GUIDANCE DOCUMENT

Control and Mitigation of Aquatic Invasive Species in Pacific Island Streams

Guidance for Control of Aquatic Invasive Species in Hawaiian Island Streams

SERDP Project RC-2447

MAY 2020

Michael Blum
Tulane University

J. Derek Hogan
Texas A&M University

Peter B. McIntyre
Cornell University

SERDP
DOD ▪ EPA ▪ DOE

Page Intentionally Left Blank

Page Intentionally Left Blank

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
28-05-2020

2. REPORT TYPE
SERDP Guidance Document

3. DATES COVERED *(From - To)*
6/2/2015 - 6/2/2020

4. TITLE AND SUBTITLE
Control and Mitigation of Aquatic Invasive Species in Pacific Island Streams

Guidance for Control of Aquatic Invasive Species in Hawaiian Island Streams

5a. CONTRACT NUMBER
W912HQ-15-C-0006

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER
RC-2447

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
Michael J. Blum, J. Derek Hogan, Peter B. McIntyre

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tulane University
400 Lindy Boggs Building
New Orleans,LA 70118

8. PERFORMING ORGANIZATION REPORT NUMBER
RC-2447

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program
4800 Mark Center Drive, Suite 16F16
Alexandria, VA 22350

10. SPONSOR/MONITOR'S ACRONYM(S)
SERDP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
RC-2447

12. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A. Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Here we describe the feasibility and effectiveness of an AIS removal protocol designed for Hawaiian Island streams that involved use of modified electrofishing techniques, including two tiers of precautionary steps to protect native species from exposure to electrofishing activity. First, all native species were exhaustively captured by hand-netting, sequestered streamside for the duration of electrofishing, and subsequently returned to their location of capture. As a further precaution, amperage and waveform combinations were set during electrofishing to reduce the likelihood of injuries and mortality of any native species potentially remaining in the removal reach. Given that effects of AIS removal on native species and habitats might manifest in a number of ways, we examined a suite of organismal and demographic variables to understand sensitivity and response capacity of at-risk native species in the study watersheds. Native species densities did not decrease following AIS removal events, suggesting negative effects of electrofishing on native species can be mitigated with the modified methodology. However, the efficacy of AIS removal varied according to key stream characteristics and targeted taxon. These results suggest that modified electrofishing can be a valuable tool for AIS management in Hawaiian Island streams and streams on other oceanic islands in the Pacific, with the understanding that users must carefully consider conditions within streams that can influence the efficacy of electrofishing as well as the importance of preventing (re)introductions of AIS to sustain long-term benefits of removal.

15. SUBJECT TERMS
amphidromy; armored catfish; *Awaous stamineus*; ecosystem restoration; goby; guppies; Hawaii; invasive species removal; oceanic islands; stream monitoring; water chemistry; watershed management

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U	U	U	SAR	39	Michael J. Blum
					19b. TELEPHONE NUMBER *(include area code)* 504-214-9964

Page Intentionally Left Blank

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

A. bisculata	Atyoida bisculata
A. stamineus	Awaous stamineus
AC	Alternating Current
AIS	Aquatic Invasive Species
DAR	Division of Aquatic Resources
DC	Direct Current
E. sandwicensis	Eleotris sandwicensis
HAR	Hawaiʻi Administrative Rules
Hz	Hertz (frequency)
M. grandimanus	Macrobrachium grandimanus
S. hawaiiensis	Stenogobius hawaiiensi
S. stimpsoni	Sicyopterus stimpsoni
TSS	Total Suspended Solids
V	Voltage

1.0 Summary

Aquatic invasive species (AIS) are consistently identified as a primary threat to biodiversity in oceanic island streams. Both intentional and accidental non-native species introductions have resulted in the loss of native species on islands across the Pacific. For example, several native Hawaiian damselfly species receive or are candidates for federal protection due to predation by fishes intentionally introduced for sport and mosquito control. Although toxicants (piscicides or poisons) (Kolar et al. 2010, Nico and Walsh 2011) and electrofishing (Murphy and Willis 1996) are the preferred methods of lethal and non-lethal nuisance fish management worldwide, these methods are generally considered unacceptable for AIS management on Pacific islands because of potential harm to sensitive native or endemic species (Britton et al. 2008, Kolar et al. 2010). As a result, few attempts have been made to eradicate, control, or mitigate AIS in Pacific island stream ecosystems and little to no effort has been made to develop protocols for tropical and subtropical Pacific islands. Nearly all documented campaigns of AIS control on Pacific islands have relied on toxicants but implementation appears to have followed general principles developed for aquatic ecosystems elsewhere (Nico and Walsh 2011). Electrofishing, however, can provide the greatest return for the least effort among fish capture methods. Additionally, adverse complications can be addressed by taking precautionary steps, including sequestration and restocking of native species before and following electrofishing. Instrument settings also can be modified for prevailing environmental conditions (e.g., conductivity) and sensitivity of native species (Holliman et al. 2003).

Here the project team assesses the feasibility and effectiveness of an AIS removal protocol in streams on Oʻahu, Hawaiʻi using modified electrofishing techniques, including two tiers of precautionary steps to protect native species from exposure to electrofishing activity. First, all native species were exhaustively captured by hand-netting, sequestered streamside for the duration of electrofishing, and subsequently returned to their location of capture. As a further precaution, amperage and waveform combinations were set during electrofishing to reduce the likelihood of injuries and mortality of any native species potentially remaining in the removal reach (Holliman et al. 2003, Snyder 2003). Given that effects of AIS removal on native species and habitats might manifest in a number of ways, the project team examined a suite of organismal and demographic variables to understand sensitivity and response capacity of at-risk native species in the study watersheds. Native species densities did not decrease following AIS removal events, suggesting negative effects of electrofishing on native species can be mitigated with the modified methodology. However, the efficacy of AIS removal varied according to key stream characteristics, (e.g., mean discharge and discharge variability) and taxon (e.g., less effective removal of smaller, mobile poeciliid species). These results suggest that modified electrofishing can be a valuable tool for AIS management in Pacific island streams, with the understanding that users must carefully consider conditions within target streams that can influence the efficacy of electrofishing and the importance of preventing (re)introductions of AIS to sustain long-term benefits of removal.

2.0 AIS Control in the Pacific

AIS are consistently identified as a primary threat to biodiversity in oceanic island streams and non-native species, both intentionally and accidentally introduced, have resulted in the loss of native species on islands across the Pacific (Brasher 2003). For example, several native damselfly species in the Hawaiian archipelago now receive or are candidates for federal protection under the Endangered Species Act due to predation by fishes intentionally introduced into streams for sport (e.g., small mouth bass) and mosquito control (e.g., guppies and other poeciliids; Polhemus 1993, Polhemus and Asquith 1996, Yamamoto and Tagawa 2000, Englund 2001). Aquatic invasive species can create conditions that result in local extinction and prevent (re)colonization of streams by native species directly, through predation or competition (Sax and Gaines 2008), or indirectly by altering ecological processes. For example, poeciliids feed on larvae and postlarvae of native amphidromous fishes and compete with adults for invertebrate prey (Holitski et al. 2013) while invasive armored catfish compete with native species for algal food resources, limit nutrient availability by sequestering phosphorus (Vanni et al. 2002), and disturb the physical environment, increasing siltation (Rowe 2007, Capps and Flecker 2013). Over 60 species of AIS from 18 families are now established on Pacific islands (Nico and Walsh 2011) and because Pacific island stream communities are naturally depauperate (McDowall 2003), the diversity of AIS is often much higher than native species diversity. In the Hawaiian archipelago, for example, the diversity of non-native fish in streams can be an order of magnitude higher than the diversity of native fish (Moody et al. 2017; Hawaiʻi Watershed Atlas, http://www.hawaiiwatershedatlas.com/).

Few attempts have been made to eradicate, control, or mitigate AIS in Pacific island stream ecosystems, and outcomes of past management actions have not been well documented (Nico and Walsh 2011). Nonetheless, several important inferences can be drawn from available records on efforts to manage non-native fishes (Englund and Filbert 1999, Nico and Walsh 2011) in tropical and subtropical Pacific island streams. AIS management in New Zealand also affords some useful perspectives, as many at-risk native fishes in New Zealand and across the Pacific islands are amphidromous. First, eradication efforts have largely been unsuccessful (Nico and Walsh 2011). Second, nearly all attempts to manage AIS have involved methods designed for continental stream conditions (Englund and Filbert 1999, Nico and Walsh 2011). Third, AIS management must account for societal concerns about species of cultural importance and erosion of cultural traditions (Englund and Filbert 1999, Nico and Walsh 2011). Finally, management that accounts for insular conditions can promote the recovery of at-risk native species (Nico and Walsh 2011).

Eradication of entire populations is not often a viable management option (Kolar et al. 2010), so it is not surprising that efforts to eradicate non-native fishes on Pacific islands have proven unsuccessful. Eradication efforts may be hindered by the type, abundance, and distribution of the target species, and can be further constrained by the size and complexity of the invaded environment (Kolar et al. 2010). Nico and Walsh (2011) identified 11 separate campaigns to eradicate non-native fishes on tropical and subtropical Pacific islands. All but one were carried out in small closed systems (e.g., ponds). Though eradication in open systems is widely considered unfeasible, the Guam Division of Aquatic and Wildlife Resources has been attempting to eliminate chevron snakeheads (*Channa striata*) from the Ajayan River on Guam. Complete eradication has not been clearly achieved in this or any other documented open system campaign (Nico and Walsh 2011).

Nearly all of the documented campaigns on Pacific islands have relied on toxicants (piscicides or poisons) that are the preferred method for eradicating nuisance fish worldwide (Kolar et al. 2010, Nico and Walsh 2011). Chemical toxicants, such as rotenone and antimycin-a, are typically diluted liquid solutions released into the water and then neutralized or left to naturally degrade over a period of days (Dinger and Marks 2007, Moore et al. 2008). Use of toxicants can fail to eradicate target species without proper evaluation, planning and implementation (Donlan and Wilcox 2007). Although toxicants have been used extensively in New Zealand for non-native fish eradication, (e.g., Lintermans 2000, Chadderton et al. 2003, Pham et al. 2013), no comparable effort has been made to develop protocols for tropical and subtropical Pacific islands. Special consideration needs to be made when applying fish poisons to rivers and streams on tropical islands with fringing or barrier type coral reefs as these chemicals can potentially harm coastal biota because of comparably short transit times in watersheds. Prior implementation appears to have followed general principles developed for aquatic ecosystems elsewhere (Nico and Walsh 2011).

