EXECUTIVE SUMMARY

Control and Mitigation of Aquatic Invasive Species in Pacific Island Streams

SERDP Project RC-2447

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ACRONYMS AND ABBREVIATIONS

AIS	Aquatic invasive species
BACI	Before-after, control-impact
d	Day
ddRAD	Double digest restriction-site associated DNA
DNA	Deoxyribonucleic acid
DoD	U.S. Department of Defense
FW	Freshwater
g	Gram
GPP	Gross primary production
LLC	Limited Liability Company
m	Meter
mg	Milligram
mm	Millimeter
O_2	Oxygen
P	Phosphorus
Q	Discharge
SERDP	Strategic Environmental Research and Development Program
SNP	Single nucleotide polymorphism
SON	Statement of Need
SRP	Soluble reactive phosphorus
TER-S	Threatened, endangered, or at-risk species

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1.0 INTRODUCTION

Ensuring the continuity and long-term sustainability of U.S. Department of Defense (DoD) activities on Pacific islands requires effective stewardship of ecosystems that have been altered by the introduction of non-native species. Species invasions are threatening the evolutionary legacies of oceanic islands across the Pacific, and key ecological processes are being compromised by the presence of non-native species or the loss of native species. Advancing DoD stewardship of Pacific island ecosystems therefore requires understanding of how ecological processes have been altered by non-native species and validating restoration approaches that foster the recovery of threatened, endangered, or at-risk species (TER-S).

Management of aquatic invasive species (AIS) in Pacific Island stream ecosystems is particularly challenging because traditional methods for control or mitigation fail to account for characteristics of insular streams and societal sensitivities associated with water resources and TER-S management. AIS are consistently identified as a primary threat to aquatic biodiversity in oceanic island streams, but little work has been done to evaluate the feasibility or outcomes of removal or mitigation. Similarly, no consistent effort has been made to develop new protocols or modify existing approaches for AIS management. Development of actionable information and innovative approaches for managing AIS, therefore, could substantively improve stewardship of stream ecosystems that cross DoD lands, especially on islands where installations harbor native species under federal or state protection.

2.0 OBJECTIVES

Herein the project team reports findings from research designed to provide DoD resource managers with the knowledge and tools to recover key ecological processes and TER-S in Pacific island streams impacted by AIS. The project team's primary research objectives were to (1) demonstrate a novel approach to AIS management in Pacific island streams (Statement of Need (SON) research interest #2); and (2) advance basic understanding of ecosystem and TER-S responses to AIS removal from Pacific island streams (SON research interest #3). Recognizing that complete removal of AIS is not always feasible, the project team also undertook complementary studies to (3) determine whether careful consideration of target species and climate-driven hydrological conditions could increase the efficacy of AIS control to promote the recovery of ecological processes and TER-S (SON research interests #2, #3, #5). Information gained from this work was then leveraged to parameterize a decision-support tool to (4) help DoD managers design interventions that minimize resource expenditures and maximize in-stream conditions favorable to native species (SON research interest #2, #3, #5).

3.0 TECHNICAL APPROACH

Experimental AIS removals were conducted in 13 watersheds spanning a hydrological gradient across the Ko‘olau Range on the Hawaiian island of O‘ahu (**Figure ES-1**), which is home to a dense array of DoD installations and at-risk native species. Following a before-after, control-impact (BACI) study design, AIS removals were executed following a protocol tailored for Hawaiian streams that included two tiers of precautionary steps to prevent collateral injury to electro-sensitive native species.

Responses to removals were tracked for a period of up to two years, with adjacent upstream and downstream reaches serving as references. The project team examined whether removals yielded conditions favorable to native species by conducting snorkel surveys of population densities and community composition, as well as mark-recapture demographic assessments of *Awaous stamineus (A. stamineus)*, a native species of conservation concern. Additionally, the project team examined *A. stamineus* otolith microchemistry to determine how life-history varies according to AIS densities and hydrology, and whether removals influenced the balance of variation in local populations. The project team also assessed whether removals elicited an increase in genomic diversity by single nucleotide polymorphism (SNP) genotyping native *A. stamineus* in each study reach. Finally, the project team conducted in-stream and stable isotope assays to determine whether removals influenced nutrient availability and trophic structure. Data from this work was used to parameterize a coupled ocean-watershed biophysical model to evaluate local-to-archipelago scale outcomes of AIS removal, with the aim of evaluating cost and benefit functions for selecting watersheds to maximize return on investments in AIS management.

