Introduction

The components of this project were the physical characterization of the stream channel, assessments of the riparian vegetation and macrofaunal surveys. In continental systems, assessments of the physical and riparian conditions of streams have been used to improve water budgets and to identify areas of risk to flooding and habitat degradation. The physical properties of the stream bed and bank are critically important determinants of habitat availability, stream discharge, and transport. Similarly, the composition of the riparian community can influence instream productivity, nutrient cycling, water level, as well as the physical structure of the river. Nevertheless, previous surveys of Guam streams have generally ignored the importance of these parameters.

Methods

Four reaches were surveyed in the Geus River between May 22 to 30, 2016 (Figures 1 & 4). These sites covered a segment of the river extending from the river mouth to 3 km upstream. The sinuosity of these reaches generally decreased with increasing elevation while mean slope increased from 0.58% in Reach 1 to a mean slope of >6% in the Reach 4. Reaches were identified based upon characteristic changes in slope, sinuosity, sediment characteristics, and riparian community composition.

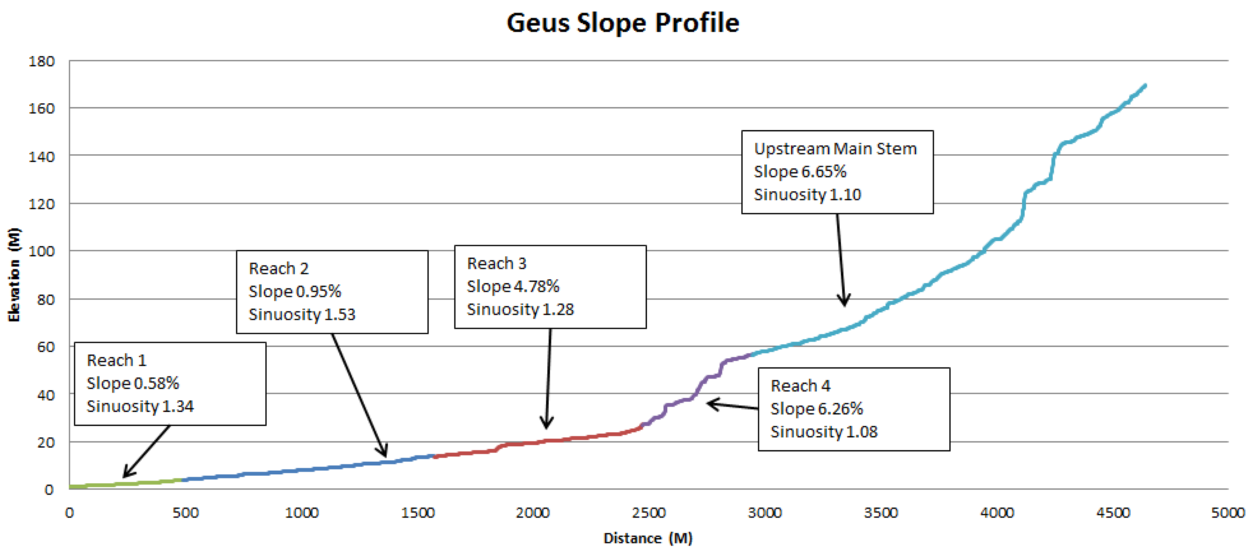

Figure 1. Geus River reaches surveyed in this study. Reaches 1 – 3 were below the first major impoundment.

Pebble counts were conducted at each reach. Briefly, an individual walked a "lazy-Z" upstream and across the channel, haphazardly sampling n=100 pebbles and measuring the size of the pebbles by hand. The gradient or slope of each reach was measured along a 100 m stretch of the river with a laser level (Spectra Precision LL300N Laser Lever, Figure 2). Finally, a depth profile was created from detailed measurements of a cross-section of the river at several sections of the reach. This profile included the bankfull depth, which is the height of the river channel that would correspond to the median (i.e., 50^{th} percentile) height during flood stage.