Electrofishing has become the foremost non-lethal capture method for freshwater fish management (Murphy and Willis 1996). Electrofishing provides the greatest return for the least effort among fish capture methods, making it especially useful for non-native fish removal. While chemical control of AIS can be relatively advantageous because it can reduce the likelihood of recolonization, mechanical methods of control, such as electrofishing, are generally no less efficient than chemical methods of control (Beric & MacIsaac 2015). The principle of electrofishing is to produce an electric field around electrodes in water to temporarily stun nearby fish (Snyder 2003). The approach has been extensively used for removal of non-native fish across North America using backpack units for small streams and barge or boat-mounted units for larger water bodies (Kolar et al. 2010). Plunge pools, boulders, and other complex habitat characteristics make electrofishing challenging on Pacific islands (Baker and Foster 1992), but studies conducted on Puerto Rico have demonstrated that electrofishing can be highly effective for capturing fish in insular streams (Kwak and Cooney 2008).

Many of the most widely used control and eradication methods, including electrofishing and chemical toxicants, are generally considered unacceptable for AIS management on Pacific islands because of potential harm to native species (Britton et al. 2008, Kolar et al. 2010). Rotenone and antimycin-a may affect native fishes and aquatic invertebrates more strongly than targeted species (Finlayson et al. 2002, Dinger and Marks 2007, Schofield and Nico 2007). Electrofishing also can result in non-target injuries and mortality (Snyder 2003), though complications can be reduced through appropriate amperage and waveform combinations (Holliman et al. 2003). Use of toxicants has been banned by some Pacific island governments, including Hawai'i where it is a felony. The State of Hawaiʻi Division of Aquatic Resources (DAR) also has established a general moratorium on electrofishing.

Though few attempts have been made to carry out AIS control or eradication on Pacific islands that have involved methods set to reflect protective controls, at least one documented case has demonstrated that precautionary steps can be taken that protect and promote the recovery of native species. As described by Nico and Walsh (2011), rotenone was used to eradicate a reproducing population of Nile tilapia in a freshwater crater lake in the Galapagos archipelago. Approximately 40,000 tilapia were removed from the lake. Before application of rotenone to the lake, aquatic invertebrates were collected and held in refuge tanks. The captive invertebrates were released back into the lake after the rotenone had degraded to promote recovery of native communities possibly affected by exposure to the toxicant (Nico and Walsh 2011). This example, and other examples of sequestering and restocking native fauna in New Zealand and elsewhere (e.g., Buktenica et al. 2013, Pham et al. 2013), indicates that AIS management can incorporate methods that reduce risks, offset potential non-target impacts, and promote the survival of at-risk species on Pacific islands.

While control and even full eradication of AIS on tropical and subtropical Pacific islands is ambitious (Leung 2002, Nico and Walsh 2011), it is nonetheless worth pursuing because favorable geomorphological and hydrological features increase the odds of success. Drainage basins on tropical and subtropical oceanic islands are relatively small, narrow, and steep by continental standards (Kinzie 1988, Brasher 2003, Covich et al. 2003, Oki and Brasher 2003). Additionally, waterfalls, rapids, or other barriers that can be surmounted by native species with the capacity to climb often restrict the spread of introduced species (Moody et al. 2017). The absence of watershed connectivity (except through marine dispersal pathways) and terminal waterfalls in many streams that function as barriers minimize the chances that AIS will recolonize following removal. This, along with consistent management prioritization of alleviating the effects of AIS on at-risk native species in Pacific island streams (Brasher et al. 2003), provide clear motivation for the development and evaluation of region-specific methods and tools for AIS management.

3.0 AIS Control Assessment

The project team assessed the feasibility and effectiveness of a modified electrofishing technique for removal of AIS in streams on Oʻahu, Hawaiʻi. Removals were conducted in 100 m long stream reaches in the lower region of 12 study watersheds across the Koʻolau Range. One hundred meter long control reaches were located upstream and downstream from the removal reaches and separated from the removal reach by 25 m buffer zones. Though the goal was to remove all AIS, the project team targeted drift-feeding poeciliids (guppies, mollies, and swordtails) and algae-eating loricariids (armored catfish) because they are among the two most abundant non-native fish groups on Oʻahu and because they impact native fishes through different ecological pathways. Since populations of native amphidromous species are depressed on Oʻahu, some to the point of extreme rarity, metrics of native species responses focused on *Awaous stamineus* (formerly known as *A. guamensis*; Lindstrom et al. 2012), which still occurs at low-to-moderate densities across the island.

Effects of AIS removal might manifest in a number of ways because invasive species have diverse impacts on the stream ecosystems and the native species. For example, poeciliids prey on native fish larvae and compete with native fish juveniles and adults for food resources. Removal of poeciliids could therefore result in a downward shift in the size structure of *Awaous stamineus* (*A. stamineus*) populations, shifts in the abundance and diversity of invertebrate prey, or isotopic shifts in *A. stamineus* tissue indicating greater consumption of invertebrate prey. Loricariid catfishes feed on algae, sequester nutrients (e.g. phosphorus), and erode stream banks. Removal of loricariids might therefore increase water clarity (i.e., a reduction in total suspended solids, total suspended solids [TSS]), increase stream substrate heterogeneity (e.g., a reduction in sediment deposition), increase stream bank stability or vegetation, and possibility lead to shifts in the abundance and diversity of benthic diatoms or isotopic shifts in *A. stamineus* tissue indicating greater consumption of basal food resources. Shifts might also occur in indicators of whole stream metabolism like rates of decomposition (i.e., of allochthonous organic material like leaf litter). Notably, a diverse range of cascading effects may differ stream to stream because removal efficacy might vary among watersheds due to differences in pre-removal invasive densities and in-stream characteristics.

The project team assessed a suite of organismal and demographic conditions to understand sensitivity and responses capacity of at-risk native species to AIS removal from the study watersheds. AIS removals involved the following steps (Figure 1):

(1) collection of water samples to assess pre-removal stream chemistry and TSS,
(2) snorkel surveys to assess pre-removal stream communities,
(3) collection and sequestration of native species by hand-netting,
(4) measurement of standard length and weight of native species,
(5) elastomer tagging of *A. stamineus* for mark and recapture to measure post-removal organismal and demographic responses,
(6) fin clipping of *A. stamineus* for genetic analysis, sacrifice of limited numbers of *A. stamineus* for otolith-based microchemical analysis of life history variation and isotopic analyses of trophic position,
(7) electrofishing to remove AIS,
(8) return of native species to their place of capture,
(9) additional collection of water samples for chemistry and TSS analysis,
(10) post-removal snorkel surveys to assess changes in AIS and native species communities.

The project team relied on water chemistry (e.g., nitrogen and phosphorus) and TSS measures to track the effects of AIS removal on in-stream conditions, and snorkel surveys were conducted to characterize changes in the structure of native and non-native communities as well as recolonization of the removal reaches by target AIS.

The project team conducted a multiyear, individual-based mark-recapture study of *A. stamineus* to assess individual growth rates as well as immigration, emigration, and mortality in response to AIS removal. The mark-recapture data was used, for example, to derive site and watershed-specific estimates of individual adult growth rate, adult population density, body condition, reproductive status, approximate age distributions, as well as apparent survival and recruitment over time. No individuals were sacrificed to complete this work.

The project team examined genetic variation of *A. stamineus* because it is inversely related to AIS presence in streams across the Hawaiian archipelago (Blum et al. 2014). Genetic variation may increase in native fish populations as a consequence of population-level responses to removal of invasive fishes. For example, variation may increase if population size increases due to greater survival that might result from greater availability of limiting resources or nutrients (i.e. greater phosphorus availability in the absence of loricariid catfish). Genetic variation might also increase if recruitment of immigrating post-larvae increases, as removal of poeciliids is expected to reduce predation on early life stages of native fishes. Accordingly the project team assessed whether removal increased allelic richness, heterozygosity, and effective population size from genome-wide analysis of single nucleotide polymorphisms based on non-lethal fin clipping of adult and juvenile *A. stamineus* (Hogan et al. 2011, Lindstrom et al. 2012).

As the previous analyses of otolith microchemistry indicated that *A. stamineus* exhibit life history variation (Blum et al. 2014, Hogan et al. 2014), where some larvae remain in natal freshwater or local near-shore environments rather than undergoing marine dispersal, the project team examined life-history variation (i.e., the proportion of migrants versus non-migrants) from post-larval otoliths to determine whether it is a plastic response to AIS removal and stream flow conditions (i.e., more larvae may remain in their natal watershed in response to more favorable local conditions). Otolith microchemistry data was used to quantify the frequency and spatial distribution of marine-dispersing and non-migrant *A. stamineus* larvae recruiting to local populations and to identify variation in marine dispersal pathways to assess the likelihood of recruitment from outside sources (Hogan et al., 2014).

Using the same individuals sacrificed for otolith analyses, the project team conducted nitrogen isotope assays to measure potential food web shifts following AIS removal. They focused on assessing the trophic level of *A. stamineus* adults, which is higher in areas with lower water quality and in areas that harbor more AIS (Lisi et al., 2018). Tissue analysis might therefore demonstrate whether improvement of local conditions reduces competition for dietary resources.

Protocols and data sheets for all portions of removal events in this study have been included as supplementary appendices. These should serve as useful resources or guidance for conducting AIS management in Pacific island streams via electrofishing, which requires standard protocols and data collection minimally for snorkel surveys, sequestering native species, and electrofishing. Practitioners may require additional protocols, such as those provided for measuring water chemistry, to assess the success and processes underlying AIS removal.