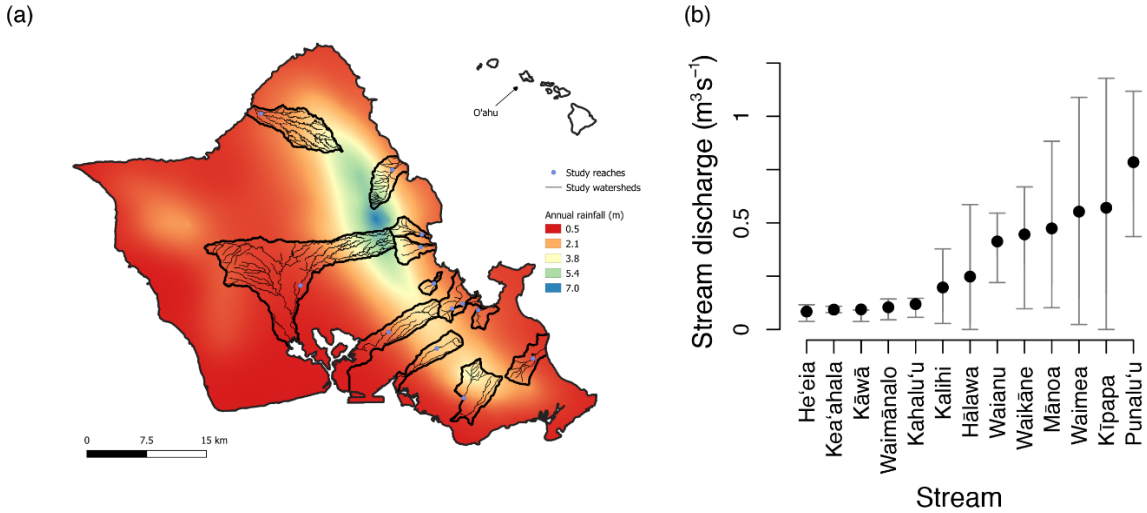


Figure ES-1. Map of O'ahu Delineating Study Watersheds and Discharge in the Study Watersheds

(A) Map of O'ahu delineating the project team's study watersheds and study reaches relative to precipitation conditions; (B) Study streams ordered by increasing median discharge (solid points, Q_{50}, $m^3 s^{-1}$). Whiskers span low (Q_{90}) and high discharge (Q_{10}) derived from standard exceedance percentiles of daily discharge.

4.0 RESULTS AND DISCUSSION

4.1 EFFICACY AND COMMUNITY OUTCOMES OF AIS REMOVAL FROM HAWAIIAN STREAMS

Preferred methods of AIS management, such as toxicants and electrofishing, are generally considered unacceptable in the region because of the potential harm to at-risk and culturally important native species. As a result, few attempts have been made to control AIS and little effort has been made to develop protocols designed to protect at-risk species in tropical and subtropical Pacific island streams.

In this study, the project team assessed the effectiveness and outcomes of implementing an AIS removal protocol tailored for Hawaiian Island streams. The protocol combines hand-capture and streamside sequestration of native species with AIS removal via three-pass electrofishing with amperage and waveform combinations set to minimize harm to remaining native species. Experimental AIS removals were conducted in 13 watersheds on the island of O‘ahu (**Figure ES-1)** following a BACI design to assess how removal efficacy and post-removal reassembly vary by surface flow and target species of interest. This approach enabled us to determine whether particular flow conditions improve removal efficacy and promote conditions that favor native species like lower AIS recolonization rates.

The project team found that removals resulted in a sharp reduction in AIS densities without changing native species densities (**Figure ES-2**). The most widespread and abundant AIS were livebearing fishes (*Poeciliidae*) and armored catfishes (*Loricariidae*), which together accounted for 66% of all AIS removed. Removal efficacy was greatest in streams with intermediate discharge irrespective of pre-removal AIS densities or target group. Long-term monitoring also demonstrated that removals led to highly persistent shifts in community composition reflecting sustained reductions in AIS densities. These findings indicate that electrofishing can be a valuable tool for AIS management in tropical and subtropical Pacific island streams, so long as steps are taken to reduce risk to native species. Careful consideration of surface flow and pre-removal biotic conditions also can help maximize desirable outcomes of removal efforts.

