Development and Use of Genetic Methods for Assessing Aquatic Environmental Condition and Recruitment Dynamics of Native Stream Fishes on Pacific Islands

SI-1646

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Brief To The Scientific Advisory Board

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Performers

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Population Genetics, Landscape Ecology

Dr. James Gilliam

North Carolina State University

Population Ecology, Mathematical Biology

Dr. Peter McIntyre

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Ecosystem Ecology, Conservation Biology

Problem Statement

DoD activities require good stewardship of Pacific island stream ecosystems.

Current assessment approaches may fail to detect impairment of oceanic island streams because:

- They rely on biological indices that are insensitive in low-diversity ecosystems
- They do not account for interactions with near-shore marine ecosystems

There is a need for new methods that meet these challenges.

Technical Objectives

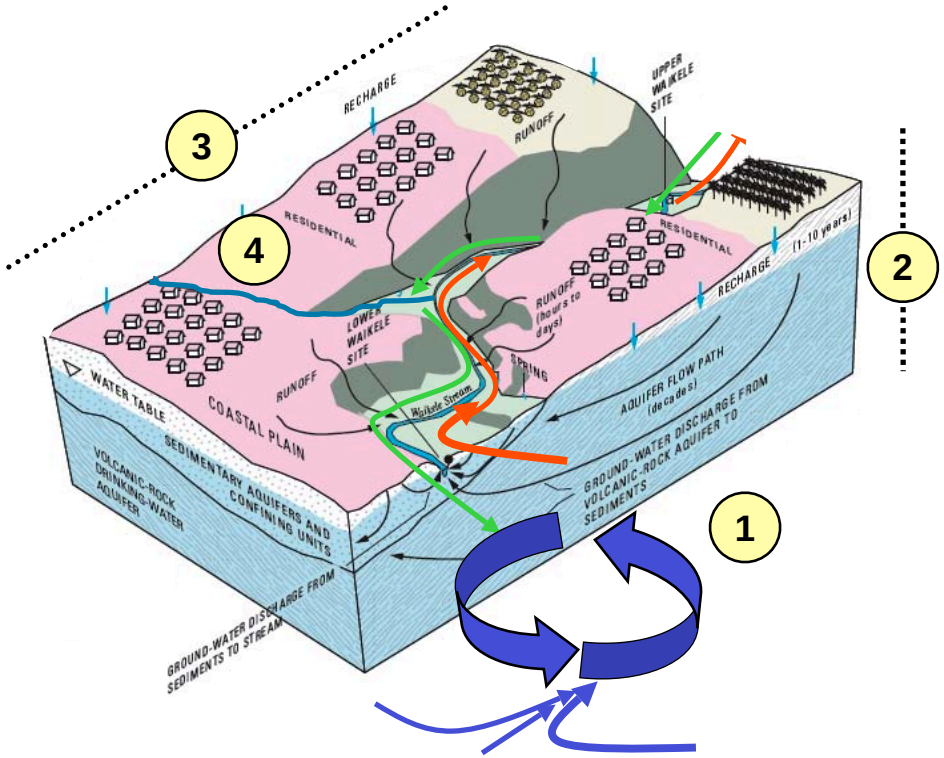
Develop genetic assays of aquatic environmental condition focusing on at-risk native fishes

- Carry out baseline studies to develop genetic assessment methods
  Genetic analysis of structure, diversity, effective population size and migration
  Demographic analysis of census population size
  Trace element analysis of migration

Use new genetic assays to assess responses of native fishes to environmental stressors and to help set management goals

- Assess effects of watershed land use and in-stream habitat on local populations

Technical Background



Model of Waikele watershed

Distinguishing aspects of oceanic island streams

Biotic:

Isolation
Low species richness
Critical connections with ocean (1)

Abiotic:

Steep elevational changes (2)
Short stream length (3)
Severe water diversions (4)
Flashy hydrology

Bioassays of stream condition must perform well in low-diversity ecosystems and account for potential marine dispersal