4.0 Traditional Electrofishing Methodology

The principle of electrofishing is to produce an electric field around electrodes in water to temporarily stun nearby fish, which can then be gathered for data collection or removal (Snyder 2003). Electrofishing is most efficient in water bodies with clear, highly conductive water and simple substrate. Properly conducted, electrofishing generally results in negligible mortality and injury rates (McCrimmon & Bidgood 1965). However adverse physiological effects can include spinal damage and hemorrhaging (e.g., Sharber & Carothers 1988; Sharber *et al.* 1994), reduced swimming stamina (Horak & Klein 1967; Mitton & McDonald 1994), and physiological and behavioral abnormalities (Mitton & McDonald 1994; Schreck *et al*. 1976; Mesa & Schreck 1989). The incidence and severity of these risks are related to the type of electrical output used (alternating current [AC], continuous direct current [DC], pulsed DC) as well as the voltage (V), Hertz frequency (Hz), and pulse width. Use of continuous or low frequency pulsed DC is thought to present the least risk of harm (Whaley *et al.* 1978; McMichael 1993; Sharber *et al.* 1994).

Electrofishing has generally been considered inappropriate for AIS management in Pacific island streams because of the dual challenges of complex substrates and the sensitivity of at-risk native species. Plunge pools, boulders, and other complex habitat characteristics common in oceanic island streams, including Hawaiʻi, can make electrofishing less effective than when used in continental streams (Baker and Foster 1992). However, studies conducted across Hawaiʻi (Maciolek and Timbol 1980), Puerto Rico (Kwak *et al*. 2007), and Indonesia (J. Derek Hogan, unpublished data) have demonstrated that electrofishing can be highly effective for capturing fish in oceanic island streams. Nonetheless, physiological sensitivities of native species make them particularly susceptible to collateral injury and mortality at electrofishing settings typically used in continental streams (Snyder 2003), adverse complications of electrofishing can, however, be addressed by taking precautionary steps, including pre-electrofishing sequestration and post-electrofishing restocking of native species (Nico and Walsh 2011) as well as modification of instrument settings for prevailing environmental conditions (e.g., conductivity) and sensitivities of native species (Holliman et al. 2003).

5.0 Modifications to Traditional Electrofishing Methodology

Working closely with the Hawaiʻi DAR, the project team developed and validated a modified electrofishing methodology that includes two tiers of precautionary steps to protect culturally important native species and to ensure electrofishing activity targets AIS species. Native Hawaiian species are more sensitive to electrofishing than many AIS species. The native aquatic macrofauna of Hawaiian streams consists of only five fishes (*A. stamineus*, *Lentipes concolor*, *Eleotris sandwicensis* [*E. sandwicensis*], *Sicyopterus stimpsoni* [S. stimpsoni], and *Stenogobius hawaiiensis* [*S. hawaiiensis*]), two gastropods (*Neritina granosa, Neritina vespertina*), and two crustaceans (*Atyoida bisulcata*, *Macrobrachium grandimanus* [*M. grandimanus*]) (Yamamoto & Tagawa 2000, McDowall 2003, Lindstrom et al. 2012). Although fish are generally less sensitive to electrofishing at smaller and larger size classes, this is not necessarily true of native Hawaiian fishes. For example, *E. sandwicensis* is considerably more sensitive than other native species and becomes more susceptible to injury at larger sizes (larger fish experience greater risk of injury and mortality at higher voltages and due to adverse effects of muscle twitch induced by pulsed current). Therefore, before electrofishing began, efforts were made to carefully capture all native fishes and invertebrates in target reaches by exhaustive hand-netting, sequestered streamside at low densities in shaded containers filled with aerated stream water, and monitored for stress during electrofishing passes. After electrofishing was completed, native species were allowed to recover from possible stress from handling and returned to their location of capture. By conducting pre-cautionary native species sequestration, potential exposure of native species to electrofishing activities was greatly reduced. As a further precaution, standard operating procedures for electrofishing were modified to reflect the hydrology and geochemistry of Hawaiian streams and the sensitivity of Hawaiian species. In particular, amperage and waveform combinations were set to reduce the likelihood of injuries and mortality of any native species potentially remaining in the removal reach during the first electrofishing pass (Holliman et al. 2003, Snyder 2003). Settings were based on a procedure developed for warm-water streams on Puerto Rico (Kwak et al. 2007) and sensitivities of Hawaiian species documented by the Hawaiʻi Cooperative Fishery Research Unit (Maciolek and Timbol 1980).

It is important to note that voltage is typically determined according to water and tissue conductivity, which can have a large effect on electrofishing efficacy. While the settings used in this study were effective for the particular study streams, conductivity should always be tested and checked against recommended baseline settings to ensure optimal efficacy. Smith-Root electrofishers, for example, can provide sufficient current at realistic power levels in potable water sources across the United States ranging in conductivity from 20 to 2000 µS/cm. Tissue conductivity and temperature of target species also must be considered when using electrofishing instruments. For example, carp conductivity varies from 372 µS/cm at 5°C to 1969 µS/cm at 25°C, which is similar to the conductivity of other common continental stream fish (e.g., trout, perch, carp, gudgeon) that ranges from 814 µS/cm to 1220 µS/cm. Species-specific conductivity has not been carefully determined for native Hawaiian fishes, although species generally expected to have higher conductivity, such as *E. sandwicensis*, appear to be more vulnerable to electrofishing. Combinations used in this study thus solely accounted for stream temperature and conductivity.

Instruments were set to minimize the risk of associated adverse impacts during initial electrofishing passes, when native species were most likely to remain in the removal reaches. Unpulsed DC current was always used for initial passes and voltage was generally set to 100V, with rare exceptions of 150 V or 200 V (as per conductivity conditions, discussed above). Continuous current presents the least risk for injury relative to other setting options. Combined with low voltage, use of unpulsed DC current reduces the likelihood of twitch reactions, the most common cause of spinal injury, particularly for larger native fishes that are at a higher risk of spinal injury from muscle twitches and spasms. Notably, capture rates are typically lower at these settings, and thus it is likely that many of the less sensitive AIS species as well as smaller fish will not be caught during initial passes.

Settings were adjusted to target the capture of smaller and otherwise less sensitive AIS species for the second and third passes, when risk to native species is significantly reduced. For both passes, the project team used pulsed current with the frequency set at 60 Hz, with a few exceptions when it was set at 30 Hz. Duty cycle was 12% except for rare exceptions when it was set to between 30% and 50%. For the second pass, voltage was generally set to 200V but ranged from 150V to 275V. For the third pass, voltage was held constant or increased slightly depending on the effectiveness of the second pass. Elevated voltage in the third pass targeted smaller fish missed in the first two passes. Performing a third pass is not always necessary; low densities and high efficacy during earlier passes can preclude the need. Using the more efficient pulsed current and increased voltage improved capture rate, but also elevated collateral risks to any native species remaining in the removal reach. Notably- relatively few native species were captured during second and third passes (Figure 2).

Following the described protocol, electrofishing was highly effective for removing AIS in the study reaches, particularly for targeted taxonomic groups (loracariid catfishes and poeciliids). Loracariid catfish included *Ancistrus temmincki* and *Hypodstomus watwata*. Poeciliids included *Poecilia mexicana, Xiphophorus helleri,* and *Poecilia reticulata.* All other AIS were considered to be a non-target group. For assessing the impact on native species, the fish species, *A. stamineus, E. sandwicensis, S. stimpsoni* and *S. hawaiiensis*, were evaluated separately, whereas the native crustacean species, *M. grandimanus* and *Atyoida bisculata* (*A. bisculata*) were considered as a single group.

Prior to electrofishing, native fish were hand-netted from the removal reaches. A total of 283 native fish were netted (252 *A. stamineus*, 5 *E. sandwicensis*, and 26 *S. hawaiiensis*) and sequestered during electrofishing passes. Though the total number of individuals that were hand-netted out of streams constituted a large proportion of all residents, native species remained and thus were removed during electrofishing passes (Figure 3). The morphology of Hawaiian streams, which varies from deep plunge pools to shallow reaches of a few inches, can complicate hand-netting by reducing catch efficacy. Additionally, native species vary in ease of capture. Whereas *A. stamineus* are slow moving and susceptible to hand-capture, *E. sandwicensis* are cryptic and more elusive. Likewise, *S. stimpsoni* are quick and alert; they also often bury themselves in soft substrates during pursuit. Both species of native shrimp (*A. bisculata* and *M. grandimanus*) are also difficult to hand net.

A total of 26,536 individuals were removed during electrofishing conducted in 12 streams, with notably higher catch counts of AIS than of native species (25,782 AIS versus 754 individuals of native species). Smaller catches of loricariids (n = 4,281) than poeciliids (n = 12,797) were consistent with observations of greater pre-removal poeciliid density (mean ± SD: loricariids = 2.6 ± 3.3 /m^2, poeciliids = 3.6 ± 5.1 /m^2). For all AIS and for the target groups, catch increased from the first to the second pass, then decreased in the third pass (Figure 2a), which followed expectations based on instrument settings. Removal of native species was low during initial passes and declined further in subsequent passes (Figure 2b). Native species comprised 4.1% of all captures during initial passes, and only 1.6% in second passes and 2.1% in third passes (Figure 3). The slight increase during third passes is likely the result of

AIS depletion, as it does not represent an absolute increase in the number of individuals that were caught during third passes.

Given expected relationships between size and electrofishing sensitivity, the project team investigated the effect of voltage and waveform on capture size at the most common settings (i.e., first pass settings of 100V DC, second 200V AC, third 275V AC) for all native species (n = 641) and the subset of AIS measured for length (n = 3,151). Length was log-transformed to account for variation in size by species and the greater number of small fish captured. Although waveform did not significantly affect length of individuals captured ($F_{1,3759}$ = 2.69, p = 0.10), size was inversely related to voltage (b = -0.0005853 mm/V, $F_{1,3482}$ = 8.632, R^2 = 0.002473, p = 0.00332; Figure 4), suggesting users can influence the size of fish targeted and associated risk across passes by manipulating voltage and waveform settings.

