble, and gravel. The upper reach site is at an elevation of about 200 ft. The substrate composition in this upper reach consists of boulder, cobble, gravel, and some bedrock.

Honomanū Stream was sampled for 3 quarters using eDNA methods. Total species detected varied among the quarters with 97 species (quarter 1), 35 species (quarter 2), and 115 species (quarter 3). Fish biodiversity in Honomanū Stream (6 species (quarter 1), 3 species (quarter 2), and 9 species (quarter 3)) was similar among sampling intervals. In contrast, invertebrate diversity (79 species (quarter 1), 28 species (quarter 2), and 97 species (quarter 3)) differed among quarters to a great degree.

Honomanū Stream at the lower reach was surveyed during November 2019 (quarter 2), February 2020 (quarter 3), and June 2020 (quarter 4). All three quarters documented *N. granosa* recruitment, and quarters 3 and 4 documented post-larvae fish recruitment. In all three quarters species observations consistently recorded the presence of *S. stimpsoni*, *A. stamineus*, and *E. sandwicensis*. Quarter 4 observations documented the presence of *M. grandimanus*.

Honomanū Estuary consists of two sub-estuaries: Honomanū riverine estuary, and Honomanū Bay. Freshwater inflow to the lower portion of the stream and estuary is maintained by coastal groundwater,

while the contribution of surface water flow is highly variable. The geomorphology of Honomanū Estuary is shaped by both flash floods and large winter swells. The cobble beach around the stream mouth expands and contracts depending on the volume of surface water flow. Groundwater flow is adequate to keep the stream mouth open however, the opening has been reduced to around 1 meter (3 feet) wide during extended dry periods. Honomanu serves as one of three reference sites for comparison in the study.

Sampled for all three quarters, Honomanū estuary had 128, 125 and 111 total species detected by eDNA methods, respectively. In the first quarter only 26 fish species were reported, compared the 42 fish species in quarter 2 and 65 species in the last quarter sampled. To record 65 fish species is the most fish species detected in a Hawaiian estuary, so far, and is at a level of diversity comparable to a Hawaiian coral reef. The low diversity of fish species in quarter one may be ecologically relevant, or, in light of the problem found for samples in the third quarter of West Wailua estuary, it may be related to another problem in the eDNA laboratory that was not detected. Invertebrates species were higher in quarter 1 (92 species) and quarter 2 (78 species) compared to quarter 3 (36 species). Two land mammals were detected in the stream and estuary, the Wild Boar *S. scrofa* and the rat *R. rattus*.

As expected, visual cast net surveys yielded more fish (N=318) than POE (N=21). For visual surveys about two thirds were introduced species (N=216) and the remainder were native species (N=102). POE methods documented only native species. A total of nine species were documented using both sampling methods, four native and one introduced species for visual methods and six native species for POE methods.

Introduced *M. engeli* was the most abundant species sampled at Honomanū using visual sampling methods (68%; N=216) and ranged from 30 - 203mm FL (Figure A- 18). Most *M. engeli* were juveniles with a median size of 46mm FL. The abundance of an introduced species in Honomanu can be explained by the accessibility by road and a large parking area that result in making it a heavily fished area. Native *K. xenura* were also recorded in high abundance accounting for 26% (N=83). Juveniles and adults were present ranging from 35 - 162mm FL however, a median size of 43mm FL indicated that many were small juveniles recruiting into Honomanū estuary. Yellowstripe goatfish *Mulloidichthys flavolineatus* was third in relative abundance (4%; N=14), occurring as juveniles (88 - 107mm FL). Other sport-fish documented included large juvenile (178mm FL) and a hefty adult (400mm FL) *M. cephalus* and a juvenile giant trevally *Caranx ignobilis*.

Species composition using POE methods at Honomanū estuary differed from visual methods. This is likely because the relatively low population size (N=21) from this method has skewed results. The most abundant species was *M. flavolineatus* (33%) occurring in size ranges similar to visual sampling methods 90 - 102mm FL). *C. melampygus*, the second most abundant species sampled (24%; 62 - 90mm FL; median 75mm FL). *K. xenura* was present however, rarely sampled using POE. Other species sampled were Pacific threadfin *Polydactylus sexfilis*, *A. insularum*, and *E. sandwicensis*.

Honokōhau. A second reference site, Honokōhau, at the northern tip West Maui. There is easy access to the lower stream and estuary from Honoapi‘ilani Highway.

Honokōhau Stream is perennial and has a total length of 20.2 miles (32.4 km). It has a stream order of 2. The watershed is made up of 94% conservation land, and 6% agricultural. This project monitors one 100-meter reach site on the lower reach of Honokōhau Stream. The lower reach site is at an elevation of

about 30 ft. The substrate composition in this lower reach consists of boulder, sand, cobble, and some gravel.

Honokōhau Stream was sampled for 3 quarters using eDNA methods. Total species detected varied among the quarters with 86 species (quarter 1), 54 species (quarter 2), and 145 species (quarter 3). Fish biodiversity in Honokōhau Stream (10 species (quarter 1), 6 species (quarter 2), and 5 species (quarter 3)) was similar among sampling intervals. In contrast, invertebrate diversity 66 species (quarter 1), 46 species (quarter 2), and 140 species (quarter 3)) differed among quarters to a great degree. The highest invertebrate biodiversity sampled was in Honokohau Stream during the 3rd quarter.

Honokōhau Stream at the lower reach was surveyed during November 2019 (quarter 2) and June 2020 (quarter 4). *N. granosa* recruitment has not been observed in Honokōhau however, post-larvae fish recruitment has been noted. Like Honomanu Stream, Honokohau Stream surveys documented a diverse population of vertebrates including three of the five 'O'opu species in Hawaiian streams; 'O'opu naniha (*Stenogobius hawaiiensis)*, *A. stamineus*, and *E. sandwicensis*. The lower reach of Honokohau stream is the only documentation of presence we have so far from our visual surveys for *S. hawaiiensis*. Observations in quarter 2 also identified the presence of *M. grandimanus*.

Two sub-estuaries, Honokōhau riverine estuary, and Honokōhau Bay, make up Honokōhau Estuary. Freshwater enters the estuary from the perennial Honokōhau Stream, while flash floods and winter storms frequently change the site's geomorphology. Benthic composition of boulders, cobble, and gravel remain constant through winter swells and flash flooding.

For the three consecutive quarters eDNA sampled in Honokōhau estuary, total biodiversity was 87, 145 and 115 species, respectively. Fish biodiversity in Honokōhau estuary was 16, 52 and 47 species, respectively. Quarter 2 with 52 fish species detected is the second highest recorded biodiversity of fish in a Hawaiian estuary, comparable to 65 fish species reported for Honomanū estuary in the third quarter. Also, like Honomanū estuary the first quarter results for fish biodiversity are significantly lower than subsequent quarters. Whether these results are ecologically significant or reflect a problem in the lab contracted to run eDNA samples needs further investigation. Invertebrate biodiversity was 62 species in the first quarter, 89 species in quarter 2 and 64 species in the last quarter sampled. For first quarter sampling, the Big horn sheep *Ovis canadensis* was detected in both the stream and estuary, while *R. rattus* was found in the stream. There were no land mammals detected at this site in the second quarter, and two rat species, the Pacific rat *Rattus exulans* and *R. rattus,* in the third quarter.

The population of fish sampled was N=232 sampled by visual cast net and N=48 by POE. All species sampled at Honokōhau estuary were native to Hawaii. Honokōhau estuary had the highest species richness of all the Maui estuaries studied with 12 species documented using cast net sampling. Importantly, this result is consistent with eDNA results for quarters 2 and 3, where high species diversity was also detected independently.

Visual cast net sampling recorded 11 different species while POE had six species. Using visual methods *K. xenura* contributed 87% relative abundance and ranged from 45 - 217mm FL (median 102mm FL). This small median size indicated that while many were juveniles, large adults greater than 200mm FL were also sampled. In contrast, the second most abundant species, *N. leuciscus*, only had a total of eight individuals documented (4% relative abundance) and included juveniles (88mm FL) and adults (185mm FL). The baitfish *A. insularum* was 2% relative abundance. The other species sampled included sport-

fish*: K. sandvicensis, M. cephalus, C. sexfasciatus, A. triostegus, K. hawaiiensis, K. sandwicensis*, and *A. sordidus*.

Again, POE sampling methods at Honokōhau resulted in a smaller population of fish sampled (N=48) from six species. *K. xenura* (73%; N=35) was the most abundant species. Fork lengths ranged from 54 - 170mm, indicating that juveniles and adults were present within the estuary. Juvenile (median 79mm FL) *N. leuciscus* accounted for 11% relative abundance. Other sport-fish species documented at Honokohau using POE sampling methods were *A. triostegus, K. sandvicensis* and *A. sordidus*.

Keālia. Located on Maui's south-central coast, Keālia Estuary is composed of two sub-estuaries: Ma'alaea Bay and Keālia Pond National Wildlife Refuge, a lagoon. Survey area includes the intermittently open lagoon-mouth. Substratum consists of mostly sand and a calcium carbonate shelf towards the southern portion of the sample location.

Keālia estuary was sampled for eDNA in quarter 1 yielding 59 species, quarter 2 with 61 species and 60 species for quarter 3. Two distinct habitats were sampled, Ma'aleaea Bay and Keālia Lagoon. Fish biodiversity for Ma'alaea Bay was 22 species in quarter 1, and 20 species each for both quarters 2 and 3. For each of the three consecutive quarters sampled Keālia Lagoon was found to have 9, 14 and 13 fish species, respectively. Invertebrate biodiversity in Ma'aleaea Bay was 22, 23 and 6 species for quarters 1, 2, and 3, respectively. In Keālia Lagoon for two invertebrate species were detected in each of the first two quarters sampled, then 33 species were found in quarter 3.

The total population sampled with both cast net sampling methods combined was N=324, visual cast net sampling accounting for most samples (N=263). The sand berm was closed during the three sampling dates. With the berm closed, no introduced species, found in large numbers inside the lagoon, were documented during visual or POE sampling methods. Cast net sampling in the lagoon takes place only when the berm is open to minimize disturbance on the endemic Hawaiian water birds. Both cast net methods each recorded six native species.

An endemic species*, K. xenura*, had the highest in relative abundance at Keālia with N=242 (92%) using visual cast net methods. The population sampled included juveniles to sub-adults (34 -115mm FL). The mean length was 69mm FL (median 70mm FL), and all *K. xenura* were below the regulated size for home consumption (127mm FL). This species is highly prized by fishers. *P. sexfilis* accounted for 4% relative abundance (N=10). All were juveniles ranging from 63 - 157mm FL. Juvenile *M. flavolineatus* were documented (median 90mm FL). All other species collected were juvenile sport-fish: *K. sandvicensis*, *A. triostegus*, and *Albula virgata* long-jaw bonefish.

Biodiversity from POE cast net sampling was comparable to results from visual cast net sampling. The most abundant species was *K. xenura* (77%; N=47), a species that ranged from juveniles (32mm FL) to sub-adults (115mm FL). The second most abundant species was *M. flavolineatus* (8%; N=5), followed by *A. virgata* (7%) as very small recruiting juveniles (median 68.5mm FL). Like visual cast net results, all other species sampled were sport-fish: *P. sexfilis, K. sandvicensis*, and ladyfish *Elops hawaiiensis*.

<u>eDNA biodiversity monitoring</u>*.*

Biodiversity measures using eDNA provide us with a tool to access and adapt our natural resource management strategies. This is particularly important for Hawaii because our islands are a global biodiversity hot spot. And management decisions and actions should include accounting for this

biodiversity. Biodiversity measures can be used as a proxy for evaluating ecosystem health and productivity. Following an ecological principle– for degraded or polluted sites support far fewer species and less productivity than can be found in more intact or natural areas.

Monitoring for biodiversity using eDNA can provide insights for spatial and temporal comparisons between a stream and its estuary; among streams and among estuaries; and as a measure of introduced species present in these ecosystems. This study evaluates how traditional sampling compares to eDNA methods in capturing species diversity in streams and estuaries. Importantly, this study examines the usefulness of eDNA as a method to monitor the responses of streams and estuaries to changes in freshwater inflow as directed by management actions.

Figure 2 is a non-Metric Multidimensional Scaling (nMDS) plot of fish species from eDNA detected in all sites for each quarter surveyed. (In nMDS figures objects, represented as circles, that are more similar to one another are ordinated more closely together, while dissimilar objects are ordinated more distantly apart from one another. Each object represents an individual site sampled at one specific time). Results of the analysis shows three clusters, with 95% confidence intervals represented by ellipticals. (Ellipticals, drawn around the clusters or groups of similar objects, represent a statistical measure of 95% confidence that objects in a group enclosed by the ellipticals belong to that group.) Streams formed the tightest cluster relative to estuaries, as expected. Estuaries with contiguous streams show a broader diversity of fish species assemblages, represented by a larger, looser partitioning. This result is reflective of the nature of fish assemblages in estuaries in the Hawaiian Islands, which include freshwater, estuarine and marine species. The third group formed around an estuarine site composed of a lagoonal estuary with its contiguous bay. In other words, each group of samples clearly represents a different ecosystem type and the analysis can distinguish among these ecosystems using only fish species data collected using eDNA methods. This result is encouraging. It is the first step needed to demonstrate that eDNA monitoring of streams and estuaries can successfully describe each ecosystem based on fish species assemblages present.

In Figure 3a, a non-Metric Multidimensional Scaling (nMDS) plot of fish species from eDNA detected in streams for each quarter, two clusters were identified. One cluster contained all streams with flow restored with one exception and the second cluster included the reference sites. Invertebrates showed similar structure between restored and reference streams (Figure 3b). Considering that sampling plans were curtailed because of Covid-19 stay-at-home policies, it is encouraging to see partitioning between the two groups. Although additional sampling for eDNA in streams should improve resolution, these results demonstrate that eDNA biodiversity monitoring in streams is a valuable approach to measure ecological responses to restoration.

Interestingly, fish assemblages from estuaries sampled with eDNA partitioned into three groups (Fig. 4a). Like streams, all estuaries downstream of streams with restored freshwater inflow formed a group. Importantly, reference sites formed two groups. One reference group included estuaries composed of streams flowing into a bay and the other reference group was an estuary with a lagoon and bay. For invertebrates the three groups were identified based on restoration status mostly (Figure 4b). However, there were some ambiguous samples. This may be resolved by additional temporal sampling. Overall, results from eDNA biodiversity monitoring in estuaries, like streams, indicate that monitoring biodiversity is a promising approach to evaluate ecological restoration.

For both streams and estuaries, reference sites had samples taken over three quarters, whereas the restored sites had only two quarters of sampling effort. The monitoring plan was designed to sample each site over four consecutive quarters. In part, this plan was intended to evaluate the level of temporal replication needed to monitor ecological restoration. Unfortunately, stay-at-home orders curtained this. Understanding how much temporal replication is required remains as a needed exercise to do in the future. It is important to return to evaluating temporal replication in streams and estuaries because both are dynamic ecosystems. Geomorphology and ecological conditions are always shifting. These changes underpin the productivity of both ecosystems.

As expected, estuaries supported higher fish diversity compared to their contiguous streams at all sites and in all quarters using eDNA measures. For example, in quarter 2 West Wailua iki stream had 6 fish species compared to 22 fish species detected in its estuary. In the West Maui watershed, Honokōhau, 51 fish species were recorded in its estuary compared to 6 fish species in the stream. In East Maui, 65 fish species were detected in Honomanū estuary; this is the highest diversity of fish species found at a site, so far. Fish biodiversity in these two estuaries is comparable to the number of fish species reported on Hawaiian coral reefs. Estuaries are expected to have higher fish biodiversity compared to streams because fish assemblages in estuaries are composed of species from freshwater and marine ecosystems as well as estuarine species.

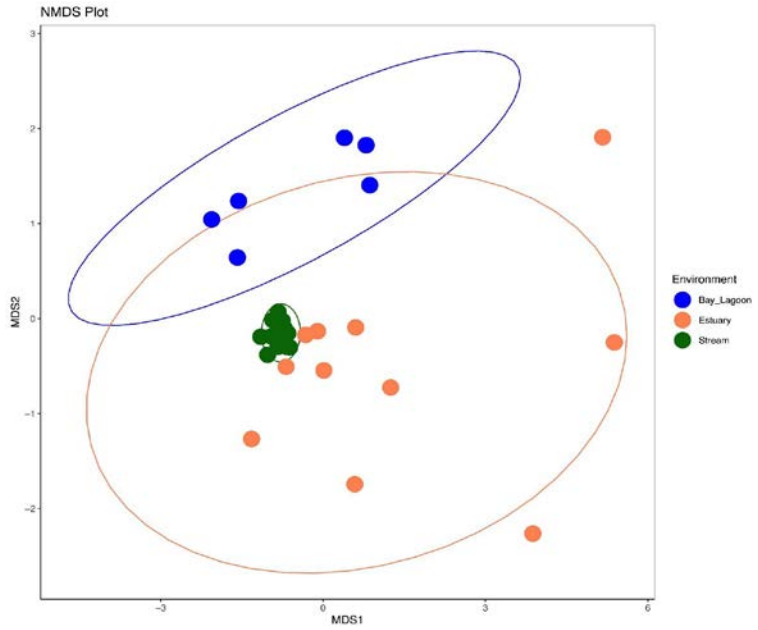


Fig. 2. nMDS revealed that fish eDNA collected from streams (green) formed one grouping, eDNA sampled from estuaries with streams (orange) are found in a second cluster and the estuarine site without a contiguous stream (lagoon with bay) grouped together in a third cluster. ellipticals=95% confidence.

In contrast to fish diversity, streams had higher invertebrate diversity compared to estuaries as detected with eDNA. Honokōhau Stream in quarter 3 had the highest invertebrate diversity of all streams sampled with 140 species. During the same sampling period Waiohue Estuary recorded 104 invertebrate species. Hawai‘i is a biodiversity hot spot. However, it is important to note that these results are likely under-reporting biodiversity for certain phyla, such as nematodes, because both taxonomic and genetic

information for certain lineages are poorly described and documented in Hawai‘i. Even after acknowledging biases associated with under-reporting for invertebrates, these results are exciting because they offer a first look at the invertebrate diversity that is linked to streams and estuaries. Many of these invertebrate species likely serve important roles at different times in the food webs of streams and of estuaries.