Technical Background

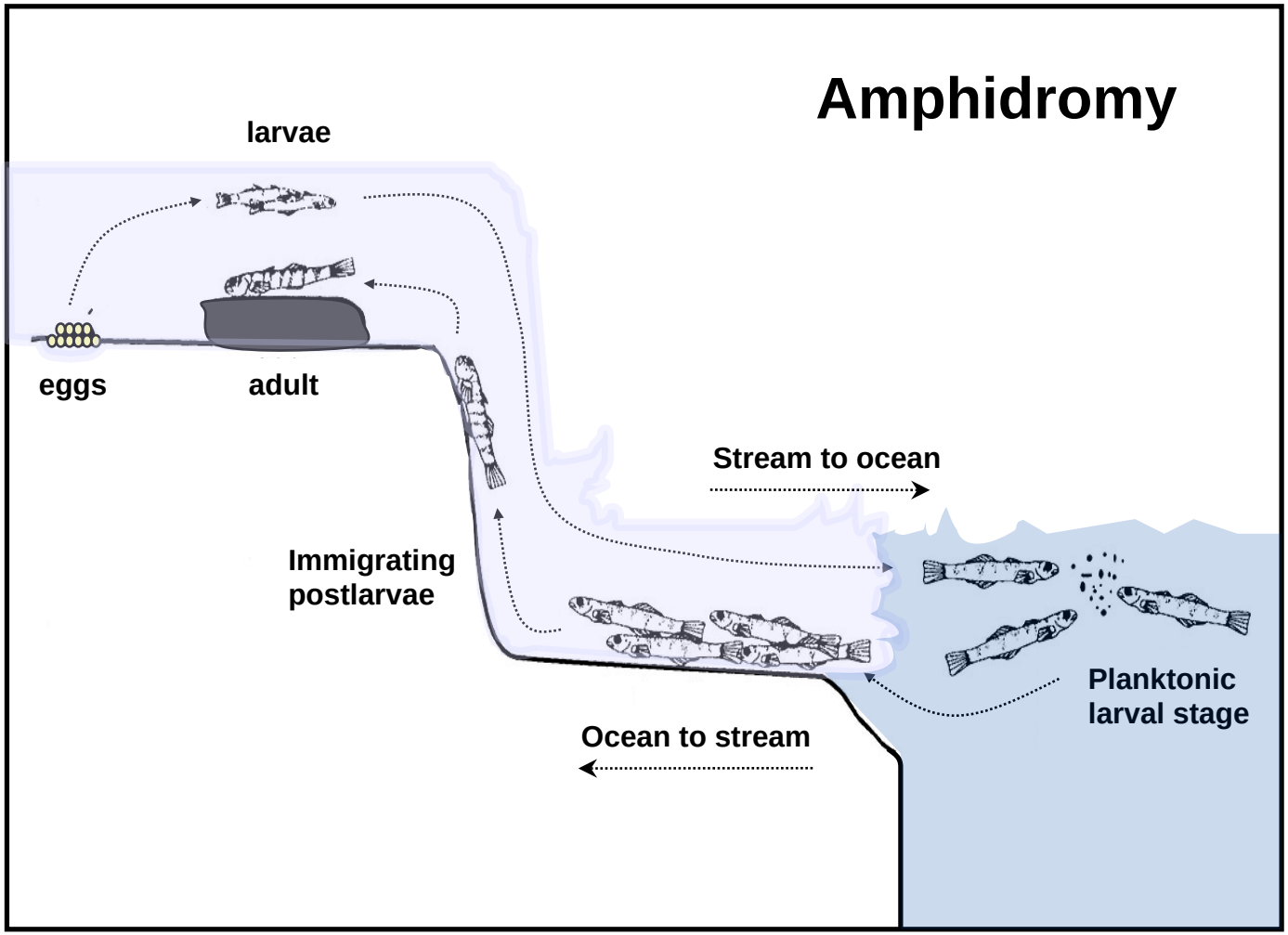
Awaous guamensis

Lentipes concolor

Eleotris sandwicensis

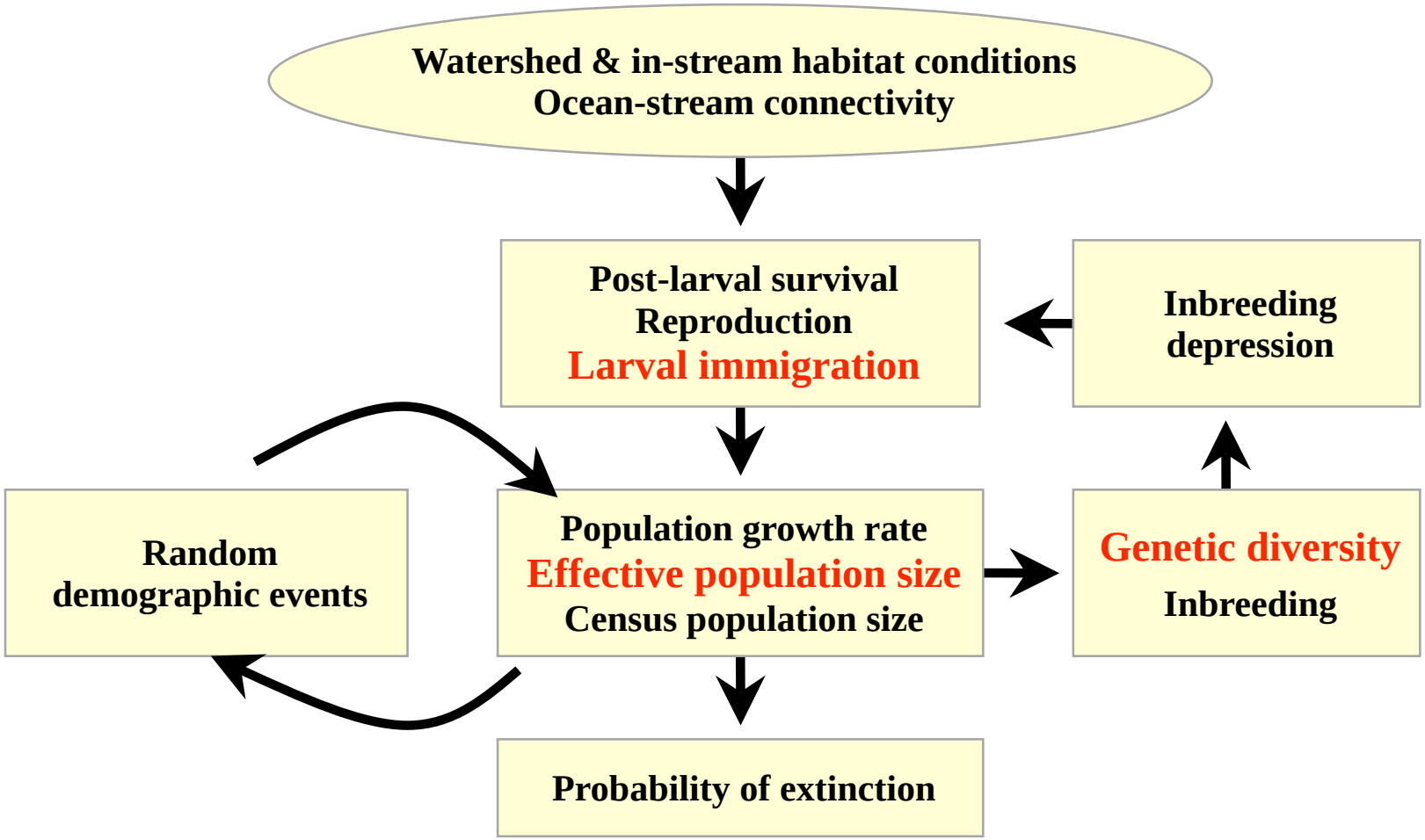
Sicyopterus stimpsoni

Stenogobius hawaiiensis



Assays of population size and immigration can characterize processes that sustain native fish assemblages

Technical Background

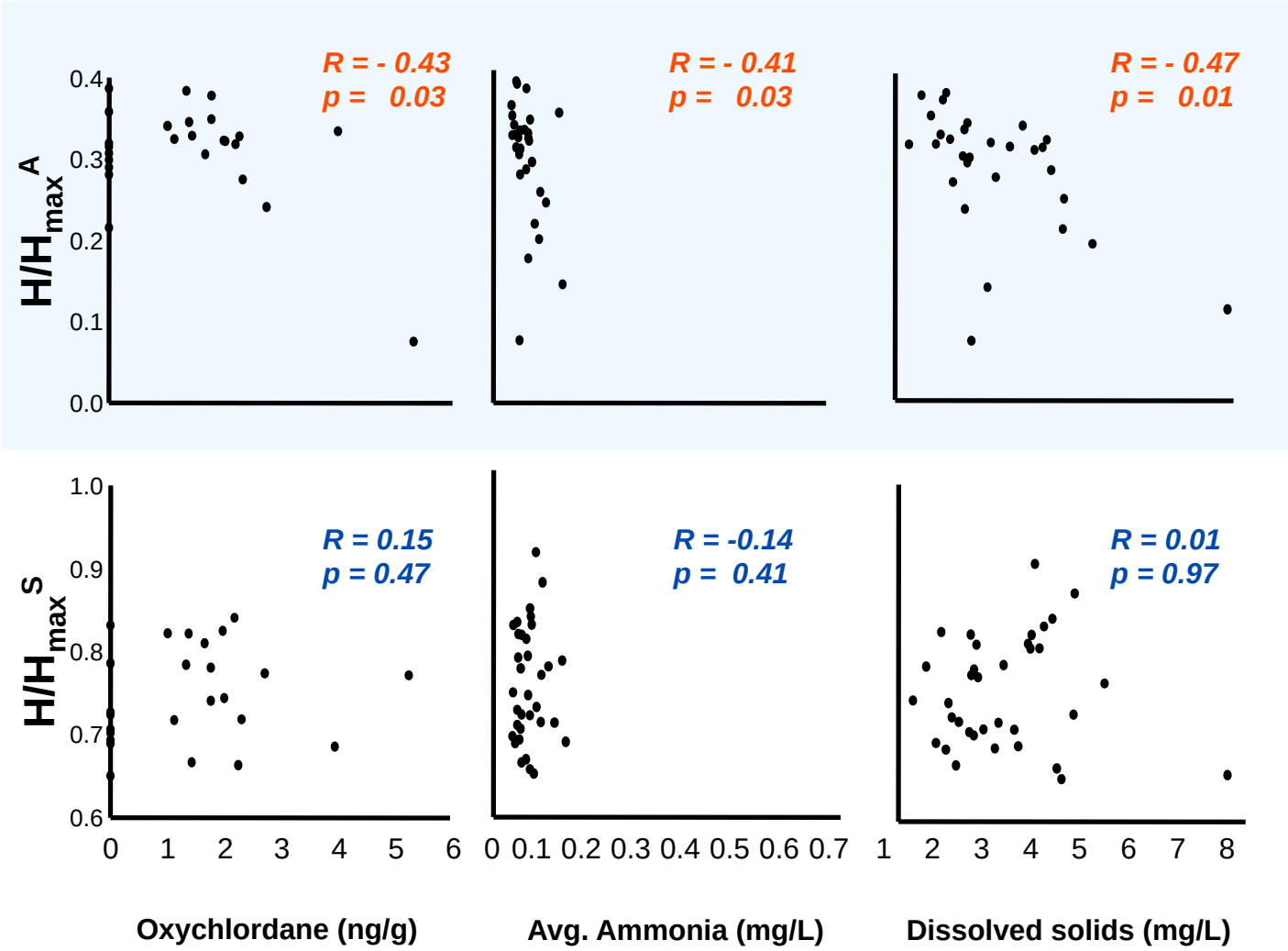


Genetic measures of diversity, population size, and immigration are promising indicators of aquatic environmental condition

Technical Background

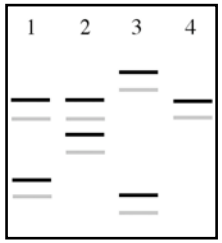
Genetic Diversity Indicator

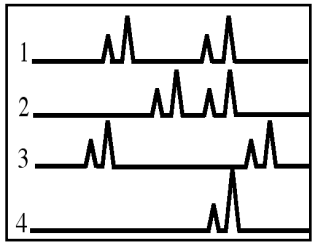
Species Diversity Indicator

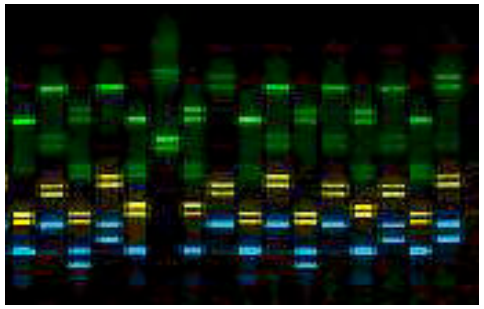


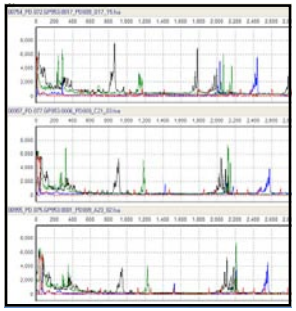
Performance of genetic indicators can match or exceed assemblage indicators

