

Division of Aquatic Resources

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

For the Commission on Water Resources Management

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October 2020

## Project 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 comparison, Honomanu (East Maui) and Honokohau streams (West 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 five 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 78 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, Honokohau and Honomanu (Fig. 1), currently operating with permitted diversions, are included for comparison. 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. Although about 35% of the eDNA data could not be processed in time to be included in this draft report, DAR has summarized findings to date. 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. For example, at one site 51 fish species were recorded; this is the highest level of fish biodiversity reported in a Hawaiian estuary to date. Invertebrate biodiversity in streams ranged from 26 species at Honokohau Stream to 45 species at Waiohue Stream. In estuaries invertebrate diversity ranged from 53 species at East Wailua iki estuary to 78 species at Honomanu estuary. 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.

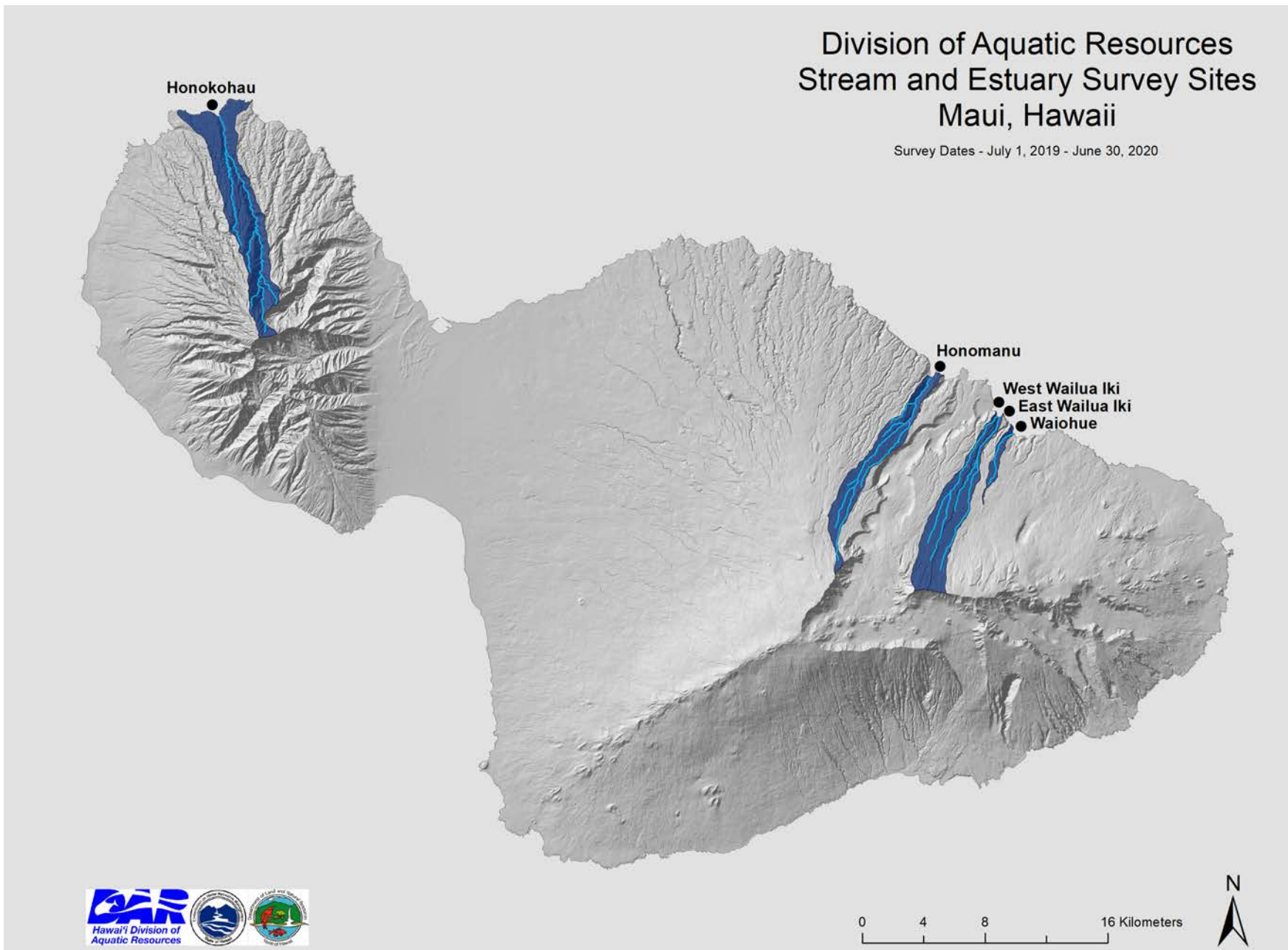


Fig. 1. The location of five Maui, Hawaii study streams and their watershed boundaries.