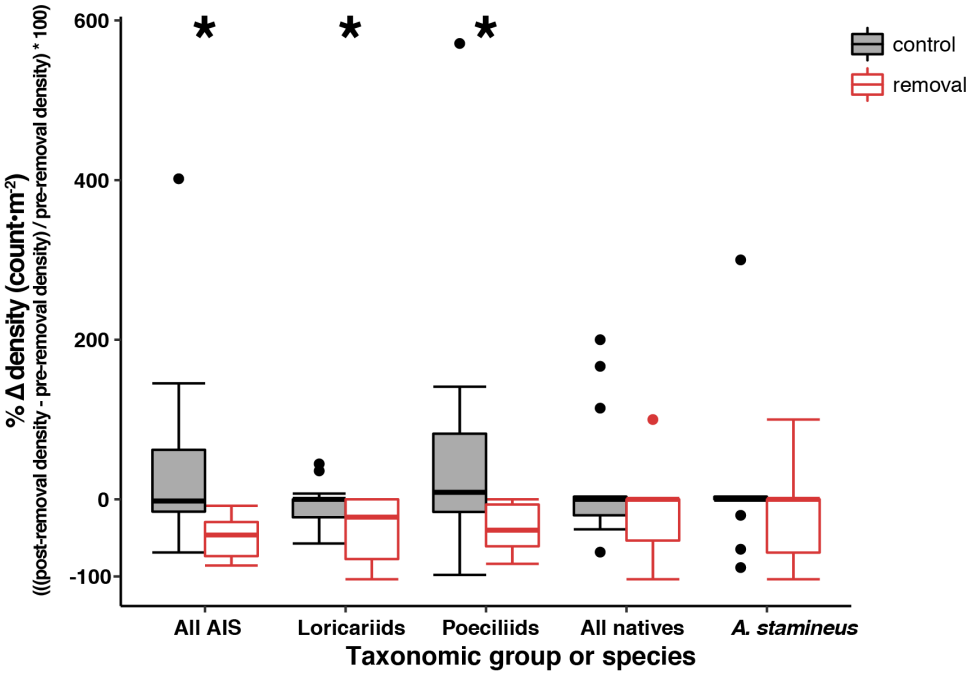


Figure ES-2. Immediate Percent Density Change Between Removal Reaches and Control Reaches

*The immediate percent density change (((post-removal density – pre-removal density)/pre-removal density) *100) for each group of interest between removal reaches (red) and control reaches (grey). Whiskers are minimum and maximum values relative to the first and third quartiles, respectively, whereas median values are denoted with bars. * = significant difference between reaches according to Welch's Two Sample t-tests.*

4.2 DEMOGRAPHIC RESPONSES TO AIS REMOVAL FROM HAWAIIAN STREAMS

Understanding ecological outcomes following AIS removal can help promote evidence-based decision-making for the recovery of TER-S. In this study, the project team assessed the demographic responses of *A. stamineus*, an at-risk native Hawaiian fish, to AIS removal in study watersheds on the island of O‘ahu. Populations of *A. stamineus* were monitored before and after the removals in both treatment and control reaches. Demographic metrics including population size, the proportion of recruits, growth rates, body condition, and in-stream movement.

Comparisons revealed that demographic measures like population size and recruitment were highly correlated between treatment and control reaches both before and after AIS removal, indicating that there was little to no demographic independence among reaches within streams. This pattern is consistent with inferences from snorkel surveys that AIS removals can create ecological “voids” (i.e., areas where resources become newly available), which are then “filled” by individuals moving in from outside of removal reaches (Koehn et al. 2000), resulting in the establishment of a new demographic equilibrium of lower overall densities in the removal and control reaches. However, the project team found that population sizes in the first six months after AIS removals were not significantly different than pre-removal population estimates. Movement of adults and juveniles into the treatment reach also did not change. However, the proportion of recruits in the populations significantly increased in the three to six months following removals. Compared to pre-removal measures, mean growth rates (measured by weight, not length) and body condition were also greater zero to three months, three to six months, and less than six months following removals (**Figure ES-3**). The magnitude of the shift in recruitment was significantly positively correlated with the removal of *Poeciliids* from the study reaches. Growth and body condition responses were positively correlated with the removal of *Loricariids* from the study reaches and negatively correlated with mean discharge. While the increase in recruitment was short-lived, the increase in growth and body condition persisted for the duration of the study.

These findings indicate that AIS removal from Hawaiian streams can promote the recovery of native fish populations. If increases in recruitment and growth rates can be sustained, then population sizes should increase over the longer term. If AIS are not extirpated, periodic maintenance of removals (i.e., every three to six months) may be necessary to sustain demographic recovery.