Technical Background

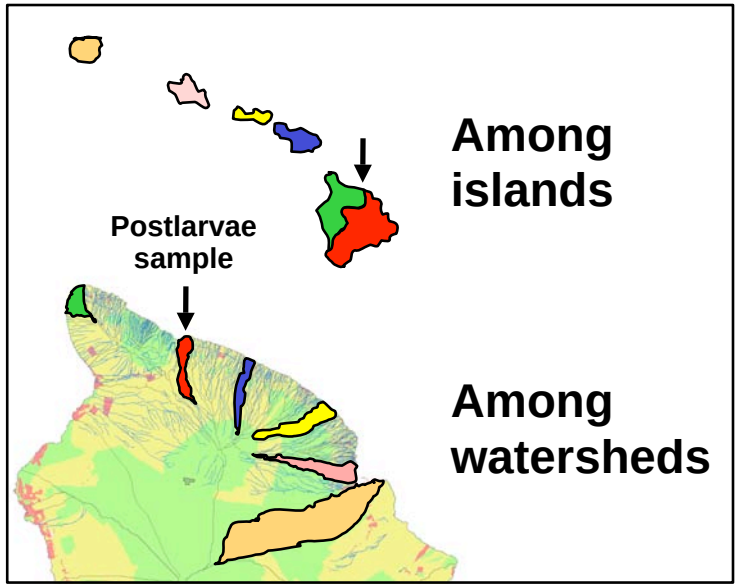


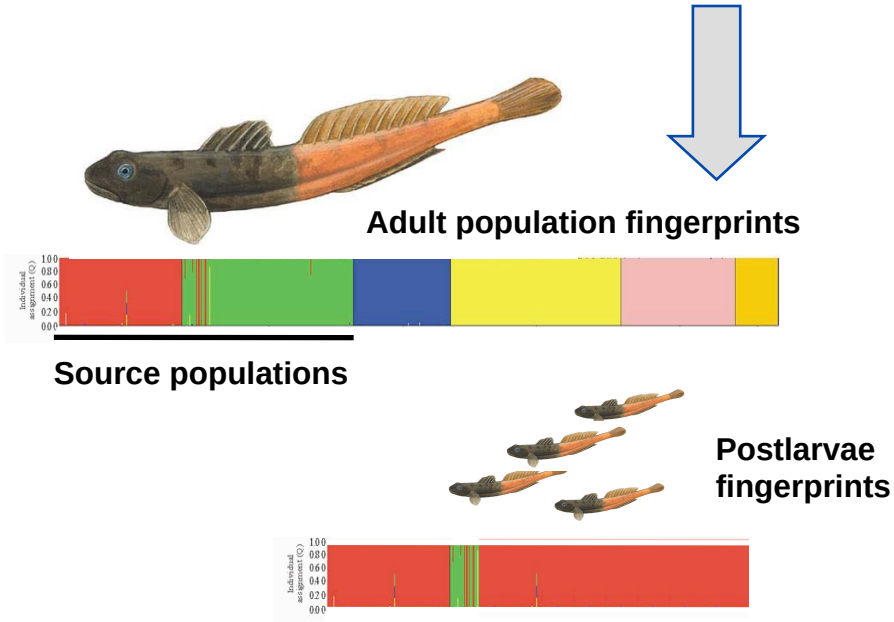






Multi-locus DNA fragment analysis and fingerprinting



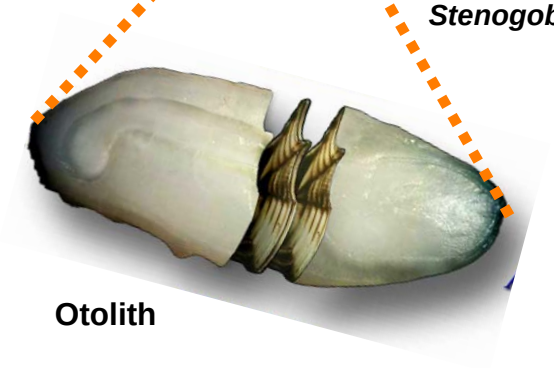


Genetic fingerprinting can be used for source tracking to determine dispersal patterns

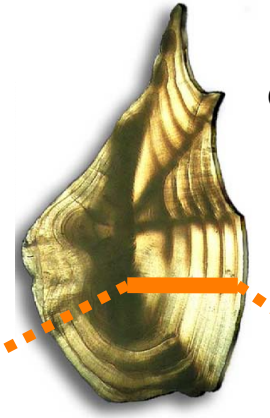
Technical Background

Otolith $CaCO_3$ incorporates Sr, Ba, Pb, Mg, & Mn from the environment

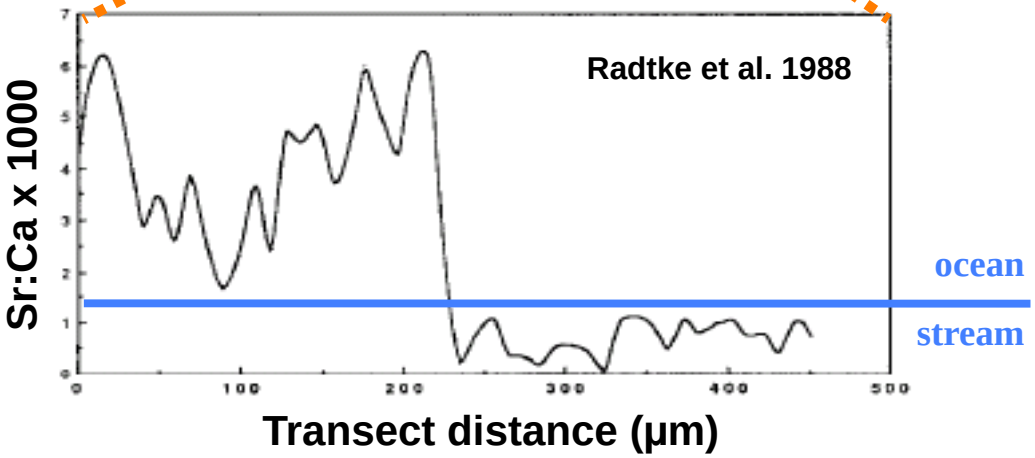
Stenogobius hawaiiensis



Otolith



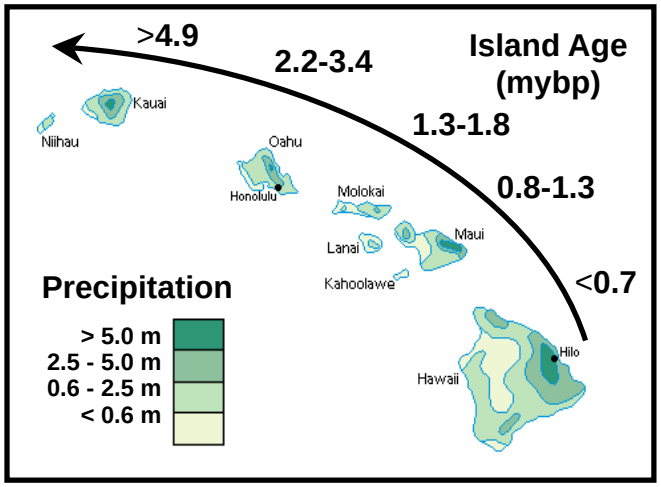
Otolith cross section



Otolith microchemistry can provide independent records of dispersal for comparison

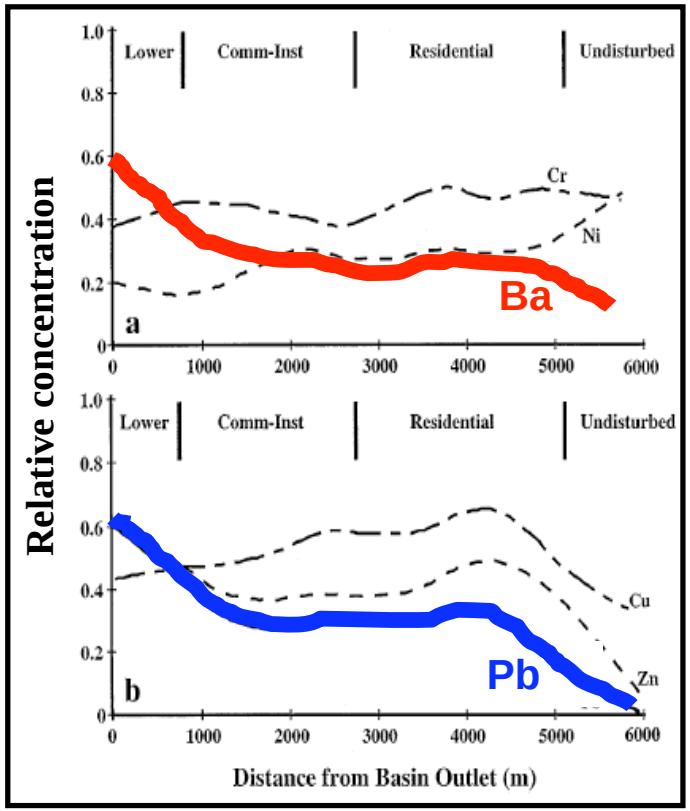
Technical Background

Rock weathering, military activity & urbanization can create distinct elemental profiles within & among islands