For more than a century streams have been diverted for commercial agriculture and other human uses, a historically predominate 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 resource 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 draft 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 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 excessive production 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 with 100% baseflow restored: East Wailua iki Stream, West Wailua iki Stream and Waiohue Stream (Table 1). Two additional sites currently operating with permitted diversions were selected by DAR, Honomanu Stream in East Maui and Honokohau Stream in West Maui. Both 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 1. 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%

In both streams and estuaries two types of sampling were performed, traditional monitoring and eDNA biodiversity monitoring. 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 draft report includes results for eDNA samples collected in the first two quarters. Third quarter results from the contractor are scheduled to be available in October/November 2020. (Note that 3 estuarine samples from the 2<sup>nd</sup> quarter required additional handling at the molecular laboratory - meaning that these delayed results will be used to update this report as those data are made available to DAR.) DAR staff will need several weeks to

complete processing and running QA/QC protocols for the data before results can be included in an updated report in November/December 2020.

This project is designed to be a timeseries study to reflect ecological conditions inherent to both Hawaiian streams and estuaries. 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. Sampling began in August 2019 (Quarter 1) and continues in Quarter 5 (July to September 2020). For the first three quarters sampling proceeded as planned, however public health restrictions affected the 4<sup>th</sup> and 5<sup>th</sup> quarters. The lack of adequate temporal sampling is reflected in interpretations of monitoring data presented in this report.

## **Results and discussion.**

*eDNA biodiversity monitoring.* Monitoring for biodiversity using eDNA provides 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. Additionally, this study evaluates how traditional sampling compares to eDNA methods in capturing species diversity in streams and estuaries.

Estuaries were found to have higher fish diversity compared to their contiguous streams at all sites and in both 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 (Fig. 2). In the West Maui diverted site, Honokohau, 51 fish species were recorded in its estuary compared to 6 fish species in the stream. The 51 fish species in Honokohau estuary was the highest diversity of fish species found at a site, so far. Additionally, comparing fish biodiversity at the family level, fish assemblages in estuaries sampled in both Quarter 1 and Quarter 2 had 16 and 28 families, respectively. This was significantly more diverse than streams, which had 7 families in Quarter 1 and 6 families in Quarter 2. 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.

In contrast to fish diversity, for invertebrate species both streams and estuaries shared similar levels of biodiversity in the two diverted sites sampled in Quarter 1 (Fig. 3). For example, Honomanu Stream had 77 species in 9 phyla detected compared to 89 species in 11 phyla reported in the downstream estuary.

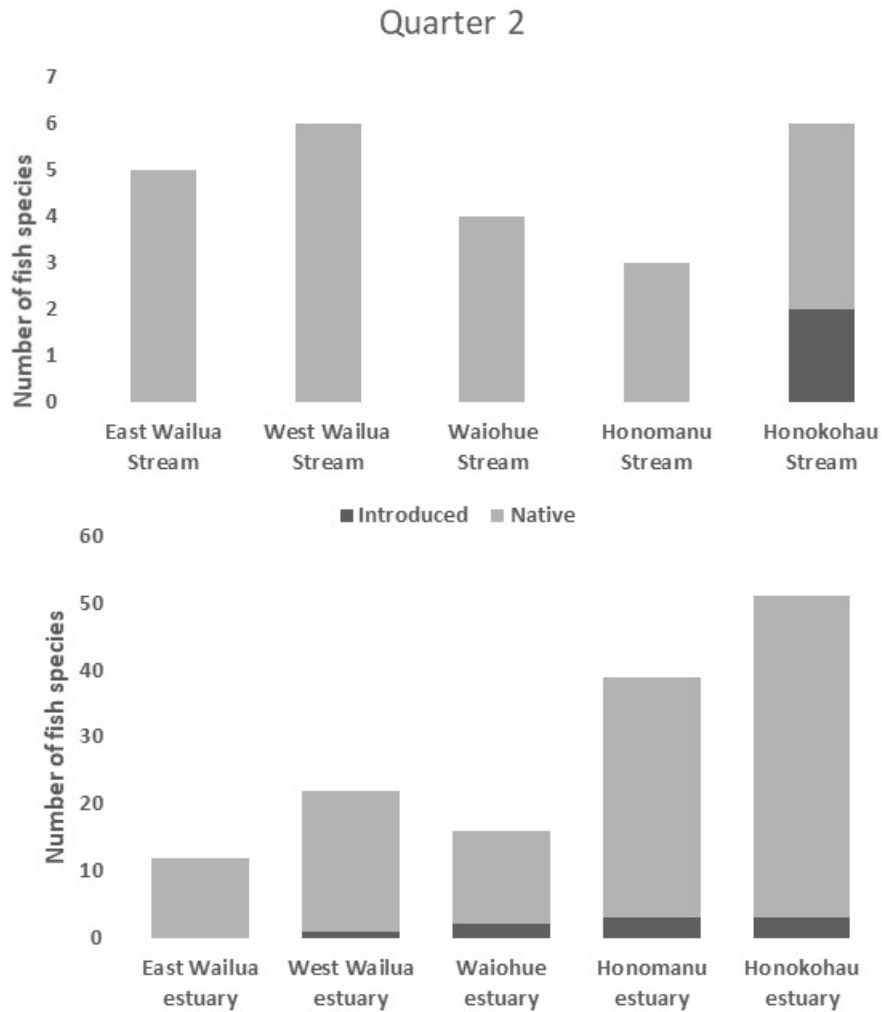


Fig. 2. A comparison of the total number of fish species, including introduced species, found in streams and estuaries as detected by eDNA sampling for Quarter 2.