Relatively few mortalities and injuries of native species occurred during electrofishing passes. Of the 329 native fish captured across 12 streams, only seven collateral mortalities occurred, likely from spinal twitch injury (e.g., one fish suffered a broken spine) or excessive tetany preventing respiration. Mortalities were distributed across three species (three *E. sandwicensis,* two *A. stamineus*, two *S. hawaiiensis*). All native fish mortality occurred during second or third passes using pulsed DC at 60 Hz and 12% duty cycle. Two mortalities occurred at 175V, two at 200V and three at 275V. The project team also detected five non-lethal injuries, distributed across two species (three *E. sandwicensis*, two *A. stamineus*). Only one injury was incurred using unpulsed DC at 200V; all other injuries occurred with pulsed current at 200V, 60 Hz, and a duty cycle of 12%. Injuries were commonly burns. Likely the result of contact with the electrodes. Future efforts might consider installing a small non-conductive screen to prevent fish from contacting electrodes. Notably, native species densities were not significantly different following removal events (all natives: n = 26, $F_{1,12}$ = 3.11, p = 0.10; *A. stamineus*: n = 26, $F_{1,12}$ = 2.23, p = 0.16; Figure 5), which further illustrates that collateral effects of electrofishing on native species can be mitigated during the targeted removal of AIS.

6.0 Factors Influencing Electrofishing in Hawaiian Streams

The project team found that the modified electrofishing protocol resulted in reduced densities both of AIS as a whole (n = 26, $F_{1,12}$ = 14.20, p = 0.003) and for the target taxa (loricariids: n = 26, $F_{1,12}$ = 5.77, p = 0.03; poeciliids: n = 26, $F_{1,12}$ = 3.40, p = 0.09; Figure 6), but that removal efficacy depended on stream conditions. Generally, more total AIS and more loricariids were removed in streams with low mean discharge (range 0.09-0.72 CFS) and discharge variability (range 0.05-5.39 discharge variability; Figure 7), although only the relationship between mean discharge and loricariid counts was statistically significant (Table 1). The number of poeciliids removed was not related to either stream characteristic. In contrast, change in the density of AIS relative to pre-removal densities was generally greater in streams with greater discharge and variability (Figure 8); the effect of discharge variability on change in all AIS density was significant and the effect on poeciliid density was significant at alpha = 0.1 (Table 1). However, the project team observed a negative relationship between mean discharge and the change in density of all AIS species (Figure 9). They also found evidence indicating that the AIS community within a stream influenced the change in relative poeciliid density, with fewer removed in reaches with greater pre-removal AIS densities (pre-removal densities 1.37-18.5 /m^2 and removed biomass 1286.38-13347.42 g; Figure 9). This relationship was only significant for pre-removal density at alpha = 0.1 (Table 2).

7.0 Conclusions

Aquatic invasive species are regularly identified as a major threat to native fauna in Pacific island streams. Despite this, AIS management remains challenging and management protocols have not been developed for the region. Electrofishing, alongside other methods that are frequently used elsewhere, is often considered unacceptable because of collateral risk to ecologically and culturally important native species. The results demonstrate, however, that electrofishing may prove to be a useful tool for AIS management in the region. When modifications are made to conventional methods, potential risks to native species can be greatly reduced while ensuring capture efficacy of targeted AIS species. Prospective practitioners of electrofishing must nonetheless consider the role of stream characteristics, including hydrology and existing AIS communities, when assessing the potential benefit of electrofishing for AIS management in the region. Further, while Pacific island streams provide excellent opportunities to attempt full eradication of AIS from watersheds connected only by marine dispersal pathways, practitioners must be cognizant that reintroductions might curtail potential benefits to native species.

Based on the findings, the project team recommends practitioners employ a specific strategy, summarized in the one-page flow chart included in the supplementary appendices, based on the premise of conducting modified electrofishing for AIS control in Pacific island systems:

- Hand collect natives prior to electrofishing and sequester captured individuals streamside.
- Conduct initial electrofishing passes using unpulsed DC current at the minimum voltage required to produce an effect, but to safely capture remaining individuals of native species from the removal reach.
- Multiple passes at these settings may further reduce chances of collaterial mortality or injury to native species.
- Subsequently, conduct an additional pass using pulsed current at an increased voltage for the targeted removal of AIS, while still accounting for potential risk to larger, vulnerable natives.
- Conduct a final pass, increasing voltage if necessary, to achieve depletion of AIS, remaining alert to ensure that electrodes do not come in to contact with native species.
- Unlike toxicants, electrofishing does not leave any lasting effects on stream habitat, therefore native species should be returned to their site of capture immediately following the final pass.

Although the methods highlighted here reduce the risk of adverse effects, electrofishing in Hawaiian streams may still harm or kill native species. Taking precautionary steps does not eliminate all risk of collateral injury and mortality. Therefore, all practitioners should seek prior agency approval, as **the use of electrofishing devices to take aquatic life is prohibited under Section 188-23, Hawaiʻi Revised Statutes and Section 13-75-6, Hawaiʻi Administrative Rules (HAR), without a permit issued under Section 187 A-6, HAR**. In addition, the project team recommends that (1) some or all electrofishing be done under the supervision of DAR (B. Nishimoto, personal communication); (2) in cases where native species are captured during electrofishing passes, practitioners cease electrofishing and tend to captured individuals to reduce further risk of stress-induced injury or mortality; and (3) if there is mortality of native species, users terminate electrofishing and contact DAR to reassess their electrofishing protocol.

The results demonstrate that collateral effects of AIS removal via electrofishing on native species can be mitigated for Hawaiian island streams, indicating that electrofishing can be a useful tool for AIS management on tropical and subtropical Pacific islands. However, results are also consistent with expectations that AIS reductions will vary among watersheds. In part, this is a result of differences in complex habitat characteristics, such as steep elevational changes, plunge pools, and boulders, which can make electrofishing in Pacific island streams more challenging than in most continental streams. It is also important to consider that hydro-geomorphological (e.g., discharge) and the physiological differences among target groups can influence removal efficacy in Pacific island streams. Indeed, stream hydrology differentially influenced removal outcomes (i.e., number of removed individuals and change in relative density) within and across species. As the smaller and more mobile of the target taxa, the project team expected that poeciliids were less likely to be caught and more likely to recolonize following removals compared to catfish. This expectation was borne out, demonstrating the value of scaling up removals to eradicate AIS from entire watersheds. It also highlights the importance of stringent regulation of (re)introductions. The work also illustrates that electrofishing is not appropriate in saline or estuarine habitats or in freshwater habitats subject to tidal influence where risk of injury or mortality for native species can be much greater, due to the need for pulsed current run at higher voltages. Finally, it is worthwhile to note that the results presented here are based on work conducted on Oʻahu; outcomes may be different elsewhere. Therefore, potential users must consider watershed and regional conditions to determine whether and where to employ electrofishing for AIS management on Pacific islands.

References

Baker JA, Foster SA (1992) Estimating density and abundance of endemic fishes in Hawaiian streams. Division of Aquatic Resources, Department of Land and Natural Resources, State of Hawaii. 50p.

Beric B, MacIsaac HJ (2015) Determinants of rapid response success for alien invasive species in aquatic ecosystems. Biological invasions 17: 3327-35

Blum MJ, Gilliam JF, McIntyre PB (2014) Development and use of genetic methods for assessing aquatic environmental condition and recruitment dynamics of native stream fishes on pacific islands. Tulane University, New Orleans

Brasher AMD (2003) Impacts of human disturbances on biotic communities in Hawaiian streams. BioScience 53:1052–1060

Britton JR, Brazier M, Davies GD, Chare SI (2008) Case studies on eradicating the Asiatic cyprinid *Pseudorasbora parva* from fishing lakes in England to prevent their riverine dispersal. Aquatic Conservation: Marine and Freshwater Ecosystems 18: 867–876

Buktenica MW, Hering DK, Girdner SF, Mahoney BD, Rosenlund BD (2013) Eradication of nonnative brook trout with electrofishing and Antimycin–A and the response of a remnant bull trout population. North American Journal of Fisheries Management 33: 117–129.

Capps KA, Flecker AS (2013) Invasive Fishes Generate Biogeochemical Hotspots in a Nutrient–Limited System. PLoS ONE 8(1): e54093.

Chadderton WL, Kelleher S, Brow A, Shaw T, Studholme B, Barrier R (2003) Testing the efficacy of rotenone as a piscicide for New Zealand pest fish species. In: Managing Invasive Freshwater Fish in New Zealand. pp. 113–130. DOC workshop, May 2001, Hamilton.

Covich AP, Crowl TA, Scatena FN (2003) Effects of extreme low flows on freshwater shrimps in a perennial tropical stream. Freshwater Biology 48: 1199–1206.

Dinger EC, Marks JC (2007) Effects of high levels of Antimycin A on aquatic invertebrates in a warmwater Arizona stream. North American Journal of Fisheries Management 27: 1243–1256.

Donlan CJ, Wilcox C (2007) Complexities of costing eradications. Animal Conservation 10: 154–156.

Englund RA, Filbert RB (1999) Flow restoration and persistence of introduced species in Waikele Stream, Oʻahu. Micronesica 32: 143–154

Englund RA (2001) Long–term monitoring of one of the most restricted insect populations in the United States, *Megalagrion xanthomelas* (Selys–Longchamps), at Tripler Army Medical Center Oʻahu, Hawaii (Zygoptera: Coenagrionidae). Odonatologica 30: 255–263.

Finlayson BJ, Schnick RA, Cailteux RL, DeMong L, Horton WD, McClay W, Thompson CW, Tichacek GJ (2000) Rotenone use in fisheries management: administrative and technical guidelines manual. American Fisheries Society, Bethesda, Maryland, USA. 212 p.

Hogan JD, Blum MJ, Walter RP (2011) Characterization of ten novel microsatellite markers in *Awaous guamensis* with comments on cross amplification in congeners and other amphidromous fish native to Hawaiʻi. Conservation Genetics Resources 3: 275–277

Hogan JD, Blum MJ, Gilliam JF, Bickford N, McIntyre PB (2014) Consequences of alternative dispersal strategies in a putatively amphidromous fish. Ecology 95: 2397-408

Holitzki TM, MacKenzie RA, Wiegner TN, McDermid KJ (2013) Differences in ecological structure, function, and native species abundance between native and invaded Hawaiian streams. Ecological Applications 23:1367-83.

Holliman FM, Reynolds JB, Kwak TJ (2003) A predictive risk model for electroshock induced mortality of the endangered Cape Fear Shiner. North American Journal of Fisheries Management 23: 905–912.