Sr, Ba, Pb, Mg, Mn, ^{87}Sr:^{86}Sr

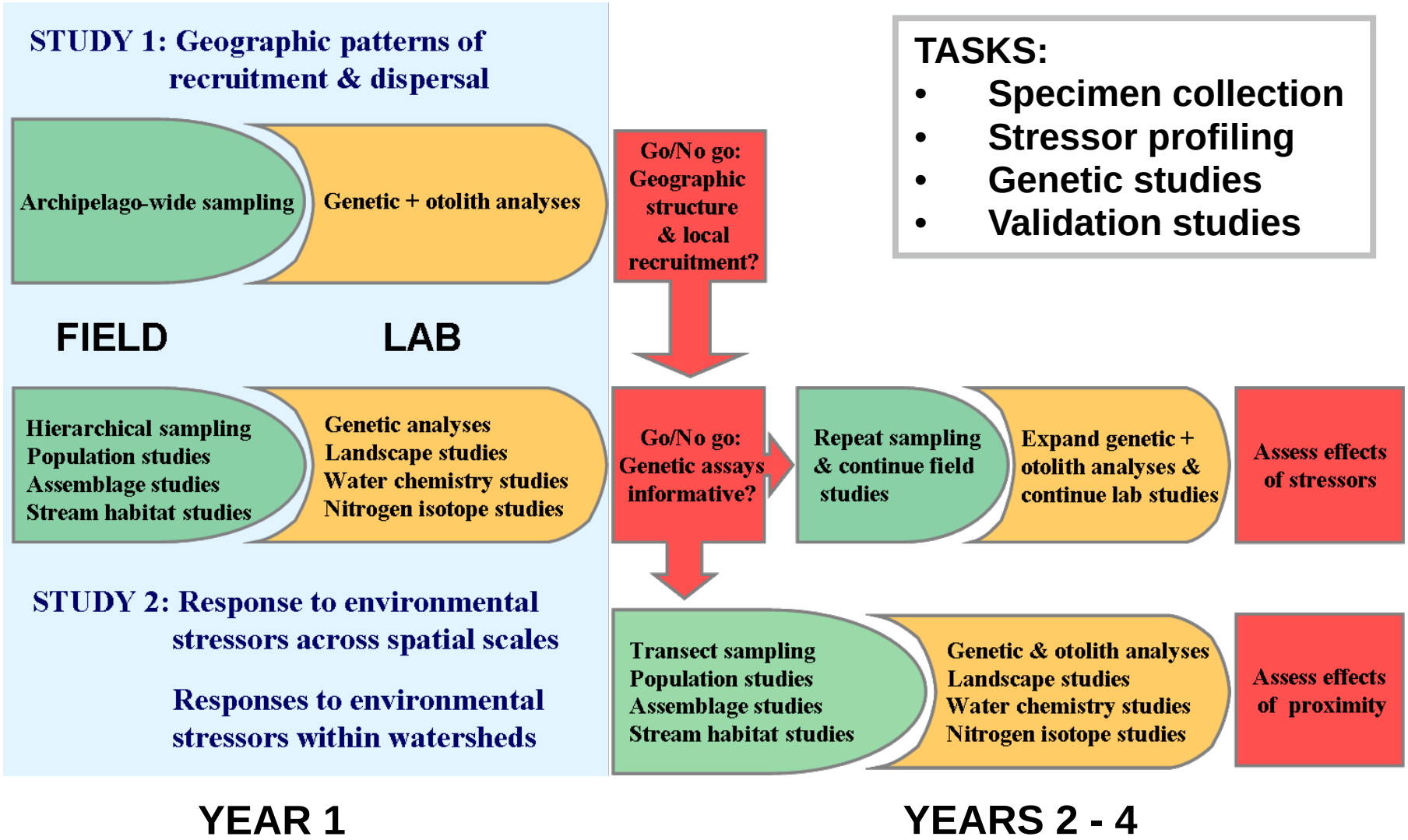
Ba, Pb



Manoa stream, Oahu (Sutherland 2000)

Otoliths record microchemical fingerprints of source, dispersal, and breeding areas that can be used to determine dispersal patterns

Technical Approach



TASKS:

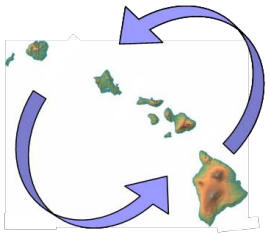
- Specimen collection
- Stressor profiling
- Genetic studies
- Validation studies

Technical Approach

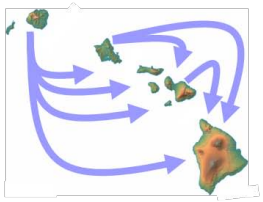
STUDY 1: Geographic patterns of recruitment and dispersal

Lentipes concolor

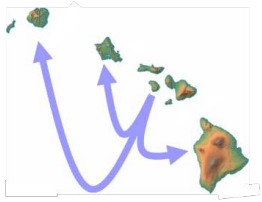
Awaous guamensis



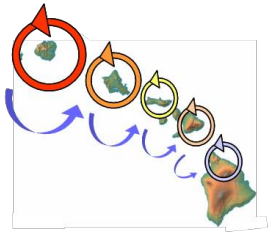
Panmixia & larval mixing



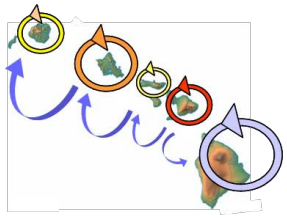
Directional IBD & larval mixing



Non-directional IBD & larval mixing



Directional vicariance & no larval mixing



Non-directional vicariance & no larval mixing

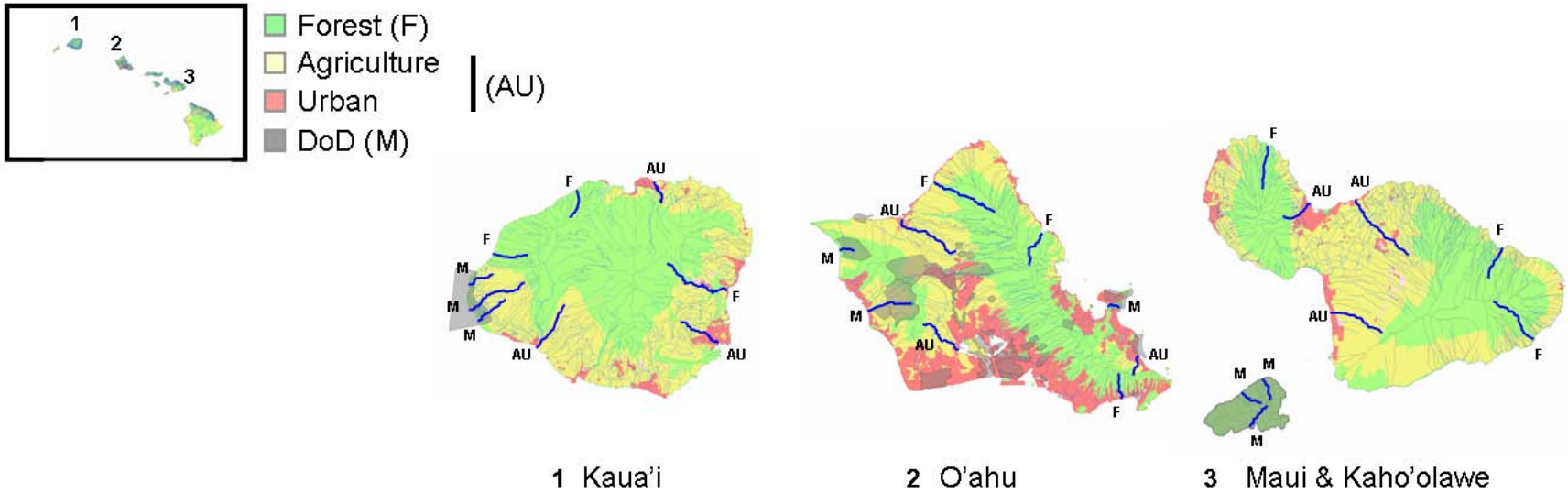
H_1: ***Local recruitment draws from mixed immigrant pools due to larval exchange***

Sample: [20 adults + 20 postlarvae] x 2 species x 5 streams x 5 islands

Goals:
- Quantify patterns of genetic variation across the archipelago
- Compare genetic profiles of postlarvae and adults
- Quantify spatial & ontogenetic patterns of otolith chemistry (*A. guamensis*)

Technical Approach

STUDY 2: Response to environmental stressors across spatial scales



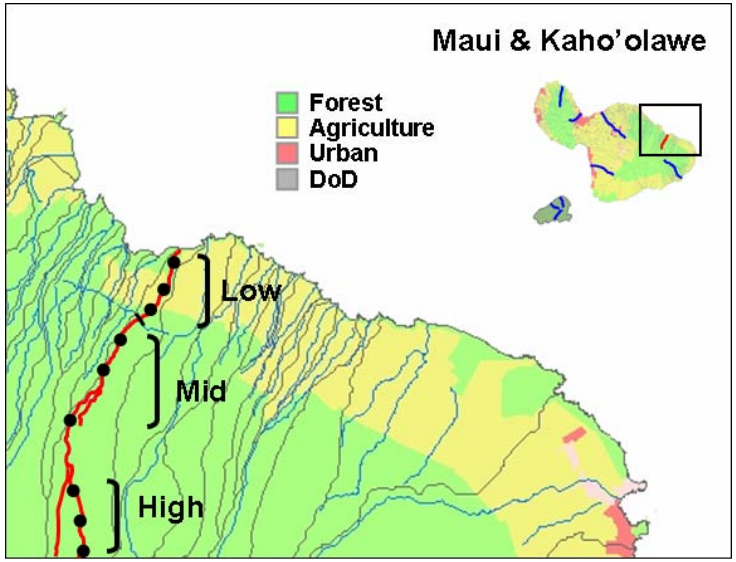
H_1: ***Population size & immigration vary according to in-stream conditions & land use***

Sample: 40 fish x 2 species x 3 islands x 3 land uses x 3 streams x 3 sites x 2 years

Goals:
- Quantify census population size and assemblage structure
- Characterize stressors (in-stream habitat, land use, nutrient loading)
- Quantify patterns of genetic variation across spatial scales
- Compare genetic profiles of postlarvae and adults
- Quantify spatial & ontogenetic patterns of otolith chemistry (*A. guamensis)*