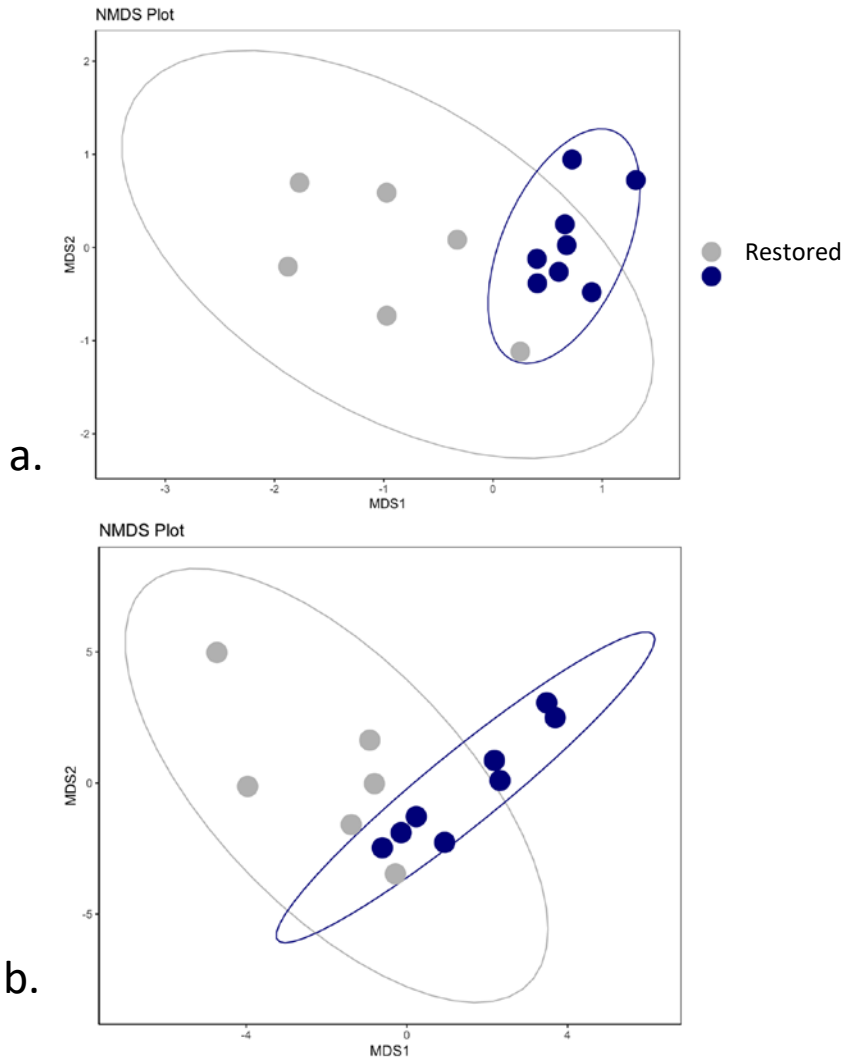


Fig. 3. nMDS plots of fish assemblages (a) and invertebrates (b) from streams sampled with eDNA.

Streams and estuaries in Hawai‘i have established populations of non-native fishes that make up a portion of biodiversity in these ecosystems. Introduced species in the first quarter were present in both streams and estuaries at Honomanū and Honokōhau. Interestingly, for the three streams with 100% baseflow sampled in the second quarter no introduced species were detected. This was not true for estuaries. Except for East Wailua iki, all estuarine sites had between 1 to 3 introduced fish species

detected. Information on introduced invertebrate species in Hawai‘i is poorly understood and will require more insights from experts to confirm many of the native ranges of species detected by eDNA biodiversity monitoring.

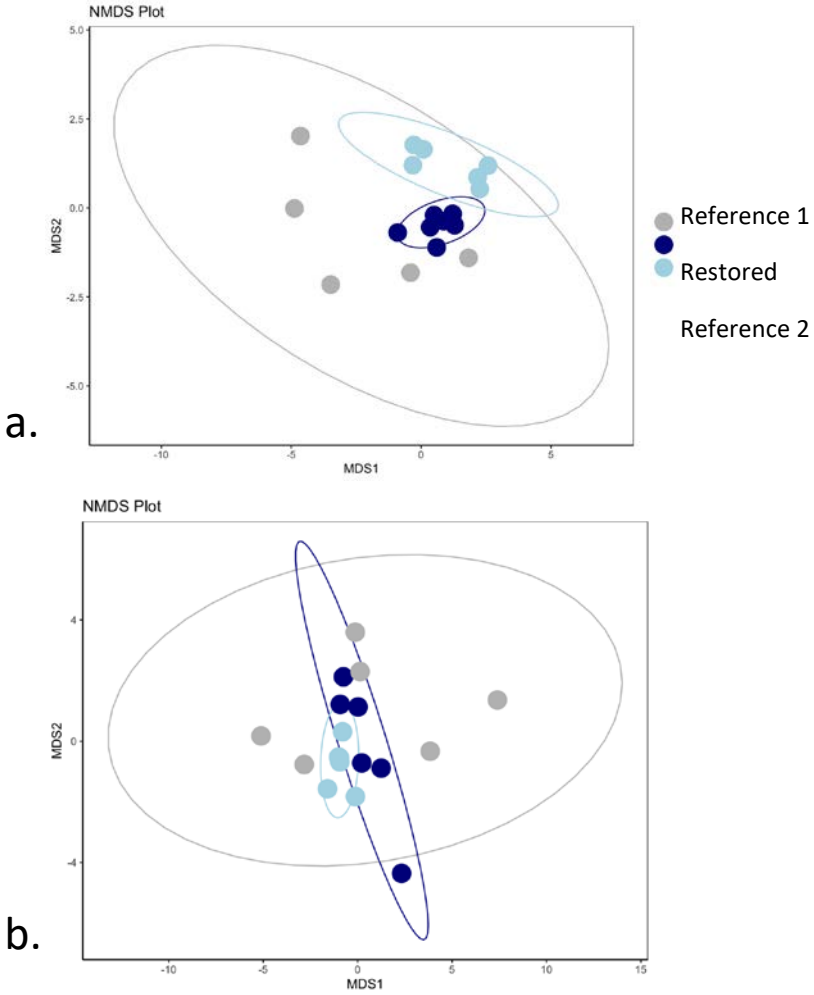


Fig. 4. nMDS plots of fish assemblages (a) and invertebrates (b) from estuaries sampled with eDNA.

Genetic markers used to detect fish species in this study are broadly applicable to other vertebrate species, such as tunicates, amphibians, birds, and mammals. Results show a broad list of introduced vertebrate species associated in streams and estuaries of Maui. Particularly notable, eDNA from introduced mammals was detected in water samples taken in both streams and estuaries. Detecting mammal eDNA in estuaries is alarming because, unlike simple linear flow found in streams, estuaries are hydrodynamically complex meaning eDNA is more diluted in estuaries compared to streams. Mammal eDNA in water samples collected in estuaries indicates that these mammals are strongly associated with the water in a watershed. Introduced mammal eDNA found in both ecosystems include the Wild Boar (*Sus scrofa*), cattle (*Bos taurus*), Big Horn sheep (*Ovis canadensis*), Black rat (*Rattus rattus*), Polynesian rat *(Rattus exulans*), and the Black-tailed rabbit (*Lepus californicus).* Using eDNA sampling to detect the presence of introduced mammals improves our understanding of potential disturbances in watersheds,

such as erosion, sedimentation, and transmission of disease or other invasive species, that negatively impact productivity and habitat quality in streams and estuaries. The presence of these mammals can indicate previously undetected stressors on these aquatic ecosystems and reveals another beneficial application of eDNA sampling in streams and estuaries.

Comparing results of fish species diversity measures from visual monitoring and eDNA biodiversity monitoring, for streams we found that visual surveys detected close to the same biodiversity as eDNA sampling except for Honokohau Stream. Importantly, eDNA sampling detected one species in Honokohau Stream that was not observed by visual surveys and that difference was an introduced species. For estuaries, the number of fish species detected by eDNA sampling was an order of magnitude greater than results from cast net surveys. These results are encouraging. DAR uses 1/4" cast nets to sample estuaries, a method well-suited for juvenile fish because it minimizes harm to fish so that samples can be measured and returned live to the estuary. As with every method, there are tradeoffs. Using a 1/4" cast net does not translate well for sampling cryptic species, benthic species, or larger, fast-moving predators. However, eDNA biodiversity monitoring offers insights into the presence of these difficult to sample species. By pairing the two methods a clearer understanding of conditions in estuaries and streams is gained.

DAR staff returns from sampling the estuary with his cast net.

Cast net sampling in estuaries. Honomanū (Appx. A) and Honokōhau (Appx. B) estuaries, the diverted stream sites, were sampled using cast nets for the first three quarters. The endemic Āholehole (*Kuhlia xenura*) was found at both diverted stream sites and in all three quarters sampled (Fig. 5). In Honokōhau estuary Āholehole was more that 75% of the relative abundance in each of the three quarters sampled. Āholehole is an important coastal food fish that uses estuaries and streams as nursery habitat. In contrast, for Honomanū estuary the introduced fish Kanda mullet (*Moolgarda engeli*) made up >40% relative abundance in each quarter. In the second quarter 48% relative abundance in Honomanū estuary

was the oama (*Mulloidichthys flavolineatus),* a sought-after bait fish. Over the 3 sampling periods 5 fish species were documented in Honomanū estuary and 10 species in Honokohau estuary.

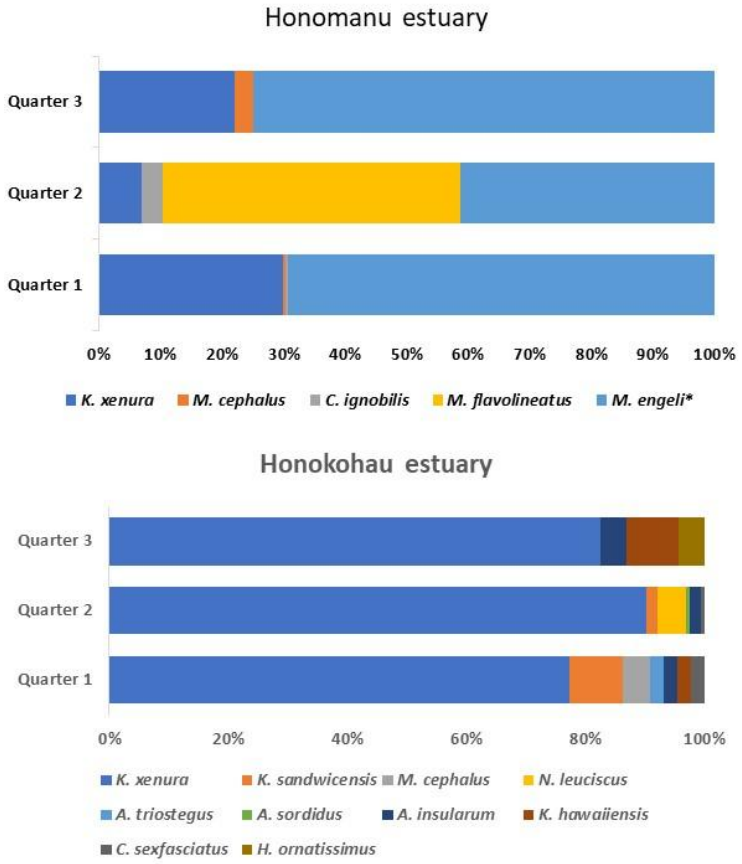


Fig. 5. A comparison of relative abundance of fish species sampled using visual cast net method in the two reference sites over three quarters. * indicates an introduced fish species.

Results from East Wailua iki (Appx. C) estuary compared to West Wailua iki (Appx. D) estuary are a study in contrast (Fig. 6). In monitoring efforts in 2012-2013 DAR staff observed that East Wailua iki stream had a cobble berm present for most of the year, while West Wailua iki Stream remained open to the bay. In this study for quarter 2 the cobble berm in front of East Wailua iki Stream had plants rooted in the berm and the stream bottom was covered in waterlogged tree branches, indicating that the stream had not been open to the bay recently. The stream mouth estuary of West Wailua iki Stream was deeper and more pronounced in both quarters in 2019-2020 than was observed in 2012-2013. This is notable because 100% baseflow has been returned to both streams since 2016. In quarter 3 East Wailua iki stream mouth had been opened to the bay by a freshet and still had an open 30 cm gap in the berm during sampling. Also, the woody debris that was observed earlier had been flushed out of the stream. Juvenile fish prefer to aggregate near sources of freshwater inflow in an estuary, such as an open stream mouth. This explains why with a mostly closed stream mouth at the East Wailua iki side of the estuary few fish were sampled (N=4) and only 3 species recorded over both quarters. With an open stream mouth, West Wailua iki side yielded more fish (N=67) and 8 species over both quarters. Together both

Āholehole species (*K. xenura* and *K. sandwicensis*) made up over 70% relative abundance for each quarter at West Wailua iki estuary.

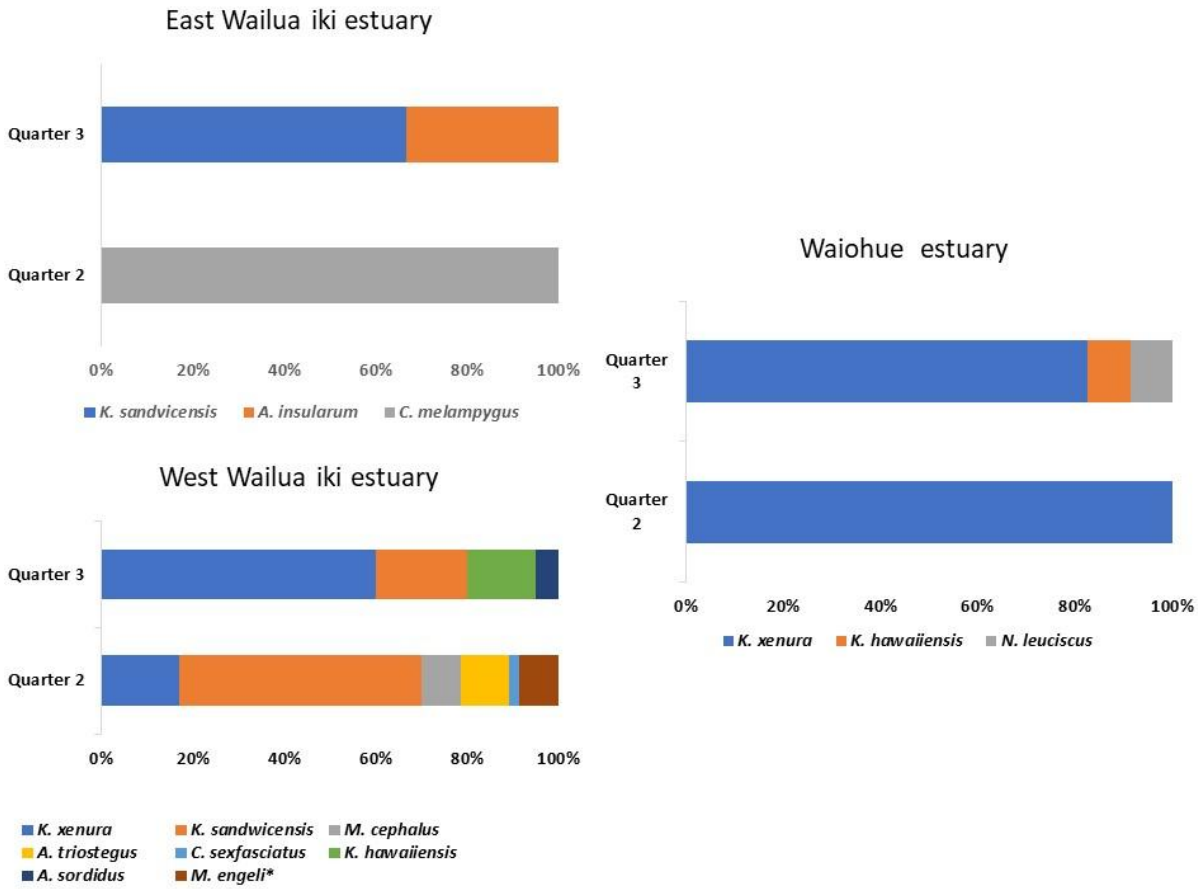


Fig. 6. A comparison of relative abundance of fish species sampled using visual cast net method in East Wailua iki, West Wailua iki and Waiohue estuaries sampled in quarter 2 and quarter 3. * indicates an introduced fish species.

The endemic Āholehole also made up most of the sampled fish at Waiohue estuary (Appx. E). West Wailua iki had the introduced fish Kanda mullet. Unlike the diverted site, Honomanū estuary where Kanda mullet was >40% relative abundance over all sampling periods, at West Wailua iki this species was recorded only in the second quarter and contributed 9% relative abundance. For other sites sampled, no introduced fish species were detected with cast net sampling.

Visual sampling in streams. In quarters 2-4 the mollusk Hihiwai (*Neritina granosa*) had the highest density of individuals of all native species in Honomanū Stream lower site (Appx. A). The highest number of adult Hihiwai was found in the second and third quarters, while quarter 4 had a higher level of recruitment for this species than previous quarters. The native stream gobies ‘O’opu Nakea (*Awaous stamineus)*, ‘O’opu Nopili (*Sicyopterus stimpsoni*), and ‘O’opu Akupa (*Eleotris sandwicensis*) were all present for all quarters sampled. Honomanu Stream had an exceptional population of ‘O’opu Nopili that was made up of post-larvae, juveniles and adults. Further, juvenile ‘O’opu Nopili was the most common fish encountered for each of the three quarters sampled. Post-larvae recruitment success for stream

species is dependent on a continuous flowing stream, which results in a healthy stream and a contiguous estuary.

In the fifth quarter at Honomanū Stream upper site ‘O’opu Nopili, again, occurred in the highest densities compared to other fish present, ‘O’opu Alamo’o (*Lentipes concolor*), ‘O’opu Nakea and ‘O’opu Akupa. Importantly, ‘O’opu Nopili populations included juveniles and adults. This upper site can be intermittently flowing with a section of stream bed above and below the highway bridge that can dry out entirely, thereby isolating this upper site from the lower one and its estuary. To better understand this, more surveys are recommended.

Honokōhau Stream (Appx. B), near the estuary, was sampled in quarters 2 and 4 (high water prevented 3rd quarter sampling). This was the only lower stream site with an absence of Hihiwai. The lack of an explanation for why this species was not found demonstrates the need for a better understanding of these ecosystems. In both quarters’ adults of ‘O’opu Naniha (*Stenogobius hawaiiensis*) and ‘O’opu Nakea both had higher densities relative to other species and size classes. In the 2nd quarter ‘O’opu Nakea and ‘O’opu Akupa were both recorded as both post larvae/juveniles and adults.

West Wailua iki Stream (Appx. D), near the estuary, was sampled in the 2nd and 3rd quarters (Fig. 7). For both surveys Hihiwai had the highest density of individuals. Hihiwai population differed between the surveys, with more adults present in quarter 2, and in quarter 3 there was more recruiting individuals detected. Three fish species were present in both quarters, ‘O’opu Nakea, ‘O’opu Nopili and ‘O’opu Akupa.

An Akupa watches as Hihiwai migrate up stream.