In the second quarter streams had similar invertebrate species diversity compared to estuaries. Invertebrate diversity detected in estuaries ranged from 53 species at East Wailua iki estuary to 78 species at Honomanu estuary. Streams ranged from 26 species at Honokohau Stream to 45 species Waiohue Stream. Also, in the second quarter for both streams and estuaries invertebrate species diversity were derived from 9 phyla. Two of the invertebrate phyla, arthropods (includes crustaceans and insects) and annelids (segmented worms), contributed over half of the species diversity in both ecosystems. Hawai'i is a biodiversity hotspot. 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.



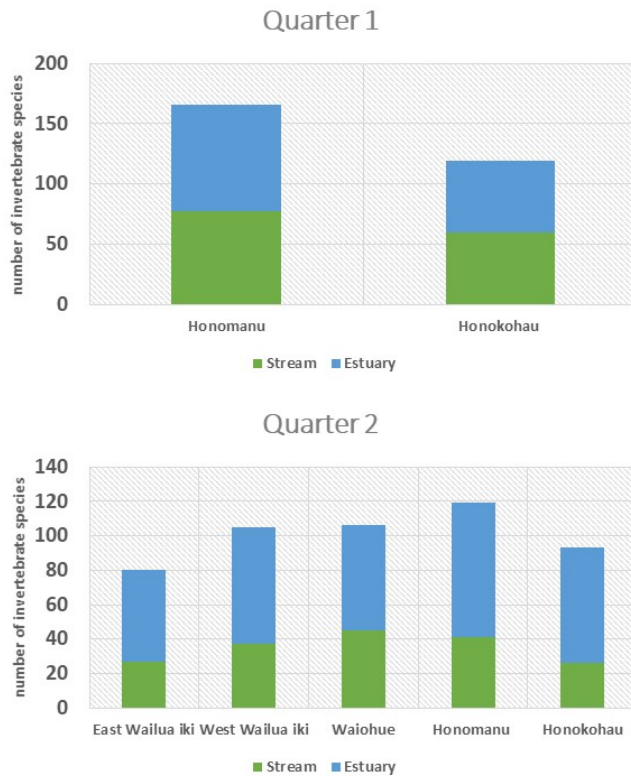


Fig. 3. A comparison of the number of invertebrate species found in streams and estuaries as detected by eDNA sampling for Quarters 1 (above) and 2 (below).

Streams and estuaries are both dynamic ecosystems because physical and ecological conditions are always shifting in these ecosystems. In streams for example, a freshet can scour algae attached to boulders and redistribute boulders downstream resulting in a reset for species present and food web structure. As juvenile fish habitat, fish assemblages using estuaries regularly shift as life histories differ among species in Hawai'i, with some species spawning year-round while others reproduce seasonally. As a result, the monitoring design for this study integrates temporal sampling for biodiversity. Results for temporal sampling are limited, for now, to the two diverted sites pending eDNA results (Fig. 4). Comparing results for fish species diversity both Honomanu and Honokohau estuaries had more fish species detected in the second quarter than the first one. Streams, on the other hand, had similar levels of fish diversity in both quarters. Invertebrate species diversity in streams differed between the quarters. For both sites more invertebrate species were detected in the first quarter than the second one. Invertebrate diversity in estuaries was comparable between the quarters for both sites. Additional sampling will improve our interpretations for temporal shifts in biodiversity.

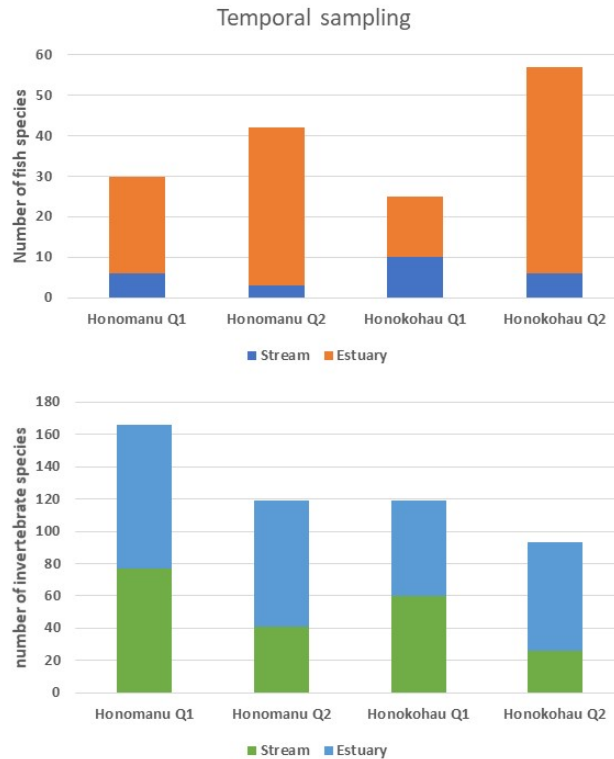


Fig. 4. A temporal comparison of biodiversity for fish species (above) and invertebrate species (below) in the streams and estuaries of Honomanu and Honokohau for the first and second quarters.