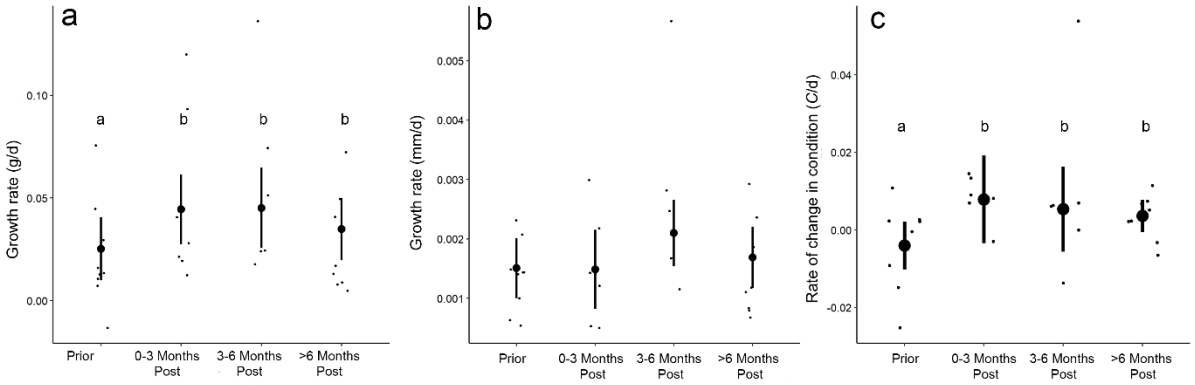


Figure ES-3. Awaous Growth Rates and Condition Prior to and Following AIS Removals

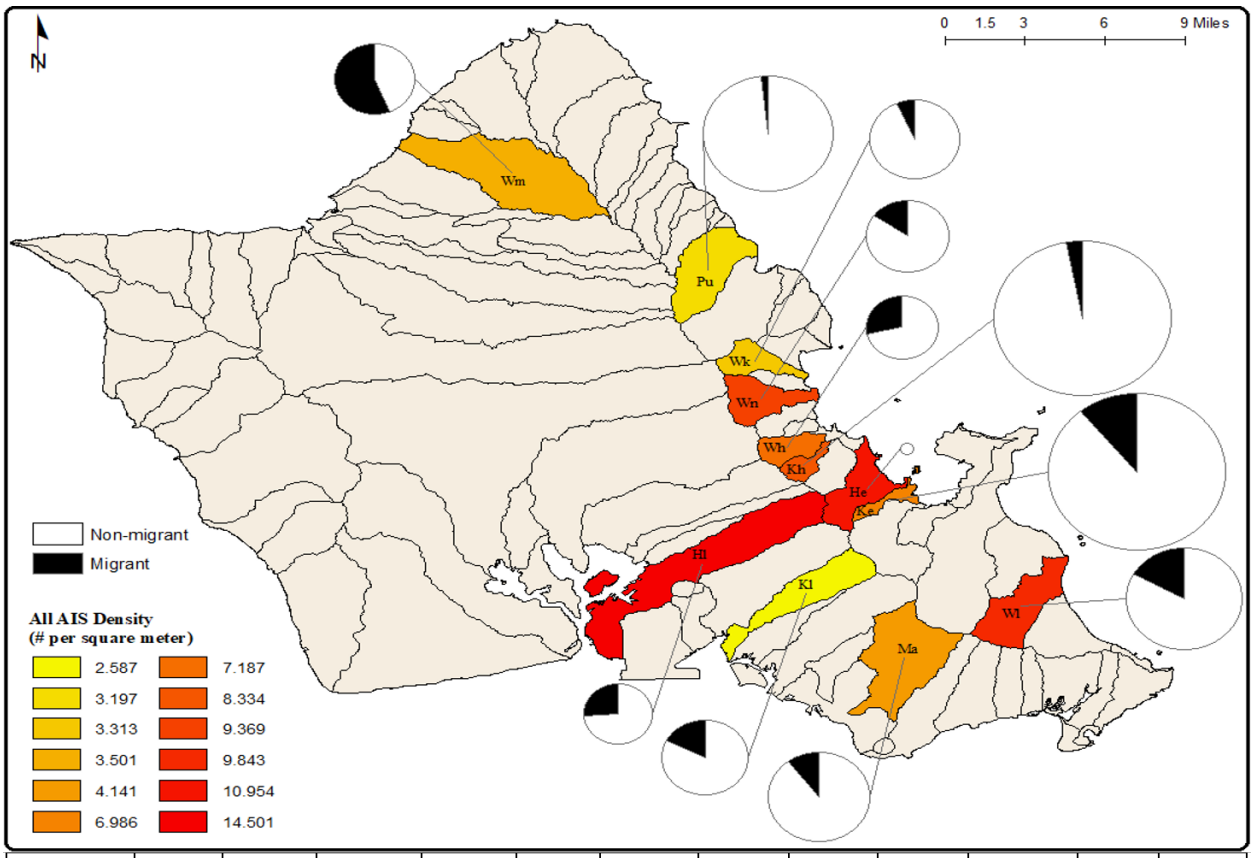
Mean growth rates (large black dots) in (a) (g/d) and (b) mm/d, as well as (c) rate of change in condition (C)/d in each study population (small black dots) prior to AIS removal, and 0-3, 3-6, and >6 months post AIS removal. Black vertical bars indicate 95% confidence intervals. Letters above each box plot indicate significant differences among periods identified by sequential contrasts post-hoc tests.