Horak DL, Klein WD (1967) Influence of capture methods on fishing success, stamina, and mortality of rainbow trout (Salmo gairdneri) in Colorado. Transactions of the American Fisheries Society 96: 220-2

Kinzie III RA (1988) Habitat utilization by Hawaiian stream fishes with reference to community structure in oceanic stream islands. Environmental Biology of Fishes 22: 179–192

Kolar CS, Courtenay WR, Nico LG (2010) Managing undesired and invading fishes. In: Hubert, W. A. and Quist, M. C. (eds.). Inland fisheries management in North America, third edition, pp 213–259. American Fisheries Society, Bethesda, Maryland, USA.

Kwak TJ, Cooney P (2008) Fishery population and habitat assessment in Puerto Rico streams. Report to Puerto Rico Department of Natural and Environmental Resources.

Leung B, Lodge DM, Finnoff D, Shogren JF, Lewis MA, Lamberti G (2002) An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of London. Series B: Biological Sciences 269: 2407-13

Lindstrom DP, Blum MJ, Walter RP, Gagne RB, Gilliam JF (2012) Molecular and morphological evidence of distinct evolutionary lineages of *Awaous guamensis* in Hawaiʻi and Guam. Copeia 2012 (2): 294–301

Lintermans M (2000) Recolonization by the mountain galaxias *Galaxias olidus* of a montane stream after the eradication of rainbow trout *Oncorhynchus mykiss*. Marine and Freshwater Research 51: 799–804

Lisi PJ, Childress ES, Gagne RB, Hain EF, Lamphere BA, Walter RP, Hogan JD, Gilliam JF, Blum MJ, McIntyre PB (2018) Overcoming urban stream syndrome: Trophic flexibility confers resilience in a Hawaiian stream fish. Freshwater biology 63: 492–502.

Maciolek JA, Timbol AS (1980) Electroshocking in tropical insular streams. The Progressive Fish-Culturist 42: 57-8

McCrimmon HR, Bidgood B (1965) Abnormal vertebrae in the rainbow trout with particular reference to electrofishing. Transactions of the American Fisheries Society 94: 84-8

McDowall RM (2003) Hawaiian biogeography and the islands' freshwater fish fauna. Journal of Biogeography 30: 703–710

McMichael GA (1993) Examination of electrofishing injury and short-term mortality in hatchery rainbow trout. North American Journal of Fisheries Management 13: 229-33

Mesa MG, Schreck CB (1989) Electrofishing mark–recapture and depletion methodologies evoke behavioral and physiological changes in cutthroat trout. Transactions of the American Fisheries Society. 118: 644-58

Mitton CJ, McDonald DG (1994) Consequences of pulsed DC electrofishing and air exposure to rainbow trout (Oncorhynchus mykiss). Canadian Journal of Fisheries and Aquatic Sciences 51: 1791-8

Moody KN, Gagne RB, Heim-Ballew H, McIntyre PB, Gilliam JF, Blum MJ (2017) Invasion hotspots and ecological saturation of streams across the Hawaiian archipelago. Cybium 41:127-56

Moore SE, Kulp M, Rosenlund B, Brooks J, Propst D (2008) A field manual for the use of Antimycin A for restoration of native fish populations. National Resource Report NPS/NRPC/NRR–2008/033, U.S. National Park Service, Fort Collins, Colorado, USA. 149 p.

Murphy BR, Willis DW (1996) Fisheries Techniques, 2nd edn., American Fisheries Society, Bethesda, MD. American Fisheries Society.

Nico LG, Walsh SJ (2011) Non–indigenous freshwater fishes on tropical Pacific islands: a review of eradication efforts. In: Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). Island invasives: eradication and management, pp 97–107. IUCN, Gland, Switzerland.

Oki DS, Brasher AMD (2003) Environmental setting and implications for water quality and aquatic biota, Oʻahu, Hawaiʻi. Honolulu: US Geological Survey. Water–Resources Investigations Report 03–4156

Pham L, West D, Closs GP (2013) Reintroduction of a native galaxiid (*Galaxias fasciatus*) following piscicide treatment in two streams: response and recovery of the fish population. Ecology of Freshwater Fish. doi: 10.1111/eff.12031

Polhemus DA (1993) Damsels in distress: A review of the conservation status of Hawaiian *Megalagrion* damselflies (Odonata: Coenagrionidae). Aquatic Conservation: Marine, Freshwater Ecosystems 3: 343–349

Polhemus DA, Asquith A (1996) Hawaiian damselflies: a field identification guide. Bishop Museum Press. 122 pp.

Rowe DK (2007) Exotic fish introductions and the decline of water clarity in small North Island, New Zealand lakes: a multi–species problem. Hydrobiologia 583: 345−358

Sax DF, Gaines SD (2008) Species invasions and extinction: The future of native biodiversity on islands. Proceedings of the National Academy of Sciences, U.S.A. 105: 11490–11497

Schofield PJ, Nico LG (2007) Toxicity of 5% rotenone to non–indigenous Asian swamp eels. North American Journal of Fisheries Management 27: 453–459.

Schreck CB, Whaley RA, Bass ML, Maughan OE, Solazzi M (1976) Physiological responses of rainbow trout (Salmo gairdneri) to electroshock. Journal of the Fisheries Board of Canada 33: 76-84

Sharber NG, Carothers SW (1988) Influence of electrofishing pulse shape on spinal injuries in adult rainbow trout. North American Journal of Fisheries Management 8: 117-22

Sharber NG, Carothers SW, Sharber JP, de Vos Jr JC, House DA (1994) Reducing electrofishing-induced injury of rainbow trout. North American Journal of Fisheries Management 14: 340-6

Snyder DE (2003) Electrofishing and its harmful effects on fish, Information and Technology Report USGS/BRD/ITR—2003–0002: U.S. Government Printing Office.149 pp.

Vanni MJ, Flecker AS, Hood JM, Headworth JL (2002) Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. Ecology Letters 5: 285−293

Whaley RA, Maughan OE, Wiley PH (1978) Lethality of electroshock to two freshwater fishes. The Progressive Fish-Culturist 40: 161-3

Yamamoto MN, Tagawa AW (2000) Hawai‘i’s native and exotic freshwater animals. Mutual Publishing. 200 pp.

Tables

Mean Discharge						
Metric	**Taxa**	***n***	***df***		***F***	***p***
			Numerator	**Denominator**		
Counts	All AIS	13	2	10	1.96	0.19
	Loricariids	13	2	10	5.92	0.02
	Poeciliids	13	1	11	0.12	0.73
Δdensity	All AIS	13	1	11	1.89	0.20
	Loricariids	9	1	7	0.86	0.38
	Poeciliids	9	1	7	0.89	0.38
Discharge variability						
Metric	**Taxa**	***n***	***df***		***F***	***p***
			Numerator	**Denominator**		
Counts	All AIS	13	1	11	0.20	0.67
	Loricariids	13	2	10	1.29	0.32
	Poeciliids	13	1	11	0.14	0.72
Δdensity	All AIS	13	1	11	7.85	0.02
	Loricariids	9	1	7	0.43	0.53
	Poeciliids	9	1	7	4.75	0.07

Table 1 Results of one-way fixed ANOVAs for effects of stream discharge mean and variability on counts of AIS removed and change in AIS density for all AIS species, loricariids, and poeciliids.

Pre-removal AIS density						
Metric	**Taxa**	***n***	***df***		***F***	***p***
			Numerator	**Denominator**		
Δdensity	All AIS	13	1	11	0.01	0.94
	Loricariids	9	1	7	0.03	0.88
	Poeciliids	9	1	7	4.03	0.08
AIS biomass removed						
Metric	**Taxa**	***n***	***df***		***F***	***p***
			Numerator	**Denominator**		
Δdensity	All AIS	13	1	11	0.01	0.94
	Loricariids	9	1	7	0.53	0.49
	Poeciliids	9	1	7	3.43	0.11

Table 2 Results of one-way fixed ANOVAs for effects of pre-removal density and biomass removed of all AIS species on change in AIS density for all AIS species, loricariids, and poeciliids.

Figures

Figure 1: Stepwise approach to AIS removal to safeguard native species and access efficacy of AIS removal via electrofishing in Hawaiian streams: (1) collection of water samples to assess pre-removal stream chemistry and TSS, (2) snorkel surveys to assess pre-removal stream communities, (3) collection and sequestration of native species by hand-fishing, (4) measure standard length and weight of native species, (5) tagging of *A. stamineus* for mark and recapture to measure post-removal organismal and demographic responses, (6) fin clipping of *A. stamineus* for genetic analysis, sacrifice of limited numbers of *A. stamineus* for otolith analysis of species life history variation and isotopic analyses of trophic position, (7) electrofishing to remove AIS, (8) return of native species to their place of capture, (9) additional collection of water samples for chemistry and TSS analysis, and (10) post-removal snorkel surveys to assess changes in AIS and native species communities.