Streams and estuaries in Hawai'i have established populations of non-native fishes that make up a portion of biodiversity in these ecosystems (Fig. 2). Introduced species in the first quarter were present in both streams and estuaries at Honomau and Honokohau. 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.

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 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.

**Cast net sampling in estuaries.** Honomanu (Appx. A) and Honokohau (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 Honokohau estuary Āholehole was more than 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 Honomanu estuary the introduced fish Kanda mullet (*Moolgarda engeli*) made up >40% relative abundance in each quarter. In the second quarter 48% relative abundance in Honomanu estuary was the oama (*Mulloidichthys flavolineatus*), a sought-after bait fish. Over the 3 sampling periods 5 fish species were documented in Honomanu estuary and 10 species in Honokohau estuary.



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

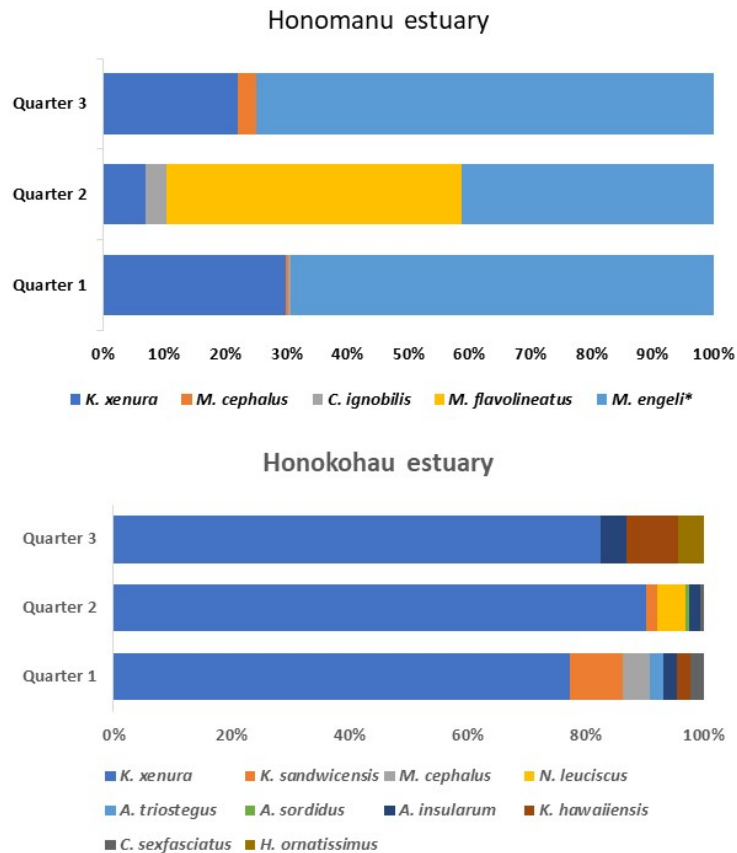


Fig. 5. A comparison of relative abundance of fish species sampled using visual cast net method in the two diverted sites over three quarters. \* indicates an introduced fish species.

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, Honomanu 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 Honomanu 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 guamensis*), ‘O‘opu Nopili (*Sicyopterus stimpsoni*), and ‘O‘opu Akupa (*Stenagobius hawaiiensis*) 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 Honomanu 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.

Honokohau Stream (Appx. B), near the estuary, was sampled in Quarters 2 and 4 (high water prevented 3<sup>rd</sup> quarter sampling). This was the only lower stream site with an absence of Hihiwai, the lack of an explanation of why this is, demonstrates the need for a better understanding of these systems. 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 2<sup>nd</sup> 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 2<sup>nd</sup> and 3<sup>rd</sup> 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.