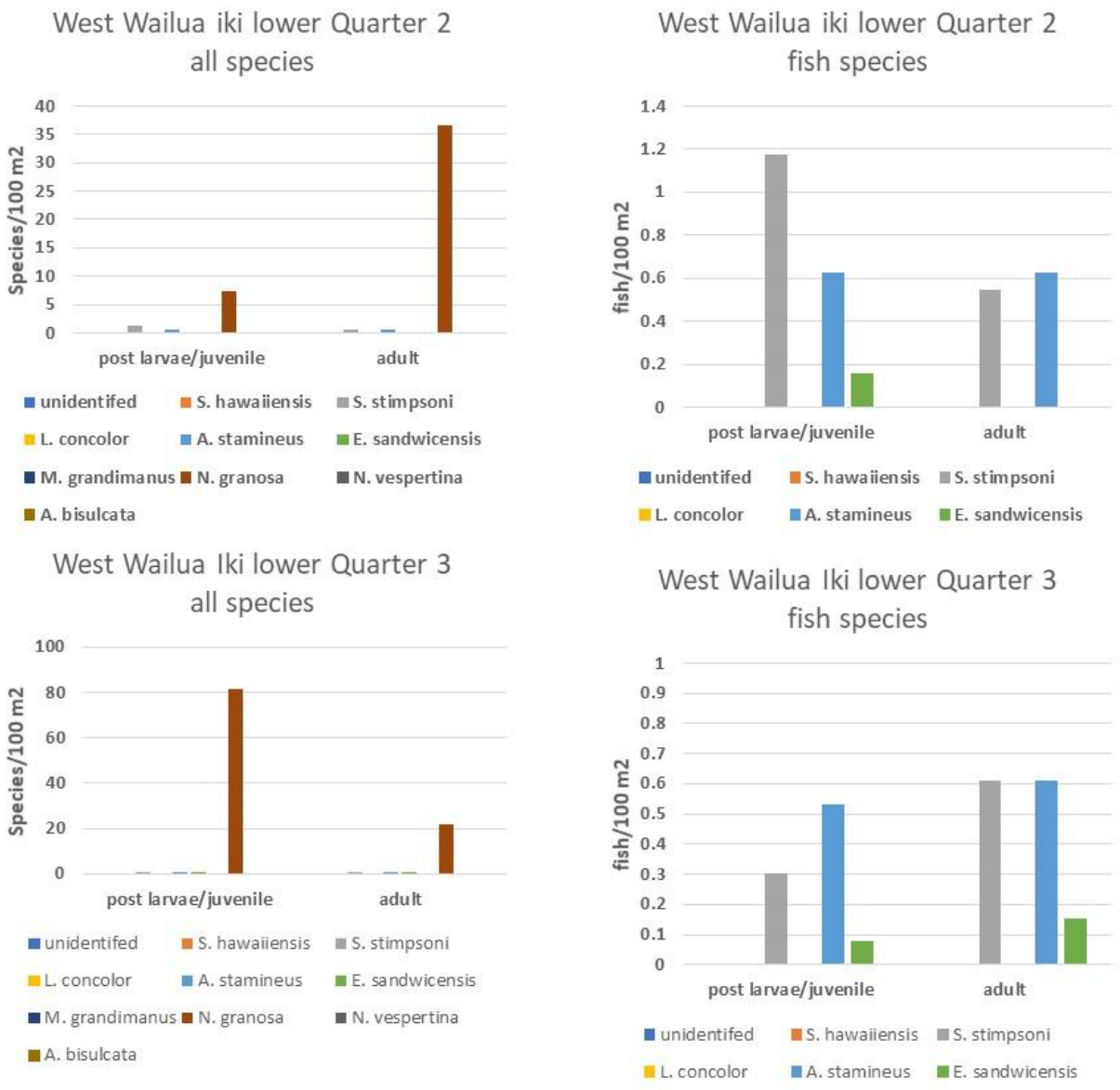


Fig. 7. A comparison of West Wailua iki Stream lower site visual surveys for quarters 2 and 3 with both fish and invertebrate species density and fish species only density.

East Wailua iki Stream (Appx. C) lower site was surveyed in quarter 3 (Fig. 8). Few juveniles of any species were observed because this stream mouth is closed by a cobble berm most of the time. Hihiwai, ‘O’opu Nakea, ‘O’opu Nopili and ‘O’opu Akupa were present. In the East Wailua iki Stream upper site, surveyed in the 5th quarter, ‘O’opu Alamo’o and ‘Opae (*Atyoida bisulcata*) were the only aquatic species found. More surveys are needed in this stream.

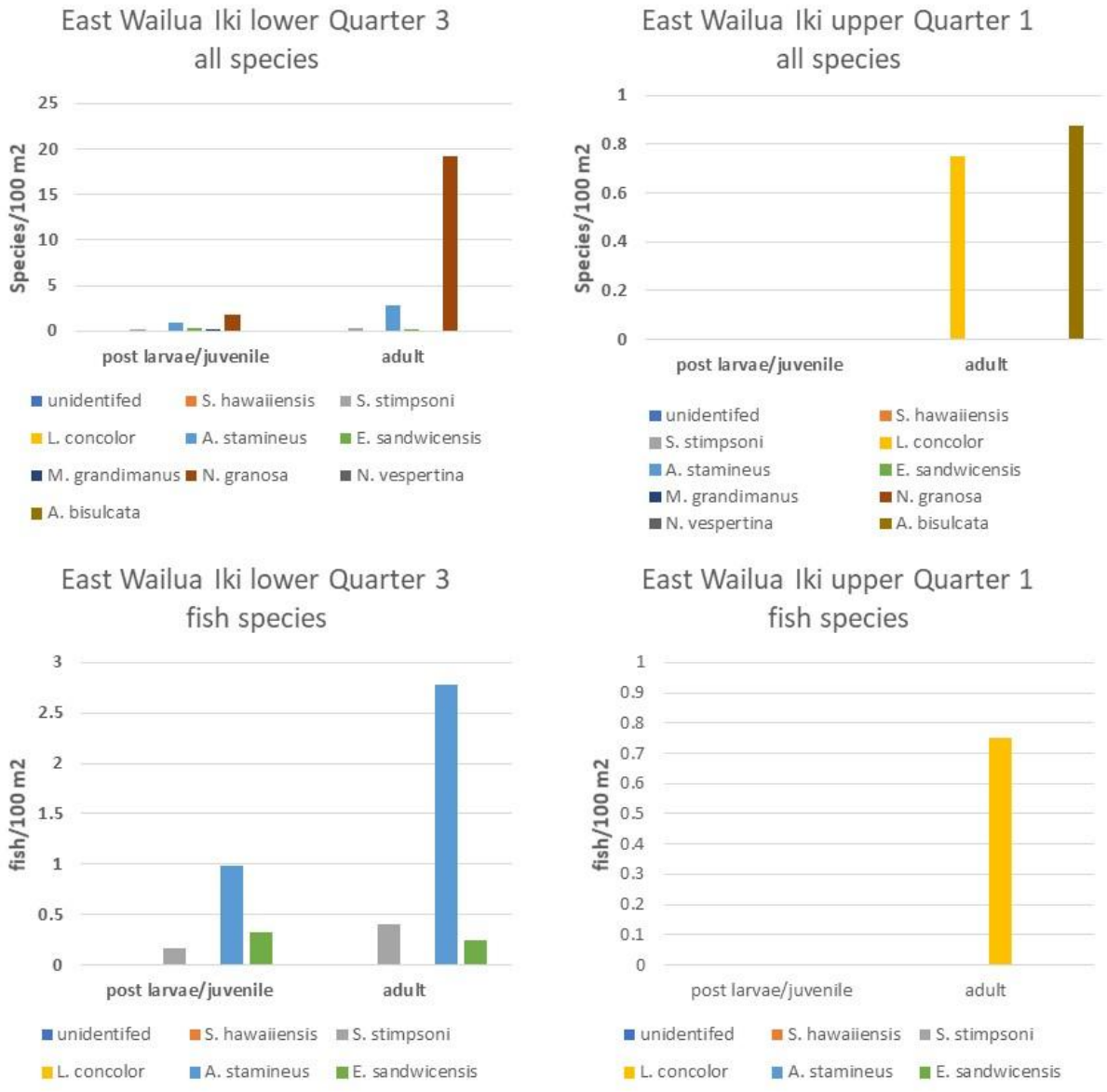


Fig. 8. A comparison visual surveys in East Wailua iki Stream lower and upper sites with both fish and invertebrate species density and fish species only density.

In quarters 2 and 3 at Waiohue Stream (Appx. E) lower site Hihiwai were the most common species observed (Fig. 9). The population of Hihiwai had more adults in the 2nd quarter compared to the subsequent one, while Hihiwai in quarter 3 were found recruiting to Waiohue Stream. Both quarters

recorded higher ‘O’opu Nopili recruitment compared to the other fish species present, ‘O’opu Nakea and ‘O’opu Akupa. In the Waiohue Stream upper site ‘O’opu Alamo’o and ‘Opae were the only species present.

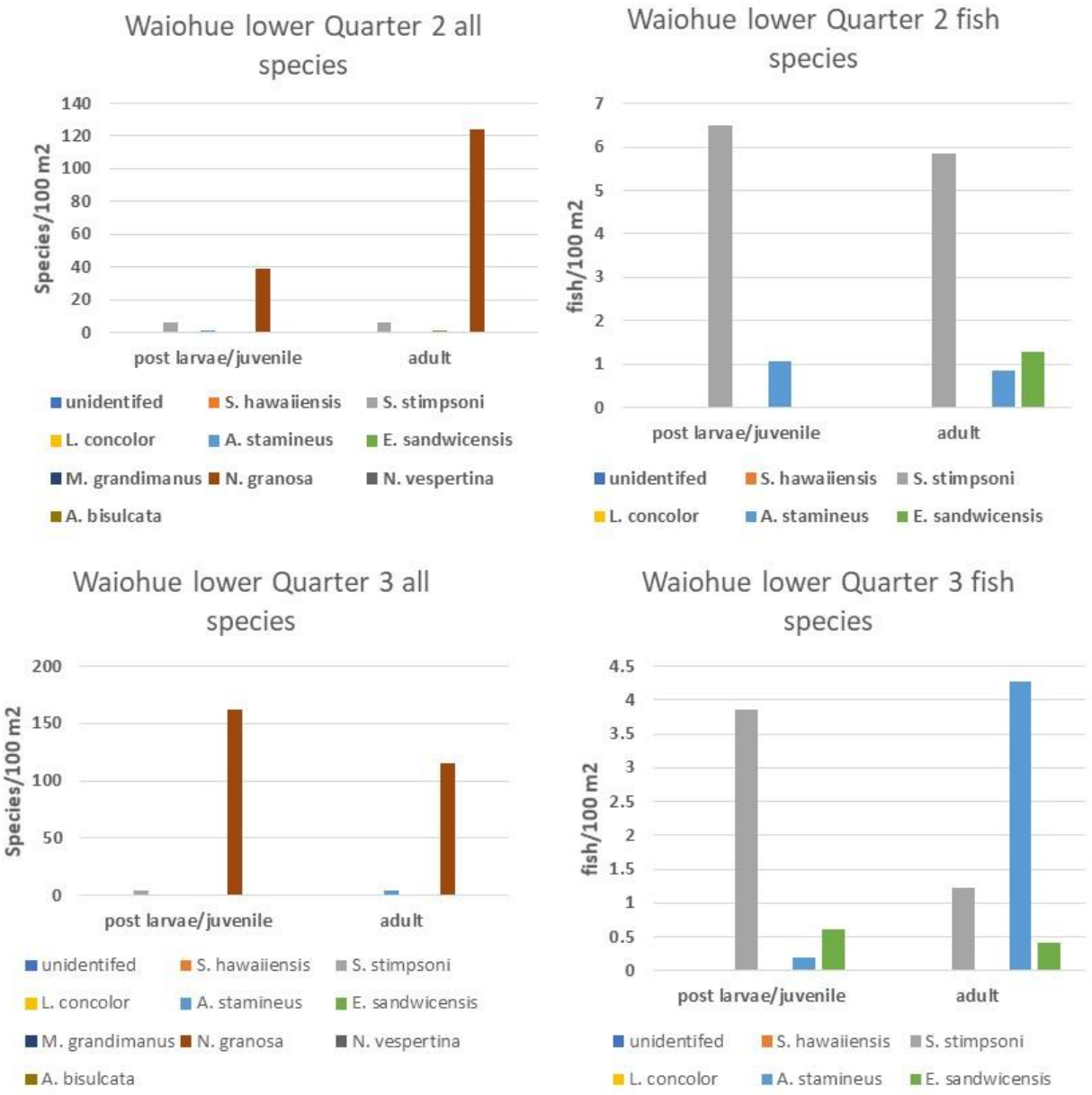


Fig. 9. A comparison of Waiohue Stream lower site visual surveys for quarters 2 and 3 with both fish and invertebrate species density and fish density only.

Physical habitat in streams. Water withdrawals to streamflow can lead to changes in aquatic habitat availability and diversity and can result in changes or shifts in community dynamics. Water withdrawals may have the largest negative effect to aquatic biota during times when flows are naturally low since withdrawals during these times would represent larger percentages of the total stream flow and occur when natural in-stream conditions are already stressed. As flows lower in a stream, the volume, area, and depth of aquatic habitat is reduced. Low flows can reduce, limit, or eliminate stream connectivity. Further reductions in low flow and subsequent reduction in flow velocity can lead to increased fine sediment accrual. Prolonged reductions in flow can lead to reduced densities of low dependent taxa and/or overall loss of species richness.

For the East Maui study streams this would typically occur during summer months however, there is high natural variability in the timing, duration, and intensity of these low-flow periods and can occur at any time during the year. Withdrawals during times of relatively high flow are presumed to have less effect to overall magnitude, duration, and frequency of flow and subsequently to aquatic biota beyond the natural variability however, they may affect fluvial geomorphic processes such as sediment delivery and channel forming and flood dynamics.

The following lists the link between low flow characteristics that affect the aquatic biota processes and patterns in streams:

1. Low flows reference the extent of physical aquatic habitat, affecting the composition of biota, trophic structure, and carrying capacity.
2. Low flows mediate changes in habitat conditions and water quality, which in turn, affect patterns of distribution and recruitment of biota.
3. Low flows can restrict connectivity and diversity of habitat, increasing the importance of refuge habitats.
4. Low flows can affect the delivery of food or resources to aquatic biota, thereby affecting ecosystem production.

These characteristics or relationships between low flow and aquatic biota response do not operate in isolation. The response to low flows likely overlaps or occur simultaneously resulting in complex effects.

Preliminary results (Table 2) show stream flows at times of sampling for all sites were lowest in quarter 1 & 3 in Honomanū Lower, and quarter 5 in Honomanu Middle. Highest flows occurred in quarter 3 & 4 in W. Wailua Iki Lower and Honomanū Lower respectively. The range of flows ranged from 0.05 to 20.65 cfs.

Table 4. Measured stream flows at the location and time of aquatic surveys for all sites.

				Discharge	
Quarter	**Sampling Date**	**Stream**	**Reach**	**MGD**	**CFS**
1	August 7, 2019	Honomanu	Lower	0.45	0.7
2	October 16, 2019	Waiohue	Lower	5.19	8.03
3	January 22, 2020	W. Wailua Iki	Lower	13.34	20.65
3	January 23, 2020	Waiohue	Lower	7.64	11.81
3	February 5, 2020	Honomanu	Lower	0.03	0.05
3	February 21, 2020	Waiohue	Upper	5.11	7.9
4	June 5, 2020	Honokohau	Lower	7.86	12.15
4	June 10, 2020	Honomanu	Lower	11.34	17.55
4	June 17, 2020	Waiohue	Upper	3.27	5.05
5	July 15, 2020	E. Wailua Iki	Upper	2.19	3.4
5	July 21, 2020	Honomanu	Middle	0.21	0.33
5	August 17, 2020	Waiohue	Upper	3.12	4.83

Some measures of physical habitat availability can be expressed as in-stream channel width, depth, volume, velocity, and substrate characteristics. The graphs below examine the relationship between discharge and average depth (Fig. 10), and discharge and wetted width (Fig. 11) across all sampling sites. Higher correlations may occur as additional data is added as successive samplings are conducted. These relationships may be used to predict reductions in available habitat for a stream under future water withdrawal scenarios.

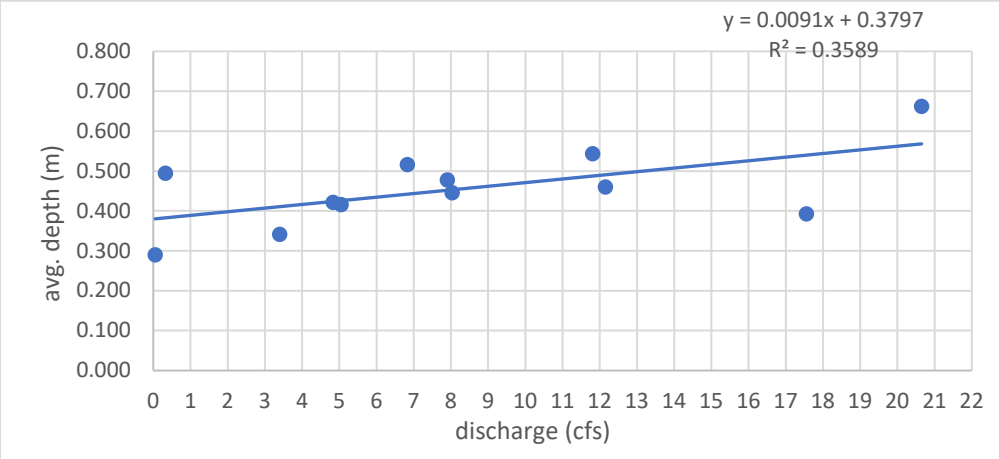


Fig. 10. Discharge and average depth relationship.

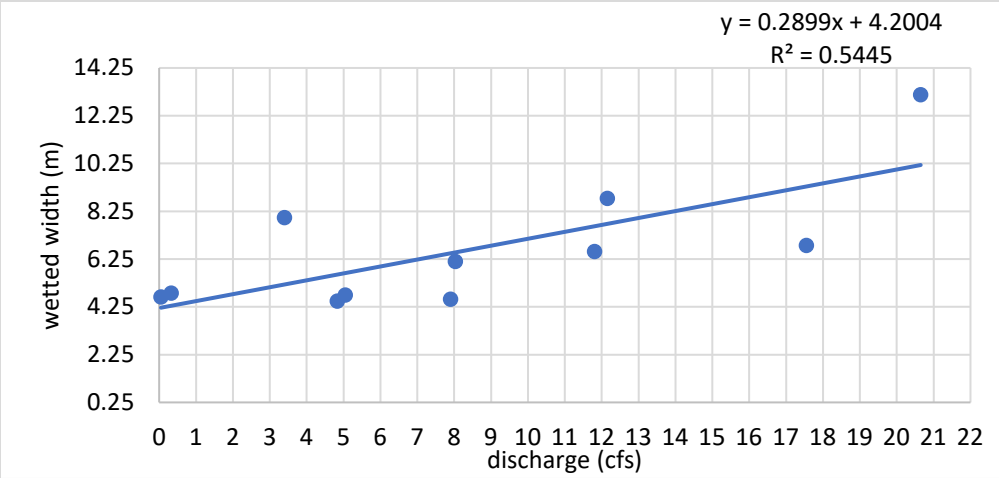


Fig. 11. Discharge and wetted width relationship.

Conclusions and lessons learned

CWRM requested that DAR collect baseline data in selected streams in East Maui restored to 100% baseflow conditions. In the future CWRM may permit water withdrawals in East Wailua iki stream up to 64% of normal baseflow. In contrast, West Wailua iki and Waiohue streams are slated to remain at 100% baseflow for the foreseeable future. Clearly, restoration of 100% baseflow in West Wailua iki and Waiohue streams goes a long way to improve ecosystems degraded for over 100 years by significantly reduced freshwater inflow. Given that these streams are receiving natural flow conditions, the next step in their ecological restoration is the removal of physical structures that are now obsolete to their original function. Obsolete structures in streams unnecessarily impede endemic species with a life history strategy that depends on connectivity. Streams are used as adult habitat, the ocean for larval development and dispersal, and estuaries serve as the gateway between the two ecosystems. Hawaiian streams are a dynamic ecosystem that is well-adapted to recover from physical disturbances. This means that streams can recover and respond favorably to the removal of physical barriers that impede connectivity. It bears repeating, productive streams feed estuaries, and healthy estuaries are needed for sustainable fishing.

Additionally, for streams where the take of water will continue, withdrawals should mimic and be within the natural range of variability with respect to all flow characteristics of magnitude, timing, frequency, and duration of a particular stream in order to minimize potential effects to aquatic biota within that stream.

DAR successfully improved our monitoring in freshwater-dependent ecosystems by including eDNA biodiversity monitoring, by extending the scope of monitoring to include estuaries alongside streams and by including diverted sites for comparison. Moreover, monitoring data from Honomanū and

Honokōhau have added value as baseline data for those streams and estuaries because these results can be used by CWRM and DAR for management as future needs dictate.