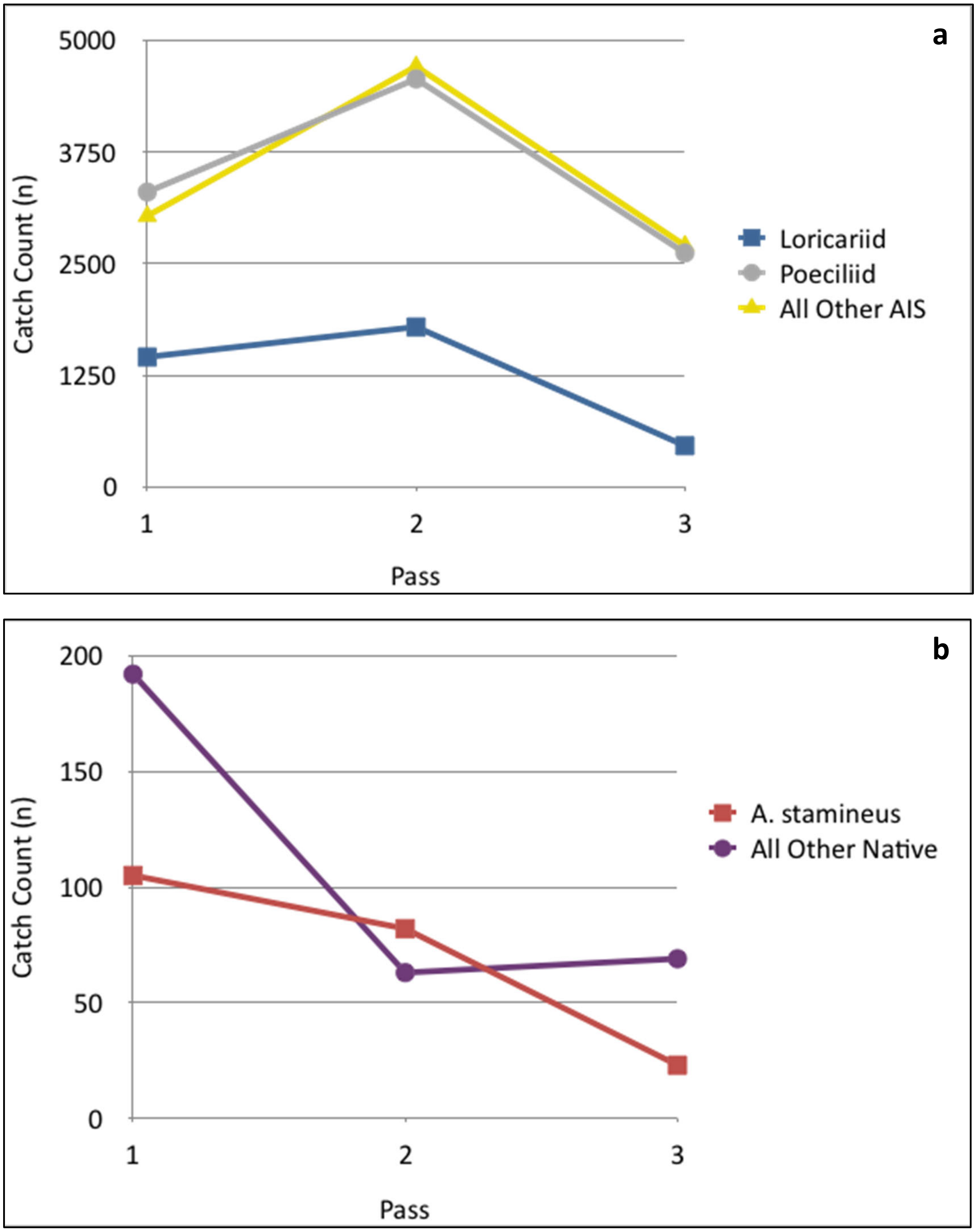


Figure 2: Number of (A) AIS and (B) native organisms caught during electrofishing by pass number.

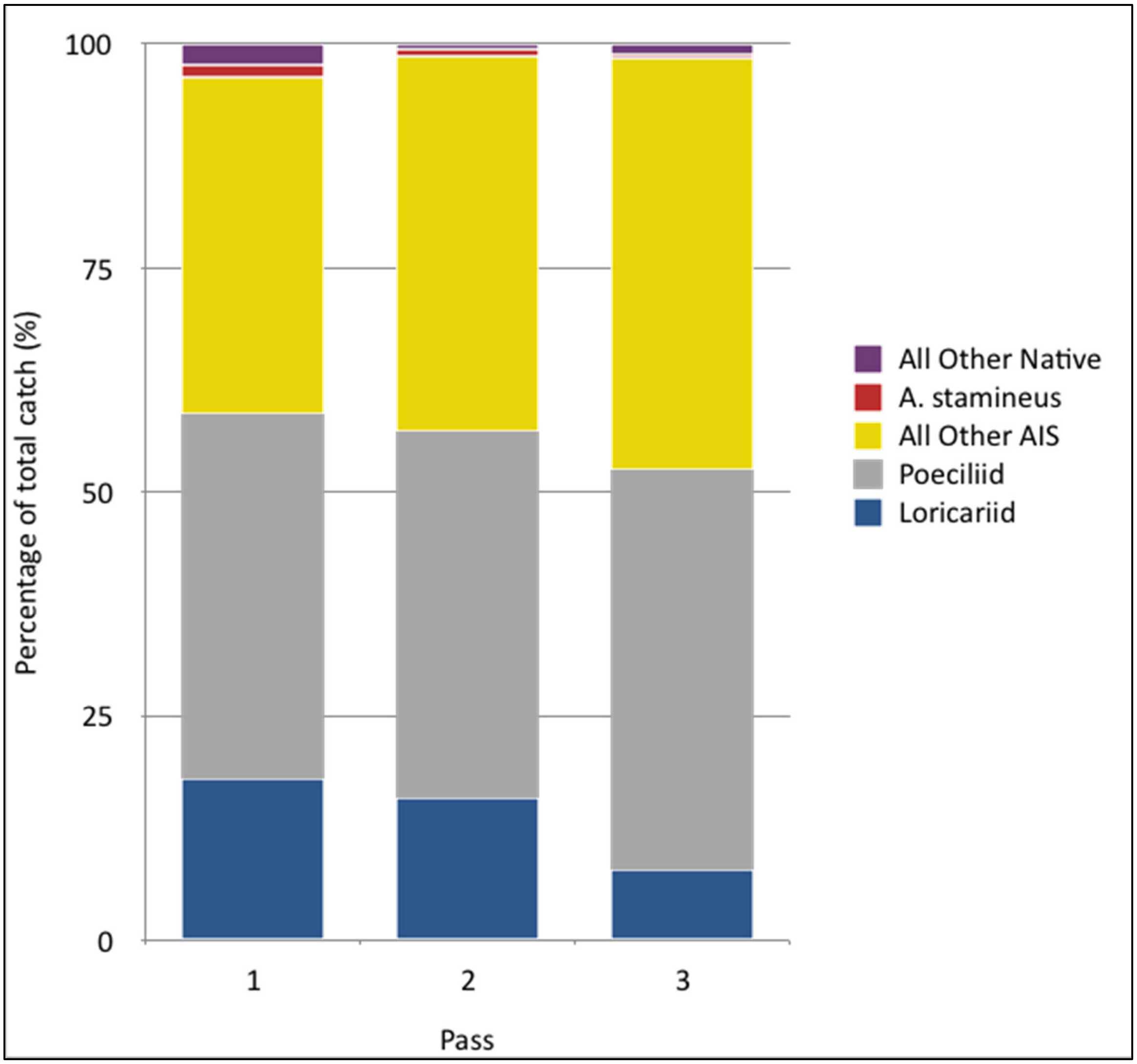


Figure 3: Percentage of catch comprised of AIS and native species per pass number.

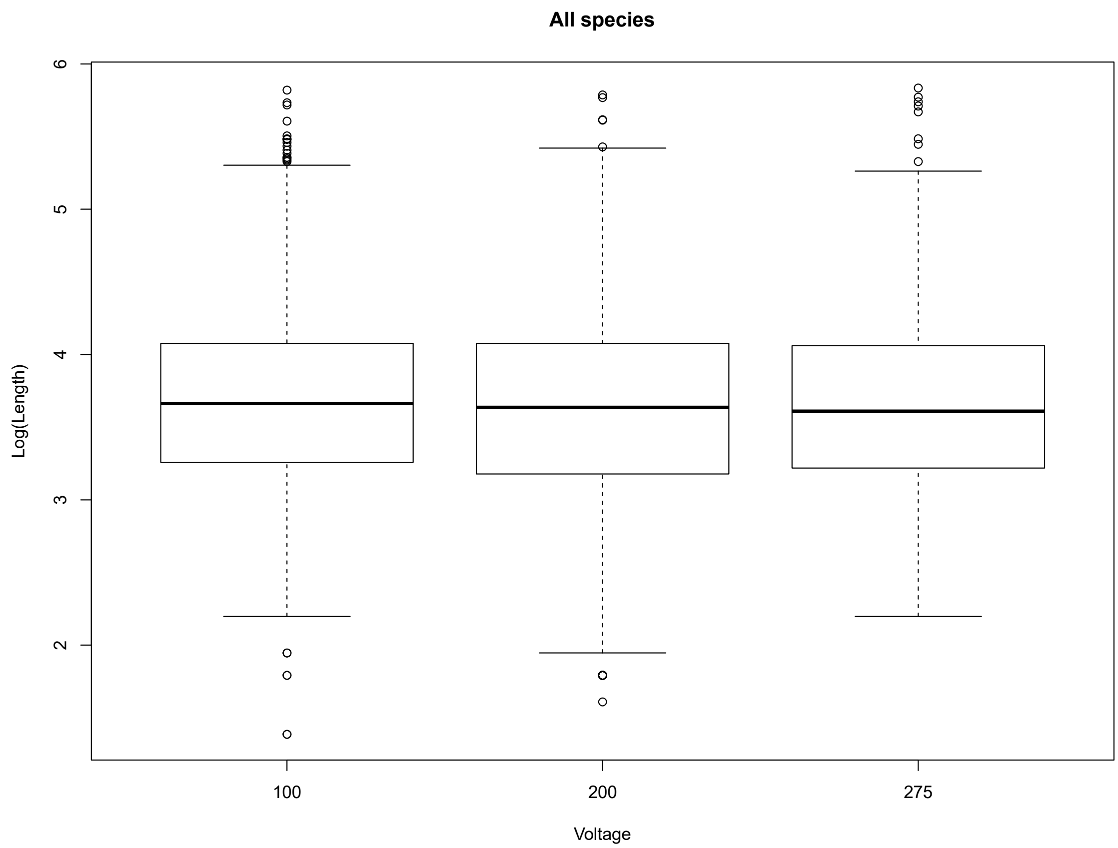


Figure 4: Length of all organisms caught by voltage setting.

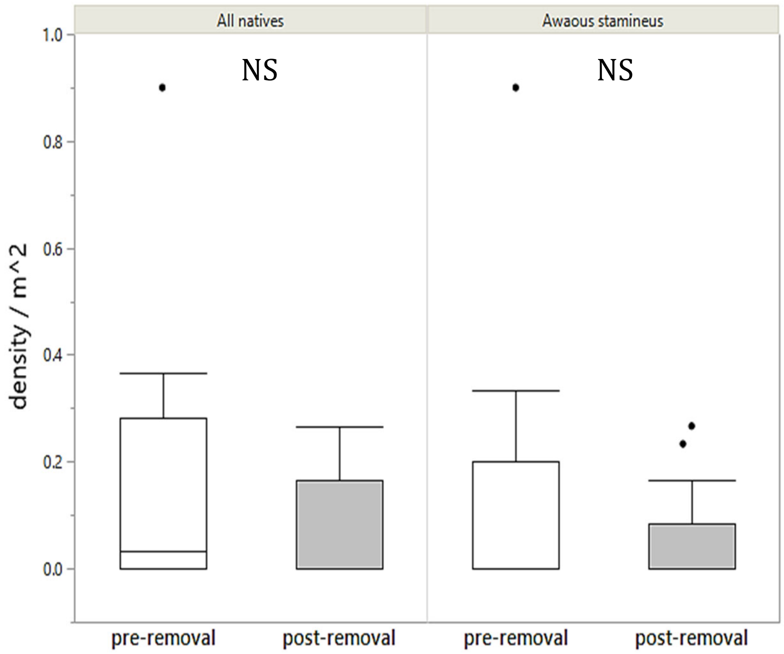


Figure 5 : Box plots of change in density from before and after AIS removal events for all native species and for *A. stamineus*. Results are of one-way fixed ANOVAs blocked by stream. NS indicates statistical non-significance.