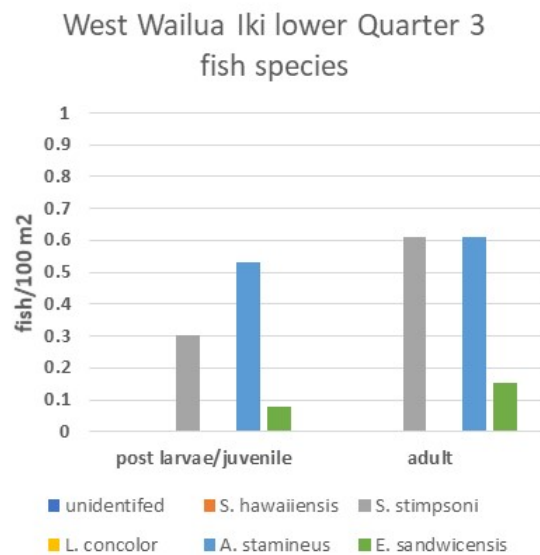
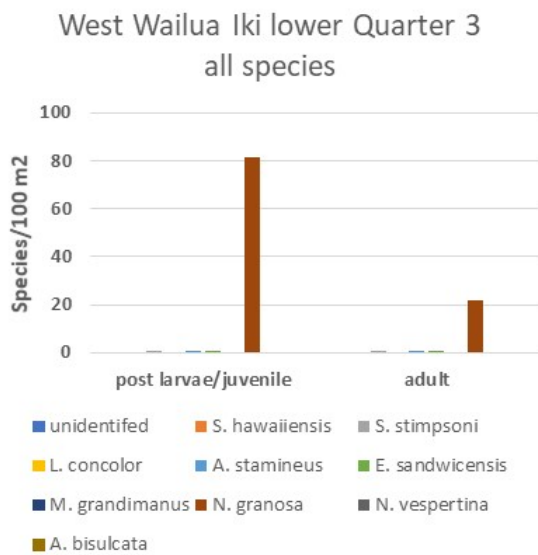
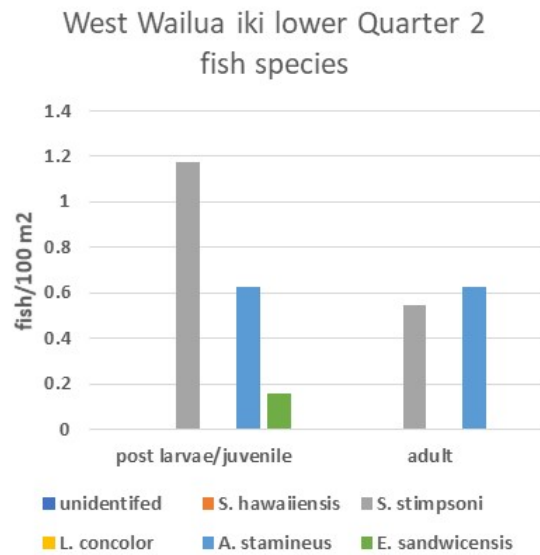
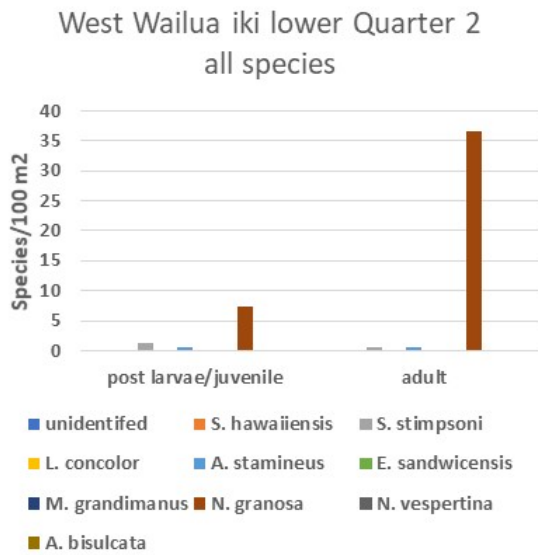


Fig. 7. A comparison of West Wailua iki Stream lower site visual surveys for Quarters 2 and 3 with both fish and invertebrate species density and fish species only density.

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 5<sup>th</sup> quarter, 'O'opu Alamo'o and Opae (*Atyoida bisulcata*) were the only aquatic species found. More surveys are needed in this stream.