Technical Approach

STUDY 2: Responses to environmental stressors within watersheds

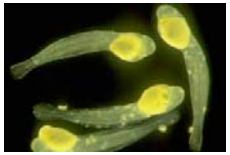


H_1: ***Population size & immigration are higher at sites closer to sources of colonization***

Sample: 40 fish x 1 stream x 3 land uses x 3 elevations x 3 sites

Goals:
- Quantify census population size and assemblage structure w/in watersheds
- Characterize stressors (in-stream habitat, land use, nutrient loading)
- Quantify patterns of genetic variation w/in watersheds
- Compare genetics of postlarvae and adults w/in watersheds
- Quantify ontogenetic patterns of otolith chemistry (*A. guamensis)* w/in watersheds

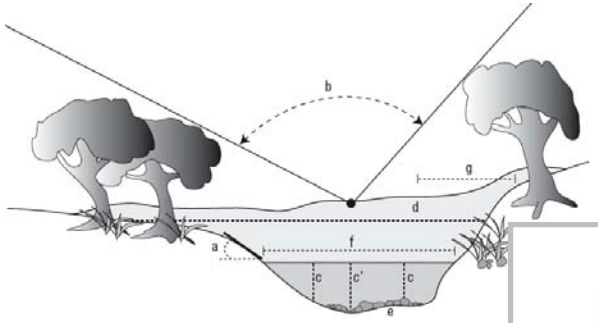
Technical Approach



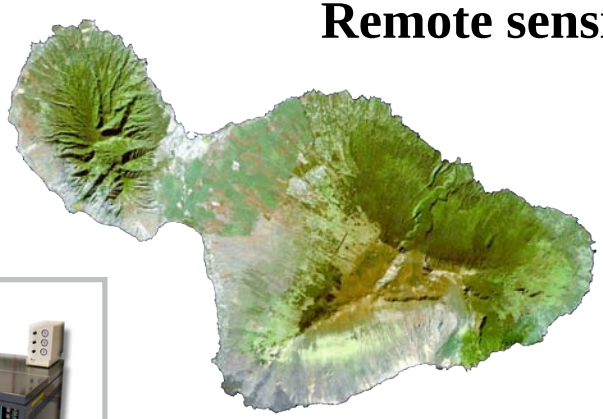
We will carry out three-pass depletion sampling to obtain specimens and to estimate census population size and assemblage structure

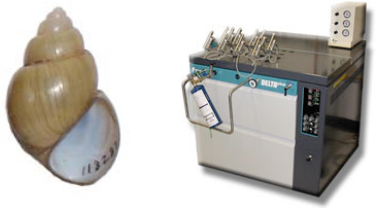
Technical Approach

Physical habitat



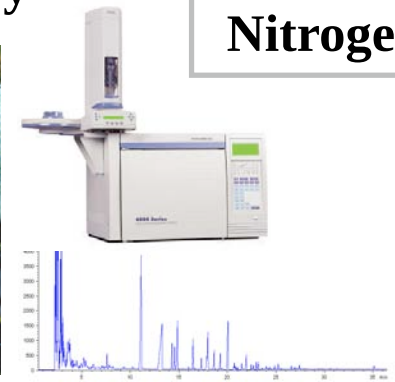
Remote sensing



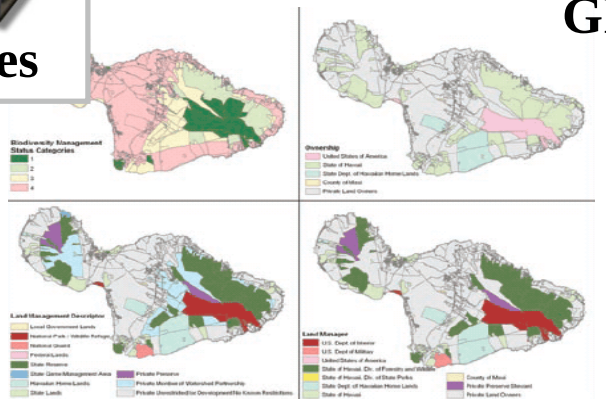


Nitrogen isotopes

Water chemistry



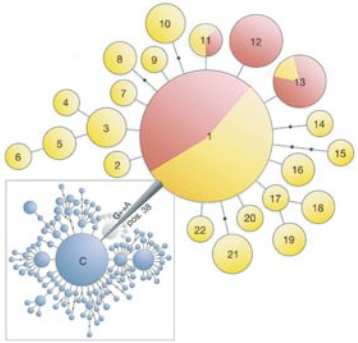
GIS

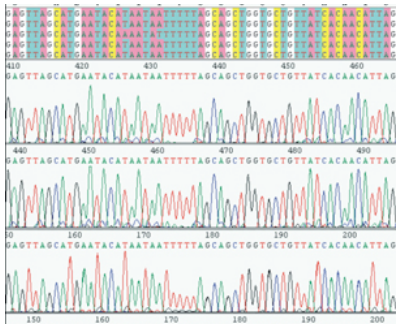


We will assess in-stream and watershed stressors via standard protocols and nutrient loading via nitrogen isotope ratios

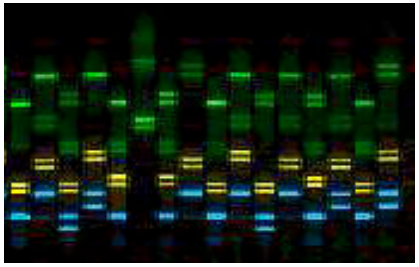
Technical Approach

Analysis of genetic diversity



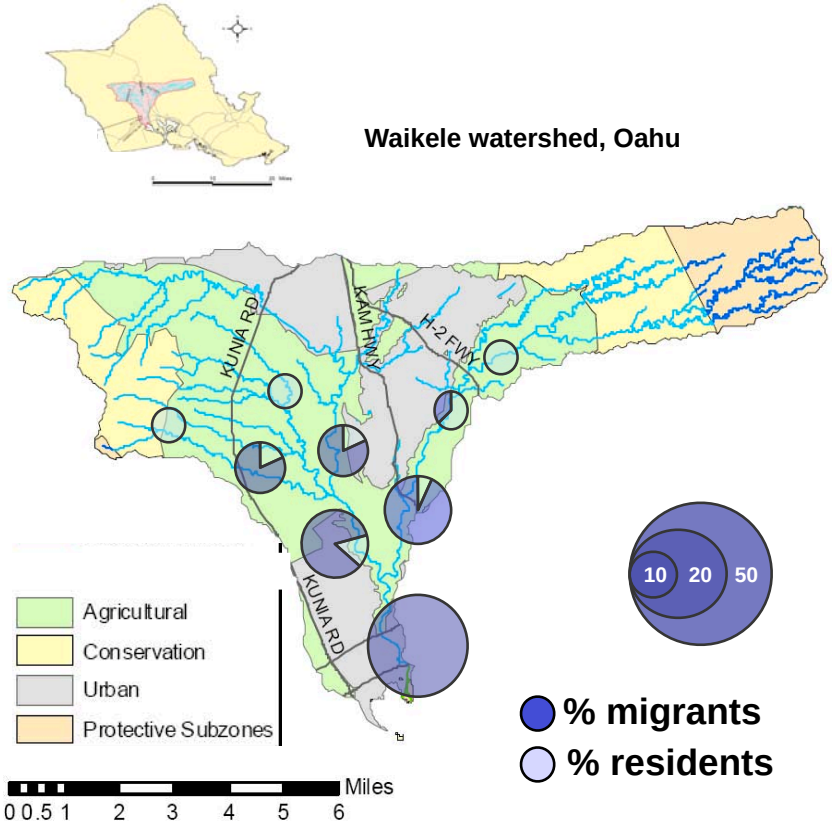


mitochondrial (mtDNA) haplotype diversity



multi-locus microsatellite genotype diversity

Analysis of effective pop. size & immigration



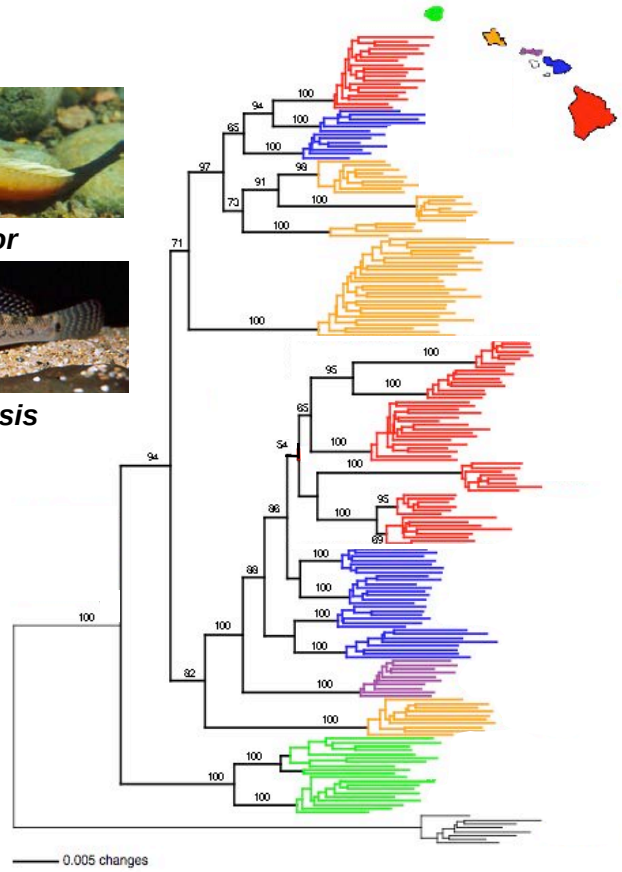
We will assess mtDNA and microsatellite variation to estimate genetic diversity, effective population size and immigration