By implementing this project CWRM and DAR are improving our working relationship to manage freshwater-dependent ecosystems. This is especially timely because global climate change in Hawai‘i means diminished rainfall, thereby reducing the amount of freshwater available for human needs and the needs of freshwater-dependent ecosystems. By working together, CWRM and DAR can develop and improve on our adaptive management strategies to face this challenge.

The application of eDNA sampling to monitor biodiversity is among the first uses of this method by DAR. In the meantime, there are several areas DAR can improve on for biodiversity monitoring using eDNA going forward. Firstly, we learned that the number of replicates needed to adequately sample biodiversity differs among ecosystems. Sampling for eDNA in wadeable streams is a straightforward exercise because this aquatic system is defined by linear flow, which acts to concentrate and direct particles for sampling. We found that two replicate samples per stream segment were adequate because all abundant species observed during traditional sampling were also found with eDNA sampling. That said, eDNA in streams was useful because it detected rare species not observed with traditional monitoring approaches. In contrast, estuaries are hydrodynamically complex ecosystems with floating particles distributed randomly. Further, estuaries in Hawai‘i are typically more speciose than streams. It is likely that we under sampled biodiversity in estuaries. Therefore, we can improve eDNA biodiversity monitoring in estuaries by increasing the number of samples taken. To determine the optimal number of replicates we can run species accumulation exercises. We also learned that there were challenges with failed PCR for estuarine samples. By taking more replicates this problem can potentially be mitigated. Finally, DAR is now working to expand the DNA library for Hawaiian fish and invertebrates found on GenBank by opportunistically sampling species encountered during routine estuary monitoring. By banking samples to be sequenced in the future, DAR is contributing to the expansion of the DNA library for Hawai‘i. Future eDNA biodiversity monitoring projects in Hawai‘i, run by DAR, other government agencies, universities, and museums will benefit from this effort.

Stream flow information from CWRM can improve our understanding and analysis of the relationship between stream flow characteristics and aquatic biota. For example, estimates for gallons per day in each stream being monitored during 100% and 64% baseflows by season, and historical estimates for gallons per day in each stream being monitored while diversions were active could provide a better understanding of the biological response to modifications to the natural hydrograph of a particular stream.

Budget Expenditures

$126,000 - UH contract, HCRI Freshwater Biologist (2+ years of salary and fringe)

$60,000 - eDNA testing thru HPU

$51,000 - Helicopter service for 9-10 trips (2 completed)

$8,500 - travel cost (includes airfare, per diem, air cargo)

$14,500 - misc. supplies, etc.

$260,000 – Total

APPENDICES

APPENDIX A

Standard Operating Procedures for Stream Surveys

A.1 Purpose and Method Summary

Acronym and Term List

DAR	Division of Aquatic Resources
eDNA	Environmental DNA
FLOW METER	Instrument that measures water discharge (stream flow)
GPS	Global Positioning System
SBN	Survey Book Number
SD CARD	Security Digital Card – Card used to store images in the stream cameras.
SONDE	Water quality measuring instrument
SOP	Standard Operating Procedures
SUBSTRATE	Referring to the stream bed composition (e.g., boulder, etc.)
WADING ROD	Attached to the flow meter to take readings at different depths

Purpose

The monitoring of these resources is necessary to assist sustainable management practices and to ensure healthy aquatic ecosystems can persist. The Department of Land and Natural Resources (*DLNR)* Division of Aquatic Resources (*DAR)* and the Commission on Water Resource Management (*CWRM)* endorse this project's monitoring of the biological, physical, and chemical parameters of east Maui streams and estuaries. The nature of this East Maui Stream Project is to establish community and environmental harmony by providing proper stewardship while supporting economic interests. This project is a collaboration within the Division of Aquatic Resources (*DAR)* to monitor the streams and estuaries of east Maui reflecting on the connection between mauka (mountain) to makai (sea), a deeply rooted principle in the Hawaiian culture.

Method Summary

Project staff will conduct quarterly surveys on five streams: Honomanu, Waiohue, West Wailua Iki, East Wailua Iki, and Honokohau. Each monitoring site will have four 25-meter transects (total 100 meters). Sites are measured out with metric measuring tape, and metal bolts are drilled into boulders and banks with colored flagging tied to them. There are a total of 9 monitoring sites. Project staff will determine if re-establishment is needed for level logger or temperature loggers at higher elevation sites.

Streams	Sites
Honomanu	lower, middle, upper
Waiohue	lower, upper
East Wailua Iki	lower, upper
West Wailua Iki	lower
Honokohau	lower

Visual surveys will be conducted by two surveyors. The stream width will be measured along with wetted edges (metric). Stream widths will be measured for the lower, middle, and upper boundaries. The substrate and habitat will be estimated by percentage for each section. Surveyors will be using masks and snorkels to count and identify stream species. Animal measurements will be total length (inches). A fiberglass ruler will be used to measure animal lengths and stream depth (centimeters). The same size classes will be used from the original datasheet. A sonde will be used to record water quality measurements. Digital photos will be taken (upstream, downstream, left bank, right bank, etc.) Images will also be captured to document habitat and stream animals seen.

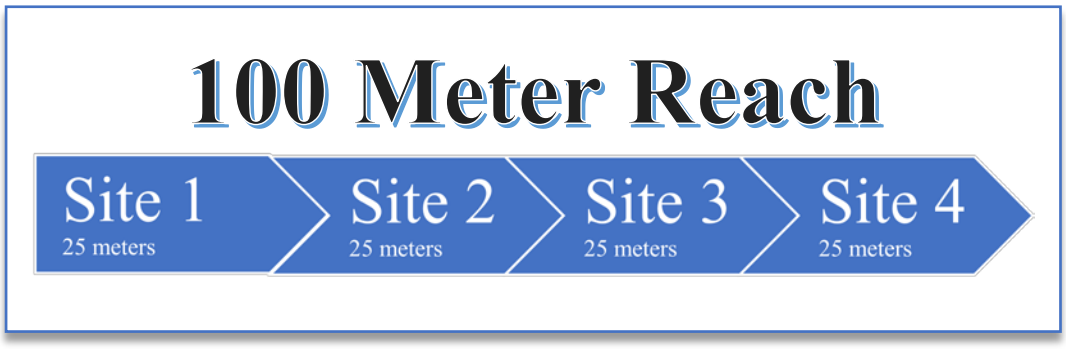
A.2 Equipment and Supply Checklist

☑	**Survey Materials**		☑
	Waterproof datasheets	Booties	
	Waterproof clipboard	DAR field shirt	
	GPS	Wetsuit (if necessary)	
	Metric measuring tape	Hood (if necessary)	
	Ruler (depth and fish measuring)	Phone to tell time	
	Snorkel	Dry bag	
	Camera	Rain jacket	
	Pen/Pencil	Sunscreen	
	Sonde (water quality)	Sunglasses	
	Bolts/flagging (2 colors)	Water and food	
	A light	First aid materials	
Flow measurements			
	Flowmeter		
	Top setting wading rod		
	Surveyor's tape		
	Streamflow waterproof datasheet		

A.3 Stream Survey Procedures

When sites are setup

1) Check the stream to make sure bolts with colored flagging marking the four 25-meter sites are still intact and visible to surveyors. If they are unable to be found, recalculate locations as needed and input new bolts and colored flagging (see instructions below for setting up new sites).
2) Delineate sites between surveyors and make sure datasheets are organized and provided to each surveyor.



Setting up new sites

1) Select a site making sure there are at least 100-meters of accessible stream reach available for survey.
2) Select a starting point for site one. Site selections should be based on factors like; safety, accessibility, ability to mark banks of the site with bolts and colored flagging, and stream condition – surveyor should be able to survey all or most of the 25-meters.
3) Pound bolts with colored flagging attached to the left and right bank of site one's lower transect. Take GPS coordinates.
4) Use the metric measuring tape to measure from one of the bolts at site one 25-meters upstream to the end of site one. Pound bolts with the same color flagging attached to the left and right bank of site one's upper transect.
5) Use the metric measuring tape to accurately measure the middle transect of site one at 12.5 meters. Pound bolts attached with a different color flagging than used for the lower and upper transect of site one into the left and right bank.
6) Repeat steps 3 through 5 three more times to set up a total of four 25-meter study sites (100-meter stream reach).

Lower transect

1) Set out the sonde for recording; degrees centigrade (◦C), millimeters mercury (mmHg), dissolved oxygen (DO), conductivity (SPC), potential of Hydrogen (pH), turbidity (FNU), total suspended solids (TSS), and depth (m). Turn on the sonde by holding the power button on the front. This step is only done at the lower transect.
2) String metric measuring tape across the stream width at the beginning of the site. Record on the site's datasheet the channel width, wetted width, and maximum depth. Record the substrate.
3) Collect measuring tape. Collect sonde data recording information on the site's datasheet and put away.
4) Take GPS coordinates and record on the site's datasheet.

Middle transect

1) The location of the middle transect will be roughly 12.5 meters from the lower transect and marked with a different color flagging. String the metric measuring tape across the middle transect line.

2) Measure and record on the site's datasheet the channel width, wetted width, and maximum depth.
3) Record substrate.

Upper transect

1) The end of the upper transect is the starting point for the lower transect of the next site.
2) String the metric measuring tape across the upper transect line reaching the bolts on either bank.
3) Measure and record on the site's datasheet the channel width, wetted width, and maximum depth.
4) Record substrate.
5) Copy recorded measurements from the datasheet of the previous sites upper transect onto the next site's datasheet lower transect.
6) Record substrate (not done by the same surveyor that recorded substrate for previous sites upper transect)
7) Repeat all transect steps for all transects three more times, except lower transect step two should already be complete and recorded on the datasheet, to complete a 100-meter sampled reach length.
8) At the end of site four, take another GPS coordinate to complete beginning and end coordinates for the 100-meter sampled reach length.

Setting up for species counts

1) Take photos, including upstream, downstream, left, and right bank.
2) Make sure the datasheet is filled out with; the date, the surveyor's name, the survey start time, the GPS coordinates and waypoint, stream name, the tributary ID, the reach type, the sampled reach length (100m), the photo numbers, the correct site number, the sampled site length (25m), and the SBN number.
    a) SBN number = (initials of surveyor) + (3-digit site number) + (m) + (-mmddyyyy).

        Example: JK001m-04062020

    b) Tributary ID = watershed code + tributary code

        Example: 64016 + 001

    c) Reach type = estuary (1), lower (2), middle (3), upper (4), or headwater (5).
3) Record the survey start time.

Visual

1) Visually scan the shallow edges of the stream while walking along the study site and take counts. Record observations in the appropriate species column and size class on the reverse side of the datasheet. Note: be aware of your shadow and movements so you don’t scare away species before your observations.

Snorkel

1) Plan your survey method ahead of time.
    a) Method 1: Follow the stream current straight up, taking species counts as you go. Once you reach the end of the survey site, loop back around to follow the stream up the sides and take species counts.
    b) Method 2: Transect diagonally across the stream back and forth, covering as much area as you can, and taking species counts as you go until you reach the end of your survey site.
2) Prepare to enter the water wearing a wetsuit and hood if necessary, with your mask and snorkel, ruler, datasheet, and clipboard.
3) Once in the water, start scanning the area for any species moving away from you and take counts and lengths recording in the appropriate species column, and size class on the back side of the datasheet then continue to conduct your survey method. For species reference, refer to species photos in the Stream Species section of this SOP.
4) When the survey is complete, make sure to record the survey end time on the datasheet and tally up all your species counts before continuing onto the next survey.

Note: Both visual and snorkel survey techniques are only applicable if habitat conditions allow the surveyor to do so. In some cases, the surveyor may only be able to perform one of these techniques and should make a note of this on the datasheet in the notes section.

A.4 Flow Measurements

1) Select a site for your flow measurement. Try to pick a location that well represents the amount of flow in the stream at that time. Try to choose a part of the stream that is not too wide, not too shallow, and not too complicated with boulders, trees, vegetation, etc. A run area with boulders on each end is ideal. If there is water flowing underground or under substrate that cannot be measured, do not select that site.
2) Once a site is selected, string the Surveyor's tape across the stream, attaching it to either side of the stream bank.
3) Record on the flow datasheet the bank full and the left and right wetted edge.
4) Take depth recordings in one-foot segments, across the entire width of the stream, starting at your left wetted edge recording.

    Example: if the left wetted edge was 5 feet, then the one-foot depth recordings start at 5.5 feet, 6.5 feet, 7.5 feet, 8.5 feet, etc.
5) Next, take the flow meter, turn it on, attach it to the top setting wading rod, and enter the stream–making sure the flow meter remains dry.
6) Measure the depth at the centerline of the one-foot segment.

    Example: If the one-foot depth recording was started at 5 feet because the left wetted edge was 5 feet, then the starting point for the centerline of the one-foot segment depth would be 5.5 feet, 6.5 feet, 7.5 feet, 8.5 feet, etc.
7) Use the centerline depth reading as the first position for flow meter calculations on the numbered top setting wading rod (this will provide numbers for middle streamflow). Take

recording after the flow meter has gone through three number calculations for more accurate recordings.

Example: centerline depth = .70, this is the number the rod is set on.

8) Multiply the centerline depth reading by two, position the rod accordingly, and record after three number calculations (this will provide numbers for surface streamflow).

Example: centerline depth = .70 x 2 = 1.40, this is the number the rod is set on.

9) Divide the centerline depth by two, position the rod accordingly, and record after three number calculations (this will provide numbers for bottom streamflow).

Example: centerline depth = .70/ 2 = .35, this is the number the rod is set on.

10) Repeat steps 6 through 9 for the entire width of the stream. Surveyor will need multiple datasheets.

End of Day Tasks

- Make sure all gear is rinsed and hung to dry (wetsuit, hoods, tabis, nets, etc.).
- Datasheets should be collected, dried, and looked over for mistakes/missing information before any project staff leaves for the day.
- Photos should be uploaded, saved, and organized with coordinating survey data.
- eDNA samples should be processed if applicable.

A.5 Animal Collection

Purpose

Stream species sampling collects biological data over time for the DAR database.

Method Summary

Species are sampled by collection with ‘O’pae nets. They are kept in a bucket under aeration pre and post measurements until they can be released back into the stream.

Equipment and Supplies Checklist

Stream Sampling	
‘O’pae net	
Tabis (booties)	
Bucket x 2	
Aerator	
Caliber	
Scale	
Pencil	
Paper for recording length and weight	
Aquarium net	

Animal Collection Procedures

1) Take recordings of start and end times and location coordinates.
2) Use an ‘O’pae net to walk upstream as you maneuver your net around the rocks.
3) Check after each net placement if you have any species in the net. If species are caught in the net transfer them to the bucket with an aerator going.
4) Once sampling is complete, use a small aquarium net to catch the species in the bucket one by one.
5) Lay the species on a towel on a flat surface as best you can and use the towel to cover over and calm if there is too much movement.
6) Quickly measure the species and then put it on the scale to quickly weigh.
7) Once you have taken a measurement and weight place in another bucket with water and aeration.
8) Once all species are measured and weighed, they are ready for release back into the stream reach they came from.

A.6 Stream Species

Hawai‘i is home to a multitude of unique resident biota, many of which are endemic meaning they are found nowhere else in the world. The importance of monitoring these freshwater and estuarine ecosystems cannot be undervalued. Freshwater is one of, if not the most, valued resource. Urban development, changing climate, and the introduction of invasive species create ecological stressors that can greatly impact aquatic populations and degrade their habitat. The East Maui Streams Project collects biological, and aquatic habitat data to assess stream quality for the following species.

<table>
<tr>
<td> 
Hihiwai Neritina granosa</td>
<td> 
Hapawai Neritina vespertina</td>
<td> 
‘O‘pae kala‘ole Atyoida bisulcata</td>
</tr>
<tr>
<td> 
Tahitian prawn M. lar</td>
<td> 
‘O‘pae ‘oeha‘a M. grandimanus</td>
<td> 
Aholehole Kuhlia xenura</td>
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<tr>
<td> 
Nopili Sicyopterus stimpsoni</td>
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Nakea Awaous stamineus</td>
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Alamo‘o Lentipes concolor</td>
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Alamo‘o Lentipes concolor</td>
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Akupa Eleotris sandwicensis</td>
<td> 
Naniha Stenogobius hawaiiensis</td>
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APPENDIX B

Standard Operating Procedures for Estuary Surveys

B. 1 Purpose and Method Summary

Acronym and Term List

BIOTA	Refers to ecological systems and functions that respond to habitat conditions, whereas key biota refers to fishes.
CWRM	Commission on Water Resource Management
DOCARE	Division of Conservation and Resource Enforcement
GPS	Global Positioning System
HABITAT CONDITIONS	Refers to physical, chemical, and thermal features, including freshwater inflow to estuaries, of contiguous stream and estuary environments.
POE METHODS	Probability of Encounter – Random cast net sampling method
SBN	Survey Book Number – Unique identifier for data entry
VISUAL METHODS	Visual cast net sampling method – targets fish species by visual search
YSI EXO 1 SONDE	Water quality measurement instrument

Purpose

The Division of Aquatic Resources (DAR) was tasked by the Commission on Water Resource Management (CWRM) to conduct baseline studies at three East Maui streams under 100% baseflow conditions. This study will address four main topics.

1) Addresses impacts of water withdrawals from Maui streams on estuarine key biota and ecological functions.
2) Compare habitat conditions in H90 and H100 restored streams on Maui.
3) Compare estuarine key biota in H90 and H100 restored streams on Maui.
4) Document and corroborate H90 and H100 rates proved by CWRM on each stream sampled.

Sampling schedule per site is four times per year and includes summer low flows.

Method Summary

Before sampling contact DOCARE office and inform them of the dates, time, location, and methods to be used for sampling.

Fixed sampling areas (stations) will be predetermined in the estuary based on data, prior knowledge, and spatial maps created from prior estuary cast net surveys. This information identifies juvenile fish "hot spots" within estuaries composed of streams entering an embayment as the stream mouth habitat. These habitat "hot spots" consist of a fixed linear distance along the shoreline that encompass areas that have consistently yielded fish captures from random net casts (> 75% probability of capture). Once "hot spots" are designated, fixed starting points and end points of sampling stations will be demarcated by GPS coordinates and documented on the data sheet. The same start and end point will be used for all subsequent surveys for these fixed sampling stations (repeated measure). Start and end point

boundaries will also be marked by visual methods such as: brightly colored marking tape secured on a plastic/metal pole implanted into the ground to visually determine start and end points. The size of the sampling stations will be 60 m linear distance at the stream mouth. Fish sampling will be conducted using a monofilament cast net 8' in length with a ¼" square mesh size. There will be two different sampling methods utilized: Visual (non-random) and Probability of Encounter (POE, systematic).

Sampling sites:

Estuary
Honomanu
Waiohue
East Wailua Iki
West Wailua Iki
Honokohau
Kealia

B.2 Equipment and Supply Checklist

Survey Materials	
One 8' length, ¼" square mesh cast net (plus 1 spare net)	Digital camera
At minimum four 3-to-5-gallon buckets (covers optional)	Extra batteries ("AA", "D")
Air pumps (minimum of 4)	Flagging tape
Measuring boards (minimum of 3)	Whirl packs/ plastic bags
Surveyors measuring tape in meters	Identification books
Small scoop nets	Tabis (non-slip booties)
Hand tally counter (minimum of 3)	DAR field shirt
GPS (Garmin GPSMap 64 or similar)	Polarized sunglasses
YSI EXO1 water quality sonde	Hat
Weighted frame for Sonde with float	Sunscreen
Clip board	Raincoat
Waterproof data sheets	Water and Food
Stopwatch	First aid kit
Extending painters pole with cm markings	Cooler with ice
Pencils, pens	

B.3 Visual Method (non-random)

The visual sampling method (non-random) is the first sampling method employed when sampling a selected station. Surveyors will visually locate and capture any schooling juvenile estuarine sport-fish species within the selected sampling station boundaries.