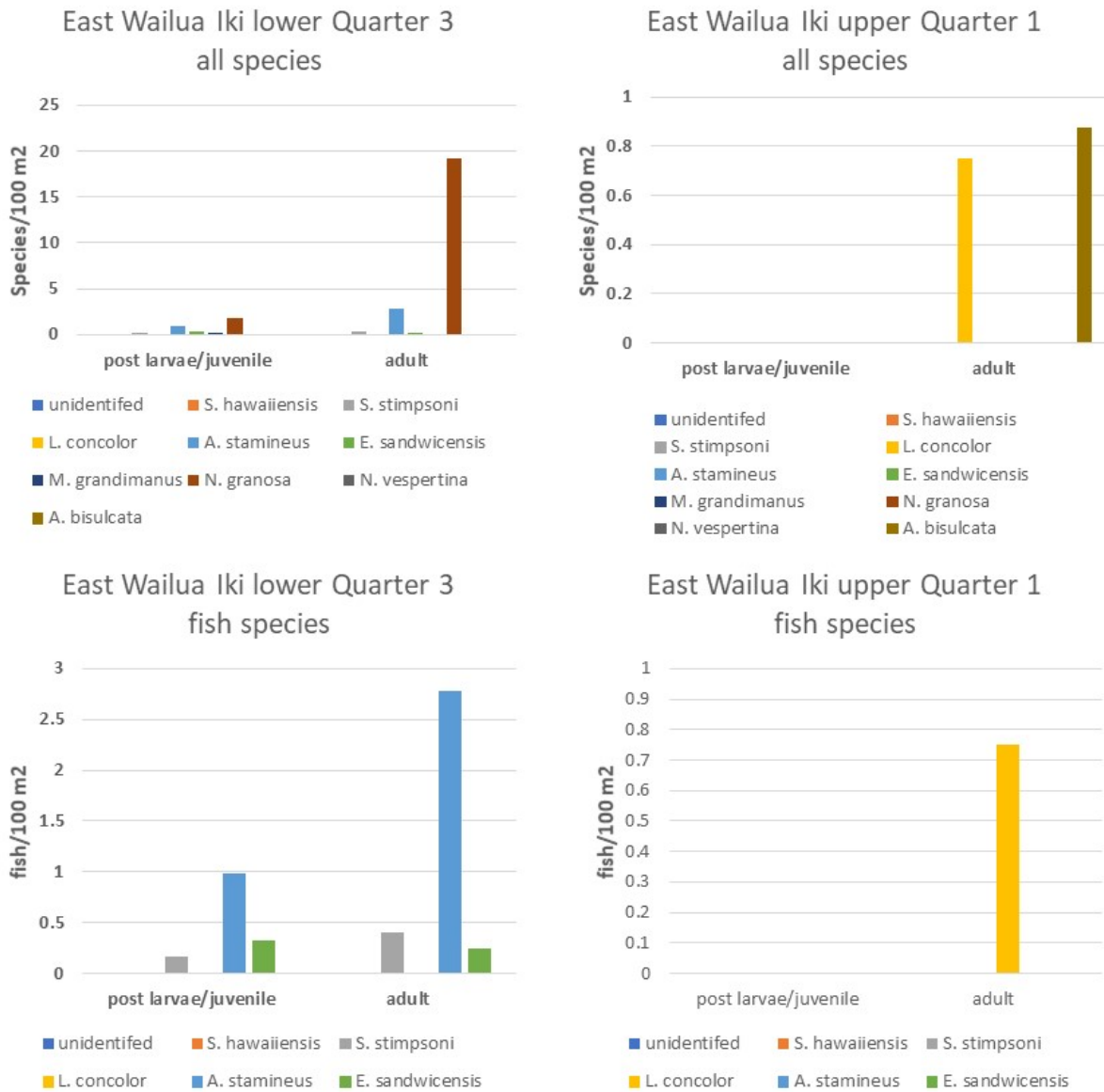


Fig. 8. A comparison visual surveys in East Wailua iki Stream lower and upper sites with both fish and invertebrate species density and fish species only density.

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 2<sup>nd</sup> 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.