Technical Approach

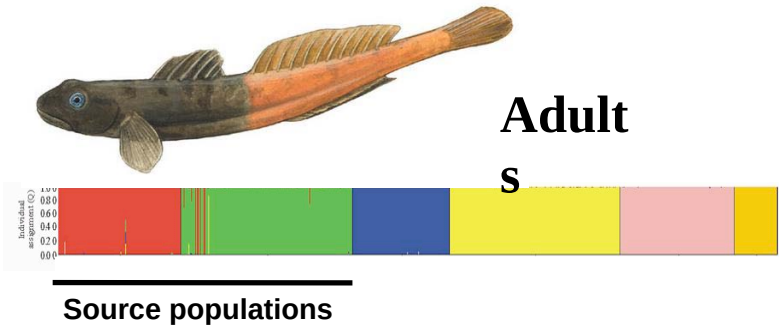
Analysis of genetic structure

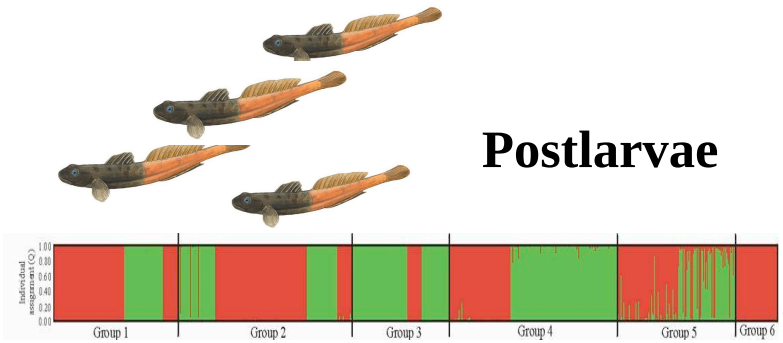
Lentipes concolor

Awaous guamensis



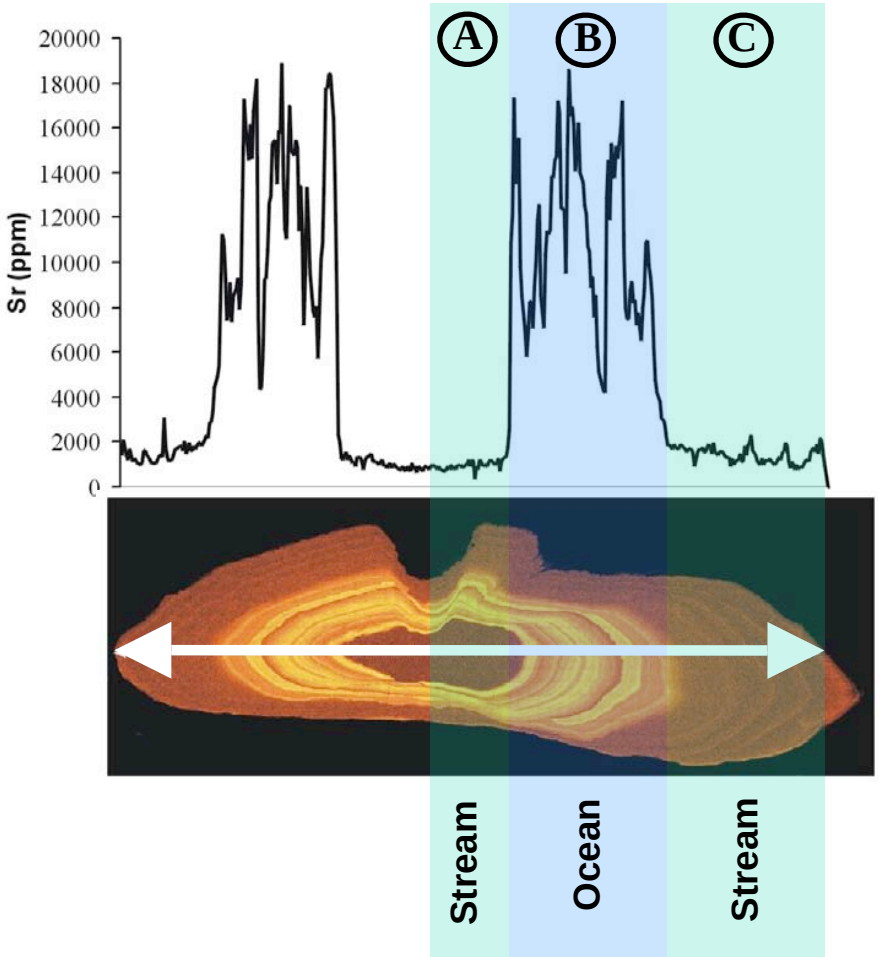
Genetic fingerprinting & source tracking





We will assess mtDNA and microsatellite variation to determine genetic structure and sources of postlarvae

Technical Approach



Analysis of:
Natal origin: (A)
Dispersal route: (B)
Source tracking: (A) vs. (C)

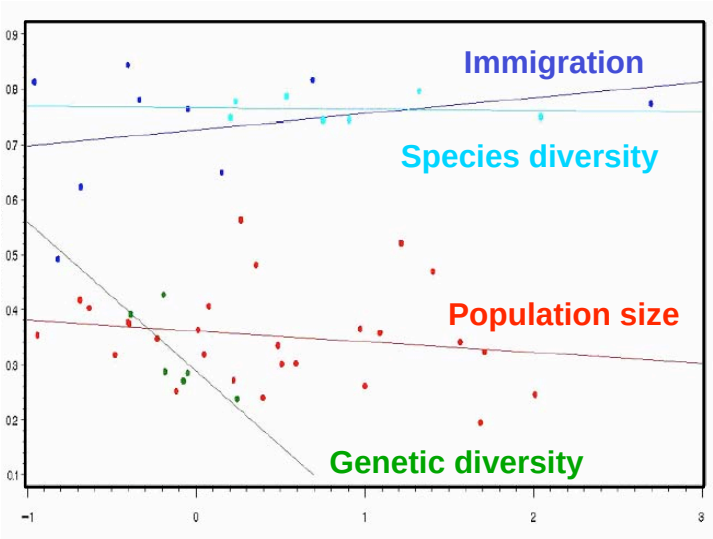
Awaous guamensis

Laser-ablation & ICP-MS across otolith for:
Ca, Sr, Ba, Pb, Mg, Mn, & ^{87}Sr:^{86}Sr

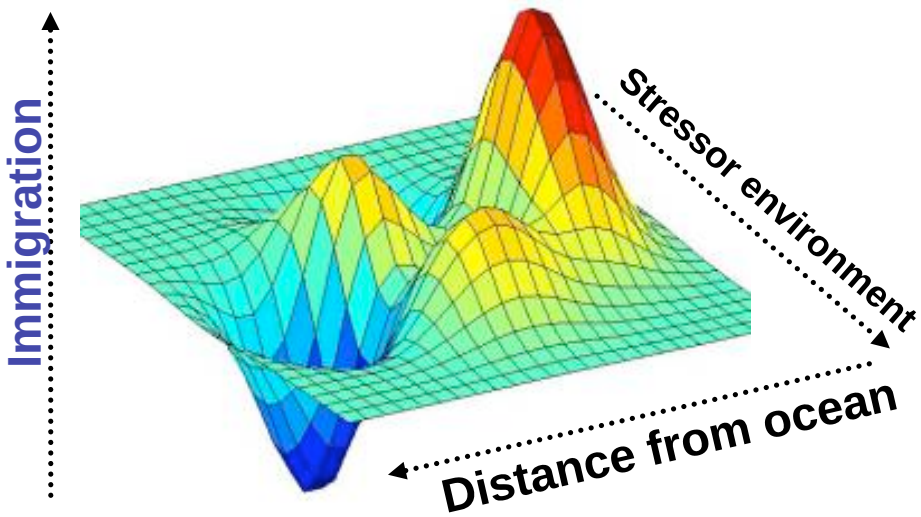
We will assess otolith microchemistry to obtain independent records of dispersal for source tracking