1) A limit on number of casts will be determined by the linear distance of the sampling station. For every 12 meters (approximately 20 paces), two (2) casts will be allowed.

- For example, if a sampling station equals 60 m linear distance, dividing that distance by 12 meters equals a quotient of approximately 5. Multiplying the quotient (5) by 2 casts equals 10 casts. Therefore, for a sampling station 60 m in linear distance, a limit of 10 non-random visual casts can be made.

1) A sampling time limit will be determined by the linear distance of the sampling station. For every 12 meters (approximately 20 paces), four (4) minutes will be allowed for sampling.
    - For example, if a sampling station equals 60 m linear distance, dividing that distance by 12 meters equals a quotient of approximately 5. Multiplying the quotient (5) by 4 minutes equals 20 minutes. Therefore, for a sampling station 60 m in linear distance, the sampling time limit would be 20 minutes.

2) If no fish are visually located and sampling time remains, haphazard casts (net casters knowledge and best guess where fish may be within the sampling boundaries) will be made until the limit number of casts is reached.

B.4 Probability of Encounter Method (POE, systematic)

After visual sampling is completed, the POE method (systematic or random) will be utilized after a 15-minute pause to let the area "settle down".

1) The first cast will be made at one end of the sampling station boundaries.
2) Each subsequent cast will be taken at a distance 20 paces away from the previous cast (ca. 12 m) until the surveyor has reached the other boundary of the sampling station.
3) When the boundary has been reached, the surveyor will then backtrack and continue sampling until reaching the starting point or until all casts have been expended.
4) The same metrics described previously will determine the number of casts and time limit for the sampling station.
    a. For a sampling station that equals 60 m linear distance, a total of 10 casts will be made within a 20-minute time frame.
    b. The net should be casted from the shoreline when the water depth at the station is >20 cm and/or rapidly increases to such water depths that can be reached with the net standing at the edge of the shoreline.
    c. The shoreline is defined as the linear boundary where estuarine habitat is constantly submerged underwater.
    d. Net should be casted immediately once the net caster is set and in position.
5) If the sampling location is characterized by shallow habitat (< 20 cm water depth) that extends more than 6 meters from the shoreline (approximate extent of a casted net) and fish are obviously absent in these sections or large boulders are present making the area un-castable (cast net will get snagged on the boulders allowing fish to escape under the net), then the net caster will be required to do random paces perpendicular to the shoreline by multiplying a random number by a factor 2 or 5.

a. The multiplying factor should be unanimously chosen by the surveying party. For example, let the random number = 5, the unanimously chosen perpendicular multiplying factor is 2, then the number of perpendicular paces from shore is 5 X 2 = 10 paces.
b. If the net caster reaches a knee-level water depth before finishing perpendicular paces from shore, the net should be casted from that spot.
c. If conditions become too hazardous before finishing paces, the net caster should cast the net from the last safest position.

6) The net caster should set their net prior to walking random perpendicular paces. However, if fish are obviously present near the shoreline in shallow (< 20 cm water depth) habitat, random perpendicular paces from shore are not required and a cast should be made from the shoreline.
7) If the net spreads <75% of its maximum spread when casted and/or gets hung up on anything that inhibits an effective sample collection, then that cast will be recorded as a botched cast.
    - A botched cast can be redone if there is a unanimous consensus amongst all surveyors that the cast was truly botched. This make up cast will be documented as such on the data sheet.
8) If something (large boulders; submerged tree branches; etc.) or someone physically prevents effective sampling at a randomly designated sampling station (e.g., fishers, swimmers, paddlers etc.), skip the designated sampling station and continue to the next nearest unimpeded location 20 paces away.

B.5 Fish and Invertebrate Measurements

1) All fish and macroinvertebrate species sampled in the cast net will be maintained in aerated buckets until they are identified to the lowest taxonomic unit, measured, and recorded in millimeters.
2) All fish and invertebrates caught with this method will be sorted and measured after each cast.
3) Fish will be measured by fork-length or total-length when no fork is present.
4) Macroinvertebrates will be measured by carapace-length or total-length depending on the classification of the specimen.
5) All fish will be measured manually with measuring boards and invertebrates will be measured with calipers.
6) Specimens will be released back into the estuary outside of the sampling station.
    a. Any mortally wounded specimens will be kept.
    b. Unidentifiable specimens will be photographed and, if needed, preserved in 5% formalin for later identification.
7) Any unusual observations will also be documented.
8) If >300 individuals of one species are caught in a single cast, then randomly measure the first 300 individuals as a subsample. The remainder of that species are to be counted only and recorded as *total count not measured*. Fork length data entered the database for *total count not measured* is calculated from mean size measured from the subsampled 300 individuals.

B.6 Physical- Chemical Water Parameters

1) Physical- chemical water quality parameters will be taken in the middle of the sampling area within stream mouth portion of the estuary, in a location that is determined to be safe for the sonde to be deployed and recovered during the sampling period.
2) Using an YSI EXO1 water quality sonde, the following parameters will be recorded: temperature (º C), conductivity (mS/cm), dissolved oxygen (mg/L), pH (units), salinity (PSS), dissolved oxygen (%), water depth (cm) and turbidity (NTU).
3) Set the data logging interval on the sonde to 15 seconds.
    a. The sonde will be placed on the bottom of the water column using a weighted frame to hold the sonde on place.
    b. A float will be attached to the sonde as a visual marker.
4) Sonde site will be geo-referenced by GPS waypoints, saved in a hand-held GPS unit and recorded on the data sheet.
5) Data collected from the sonde will be downloaded into a file located in Dropbox.
6) The tide stage will be calculated after sampling using tide calculating software.
7) YSI EXO1 sonde will be calibrated according to YSI's recommended intervals and using YSI calibration solutions.

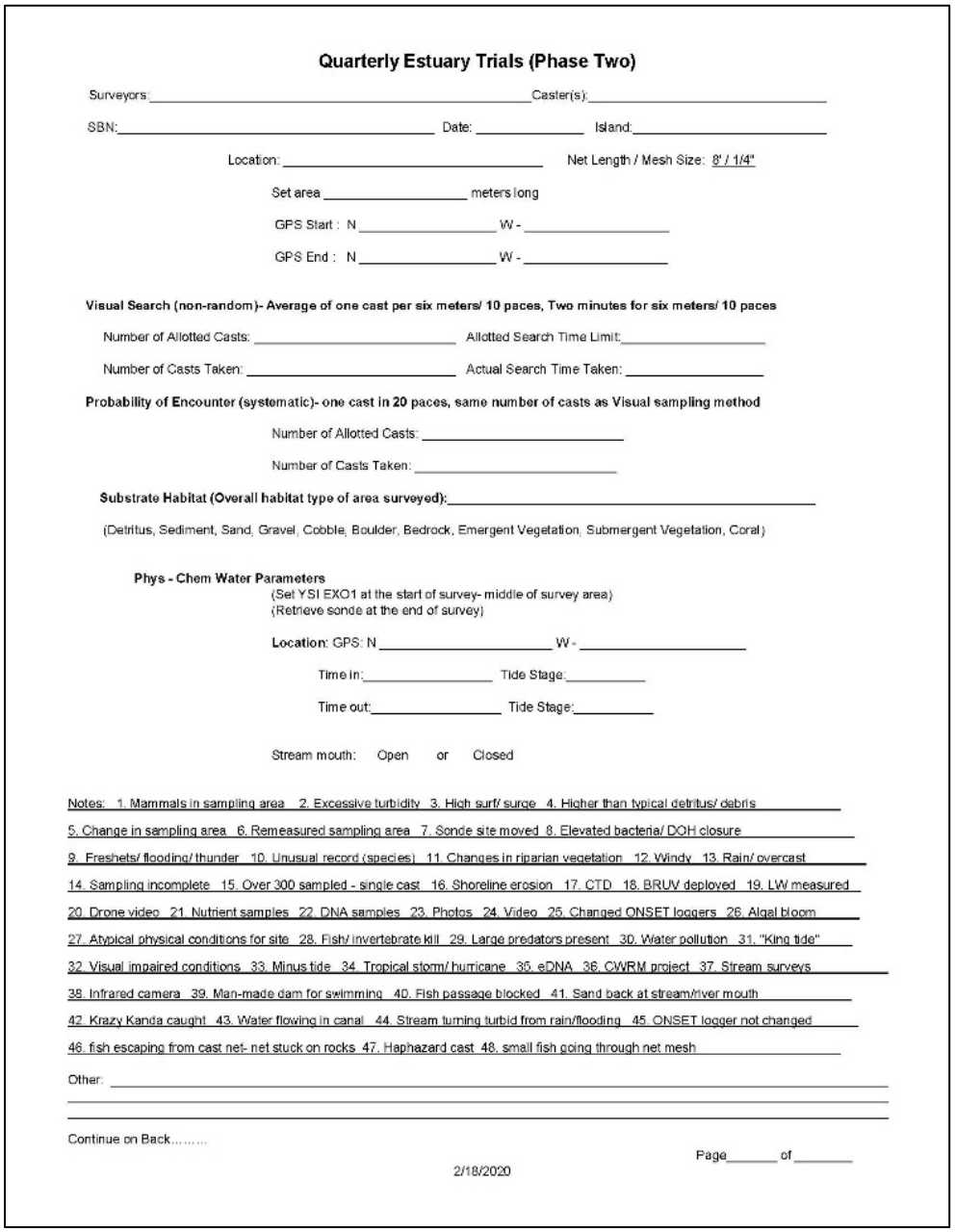
Sonde with weighted frame and attached float.

B.7 Substrate Habitat

1) Document the general substrate habitat of the entire area surveyed, i.e. if the area consists of mostly detritus, sediment, sand, gravel, cobble, boulder, bedrock, emergent vegetation, submergent vegetation, coral, or concrete.

Sampling will be rescheduled for another day if hazardous conditions are present (i.e., high surf, hazardous terrain, thunderstorms, unruly people) or if conditions preventing effective sampling (i.e., high human traffic, ocean-user events etc.) are present.

B.8 Estuary Sampling Procedures- Data Sheet Instructions



Quarterly Estuary Trials (Phase Two)

Surveyors:_____ Caster(s):_____

SBN:_____ Date: _____ Island:_____

Location: _____ Net Length / Mesh Size: 8' / 1/4"

Set area _____ meters long

GPS Start : N _____ W - _____

GPS End : N _____ W - _____

Visual Search (non-random)- Average of one cast per six meters/ 10 paces, Two minutes for six meters/ 10 paces

Number of Allotted Casts: _____ Allotted Search Time Limit:_____

Number of Casts Taken: _____ Actual Search Time Taken: _____

Probability of Encounter (systematic)- one cast in 20 paces, same number of casts as Visual sampling method

Number of Allotted Casts: _____

Number of Casts Taken: _____

Substrate Habitat (Overall habitat type of area surveyed):_____

(Detritus, Sediment, Sand, Gravel, Cobble, Boulder, Bedrock, Emergent Vegetation, Submergent Vegetation, Coral)

Phys - Chem Water Parameters
(Set YSI EXO1 at the start of survey- middle of survey area)
(Retrieve sonde at the end of survey)

Location: GPS: N _____ W - _____

Time in:_____ Tide Stage:_____

Time out:_____ Tide Stage:_____

Stream mouth: Open or Closed

Notes: 1. Mammals in sampling area 2. Excessive turbidity 3. High surf/ surge 4. Higher than typical detritus/ debris
5. Change in sampling area 6. Remeasured sampling area 7. Sonde site moved 8. Elevated bacteria/ DOH closure
9. Freshets/ flooding/ thunder 10. Unusual record (species) 11. Changes in riparian vegetation 12. Windy 13. Rain/ overcast
14. Sampling incomplete 15. Over 300 sampled - single cast 16. Shoreline erosion 17. CTD 18. BRUV deployed 19. LW measured
20. Drone video 21. Nutrient samples 22. DNA samples 23. Photos 24. Video 25. Changed ONSET loggers 26. Algal bloom
27. Atypical physical conditions for site 28. Fish/ invertebrate kill 29. Large predators present 30. Water pollution 31. "King tide"
32. Visual impaired conditions 33. Minus tide 34. Tropical storm/ hurricane 35. eDNA 36. CWRM project 37. Stream surveys
38. Infrared camera 39. Man-made dam for swimming 40. Fish passage blocked 41. Sand back at stream/river mouth
42. Krazy Kanda caught 43. Water flowing in canal 44. Stream turning turbid from rain/flooding 45. ONSET logger not changed
46. fish escaping from cast net- net stuck on rocks 47. Haphazard cast 48. small fish going through net mesh

Other: _____

Continue on Back.....

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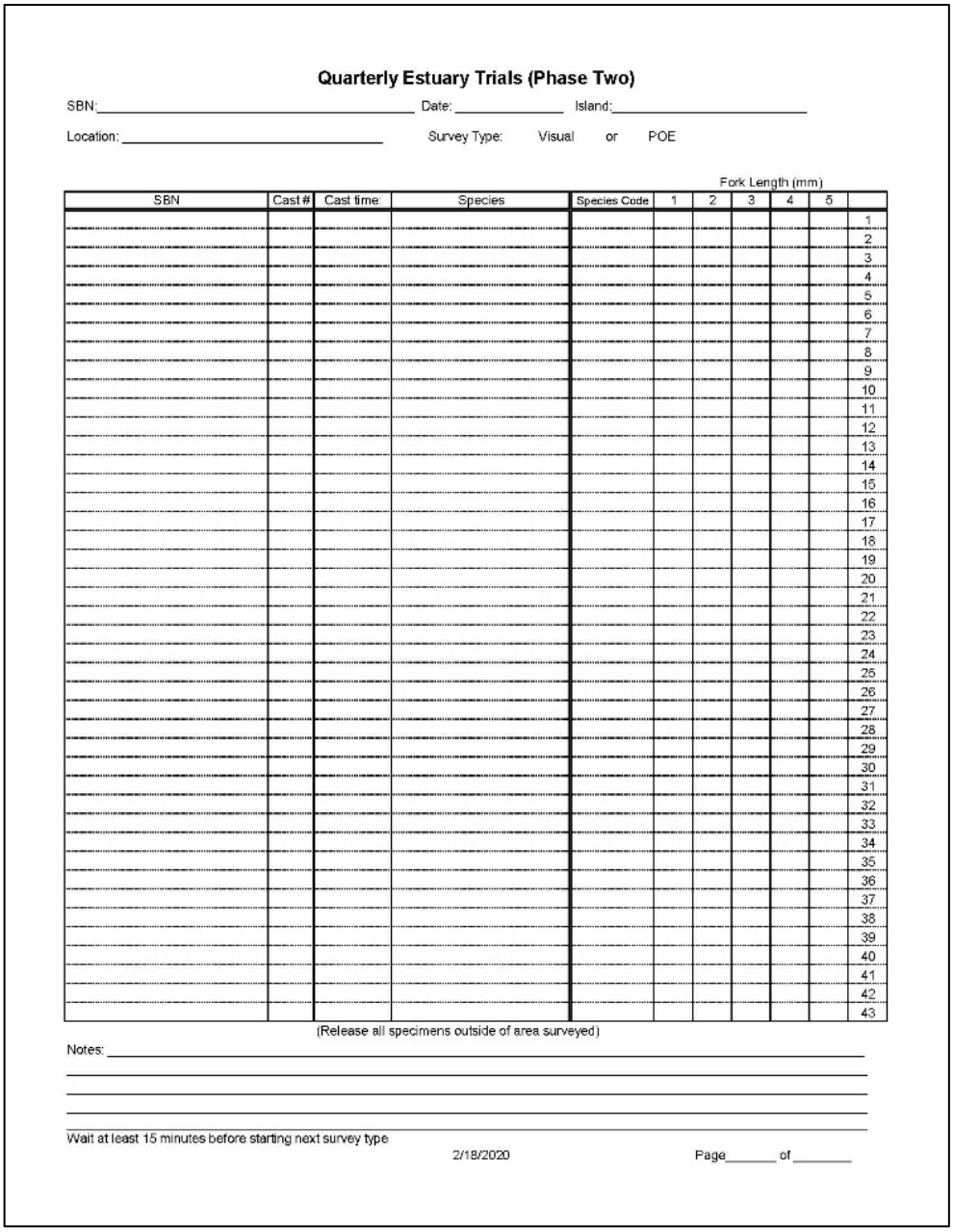
2/18/2020

Estuary Datasheet- Front



Quarterly Estuary Trials (Phase Two)

SBN:_____ Date:_____ Island:_____

Location:_____ Survey Type: Visual or POE

SBN	Cast #	Cast time:	Species	Species Code	Fork Length (mm) 1	2	3	4	5	
										1
										2
										3
										4
										5
										6
										7
										8
										9
										10
										11
										12
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										39
										40
										41
										42
										43

(Release all specimens outside of area surveyed)

Notes: _____

Wait at least 15 minutes before starting next survey type

2/18/2020 Page_____ of _____

Estuary Datasheet- Back

1) On the data sheet, record all surveyors' initials and record initials of person(s) who will be casting the net.
2) Write the Survey Book Number (SBN): (initials of recorder followed by date- XX-mmddyy) example- TS-030221 where TS is the recorders initials followed by the survey date March 03, 2021.
3) Record the date, island, and location being sampled.
4) Measure and record the set area to be sampled.

a. To determine the sampling area boundaries, use the surveyors measuring tape and measure off a linear distance of 60 meters going from one bank of the stream mouth to the other side.
b. The boundaries will then be marked with brightly colored surveyors marking tape secured on a stick/pole implanted into the ground or any other means of visually demarcating the boundary limits.

5) Record the GPS start and end coordinates
6) Determine and record the number of casts that will be taken as well as the total allowable search time.
7) Record the general habitat of the entire survey site
    - Habitat segregated by the following categories:
      Detritus, Sediment, Sand, Gravel, Cobble, Bedrock, Emergent vegetation, Submergent vegetation, Coral, and Concrete

8) Physical- Chemical water parameters
    a. Before sampling starts, deploy YSI EXO 1 with weighted frame and float for the entire survey- set at the midpoint of the survey area at a minimum depth of 0.61 meters (two feet).
    b. Record the coordinates using the GPS
    c. Record the time of deployment
    d. The sonde will record the following measurements: Temperature (ºC), Conductivity (mS/cm), Dissolved Oxygen (mg/L), pH (units), Salinity (PSS), Dissolved Oxygen (%), Turbidity (NTU), and Depth (m).
    e. The sonde will be retrieved at the end of sampling, record time of retrieval.
9) Tide stage will be calculated after sampling using tide calculating software using water quality parameter start and end times.
10) Fill out any unusual observations or thing of note in the "notes" section.
11) Visual (non-random) search – capture schooling/targeted species
    a. Circle Visual on the data sheet for Visual Methods
    b. Capture species within boundary area under allotted time frame and allowable casts
    c. The recorder will keep track of time using the stopwatch. Time starts when the net caster is actively looking for target fish species and stops when the net has been casted.
        i. Record cast time (time when the net hits the water) for each cast.
        ii. Write the corresponding cast net cast number
    d. Fill in Cast SBN for each cast: recorder initials + two digit cast number + E (estuary) **V** (Visual) – mmddyy (Example: TS01E**V**-030221)
    e. For successful catches, place in a bucket with air pumps.
    f. Record species/ species code and measure all fish and invertebrates.
        i. Release back unharmed outside of the survey area boundaries.
        ii. Bag and keep any mortally wounded specimens.
        iii. If no catch record 0 (zero).
        iv. If >300 individuals of one species are caught in a single cast, then randomly measure the first 300 individuals as a subsample. The remainder of that species are to be counted mean size measured from the subsampled 300 individuals.

v. Time starts again when the net caster is actively searching.

g. Visual sampling stops when all casts and search time have been expended.