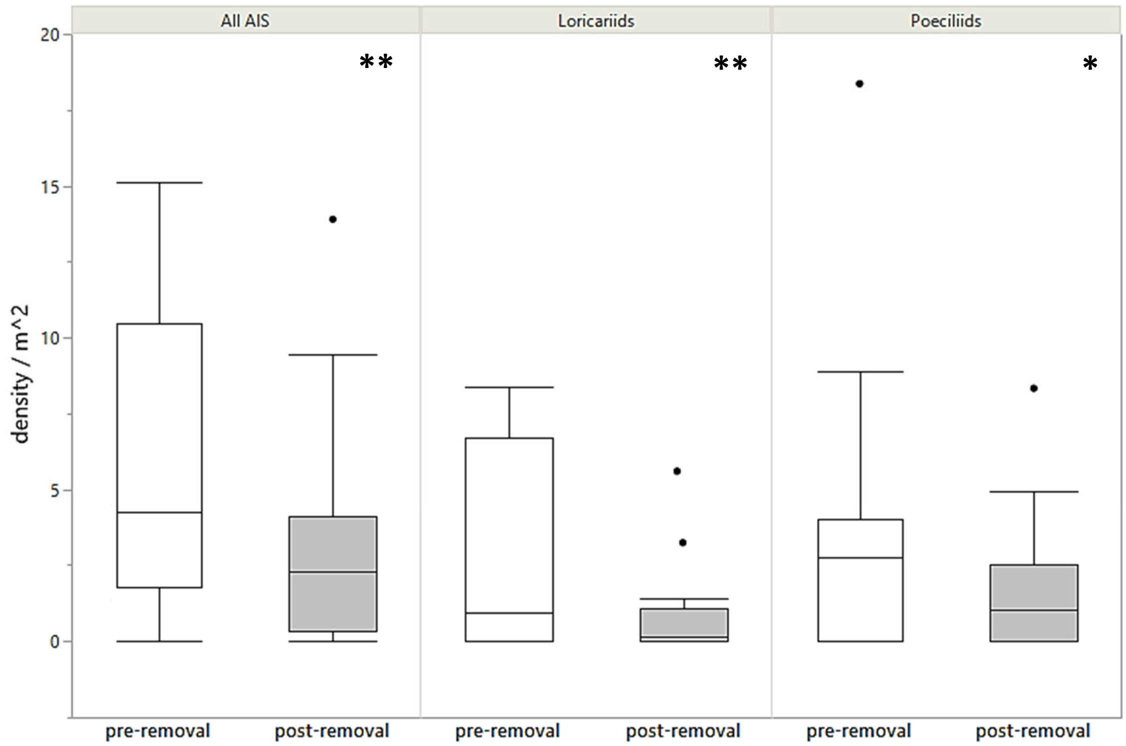


Figure 6 : Box plots of change in density pre- and post-AIS removal events for all AIS species, loricariid species), and poeciliid species. Results are of one-way fixed ANOVAs blocked by stream; * 0.05 ≤ α ≤ 0.1; ** α ≤ 0.05.

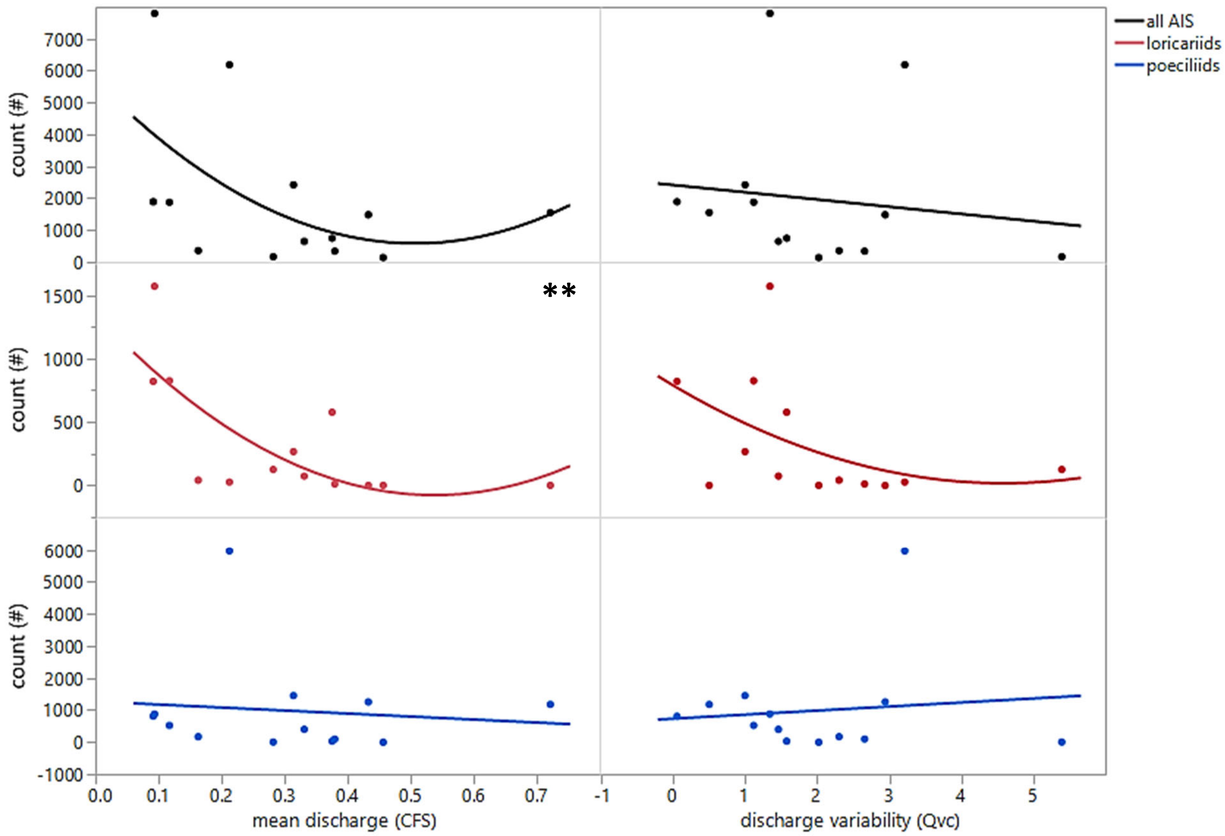


Figure 7 : Relationships between the number of individuals removed versus mean stream discharge and discharge variability for all AIS species, loricariid species, and poeciliid species. The fitted lines illustrate the heuristic nature of the depicted relationships. Statistical significance is reported according to the results of one-way fixed ANOVAs provided in Table 1. ** $\alpha \le 0.05$.

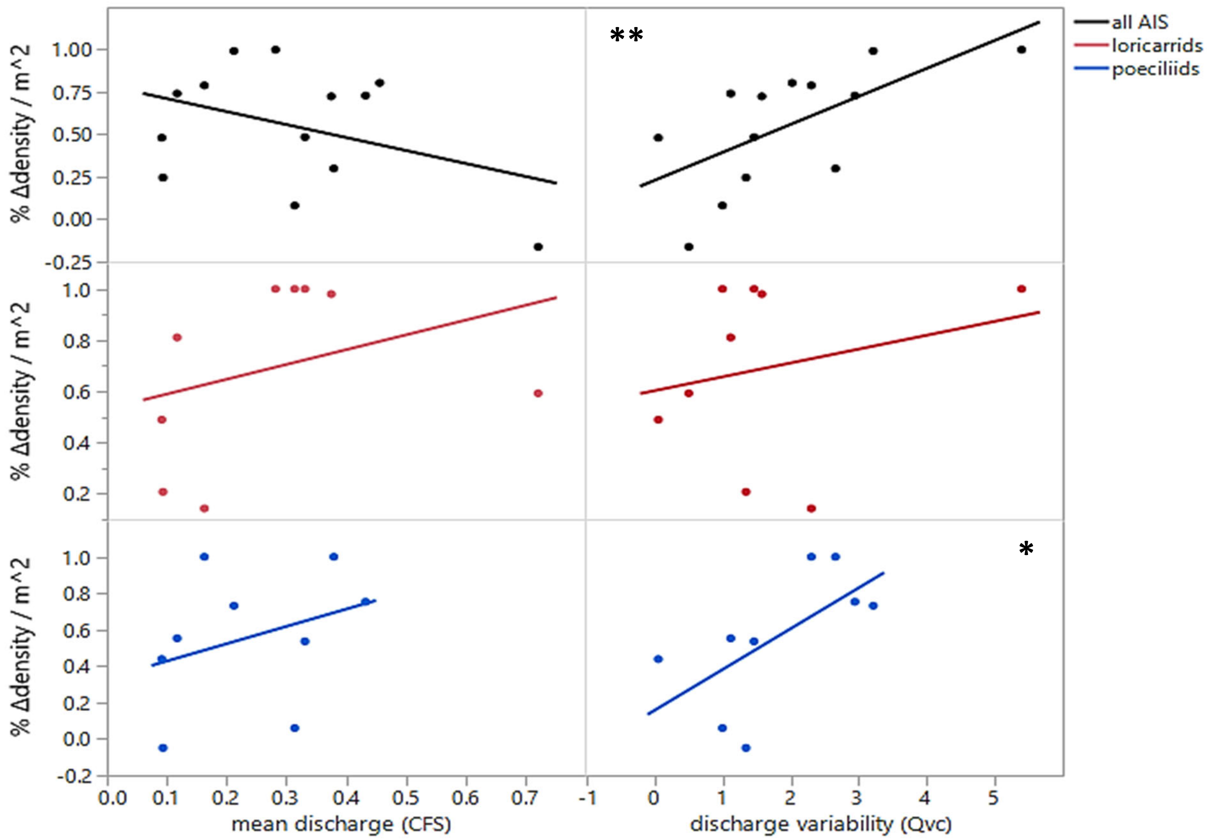


Figure 8 : Relationships between the percent change in density before and following removal events versus mean stream discharge and discharge variability for all AIS species, loricariid species, and poeciliid species. The fitted lines illustrate the heuristic nature of the depicted relationships. Statistical significance is reported according to the results of one-way fixed ANOVAs provided in Table 1. * 0.05 ≤ α ≤ 0.1; ** α ≤ 0.05.