Technical Approach

Comparative analysis of assay sensitivity



Multivariate analysis

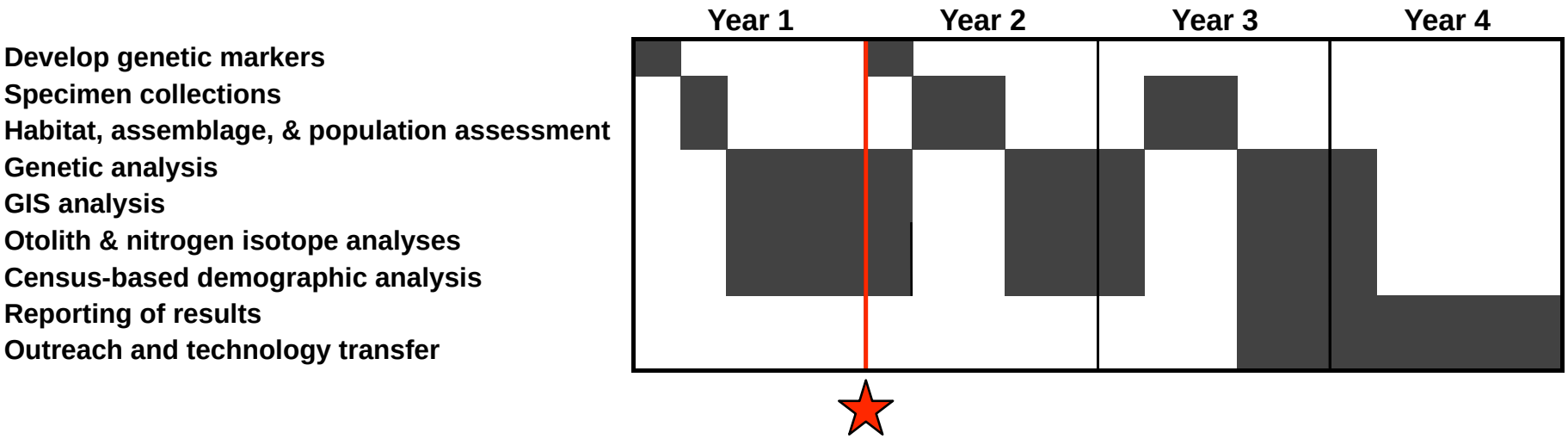


We will use multivariate statistics to compare assay sensitivity and determine the effects of stressors and proximity

Year 1 Project Plan

CATEGORY	COST ($K)
Field research	136
Genetic & GIS analyses	170
Otolith & isotopic analyses	33
Total	**339**

Overall Project Plan



GO/NO GO Decision: **Continuation of project will be contingent upon finding sufficient geographic structure and assay sensitivity to carry out assessments.**

Project Funding

	SERDP ($K)
Year 1	339
Year 2	238
Year 3	472
Year 4	42
Tota	1091

Deliverables

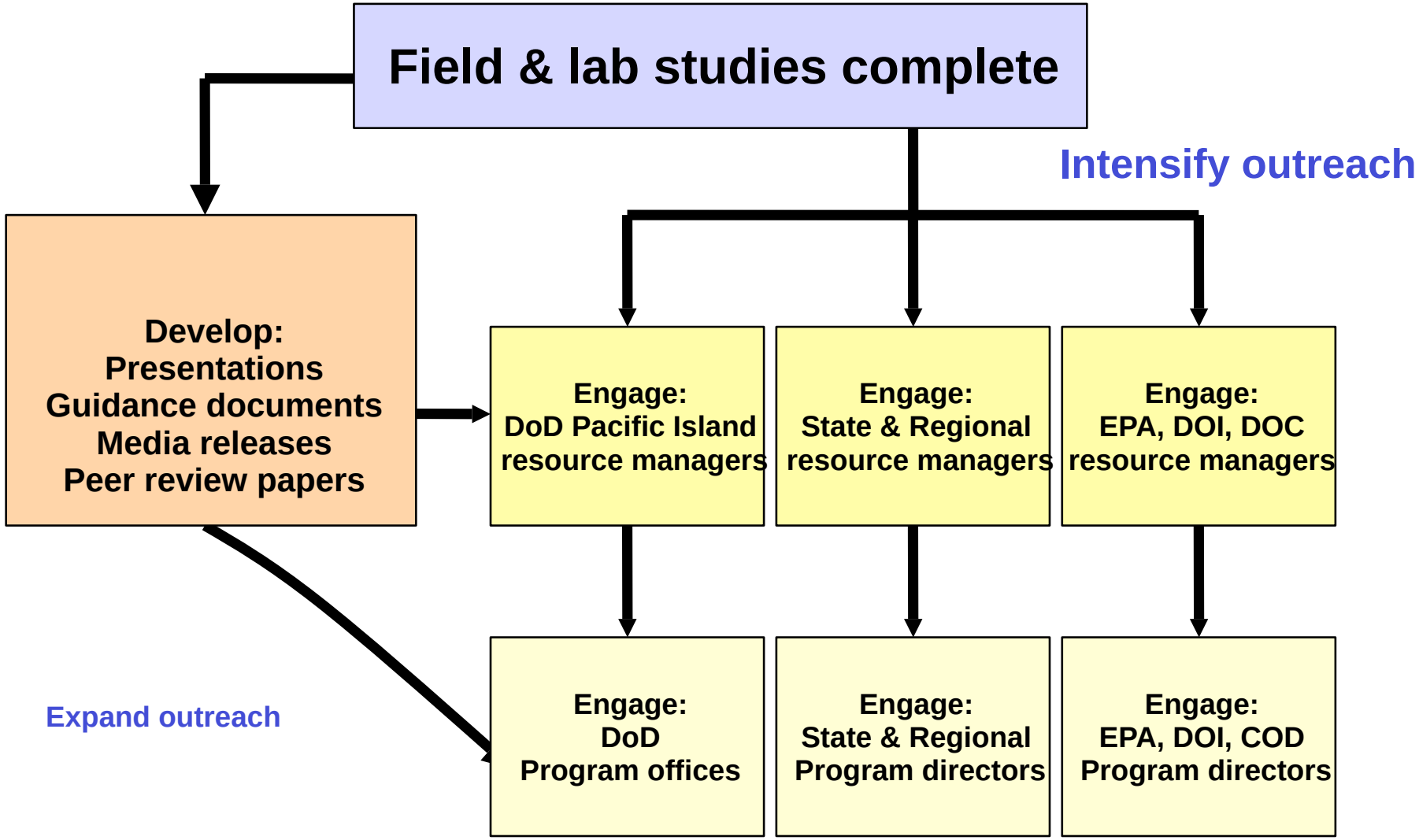
Products

- Greater understanding of oceanic island stream ecosystems & at-risk fauna
- New genetic methods for assessing the condition of oceanic island streams
- 8 peer reviewed articles anticipated
- Seminars & reports to guide technology transfer, management & conservation

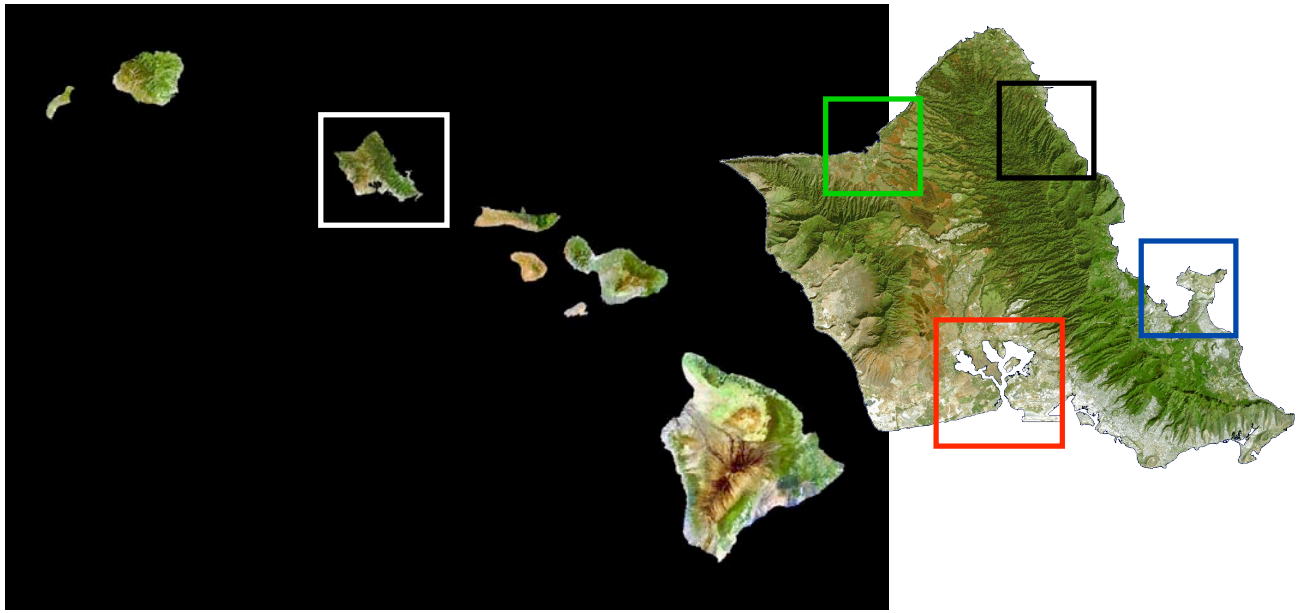
Training

- 2-4 PhD students
- 3 Postdoctoral researchers

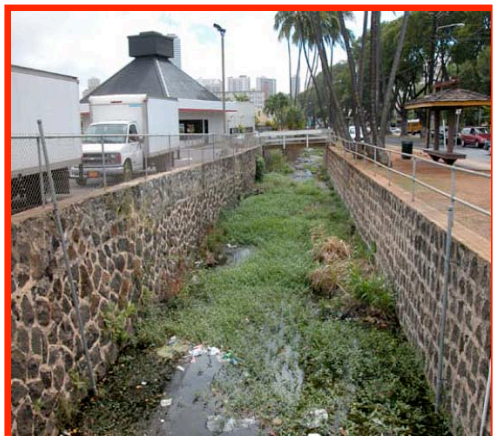
Transition Plan



Supplemental



Forest stream



Urban stream

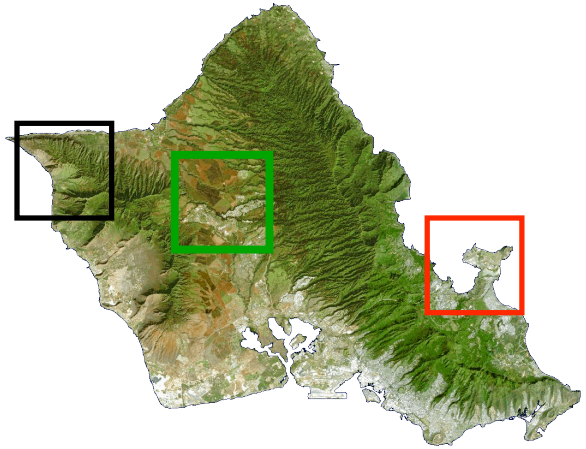
Stream diverted for ag

?