4.3 LIFE HISTORY VARIATION IN RELATION TO BIOTIC AND ABIOTIC GRADIENTS ACROSS HAWAIIAN STREAMS

Altered water quality, reduced habitat, as well as competition and predation from AIS can impose pressures that moderate the life history of native migratory species in oceanic island stream ecosystems. To properly manage oceanic island stream ecosystems, it is necessary to understand how AIS and other anthropogenic pressures influence the balance of migratory life history variation in at-risk native species. For partially migratory amphidromous species like *A. stamineus*, early life-stages may be spent in either a marine or natal freshwater (FW) environment, raising the possibility that the balance of "migrants" to "residents" (i.e., fish that remain in their natal habitat for the duration of their life cycle) in local populations might vary according to in-stream conditions.

The project team examined life history variation in populations of *A. stamineus* across in-stream biotic and abiotic gradients on the island of O'ahu. The project team found that populations exhibited reduced proportions of residents when stream flow was low and where nutrients were limited (**Figure ES-4**). This suggests that inorganic nitrate, flow variability, and soluble reactive phosphorus (SRP) likely constrain the survival of resident larvae. Similarly, the project team found that populations exhibited reduced proportions of residents in streams with high densities of non-native *Poeciliid* predators (**Figure ES-4**).

These findings suggest that improvement of in-stream conditions would likely increase the proportion of residents in local populations of *A. stamineus*, possibly increasing population stability and persistence. Steps could be taken, for example, to sustain discharge or restore natural flow regimes. Likewise, moderating nutrient availability and removing AIS would likely promote survival and persistence of *A. stamineus* and other native migratory fishes that are of conservation concern.



Watershed	Kalihi (Kl)	Punaluu (Pu)	Waikane (Wk)	Waimea (Wm)	Manoa (Ma)	Keaahala (Ke)	Waihee (Wh)	Kahaluu (Kh)	Waianu (Wn)	Waimanalo (Wl)	Heeia (He)	Halawa (Hl)
N	28	59	30	25	38	101	21	100	26	48	1	19
Non-migrants	23	58	28	11	34	90	15	97	22	40	1	14
Migrants	5	1	2	14	4	11	6	3	4	8	0	5
Poecillid Density	1.428	2.95	2.63	0.001	1.225	4.268	3.406	4.604	9.114	7.153	3.66	13.943
Loricariid Denisty	0.883	0.001	0.0331	0	0.499	2.577	3.628	3.45	0	0.065	5.83	0

Figure ES-4. Proportions of Migrant and Resident Awaous Relative to AIS Densities

Proportions of migrant and non-migrant (i.e., resident) A. stamineus relative to overall invasive species density (#/m^2) in each study watershed, with reference to sample size (N), Poeciliid density, and Loricariid density.

4.4 GENOMIC AND LIFE HISTORY RESPONSES TO AIS REMOVAL FROM HAWAIIAN STREAMS

Theory predicts that management interventions can trigger genetic rescue -- with or without demographic rescue -- which can bolster the potential for at-risk populations to persist via increases in immigration. The project team tested this prediction by assaying genomic and life history responses for comparison to demographic responses of the facultative amphidromous goby, *A. stamineus*, to experimental AIS removals. This involved comparing snorkel survey and mark-recapture based estimates of demography with double digest restriction-site associated deoxyribonucleic acid (DNA) (ddRAD) SNP assays of genomic variation and otolith microchemistry analysis of migratory life history. This approach allowed us to infer the nature and magnitude of responses to AIS removals as well as possible underlying ecological and evolutionary pathways of rescue.