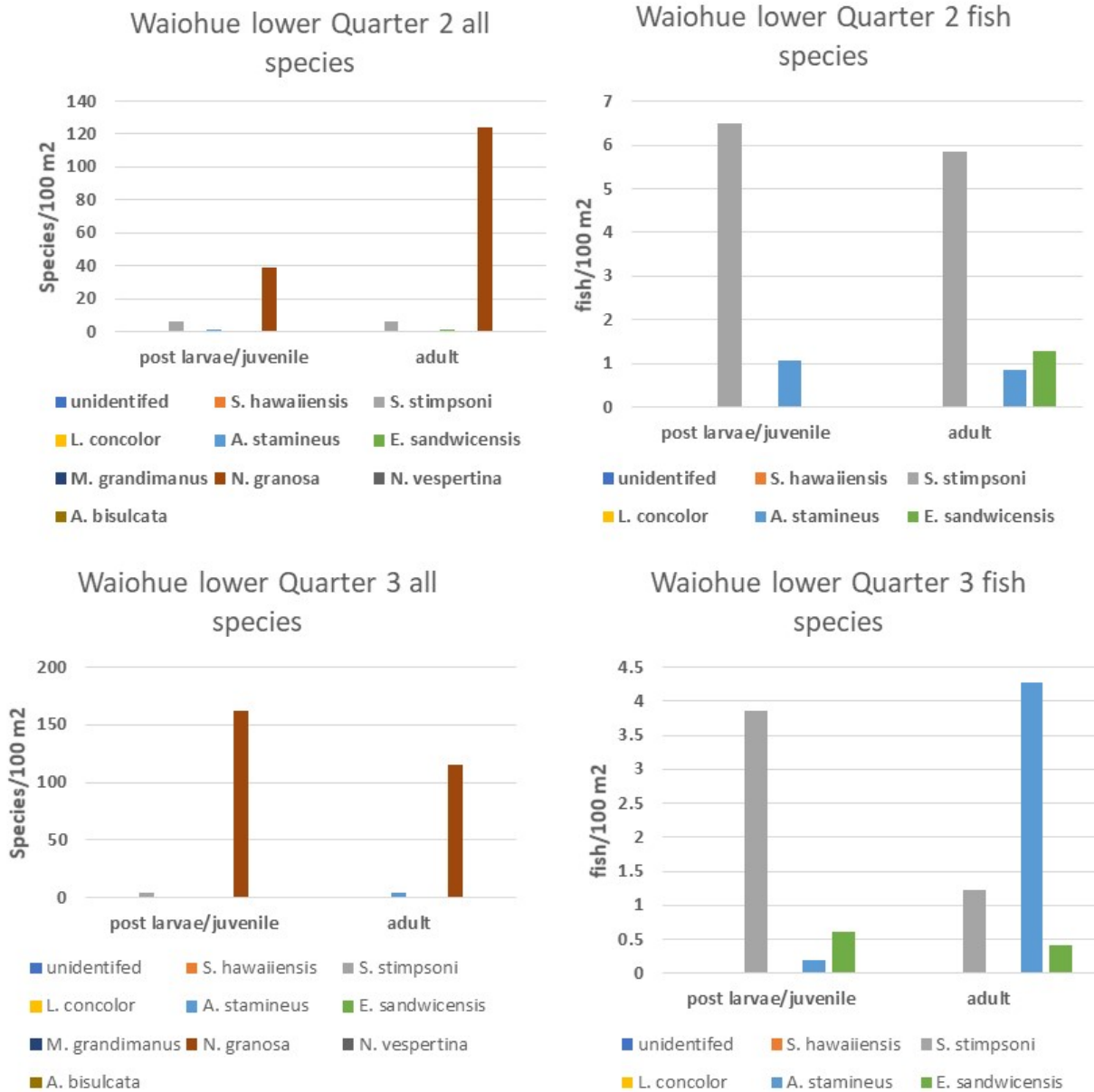


Fig. 9. A comparison of Waiohue Stream lower site visual surveys for Quarters 2 and 3 with both fish and invertebrate species density and fish density only.

**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 control 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 Honomanu Lower, and quarter 5 in Honomanu Middle. Highest flows occurred in quarter 3 & 4 in W. Wailua Iki Lower and Honomanu Lower respectively. The range of flows ranged from 0.05 to 20.65 cfs.

Table 2. Measured stream flows at the location and time of aquatic surveys for all sites.

Quarter	Sampling Date	Stream	Reach	Discharge	
				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.

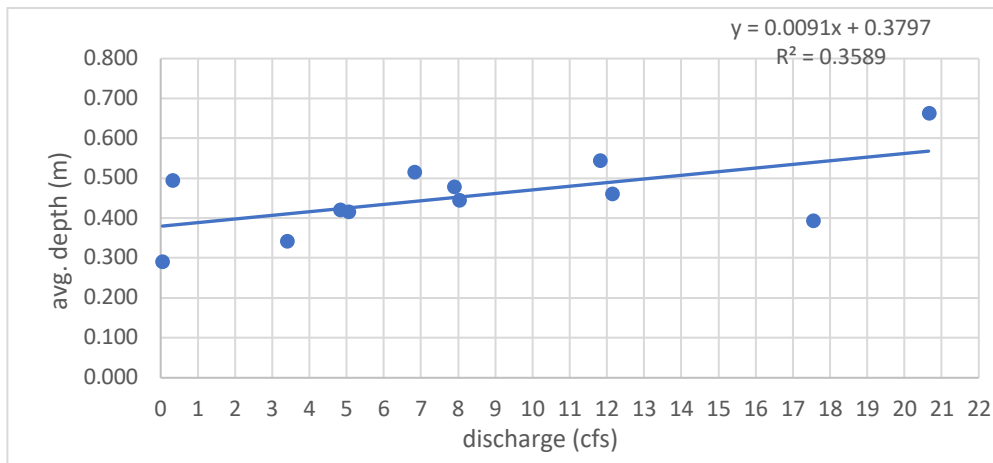


Fig. 10. Discharge and average depth relationship

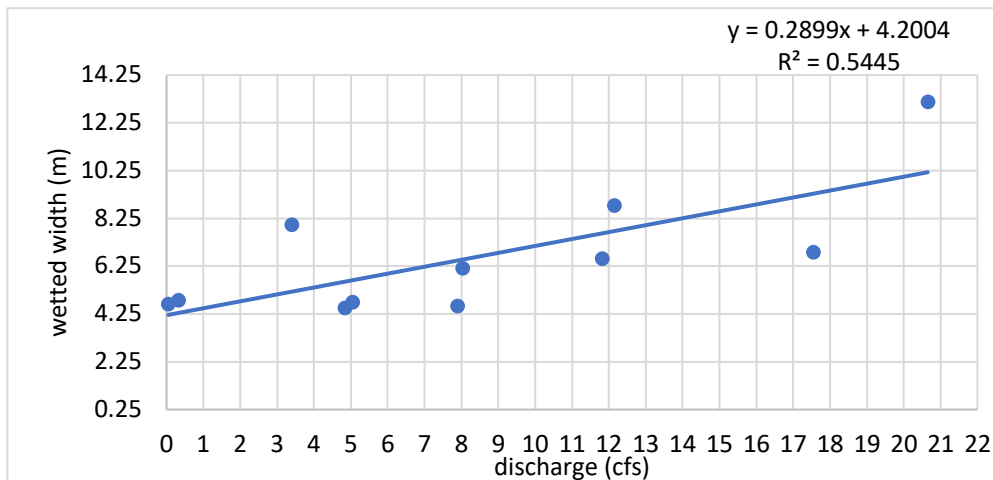


Fig. 11. Discharge and average depth relationship

### 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 Honomanu and Honokohau 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 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 wadable 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 understand 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

Appendix A. Sites for cast net sampling in estuaries and visual sampling in streams at Honomanu.