12) After Visual sampling is complete, wait at least 15 minutes before starting POE sampling.

13) Probability of Encounter Methods (POE, systematic) – 1 cast every six meters/ 10 paces (same number of casts as in visual sampling methods).
    a. Start at beginning of boundary area and make the first cast.
    b. Next cast is 20 paces away from the first cast.
    c. Each subsequent cast is made 20 paces away from the last cast. When the end boundary is reached, turn around and sample back to the starting point. Sampling is finished when the allotted number of casts are expended).
    d. On the data sheet, circle Probability of Encounter (POE).
    e. Write the corresponding cast number
    f. Record the cast time (time when the net hits the water) for each cast.
    g. Fill in Cast SBN for each cast: recorder initials + two digit cast number + E (estuary) **P** (POE) – mmddyy (Example: TS01E**P**-030221).
    h. For successful catches, place in a bucket with air pumps.
    i. Record species/ species code and measure all fish and invertebrates.
        i. Release back unharmed outside of the survey area boundaries.
        ii. Bag and keep any mortally wounded specimens)
        iii. If no catch record 0 (zero).

14) When POE methods are finished, retrieve Sonde and record time retrieved.

End of Day Tasks

- Make sure all gear is rinsed and hung to dry (net, tabis, buckets, etc.).
- Sonde should be thoroughly cleaned and rinsed.
- Datasheets should be collected, dried, and looked over for mistakes/missing information before any project staff leaves for the day.
- Photos should be uploaded, saved, and organized with coordinating survey data.
- Data sheets should be scanned as soon as a scanner is available.

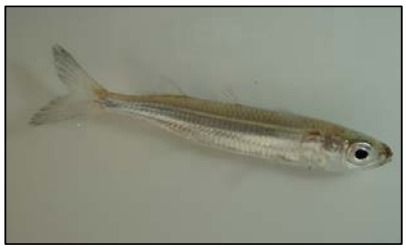
B.9 Estuarine Species

Āholehole *Kuhlia xenura*

Weke *Mulloidichthys flavolineatus*

Nenue *Kyphosus hawaiiensis*

Manini *Acanthurus triostegus*



ʻIao *Atherinomorus insularum*

ʻŌmilu *Caranx melampygus*

ʻAma ʻama *Mugil cephalus*

Uouoa *Neomyxus leuciscus*

Kanda *Moolgarda engeli*
(Introduced)

Examples of common estuarine fish species

APPENDIX C

Standard Operation Procedures for eDNA Collection

C.1 Purpose and Method Summary

Purpose

To provide an independent species assessment of East Maui streams in support of the 2020 East Maui Streams and Estuaries baseline monitoring project.

Method Summary

East Maui environmental DNA (eDNA) sampling will be conducted in accordance with quarterly surveys conducted on five streams: Honomanu, Waiohue, West Wailua Iki, East Wailua Iki, and Honokohau.

C.2 Equipment and Supply Checklist

eDNA Field		eDNA Lab		Filter Cups	
eDNA bottles x2 per sample		Sanitized and assembled filter cups		0.45 filters	
Field control bottle		10% bleach solution		Sanitized filter cups (tops and bottoms)	
Gloves: One pair per sample and one pair for field control		Vials and rack		Special tweezers	
eDNA waterproof data sheet		Paper towels		Pair of nitrile gloves	
Ice for keeping samples cold		Box of nitrile gloves		10% bleach solution	
1.5 liters bottled water (not bottled in Hawai‘i)		Tweezers			
		Pump x2			
		Water container x2			
		Sample holder x2			

C.3 Taking eDNA Samples (Streams)

Stream eDNA is most often taken right above the end of site four after completing the survey transect measurements.

1) Set the sonde in the stream location where the eDNA will be taken.
2) Prepare an eDNA datasheet filled out with the following information: date, collector name, SBN number, start time, GPS coordinates and waypoint, stream name, reach type, sampled reach length, and photo numbers.
    a. SBN number = (collector initials) + (3-digit site number) + (m) + (-mmddyyyy)
        i. Example: JK005m-04062020
        ii. eDNA site numbers should be site five if taken above survey site four, otherwise number relative to survey site location.
    b. Sampled reach length is the same as the surveys.
    c. Photos are not required for eDNA, but they are recommended.
3) Record sonde data on the eDNA datasheet and put sonde away.

4) One surveyor puts on clean gloves and grabs a clean eDNA sample bottle while the other surveyor records the time, bottle number, and GPS coordinates.
    a. NOTE: When taking the eDNA sample make sure to submerge the bottle and cap in the stream location of sample three times, filling and dumping it, before filling the bottle for a sample.
    b. When filling the eDNA bottle for a sample in the streams, make sure the bottle is not submerged too far into the water column.
5) The surveyor repeats steps five and six for the duplicate sample. It is acceptable to use the same gloves for duplicate samples.
6) The surveyor taking the sample puts the stream samples in a bag and keeps them cool by keeping them submerged in the stream water.
7) The surveyor puts on new clean gloves and pours the 1.5-liter bottle of water (not bottled in Hawai‘i) in the empty control sample bottle, first rinsing the bottle and cap three times before filling for a sample. The other surveyor records the time the sample is taken and the control bottle number. The control is marked as control on the datasheet.
8) Samples are kept as cool as possible until processed.

C.4 Taking eDNA samples (Estuary)

Estuary eDNA is taken before the estuary survey starts. Samples are taken at right side of the estuary (embayment), in the middle of the stream mouth where fresh and saltwater converge, and at the left side of the estuary (embayment). Control samples are taken before eDNA collection begins and after when collection is finished.

11) A control sample is taken before eDNA sampling. The surveyor puts on new clean gloves and pours the 1.5-liter bottle of water (not bottled in Hawai‘i) in the empty control sample bottle, first rinsing the bottle and cap three times before filling for a sample. The other surveyor records the time the sample is taken and the control bottle number. The control is marked as control on the datasheet.
12) One surveyor puts on clean gloves and grabs a clean eDNA sample bottle while the other surveyor records the time, bottle number, and GPS coordinates.

NOTE: Before taking the eDNA sample make sure to submerge the bottle and cap in the water, filling and dumping it, three times before filling the bottle for a sample.

When filling the eDNA bottle for an estuary sample, the bottle can be submerged into the water column, but make sure to dip and swish the bottle as you fill it, to increase the amount of surface area collected. Avoid collecting any large bit of detritus in the water sample.

13) The surveyor repeats step two for the duplicate sample. It is acceptable to use the same gloves for duplicate samples.
14) The surveyor taking the sample puts the samples in a bag and keeps them cool by keeping them submerged in the stream water.
15) A control sample is taken at the end of eDNA sampling. The surveyor puts on new clean gloves and pours the 1.5-liter bottle of water (not bottled in Hawai‘i) in the empty control sample bottle, first rinsing the bottle and cap three times before filling for a sample. The other surveyor records the time the sample is taken and the control bottle number. The control is marked as control on the datasheet.

16) Samples are kept as cool as possible, preferably on ice, until processed.

C.5 eDNA Lab processing

17) Use a 10% bleach solution to clean the processing area and all the equipment. Let the bleach sit a few minutes before wiping down with paper towels.
    a) While bleach is soaking the surfaces, get all the material set out onto a sanitized accessible surface. Make sure to crack open material bags so materials can easily be accessed. Materials include tweezers, a box of nitrile gloves, sanitized and assembled filter cups, 10% bleach solution, vials, and paper towels.
18) Wearing nitrile gloves, assemble the processing equipment. Make sure the hoses are securely attached to the pump, water container, and the sample holder.
    a) Connect one hose from the pump to the water container and connect the other hose from the sample holder to the water container.
19) Still wearing gloves, spray down a paper towel with a 10% bleach solution and retrieve the samples from their storage location. Spray all the samples down with the 10% bleach solution, let it sit for a few minutes, then wipe them all down thoroughly with paper towels.
20) Discard gloves, clean hands, and prepare the vials to process the selected samples. Put the samples on vial rack at the processing station in the order in which they will be processed.
21) Put a paper towel down in front of processing station.
22) Put on a clean pair of nitrile gloves.
23) Grab two assembled filter cups, tweezers, and put them at the processing station (placing filter cups on sample holder).
24) Grab sample replicates and place them on either side of the sample holder. The sample numbers should be placed in correlation with what side the vials on the vial holder are.
    a) Example: if vial 59 is placed in the vial holder on the right, then the sample bottle 59 should be placed on the right side of the sample holder to avoid sample confusion.
25) Begin running the samples by pouring each replicate into the filter cups up to the 300 ml line. Set samples down on either side of the holder with the lids covering them. Make sure the sample holder base switch is turned up (open) and turn on the pump.
26) The sample water level should begin to reduce. Keep track of the ml's processed. (This is in case the filter blows through or there are other complications with the sample).
27) Continue to refill the sample filter cups until the whole 1-liter sample bottle is processed.
28) There will be a water ring around the edge of the cup, wait for it to disappear, then turn off the pump once the water is completely gone from the filter cup.
29) Leaving the filter cup on the sample holder, carefully remove the cup from the base with the filter on it. Use the sanitized tweezers that are sitting on the paper towel to fold the filter in half once, twice, and then three times, making the shape of a pizza slice. Note; if the filter falls or is contaminated somehow, make a note of it, and continue with processing. Do not discard or start over because there is a limited amount of sampling.
30) Leave the folded filter on the cup base while grabbing the appropriately numbered vial.

31) Use the tweezers to pick up the filter and place it in the vial, making sure the filter is submerged in the vial's preservative solution.
32) Clean the entire processing area before starting on the next samples; discard the filter cups, tweezers, and gloves, then spray a paper towel with the 10% bleach solution and wipe down all equipment touched during processing.
33) Repeat steps 5 through 16 for all sample pairs.

End of Processing Tasks

1) Rinse the equipment, sample holders, and water containers.
2) Spray and wipe down all equipment with the 10% bleach solution and store in designated storage areas.
3) Gather all used reusable materials into one big bag and set aside for sanitizing.
4) Put all clean, unused processing materials back into the processing supplies container and put in storage.
5) Make sure data and filter samples are secured.

C.6 Putting Together Filter Cups

1) All equipment should be washed, sanitized, and put away in labeled storage bags.
2) Clean the area with the bleach solution, wipe the surfaces down, and layout fresh paper towels for a clean working area.
3) Set out materials (filters, cups, tweezers) on a clean surface.
4) Put on a pair of gloves, use a pair of tweezers to carefully pick up a filter and place it on the base of a filter cup. (Make sure the filter is placed on the cup with the same side down as it was in the container).
5) Take a cup and press it onto the base firmly to feel a pop, connecting the filter cup and its base.
6) Place the assembled filter cup in a clean bag labeled sterilized with the date.
7) Repeat steps 1 through 6 for as many times as needed.

C.7 Cleaning Supplies

Purpose

To clean reusables to ensure that negative controls are negative so that no carryover DNA can be detected.

Methods

eDNA protocols are intentionally sensitive to the presence of minute amounts of DNA in the environment. While there are several causes for DNA to be present in negative controls, this protocol addresses problems associated with carryover DNA. Carryover DNA is DNA remaining from earlier uses of the reusables (sample bottles, filter cups and forceps). Proper cleaning of all reusables will greatly reduce the presence of carryover DNA and goes a long way to ensure that DNA is not detected in negative controls.

If DNA is detected in a negative control, then it is treated like an eDNA sample and sequenced to identify all species present in the contaminated negative control. All species that are identified in negative controls from a sampling date are removed from all eDNA samples collected on that same sampling date before data analyses.

Note: This SOP is an upgrade to an earlier SOP. This upgrade in procedures is needed because carryover DNA has been detected in negative controls and the number of negative controls with carryover DNA increased with each successive sampling date, meaning that carryover DNA is accumulating in/on reusables. Species diversity has been negatively impacted as a result.

1) Prepare 30% bleach solution. Ensure that the volume of bleach solution is adequate to submerge all reusables. Wear gloves and eye protection as well as have good ventilation when preparing and working with a bleach solution.
2) Use bottle brushes to clean each sample bottle and cap. Inspect each bottle taking care to dislodge and rinse out any sediment or other material that may remain and carryover DNA as contamination.
3) Use a scrub brush on each filter cap and holder to dislodge any remaining DNA.
4) Use small scrub brush on the inside and outside of each pair of forceps, moving them back and forth to dislodge any remaining DNA.
5) Soak sample bottles, sample bottle caps, filter cups and forceps in 30% bleach solution for 20 minutes minimum. Make sure that bottles are filled with bleach solution and that caps, filter cups and forceps are completely submerged. Agitate every 5 minutes to ensure proper exposure of surfaces to bleach solution and breakup bubbles that can create a barrier to the bleach solution as it degrades carryover DNA.
6) Rinse with Deionized water and air dry.
7) Relabel numbers on sample bottles that were faded by bleach solution.
8) Package reusables in plastic bags and seal to prevent exposure to eDNA before use.

APPENDIX D

Standard Operation Procedures for Stream Cameras

D.1 Purpose and Method Summary

Purpose

To improve interpretation of source(s) of freshwater inflow to estuaries and track the time a stream is at a H rating (H0; H50; H90; H100, etc.) trail cameras were mounted in upstream positions to photograph streambed state. Image captures would provide visual baseline data on specific East Maui stream conditions and freshwater inflow to estuaries over time.

Method Summary

Two cameras per site is needed in the event one camera is lost or defective. Place and/or identify a minimum of three reference points within camera view to use for estimating wetted edge and other metrics used to score H rating. Dry streambeds can be documented, as well as wetted edge measurements extracted from the imagery. Cameras will be programmed to take photographs during daylight hours every 60 minutes on the hour. Cameras can be inconspicuously mounted to a tree on the high bank side of the stream in an area with a slope. This will protect the camera from most freshets and vandalism. After deployment and before changing batteries, measure the wetted edge of the stream in the camera view to normalize data for determining H rating. Photographs will be downloaded, and batteries changed at regular (1-3 month) intervals. Data from the photographs would yield: time at each H rating. For example, imagery could be used to produce data such as: 1765 hours at H0 (meaning streambed is dry), 250 hours at H90 (target rating), and 1-hour H100+ (freshet) from August to October 2019. Data from water level loggers will be used to interpret imagery. For estuaries this method will also help to estimate the contribution of surface water relative to ground water for freshwater inflow into the estuary.

Field data is recorded on a Stream Camera Data Sheet. Photos are scored to indicate stream condition. Scores are recorded in and Excel spreadsheet as; 1=run present, 2=dry bed, 3=isolated pools, 4=freshet, 5=camera not working, 6=image quality too poor to rate. Two independent scorings of the same photos are done to ensure scoring accuracy and account for errors. Once images have been scored, the monthly tally totals are added, and monthly stream condition percentage comparisons are made.

D.2 Stream Camera Setup Steps

Position the camera at an acceptable height and distance from the stream to clearly view a section of the entire stream channel. If the stream channel is too wide for one camera, deploy two cameras. Use plastic shims, cable ties, and locks to secure the camera to a tree. Choose locations where the camera is not apparent to a passerby.

Camera Settings:

Game camera: Digital Trail Camera SL112 DSP.SL20190513MCU.V85
Advanced settings used for the first deployment:
Mode: Camera
Photo Size: 16 MP
Picture Number: 01 photo
Interval: 60M
Sense level: Low
Set Date/Time: MM/DD/YYYY
Date Stamp: Date/Time
Timer: ON 06:00 to 18:30
Time Lapse: ON every 15 minutes
Serial Number: ON start with 1000, 1001, 1002, ECT. Label with Sharpie inside and outside of the housing
SD Card Storage: Stop saving when full
Format: NO
Default Setting: NO
Version: DSP.SL112C.20190513 MCU.V85
Memory card: SDHC UHS-I 32 GB labeled with Sharpie beginning with M1; M2; M3; ECT
To Change Advanced Settings:

1. Side button to SET.
2. Press the MENU button.
3. Setup menu is displayed.
4. Use *Up Down* arrows to scroll through the MENU.
5. *Left Right* arrows work only when changing settings, not in the main menu screen.
6. When the parameter is highlighted, press the OK button, then toggle up or down to change, then select OK to set. Select MENU to exit.

Note: Use the SET button to scroll through photos and check placement before locking up the unit.

D.3 Equipment and Supply Checklist

Field		Analysis	
Datasheets		Full SD cards	
GPS		SD card reader	
Batteries		Computer	
Camera lock keys			
Camera wedges			
Cleared SD cards			
Pen/pencil			
Olympus camera (optional)			
Step ladder (if necessary)			
Large black zip ties			

D.4 Deployment and Recovery Procedures

Deployment refers to when the camera is loaded with new batteries and a blank SD card. Recovery refers to the recovery of the SD card.

At the site, fill out a Stream Camera Deployment and Recovery Datasheet with the following:

- Date
- Time
- GPS position
- Elevation
- Stream and site
- Action (deployment or recovery)
- Camera orientation – facing upstream; downstream; other
- Bank mount position – right or left bank
- Stream conditions: run, isolated pools, dry, etc.

During deployment and recovery, make sure to record the following on the datasheet:

- Double-check settings for:
    - Interval programed
    - Photo size
    - Camera serial number
    - SD card unique ID
    - Camera date stamp
    - Camera time stamp
    - Actual date
    - Actual time
    - Note any changes made to the camera settings
    - Check that Camera Settings are as programmed, especially if batteries have been removed/replaced. Time and Date stamps may need to be adjusted. Also, check that Time and Date stamps don't drift during a deployment. Synchronize with internet time from cell phones, not from a watch. Note camera serial number, SD card unique ID, camera time and date of both deployments and recoveries, actual time, and date to help with post-processing, and note any changes made to camera settings.

Stream Conditions

Run present

(11/20/2019 Time: 11:16)

Dry bed

(10/18/2019 Time: 14:16)

Isolated pools

(12/10/19 Time: 10:35 (taken with Olympus camera))

Freshet

(11/23/19 Time: 14:46)

Camera not working

Image quality too poor to rate

(11/25/19 Time: 6:00)

Scoring Codes: 1=run present, 2=dry bed, 3=isolated pools, 4=freshet, 5=camera not working, 6=image quality too poor to rate

D.5 Saving Data and Photos

1) First, you will need full SD cards, SD card USB converter, and a computer.
2) In a folder labeled *Stream Camera Project*, data and photos are stored in folders labeled by year.

   Example: 2020_StreamCams
3) In yearly folders are folders labeled *data* and *photos*.

   The *data* folder contains two excel spreadsheets, one for that year's stream camera photo scoring *(2020_Stream_Cam_Data)* and one for that year's stream camera analysis *(2020_Analysis).*

2020_Stream_Cam_Data

Label all datasheets with the date, deployment or recovery #, camera serial number, and SD card number. There will be two scorings of the photos done; therefore, there will be a deployment and recovery sheet for every camera serial number and SD card.