The project team detected increased genomic diversity following AIS removals (**Figure ES-5**) as well as an increase in population densities corresponding to the reproductive cycle of *A. stamineus*. The project team also found that immigrant post-larval juveniles (i.e., new recruits) had higher genomic diversity compared to non-migrant post-larval juveniles (**Figure ES-5**), which suggests that an increase in immigration might be driving observed genomic and demographic shifts. However, contrary to this prediction, the project team detected an increase in the number of resident, not immigrant, post-larval juveniles following AIS removals. This indicates that, at least for *A. stamineus*, AIS control can result in genetic rescue via local demographic gains in resident populations rather than gains due to an increase in immigration. If so, then conservation management strategies aiming to promote the well-being of resident populations may be as or more effective than elevating gene flow in at-risk species with flexible migratory life histories.

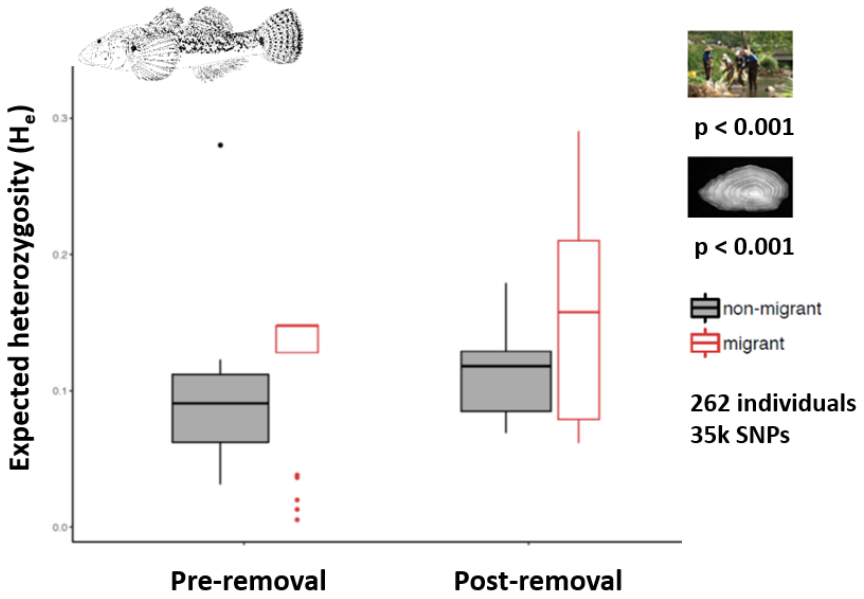


Figure ES-5. Awaous Genomic Diversity Before and After AIS Removals by Life History

Overall, A. stamineus migrants exhibit higher genomic diversity, and genomic diversity increased after AIS removals. Non-migrants exhibited a greater increase in diversity, however, which is consistent with demographic evidence of a post-removal rise in recruitment and an increase in the proportion of residents in the study populations following AIS removals. Whiskers are minimum and maximum values relative to the first and third quartiles, respectively, whereas median values are denoted with bars. Significant differences according to AIS removal (i.e., time period) and migratory life history determined according to Welch's Two Sample t-tests.

4.5 ECOSYSTEM RESPONSES TO AIS REMOVAL FROM HAWAIIAN STREAMS

AIS have reconfigured the structure and diversity of stream communities across the Hawaiian archipelago. Biotic transformation is often accompanied by altered ecosystem process rates, but it can be difficult to determine whether AIS are directly responsible for ecosystem changes or simply coincide with anthropogenic physical and chemical disturbances. In this study, the project team quantified the direct effects of AIS on nutrient and carbon dynamics by assaying ecological associations and experimentally removing AIS from 13 streams circumscribing broad gradients of hydrological dynamics, habitat conditions, and nutrient pollution on O'ahu (**Figure ES-1**).

Prior to AIS removals, ecosystem gross primary productivity was suppressed by introduced Loricariid armored catfishes even when there was an abundance of light and nutrients (**Figure ES-6**). Bioassays indicated that algae in several streams became nutrient-limited following removal of AIS, but otherwise the project team did not detect a consistent shift in nutrients that limit algal growth, concentrations of dissolved nutrients, leaf-litter breakdown rates, or whole-stream metabolism. However, differences across streams in effect sizes on ecosystem processes were related to the AIS biomass removed, background nutrient concentrations, stream size, and watershed land use. Evidence that contextual biotic and abiotic variation can mitigate or mask ecosystem responses to costly AIS removal efforts suggests that pre-removal assessments should be conducted to help prioritize restoration sites. The project team's results also indicate that a combination of interventions, such as improving water quality and habitat alongside AIS control, may be required to restore ecosystem conditions that favor native species.