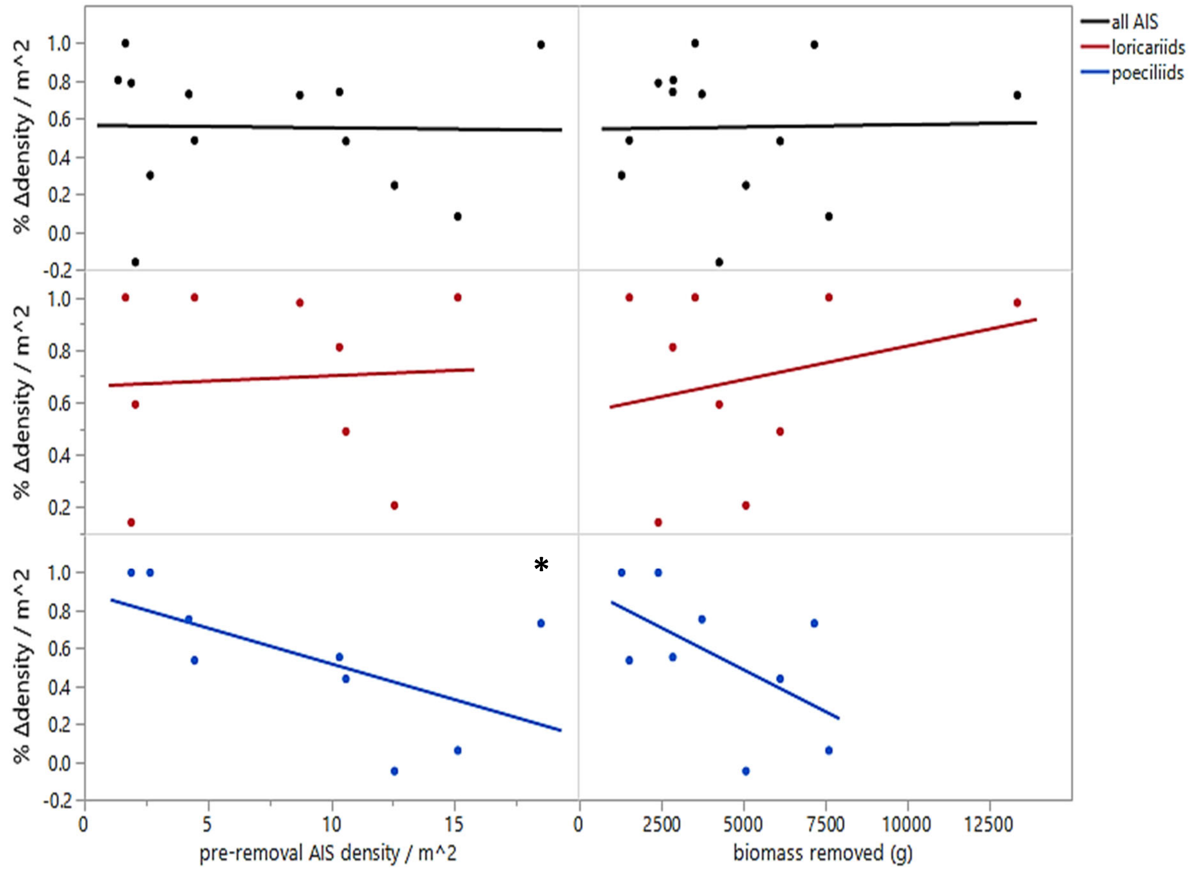


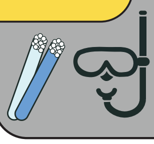
Figure 9 : Relationships between the percent change in density before and following removal events versus pre-removal AIS density and AIS biomass removed for all AIS species, loricariid species, and poeciliid species. The fitted lines illustrate the heuristic nature of the depicted relationships. Statistical significance is reported according to the results of one-way fixed ANOVAs provided in Table 2. * $0.05 \le \alpha \le 0.1$.

Appendices

Stop! Under the Hawaiʻi Revised Statutes Section 118-23 and Hawaiʻi Administrative Rules Sections 13-75-6 and 187A-6, it is illegal to conducting electrofishing in the State of Hawaiʻi. Protocol approval and permitting from the State of Hawaiʻi Division of Aquatic Resources is required for electrofishing. Do not proceed unless the proper permits have been aquired.

Aquatic Invasive Species Removal via Electrofishing in Hawaiian Streams

Day 1



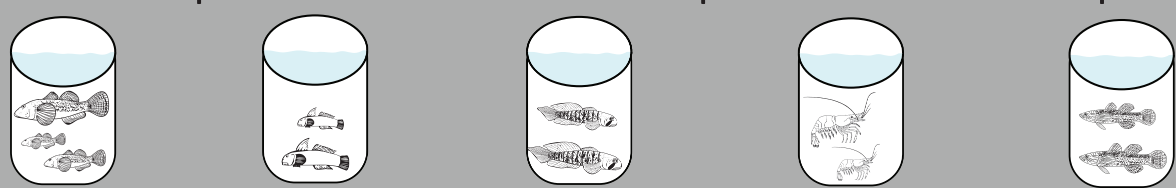
- Collect TSS and water chemistry data to access pre-removal stream conditions.
- Conduct point-quadrat snorkel surveys to quantify pre-revomal biotic composition.

Day 2

Pre-electrofishing:

- Collect native species via hand-netting and place in stream side sequestration tanks labelled with collection location.
  - Place weighted, labelled flags at collection location.

*Remember to place *Eleotris sandwicensis* in tanks separate from the other native species.



Electrofishing:

- Measure water temperature and conductivity of stream.
- Program electrofishing unit to the appropriate conditions following the chart below.
- Place AIS in stream-side collection buckets (separate from the native species).
- Place any native species captured during electrofishing in individual holding tanks, photo document injury (if occurred), and monitor for recovery.

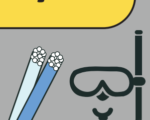
	Pass 1	Pass 2[a]	Pass 3
Waveform	Direct current	Standard pulse	Standard pulse
Voltage (V)	100	175-200 (decrease with higher conductivity)	175-200 (decrease with higher conductivity)
Frequency (Hz)	N/A	60	60
Duty Cycle	N/A	12%	12%

[a]If a high number of native species are still present in the removal area, repeated pass 1 conditions.
*If native faunal injures occurs, reduce voltage immediately and change to direct current.
**If native faunal mortality occurs, discontinue electrofishing and contact DAR immediately.

Post-electrofishing:

- Return native species back to collection locations.
- Euthanize AIS with MS-222, store and freeze for further analysis.

Day 3



- Collect TSS and water chemistry data to access post-removal stream conditions.
- Conduct point-quadrat snorkel surveys to quantify post-revomal biotic composition.

Date /time	Stream	Reach	Nutr. Rep.	air (°C)	water (°C)	DO mg/L	DO %Sat	Cond. µs/cm	TSS mL	TSS filter numbr	TSS filter mass	dry mass (mg)	ashed mass (mg)

Stream Code: ___________________________ **Reach:** ________________ **Visit #:** ______

Observer: _____________________ **Date:** ___________________ **Time:** ______________

Weather: ________________________ **Reach-wide notes:** _____________________________

Nat Fish: Ag, Es, Lc, Ss, Sh, Ks, Mc **Nat Inverts** Ab, Mg, Ng, Nv	**NN Fish:** An, At, Ca, Cf, Ga, Hc, He, Koi, Lm, Md, Pr, Psp, Poec, Til, Xh, **NN Inverts:** Cor, Ml, Mt, Nds, Pc
Sub: sed, sand, grav, cob, rub, bould, bed, conc, veg **Vis:** clear, turbid, muddy, stained, (<0.1, <0.5, <1, >1m), veg **Flow:** slow, med, fast **Type:** run, riff, casc, chute, eddy, pool, spool, ppool	

X/Y Dist		Plot#	Obs.	Species and Size (mm)		Sub	Vis	Flow	Type	Dep (m)
1	5									
1	3									
1	3									
4	8									
4	3									
2	4									
3	8									
3	7									
1	8									
1	5									
1	5									
1	6									
1	1									

2	8									
2	5									
3	7									
1	2									
1	2									
1	2									
1	4									
1	2									
2	7									
2	2									
2	2									
4	1									
1	7									
4	1									
3	1									
2	1									
4	3									
1	1									
1	5									

4	4									
2	5									
3	5									
4	8									

DATASHEETS: ANESTHETIZATION AND EUTHANIZATION

Date of Use	**Stream Name**	**Date of Make** (on bottle)	**Volume Made** - Concentration: (on bottle) 20g MS2222 + 50gNaHCO3 / literH20	**Use:** Knock-out / Kill (#)	**Starting Concentration:** Stock / Stream H20	**Ending Concentration:** Stock / Stream H20	**Notes:**
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			
				( )			

DATASHEETS: MARK AND RECAPTURE, GENETIC SAMPLING

Stream: ____________________________ **Date:** ________________________ **Visit/Cycle:** ________ /________

Observers: __________________________ **Reaches Fished:** __________________________ **Time Start/End:** _________ /________

Reach	# (Taken #, Smple #)	Status (Recap?)	Size (mm)	Weight (g)	Sex (m, f, f(g), u)	Sample # (Genetics)	Tagged?	Tag ID / Color code	Photo	Recovery Notes	Additional Notes	Time In/Out	Tagger
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	
							Yes / No					___ /___	