Example: *31-Jan Deploy 4 (1000, M1), 31-Jan Recovery 4 (1000, M1)*

2020_Analysis

Label all sheets with the date range, camera serial number, and SD card number.

Example: *Dec-Jan (1002, M5)*

Label the stream reach totals sheets with the date range, stream, camera serial number, and the reach type.

Example: *Sep-Jan Honomanu (1000) Lower*

- The *photos* folder contains photo folders for every set of SD card images. These folders are labeled with the deployment date, the deployment number, the camera serial number, and the SD card number.

  Example: *31-Jan Deploy 4 (1000, M1)*

4) After field collection of SD cards, save the photos in the appropriate photos folder and make sure to backup data. Delete the images off the SD cards only when sure there are two saved copies of the photos.

D.6 Scoring Photos

1) Data and photo saving files should be set up appropriately (see *saving data and photos*).
2) Data is listed on sheets as follows.
   a) Recovery or deployment data:

      Trib ID, island, stream name, date, time, site name, action, latitude, longitude, elevation, camera orientation, bank mount, interval timer, photo size, camera serial number, SD card unique identifier, observer name, stream condition at time of deployment or recovery, date stamp camera output, timestamp camera output, date actual, time actual, notes.

   b) Photo scoring data:

      Date of stream condition scoring, photo date, photo time, picture number camera output, interval timer photograph, movement triggered photograph, water stream rating, stream condition codes (1=run present, 2=dry stream, 3=isolated pools, 4=freshet, 5=camera not working, 6=image quality too poor to rate), other observations.

4) Fill the first half of the sheet in with the deployment or recovery data. The first set of scored photos will have the deployment data with the photo scores. The second time the images are scored, they will have the recovery data with the photo scores.
   a) Deployment example; 64009 Maui Honomanu 31-Jan-20 10:04 lower deployment 4……
   b) Recovery example; 64009 Maui Honomanu 18-Mar-20 10:57 lower recovery 4……
5) For the first round of photo scoring go through photos scoring them accordingly on the excel spreadsheet, marking the stream condition code number in the water stream rating column and tallies in the stream condition code columns.

6) For the second round of photo scoring, start with the last photo and score photos working backwards for independent assessment. Go through photos scoring them accordingly on the excel spreadsheet, marking the stream condition code number in the water stream rating column and tallies in the stream condition code columns. Images should not be scored twice in the same day.

Photo scoring rules to follow

1) Photos present a continuum between scores, therefore:
    a) Scorer's point decision is based primarily on the quality of suitable fish habitat.
    b) Run present (1) to freshet (4) decision based on water height and color.

        Ex. If the scorer can see the bottom of the stream and rocks in the stream are still above the height of the water, then the stream is scored as run present (1). If the bottom of the stream is not visible, the color of the water is not clear, and no rocks are visible above the waterline, then the stream is scored as a freshet (4).
    c) Too dark (6) determined by visibility of stream reach
        i) Ex. If the photo is grey, but the entire stream reach is visible, and it can be given a score, then scored as such.
        ii) Ex. If the photo is dark grey and the entire stream reach is not completely visible, and there could be questioning of the stream condition scored, then score the image as too dark (6).
    d) Run present (1) to isolated pools (3)
        i) Ex. If there is still stream connectivity and photos appear to have a flow, then score photos as run present (1).
        ii) Ex. If there is not visible flow downstream and water is pooled, then score photos as isolated pools (3).
    e) Isolated pools (3) to dry bed (2)

        Ex. If there is no water visible in the stream or small pools of water are visibly drying up, then score the photos as dry bed (2).
2) The scorer does not see level logger data until after photos are scored twice.

D.7 Monthly Comparisons

3) Go through all scored photos and add up all tallies in stream condition scoring columns by month. Input these monthly totals for each stream condition into the appropriately designated sheet in the analysis spreadsheet.

    a) Example:

March. 2020 (1000, M1)	**Stream Condition Codes**					
	1=run present	*2=dry bed*	*3=isolated pools*	*4=freshet*	*5=camera not working*	*6=image quality too poor to rate*
1-Mar-20	25			20		5
2-Mar-20	39			7		4
3-Mar-20	46					4
4-Mar-20	46					4
5-Mar-20	47					3

4) Add each column, giving the total number for each stream condition for the month. Divide these sums by the total sum of recorded stream conditions for the month. This provides a rough percentage of monthly stream conditions for that stream reach.

    Ex. If the total number of photos taken in the month is 1,135 and the total monthly scoring for run present is 153 photos divide 153 by 1,135 to get the monthly percent of 13.48% that there was a run present.

APPENDIX E

Standard Operating Procedures for Water Level Loggers

E.1 Purpose and Method Summary

Acronym and Term List

HOBOware	Software used to calculate water depth from pressure and temperature data
I:Drive	State of Hawaii Network Hard drive
Onset Hobo Water Level Logger	Brand of water level logger

Purpose

To approximate freshwater inflow as surface water into estuaries and record number and duration of freshets Onset Hobo Water Level Loggers (Part Number U20L-01 with range 0 to 9m) will be deployed at each site.

Method Summary

Loggers will be programed to record water temperature and water pressure in 15-minute intervals continuously. At regular intervals water level loggers will be swapped out to download data, check battery life, clean debris around logger, and maintain logger case, spike and cable ties. For each swap on a quarter hour interval (00:00; 0015; 00:30; 00:45) time and water depth (cm) is recorded using a ruler placed temporarily near logger. For the logger to be removed this occurs prior to its removal and for the logger being deployed it occurs after placement and allowing at least five minutes for the logger temperature to acclimate to water temperature. Logger data is downloaded into the HOBOware version 3.7.22 software and data assistant are used to calculate water depth from pressure and temperature data. Data are saved to Dropbox folder. Copies of the folder will be housed in the I:Drive.

E.2 Equipment and Supply Checklist

Water Level Logger Materials	
Water level logger	gloves
Water level logger housing	Bucket
Concrete forming peg(s)	Shovel
Zip ties	Sledgehammer
Scissors	nitrile gloves
Stainless steel hose clamp	2-part Marine epoxy
Screwdriver/ socket wrench	Paint can opener
Folding ruler	Putty knife (plastic)
Watch/Phone	Plastic board
Pencil	Drill/ drill bit
	Scrub pads

E.3 Water Level Logger Set-up

1) Install HOBOware software according to the instructions.
2) Attach the USB optic base station or waterproof shuttle to a USB port on the computer
3) Turning counterclockwise, unscrew to black plastic end cap from the logger.
4) Attach the couple to the optical base station or waterproof shuttle
5) Align the raised notch on the logger with the arrow on the coupler. If correctly seated a green light on the optical base station/waterproof shuttle will turn on indicating a connection has been made.
6) From the Device menu in HOBOware, select launch.
    a. Make sure the battery state is "good"
    b. Make sure that Absolute Pressure and Temperature are selected.
    c. For logging interval select 15 minutes.
    d. Next select the date and time that you want the logger to start logging data.
    e. Click Delayed Start
    f. To confirm logging interval and Delayed start date and time, click on the Device menu and select Status.

E.4 Deployment Procedures

1) At the base yard, using a drill and drill bit, drill out the nail holes in the concrete forming peg so a zip tie can pass easily through.
2) In the field: Select a location within the stream/ estuary where the level logger would be protected from freshets, debris, or human intervention.
3) Using a small sledgehammer, pound the concrete forming peg into the ground.
4) Attach the logger housing to the concrete forming peg using zip ties. Pass a few zip ties thru the drilled-out nail holes in the concrete forming peg and around the logger housing.
5) Use as many zip ties as needed to secure the housing to the forming peg so it is sturdy and doesn't move side to side or up and down.
    a. Stainless steel hose clamps can be used instead of zip ties to secure the housing to the forming peg. This doesn't require the holes of the concrete forming peg to be drilled out.
    b. Tighten the stainless-steel hose clamps using a screwdriver or socket wrench
6) Make sure the housing will stay submerged under water even during low tide.
7) Place a zip tie on the black plastic end cap of the level logger. This will aid in visually seeing the level logger during recovery.
8) Place the level logger into the housing. Place the zip tie tail end thru the hole in the housing end cap. Screw on the end cap being careful not to cross the threads.
9) Allow the logger at least five minutes to acclimate to the water temperature.
10) The logger will record data on the quarter hour. On the nearest quarter hour interval (00:00; 0015; 00:30; 00:45) record the time and water depth (cm) using a ruler placed near logger.

Note: In some instances, a logger may be hidden under a stone structure, using stones to conceal the logger housing. The logger housing is secured to a shortened concrete forming peg. The peg, housing, and logger are then placed in the stone structure. Rocks are then epoxied over the whole

assembly to hide it. Open the 2-part epoxy containers using a paint can opener or a flat head screwdriver. Use disposable nitrile gloves to mix the 2-part marine epoxy in a 1:1 ratio. Use a plastic putty knife to scoop out the desired amount of epoxy hardener. Use a different plastic putty knife to scoop out the same amount of epoxy resin. Place both parts on a plastic board and mix till a uniform color is achieved. Using a scrub brush, scrub off any algae from the rocks where epoxy will be placed. The cleaner the surface, the better the epoxy will bond. Place some epoxy on a suitable rock to cover the logger assembly and set in place. When the epoxy has cured, a sledgehammer will need to be used to dislodge the epoxied rocks to get access to the logger.

E.5 Recovery Procedures

1) At regular intervals, the water level loggers will be swapped out to download data, check battery life and maintain the housing.
2) Set up the replacement logger as described previously. Double check the battery life, logging interval, and set date and time.
3) Place the replacement logger into the water to acclimate.
4) On the nearest quarter hour interval (00:00; 0015; 00:30; 00:45) record the time and water depth (cm) using a ruler placed near the logger to be replaced.
5) After recording the time and water depth, unscrew the logger housing end cap and remove the logger.
6) Clean out any debris from the inside of the logger housing. Check if cable ties are secure and add new ones if needed.
7) Place a zip tie on the black plastic end cap of the replacement level logger. This will aid in visually seeing the level logger during recovery.
8) Place the level logger into the housing. Place the zip tie tail end thru the hole in the housing end cap. Screw on the end cap being careful not to cross the threads.
9) On the nearest quarter hour interval (00:00; 0015; 00:30; 00:45) record the time and water depth (cm) using a ruler placed near logger.

E.6 Water Level Logger Download Procedures

1) Launch the HOBOware program.
2) Attach the USB optic base station or waterproof shuttle to a USB port on the computer
3) Turning counterclockwise, unscrew to black plastic end cap from the logger.
4) Attach the couple to the optical base station or waterproof shuttle.
5) Align the raised notch on the logger with the arrow on the coupler. If seated correctly a green light on the optical base station/waterproof shuttle will turn on indicating a connection has been made.
6) From the Device menu in HOBOware, select Readout.
7) HOBOware will then prompt a Save in screen. Select the folder where the file would be saved and type in a file name. Click save.
8) A Plot setup pop up menu will appear.
    a. Click on the Process button
    b. A Barometric Compensation Assistant pop-up menu will appear.
        i. In Fluid Density select Brackish water

ii. In Barometric Compensation Parameters, Check “Use a Reference Water Level”.
iii. In the Reference time box, use either the set up/ deployment date and time or the Recovery date and time. Click on the arrow and scroll to find the corresponding date and time.
iv. Next fill in the Reference Water Level box. Change the units from feet to meters.
v. When finished, click on Create New Series.
vi. A graph of the data will now appear.
vii. The data can then be saved as an Excel file.

APPENDIX F

Standard Operating Procedures for Data Entry

F.1 Purpose and Method Summary

Purpose

Data entry into a database is a crucial step for processing and analyzing raw data captured on field data sheets. Large amounts of data must be managed efficiently in a timely manner with a focus on accuracy.

Method Summary

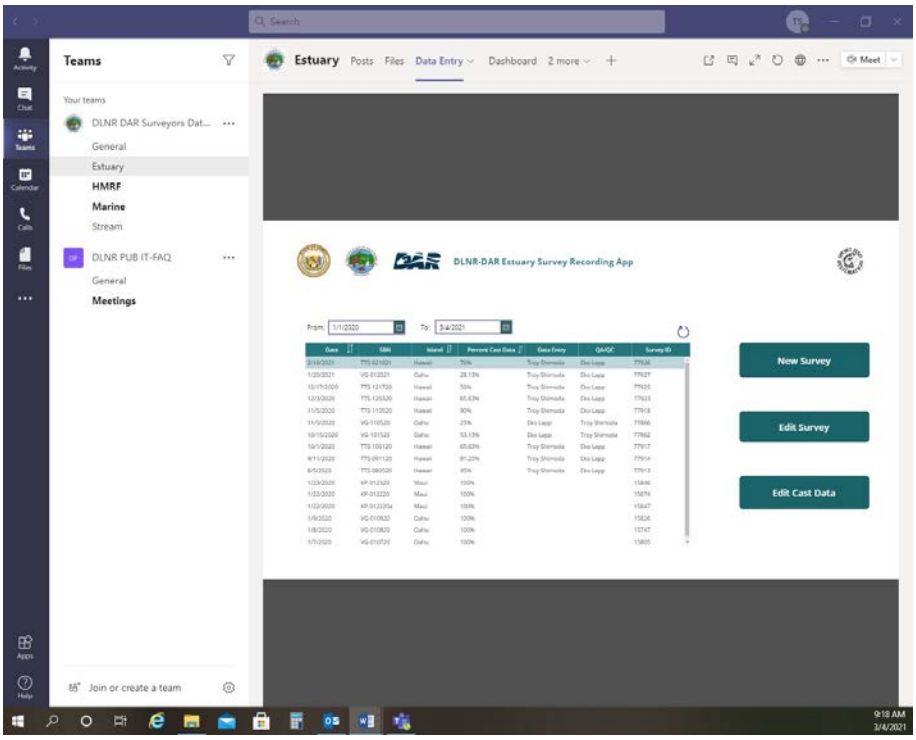
Data sheets are checked by recorder for completeness of data and legibility of writing at the end of each day. Original data sheets are turned into staff members designated by the Principal Investigator for entry of data into database. Assigned staff will maintain estuary and stream data sheets and are also responsible for data entry. Entered data will be QA/QCed and then sent to a database staffer in the Main Office (MO) building on Oahu. Entered original data sheets for estuaries are held in Hilo for reference and for streams in the Maui office. Photocopies and scans of all datasheets will be made at the end of each day or whenever a scanner is available and sent to database staff in MO for backup.

F.2 Data Entry Procedures

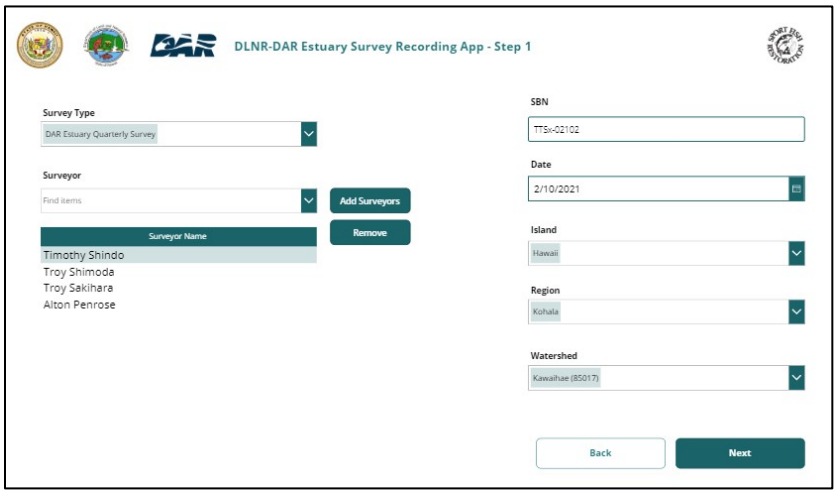
After data sheets are checked for completeness of data, legibility and scanned, data entry into the database can begin.

NOTE: To enter data into the you must have a State of Hawaii Office 365 account, State of Hawaii Microsoft Teams access, and administrator approval.

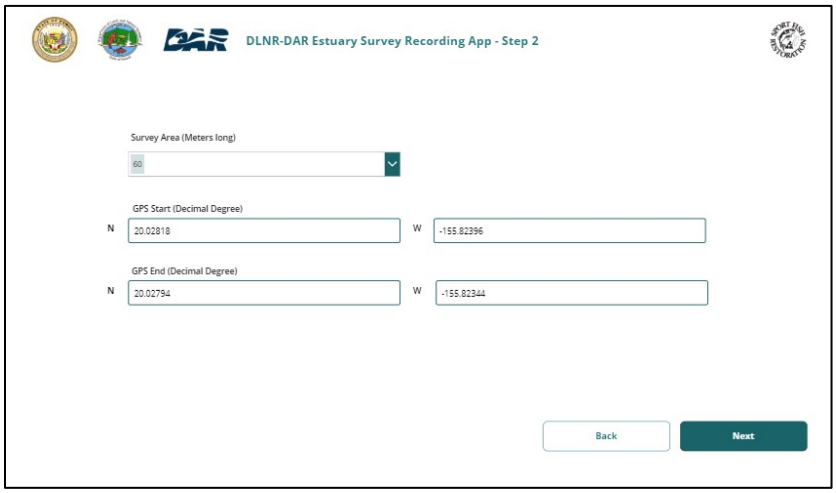
1) Log in to Office 365 and click on the “Teams” icon. To make it easier, create a “Teams” shortcut on the desktop.
2) Under the DLNR DAR Surveyors Data Entry and Reporting Portal, on the left side of the screen, click the corresponding Application which data will be entered in. The Estuary App will be used as an example. The Stream App will have a similar process for entering data.
3) To start entering data click on the “New Survey” icon.



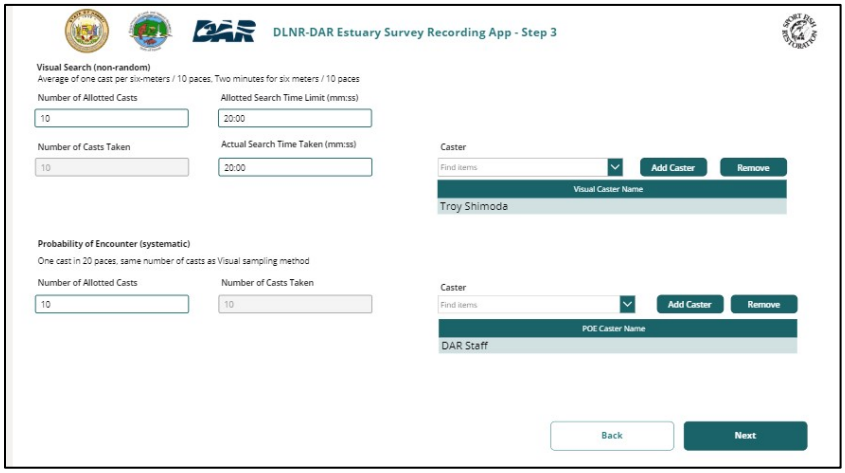
4) Step1. Fill in all the pertinent information.
    a. Survey Type
    b. From the dropdown list select the surveyors name and click “Add Surveyors”. Do this for all surveyors who were present on that day of sampling.
    c. SBN will be generated.
    d. To change the date, click on the calendar icon. Select the date and click OK.
    e. Enter the island, region, and watershed.
    f. Click “Next”.



5) Step 2. Fill in all pertinent information.
    a. Survey area. Select either 60m or 100m.
    b. Enter the GPS coordinates
    c. Click “Next”.