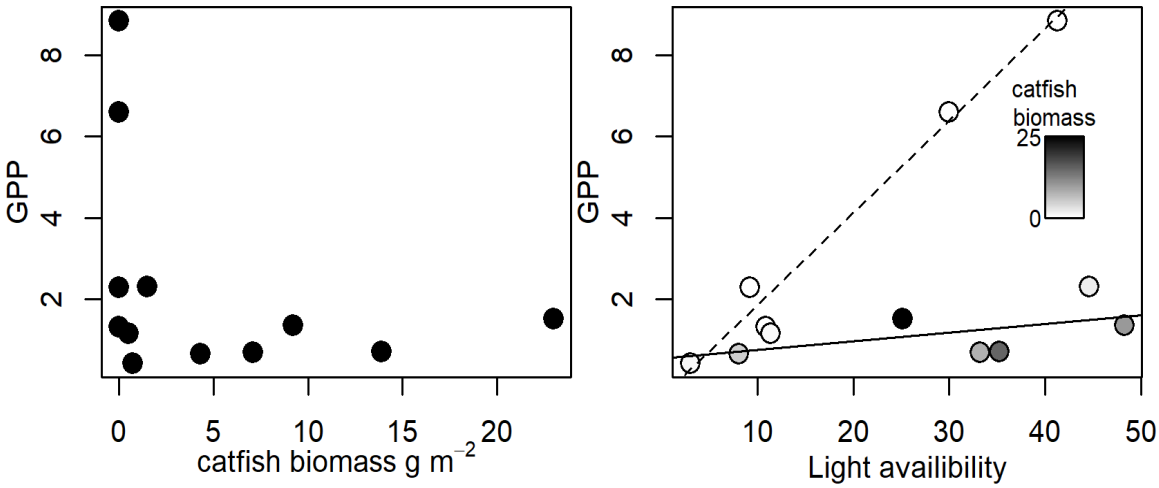


Figure ES-6. GPP in the Removal Reach Before AIS Removals by Loricariid Presence and Biomass

(A) Reach-scale gross primary production (GPP) measured before AIS removals (0.27 to 9.82 mg O_2 m^2 d^{-1}), as a function of armored catfish (catfish) biomass (0 to 23 .0 g m^{-2}) in removal reaches. (B) GPP as a function of percent light availability in the removal reaches (inverse percent canopy cover). Pearson correlation analysis demonstrated that GPP is positively associated with percent light availability, and that the association was stronger in streams without armored catfish (open points, dashed line) compared to streams with armored catfish (points shaded, solid line).

4.6 COUPLED OCEANIC-WATERSHED MODELING OF WHOLE-WATERSHED AIS REMOVAL

Even though AIS are consistently identified as a primary threat to aquatic biodiversity in oceanic island streams, little guidance is available to prioritize control or eradication of the most harmful invaders. It thus should come as no surprise that eradication attempts often fail because management campaigns do not adequately scale suppression efforts or do not adequately target high-impact populations or areas where interventions might yield disproportionate benefits beyond local populations.

Understanding that deep-rooted evolutionary differences in life history traits can sometimes buffer native species against AIS control measures; the project team has conducted coupled ocean-watershed model simulations showing that differences in life history can be strategically leveraged to not only restore local populations but also achieve metapopulation-wide benefits through demographic spillover.

The endemic stream fauna of the Hawaiian archipelago and other tropical oceanic islands is dominated by amphidromous species that naturally recolonize streams following oceanic larval dispersal. In contrast, the non-native fauna in Hawaiian streams (and insular streams elsewhere) is dominated by non-migratory species that are obligately bound to FW conditions. Accounting for this dichotomy, modeling showed that removal of both invasive (lethally) and native (lethally or non-lethally) species throughout a selected watershed can reliably convert demographic sinks into productive source populations that provide recruits to streams across the archipelago (**Figure ES-7**). Model simulations also indicate that this approach is both low-risk (local increases in natives always occur quickly) and high-reward (spillover somewhat enhances all other populations), but requires targeting removals to large, highly-invaded watersheds that are well connected by ocean currents (**Figure ES-7**).

While the project team's model has been parameterized to illustrate restoration outcomes across Hawaiian watersheds, the proposed 'clean-slate' restoration approach is broadly applicable to situations where native species can recolonize more easily than invaders following mass mortality stemming from control measures.