Appendix B. Sites for cast net sampling in estuaries and visual sampling in streams at Honokohau.

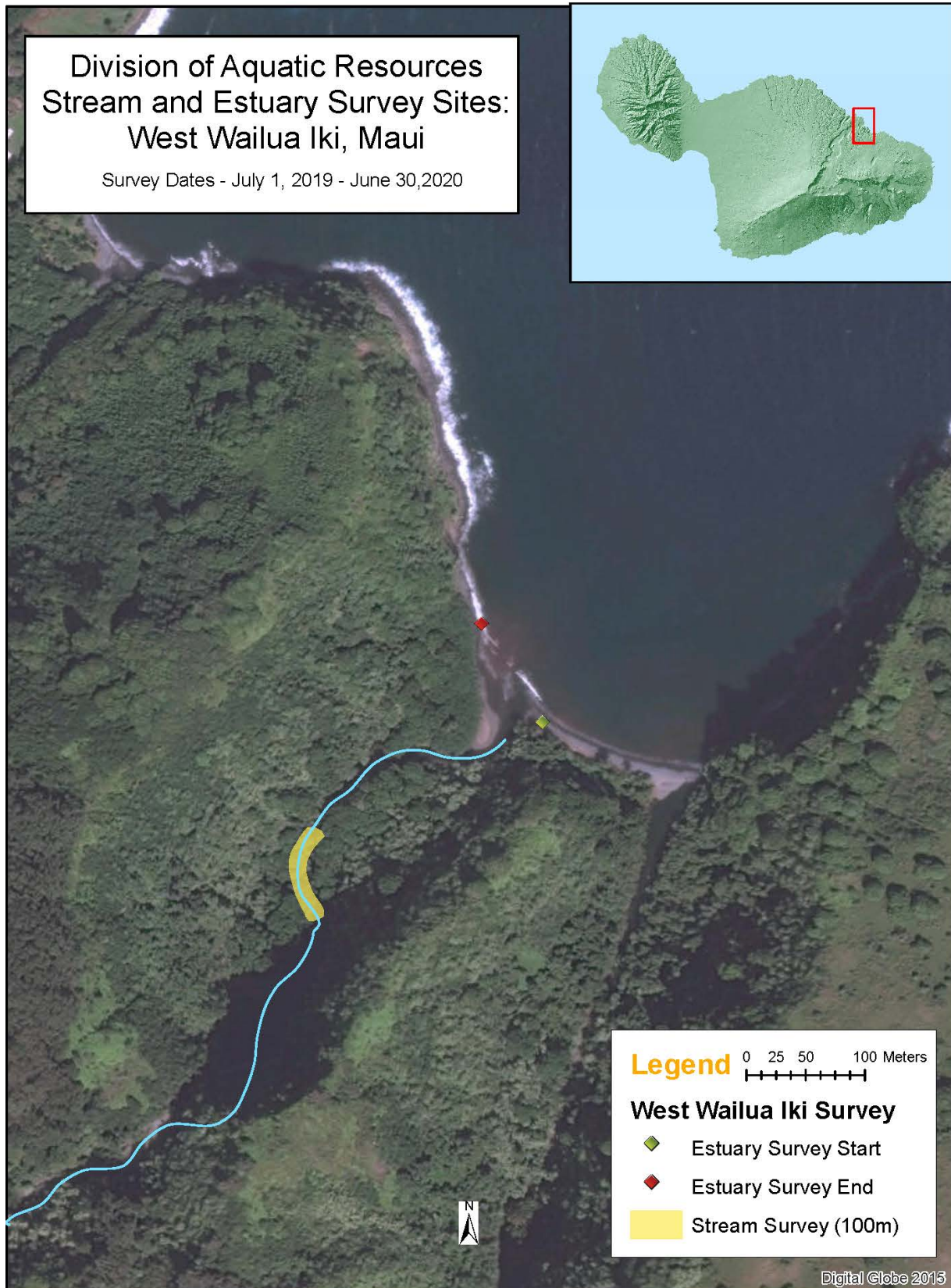


Appendix C. Sites for cast net sampling in estuaries and visual sampling in streams at East Wailua iki.





Appendix D. Sites for cast net sampling in estuaries and visual sampling in streams at West Wailua iki.



Appendix E. Sites for cast net sampling in estuaries and visual sampling in streams at Waiohue.