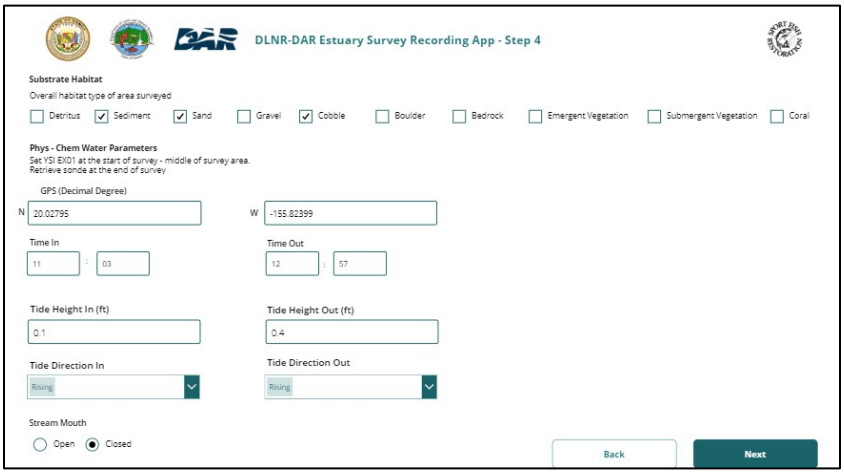


6) Step 3. Fill in all pertinent information.
    a. For Visual search - Fill in number of allotted casts, allotted search time.
    b. For Visual search - Fill in number of casts taken, actual search time.
    c. For Visual search – Find the net casters name and click “Add Caster”.
    d. For POE – fill in number of allotted casts and number of casts taken.
    e. For POE – Find the net casters name and click “Add Caster”.
    f. Click “Next”.

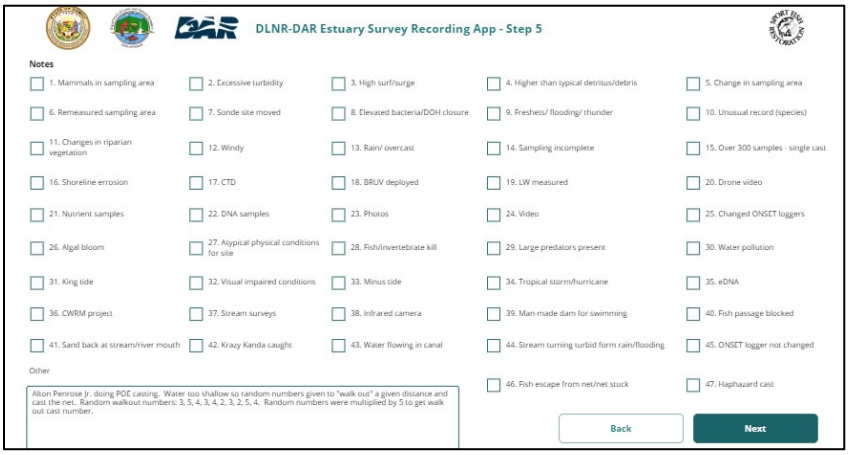


7) Step 4. Fill in all pertinent information.
    a. Check Substrate Habitat that was present during the survey.
    b. Enter the Phys – Chem GPS coordinates.
    c. Fill in Time in and Time out.
    d. Enter tide height in and tide direction in.
    e. Enter tide height out and tide direction out.

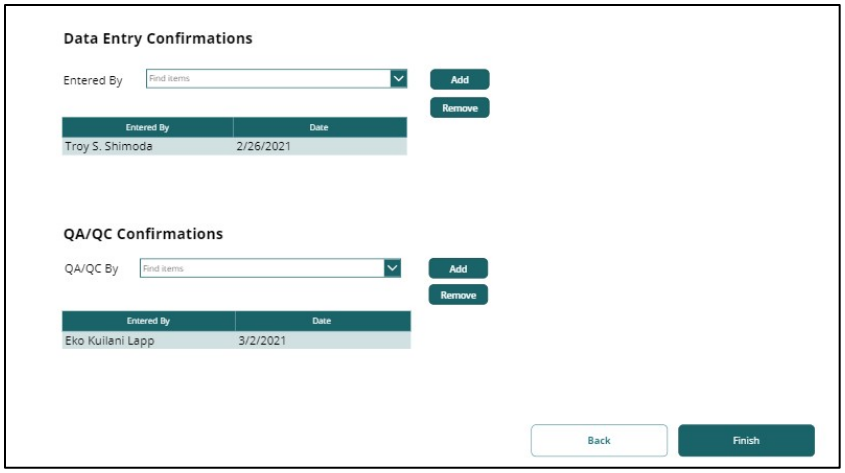
f. Indicate if the stream mouth was open or closed.
g. Click “Next”



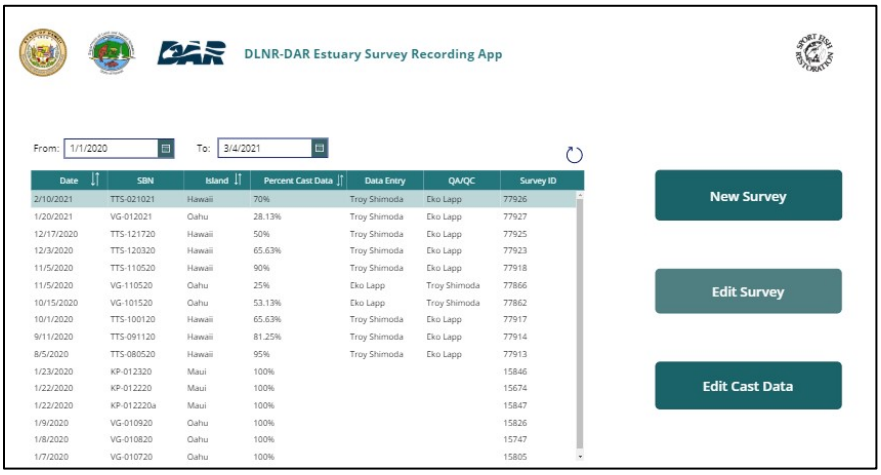
8) Step 5. Fill in all pertinent information.
   a. Check all pertinent notes. If needed additional Notes can be written in the “Other” box located in the lower left corner.
   b. Click “Next”



9) Step 6. Fill in all pertinent information.
   a. Scroll down using the down arrow or type in the person’s name doing the data entry and click “Add”.
   b. Follow the same step above for the person doing the QA/QC.
   c. Click “Finish”
   d. The App will now save all the information entered and will take you back to the original page. You can now add cast net data.

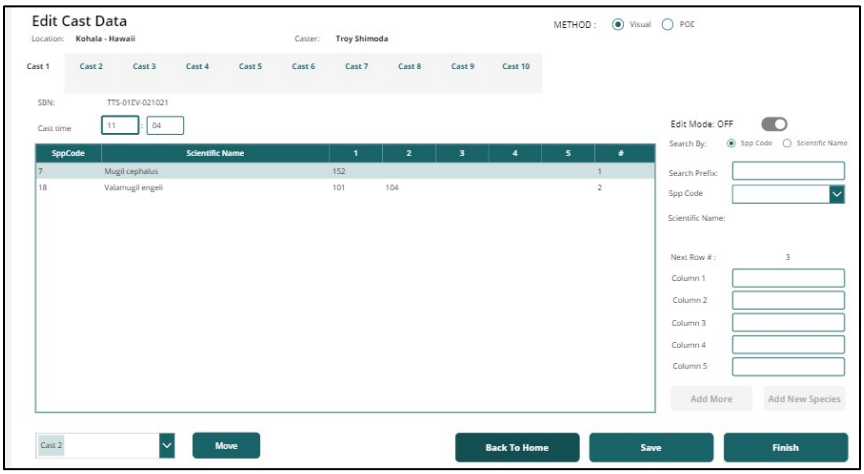


10) Step 7. Fill in all pertinent information.
    a. Select the record you want to add cast net data to and select “Edit Cast Data”.



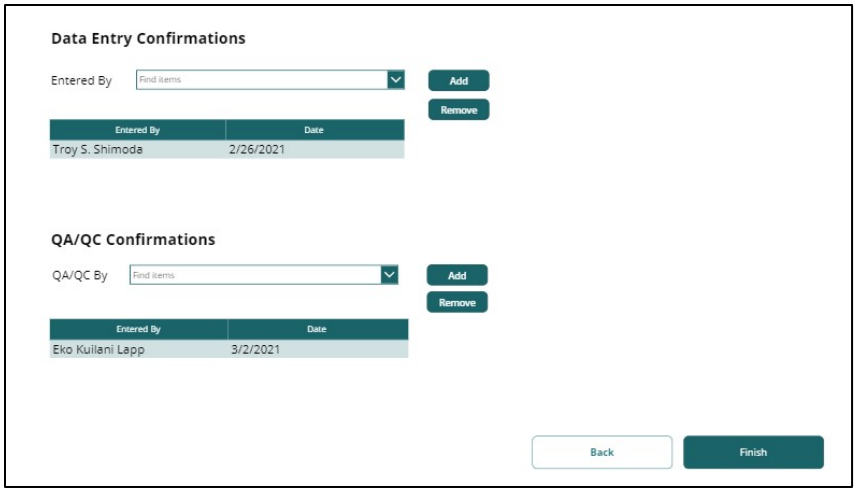
11) Step 8. Fill in all pertinent information.
    a. Double check the Location, Caster, and SBN to make sure you are entering the correct data.
    b. For Visual surveys make sure Visual is clicked in the METHOD box in the top right corner.
    c. The ribbon on the top will indicate the cast data will be entered. The cast number that is not greyed out is currently the one data is being entered.
    d. Fill out the cast time
    e. Type in species code or start typing in species name in the Search Prefix box.
        i. If typing in species code, the species code will pop up in the Spp. Code box.
        ii. The scientific name will then pop up in the Scientific Name box. Double check that it is correct.
        iii. If typing in the start of the scientific name, the first few letters of the name would suffice. Click enter. Small dots will go across the screen. In the drop-down box below, you will see the option based on what was typed. Select the correct species.

f. Once species code is correct, sizes can be entered in the column boxed 1 to 5 on the right side of the screen.
g. You can then choose to “Add More” of the same species code or “Add New Species” to switch to a different species. After one of the two buttons are hit, all the species you just entered should show up on the table in the center and will move down to the next row.
h. When finishing entering all the data for the associated cast, you MUST HIT SAVE. To save the data.
i. To switch to another cast, click on the desired cast number tab. It will then be white and not greyed out.
j. Repeat steps d. to h. until all cast data for the Visual survey is done. Save regularly.
k. To enter POE survey data, click on the POE check box in the METHOD box in the top right corner. Follow steps d. to h. until all the data for the POE survey is done. Save regularly.
l. When all data is entered, click on the “Finish” box.



12) Step 8. Fill in all pertinent information.
a. Scroll down using the down arrow or type in the person’s name doing the data entry and click “Add”.
b. If doing QA/QC follow the steps above and click “Add”

Click “Finish”. You will now be back to the original start page and will be able to Enter a New Survey, Edit a Survey, or Edit.



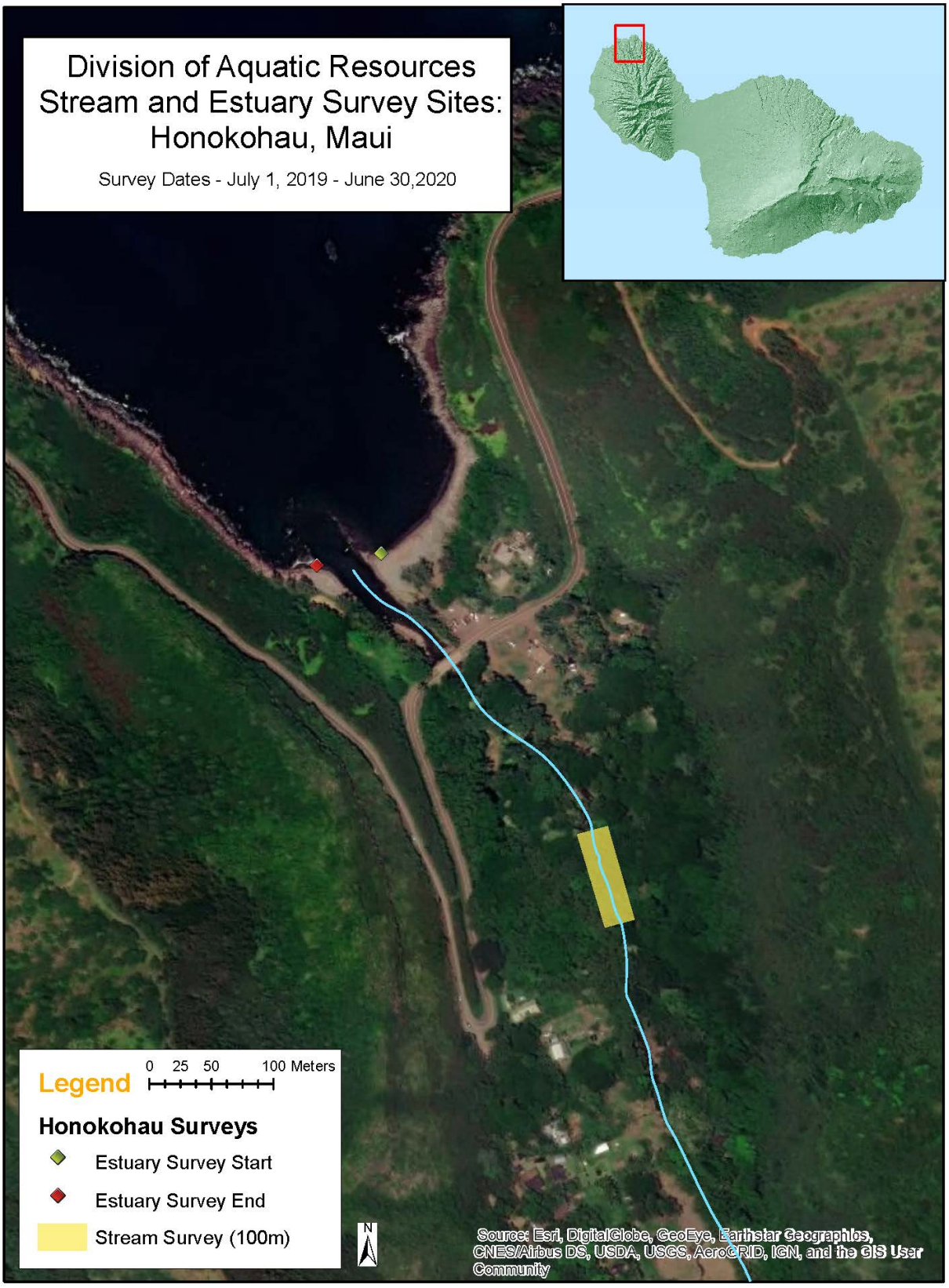
NOTE: To QA/QC the Survey data, click on “Edit Survey” and check to see if the data entered is correct. Click “Finish” when done checking. To Edit Cast Data, click on “Edit Cast Data”. If a mistake is discovered, the Edit Mode button located above the Search by Spp. Code or Scientific Name selection button must be turned ON. After enabling Edit Mode, changes can be made to the entered data. When finished correcting the data, click on “Update”. This will update the data on the table in the middle of the screen. Hit “Save”. When done editing, TURN OFF the Edit Mode button.

APPENDIX G

Detailed images of survey sites for both streams and estuaries.

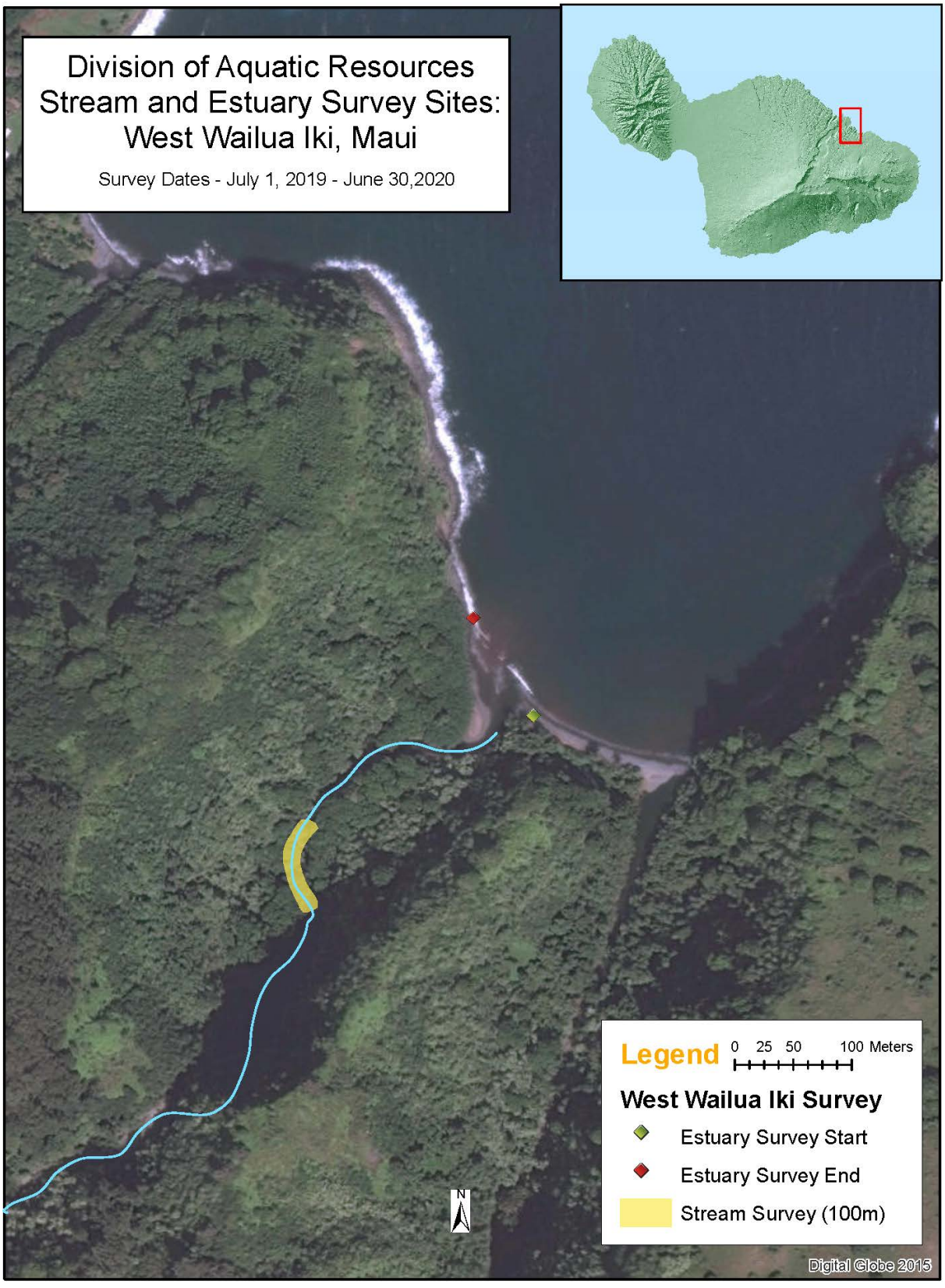
G.1. Sites for cast net sampling in estuaries and visual sampling in streams at Honomanū.

G.2. Sites for cast net sampling in estuaries and visual sampling in streams at Honokōhau.



G.3. Sites for cast net sampling in estuaries and visual sampling in streams at East Wailua iki.

G.4. Sites for cast net sampling in estuaries and visual sampling in streams at West Wailua iki.



G.5. Sites for cast net sampling in estuaries and visual sampling in streams at Waiohue.