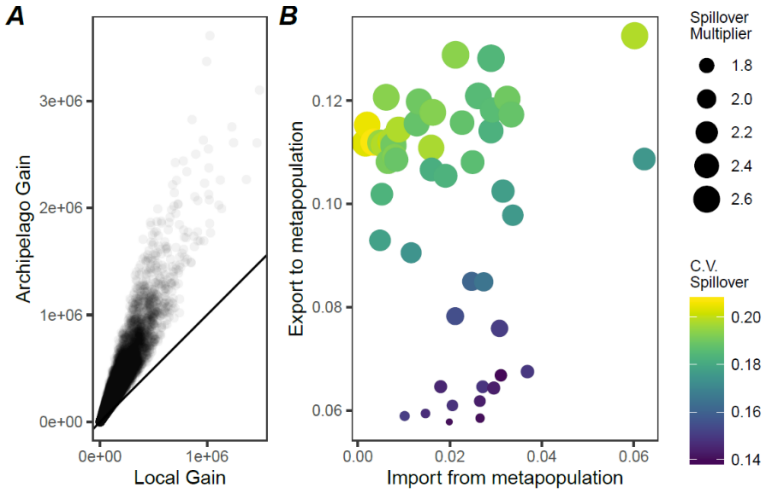


Figure ES-7. Metapopulation Spill Over from "Clean-Slate" AIS Eradication

(A) Following extermination of invasive species from a single watershed, growth of the metapopulation across the Hawaiian archipelago greatly exceeds local gains alone. In no case does the metapopulation decline. Spillover from "clean-slate" restoration is a robust outcome when populations are recruitment limited: it occurs in all study streams. If recruitment is not limited, no spillover occurs, and all points fall on the black line, indicating that gains are solely local. (B) Connectivity via larval dispersal varies widely among watersheds, which strongly affects spillover (ratio of archipelago:local population gains). Fitted to simulation results depicted in (A), each of which has a distinct combination of the import (proportion of all successfully dispersing larvae arriving at a given watershed) and export (proportion of all larvae from a given watershed that successfully disperse to any watershed) values estimated from a passive dispersal model applied to observed ocean currents from 2007-2011. Differences in spillover among streams depend primarily on export, so long as import is sufficient to ensure growth in the local population following eradication of invasive species. Spillover tends to be highest with high export.

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

Development of innovative approaches and actionable information for managing AIS can substantively improve DoD stewardship of stream ecosystems that cross DoD lands, especially on islands like O‘ahu where installations harbor native species under federal or state protection. This project not only empirically demonstrated that modified electrofishing can be a valuable tool for AIS control in Hawaiian streams, it also illustrated how managers can maximize benefits of AIS control through careful consideration of target species and climate-driven hydrological conditions. Likewise, the project delivered a model-based decision support tool that can serve as a defensible and transparent analytical framework for prioritizing interventions according to local, regional, and archipelago-scale gains.

Development of innovative approaches and actionable information for managing AIS has yielded several key findings and important conclusions, including:

- **AIS control is feasible and effective** with a tailored electrofishing protocol that minimizes collateral harm to TER-S;
- **Control efforts can result in sustained reductions** in overall AIS densities and densities of targeted AIS (where removal efficacy is mediated by AIS densities and hydrology);
- **AIS removal can increase recruitment**, growth, and body condition of TER-S;
- **AIS removal can increase genomic diversity** and composition of local TER-S populations (where gains are mediated by migratory life history); and
- **Whole watershed “clean-slate” restoration can lead to demographic spillover** that benefits populations elsewhere in the archipelago (leveraging life history differences between AIS and TER-S)

With an expanded knowledge base and toolkit, DoD resource managers and partner organizations should be able to achieve more effective watershed management and conservation of at-risk aquatic species to ensure the sustainability of military operations in the Hawaiian Islands and other oceanic islands in the Pacific.

Several complementary approaches have been taken to transfer information and tools to practitioner communities in Hawai‘i and elsewhere. To initiate the translation process, a core group of O‘ahu-based resource managers were engaged to demonstrate new methods for AIS control in coordination with ongoing AIS management activities. This involved, for example, conducting experimental AIS removals in study reaches located in management areas. This also involved conducting field demonstrations with federal and state resource managers. To support future, independent endeavors, the project team have developed a technical “user” guide for resource managers to undertake AIS removals in Hawaiian streams. The guide not only offers summary and detailed overviews of protocols, it also provides supporting technical documents, such as template data sheets. Finally, the project team also have disseminated project findings to the broader scientific community and general public through publication of peer-review papers and seminars at regional workshops and national conferences, as well as through popular press articles and community outreach.

6.0 LITERATURE CITED

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