Military stream

Supplemental

Makua Military Reservation



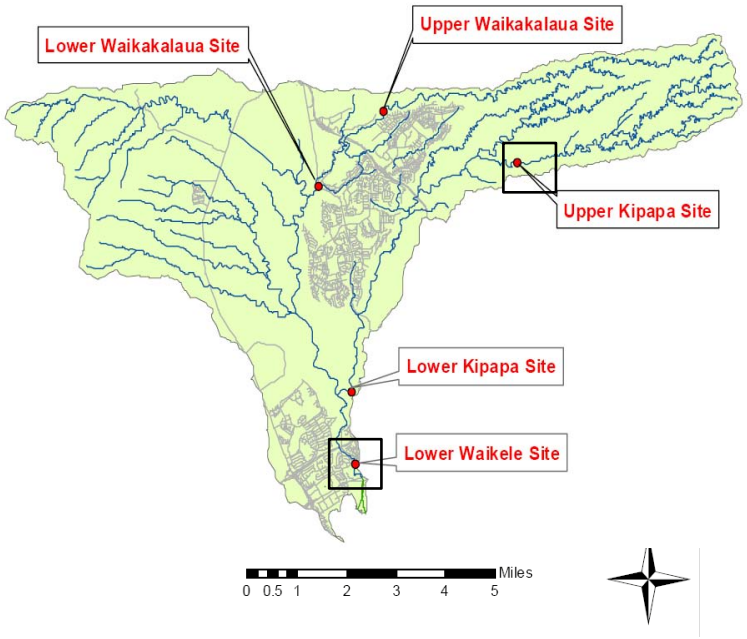
Kaneohe Bay Marine Corps Base

Schofield Barracks

Gradient of military land use and development

Supplemental

The presence and diversity of native fishes are not sensitive ecological indicators



Bioassessment of Waikele watershed (Henderson 2003)

Upper Kipapa site

"High Quality" habitat
No native fishes

Agriculture/conservation
700 ft elevation

Lower Waikele site

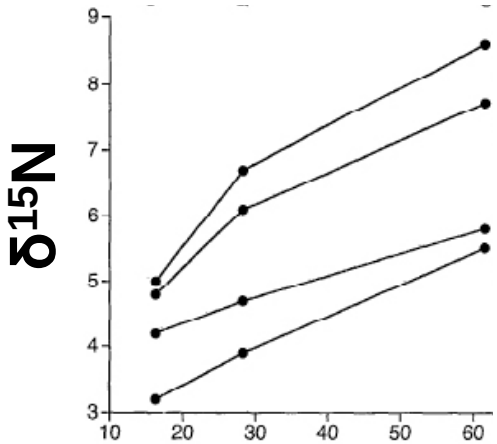
"Medium Quality" habitat
One native fish species

Urban
40 ft elevation

Supplemental

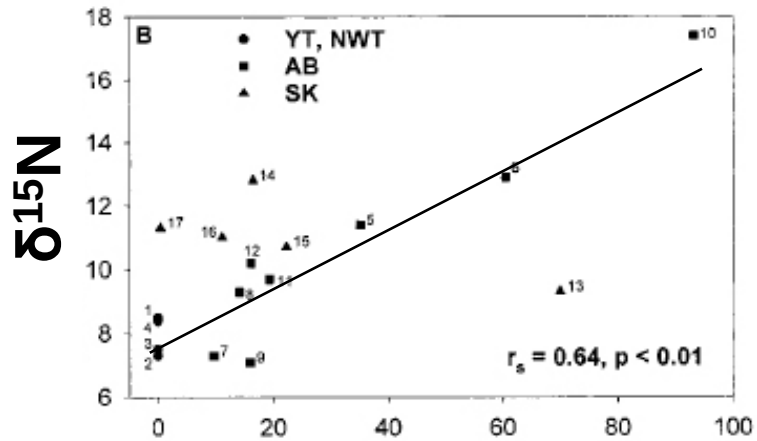
Nitrogen isotopes: an integrative measure of nutrient loading

Aquatic plants



N input from wastewater (%)
(McClelland et al. 1997)

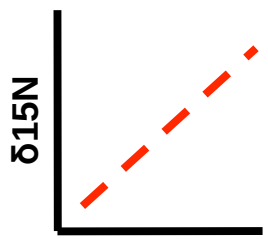
Aquatic animals



Agriculture in watershed (% area)
(Hebert & Wassenaar 2001)

Snail muscle integrates
N sources through time

Mass spectrometry



Land use

Reviewer Comments

Genetics

Comment: Use the mtDNA control region instead of cyt *b*?

Response: Both of these regions of the mtDNA genome evolve at similar rates, and therefore should provide similar levels of information on geographic structure and source tracking.

However, we have chosen to use cytochrome *b* because it is a protein-coding region, which allows use of more sophisticated models of molecular evolution for interpreting patterns of DNA sequence variation.

Reviewer Comments

Genetics

Comment: Use of microsatellites from other gobies likely to fail?

Response: Recent tests of goby microsatellite loci have found cross-amplification success rates of 100% for species within the same genus and 70% for species in different genera.

Trials are currently underway in Dr. Blum's laboratory to identify a suite of loci that cross-amplify in both focal species

Reviewer Comments

Genetics

Comment: Enough power to resolve the questions being asked?

Response: The minimum F_{ST} value that can be detected is equal to 1/ sqrt(*nm*), with *n* being the number of individual sampled and *m* being the number of molecular markers used.

For Study #1, analysis of 200 samples per island using eight microsatellite loci would enable detection of FST values as small as 0.0006 between islands.

For Study # 2 we will be able to detect minimum F_{ST} values of 0.0005-0.001 among watersheds and minimum values of 0.001-0.003 among sites within each watershed.

For comparison, Waits et al. (in press) found an average F_{ST} value of 0.008 among populations of a highly mobile freshwater fish collected at sites within a small watershed in southwestern Ohio.

Reviewer Comments

Otolith microchemistry & stable isotopes

Comment: Sources of nitrogen inputs cannot be distinguished.

Response: We are using nitrogen isotopes as an integrative measure of anthropogenic nitrogen loading according to broad land use categories (forest, military, urbanization/agriculture) rather than attempting to resolve specific nutrient sources.

Our application of isotopic methods will provide an in-stream counterpart to remotely-sensed measures of land use.

Reviewer Comments

Otolith microchemistry & stable isotopes

Comment: Variation in otolith microchemistry could be too limited to offer much spatial resolution.

Response: We are optimistic about the potential for success because isotopes likely vary across Hawaii due to differences in rock weathering (age & precipitation) and land use (urban & military). We also are optimistic because prior work has shown that informative variation can occur on spatial scales of tens of kilometers.

Our application of otolith microchemistry is cutting-edge science that has some attendant risk of failure but great potential for guiding management of oceanic island stream ecosystems.

Reviewer Comments

Study design & logistics

Comment: Recommend collaborating with Hawaiian researchers.

Response: Collaborations have been developed with:

Dr. J. Michael Fitzsimons, professor emeritus of Louisiana State University, who carried out all prior genetic studies of Hawaiian gobies.

Dr. Robert Kinzie of the University of Hawai'i, a leading expert on the population ecology of Hawaiian gobies.

Dr. Robert Nishimoto of the Hawai'i Division of Aquatic Resources, a leading expert on the ecology of oceanic island streams.

Dr. Reuben Wolff of the US Geological Survey (Honolulu, Oahu), a leading expert on the ecology of oceanic island streams.

Dr. Christine Thacker of the Natural History Museum of Los Angeles County, a leading expert on the phylogenetics of tropical gobies.

Reviewer Comments

Study design & logistics

Comment: Will adverse field conditions (e.g flooding/drought) in Year 1 prevent "work as planned"?

Response: Neither flash flooding nor cyclical drought conditions will compromise the success of proof-of-concept studies or the overall project.

Drought favors capture of stream fishes because individuals tend to congregate in residual pools. Flooding has little effect on adult gobies, but can delay upstream migration of postlarvae.

Only minor scheduling changes would be needed to allow for the recovery of upstream migration or to compensate for delays due to the difficulty of working in extreme flows.