Figure 2. Laser level survey on Reach 2 of Geus River survey.

Surveys of the riparian community were made along 25 m line transects of the river bank. The linear coverage of each plant species was estimated at several sections below and above the first major waterfall (Figure 1). Plants were identified as either native or non-native based upon a species list from the UOG Herbarium database.

Visual surveys of instream macrofaunal densities were conducted between June 16 to June28, 2016 according to methods described in Baker and Foster (1992). Briefly, densities of streamfish and prawns (*Macrobrachium lar*) were estimated within a particular area of the streambed. The size of each area was variable with a mean area of 0.72 ± 0.2 m^2. A total of 59 sites were surveyed. Sites were chosen randomly and were stratified by stream reach. Surveys extended from the river mouth to within 300 m of the terminus of the main river channel in the headwaters (Figures 1 & 4).

Results

Fluvial Geomorphology

Reach 1 was a low gradient segment of the Geus River upstream of the main roadway and bridge. This portion of the river flows through a lightly populated urban area with portions of the riverbank stabilized through concrete and rip rap. The relatively high entrenchment ratio and moderately high sinuosity index are consistent with the classification of this reach as a Rosgen stream type C (Table 1).

Table 1. Geomorphic and carbon data for cross-sections 1.1-1.3

Cross-Sections	Rosgen Stream Type	Sinuosity	Entrench Ratio	W:D*	Slope	Detritus Cover	Filament Algae Cover
1.1			1.11	12.7	0.76%	S	VD
1.2			1.11*	7.7	0.34%	D	S
1.3			1.61	4.5	1.05%	VS	M
Mean	C	1.34			0.58%		

*Estimated, N- <10%, VS- 1-10%, S- 10-40%, M- 40-70%, D- 70-90% VD- >90%.

Reach 2 flowed through an extensive wetland area as part of an alluvial floodplain with an entrenchment ratio >1. This area was characterized by deep pools and bamboo dams. There was evidence from incised banks that the river channel is dynamic in this area, with exposed older river beds visible in the strata. Interestingly, this reach only had modest sinuosity, despite the evidence that the stream may meander in this section. Nevertheless, erosive bank features, such as cut banks and point bars, were common in this reach. The low gradient (mean slope = 0.95%) and moderate sinuosity support the classification of this reach as a Rosgen stream type C (Table 2).

Table 2. Geomorphic and carbon data for cross-sections 2.1-2.3

Cross-Sections	Rosgen Stream Type	Sinuosity	Entrench Ratio	W:D*	Slope	Detritus Cover	Filament Algae Cover
2.1			1.47*	12.4	0.85%	S	M
2.2			2.27	3.9	3.39%	D	M
2.3			1.38*	13.6		M	M
Mean	C	1.28			0.95%		

* Estimated, N- <10%, VS- 1-10%, S- 10-40%, M- 40-70%, D- 70-90% VD- >90%.

Reach 3 was located in an area of transitional substrate composition and gradient. Portions of this reach flowed over exposed basalt bedrock and small cascades. In some areas, the river was impounded by basalt-lined channel walls. This area also had evidence of historical and recent channel modification, including fresh bank-cutting. The remains of an older, dilapidated concrete water impoundment formed an extensive debris-field in the main river channel. This impoundment presumably once served as a municipal water source. The river channel was much shallower compared to Reach 1 and 2 (mean width:depth ratio = 12.7 ± 4.8; Table 3). This reach was classified as Rosgen stream type C.

Table 3. Geomorphic and carbon data for cross-sections 3.1-3.3

Cross-Sections	Rosgen Stream Type	Sinuosity	Entrench Ratio	W:D*	Slope	Detritus Cover	Filament Algae Cover
3.1			1.78*	4.4	1.06%	M	S
3.2			1.26*	21.0	1.36%	S	M
3.3			1.99	13.0	1.16%	M	S
Mean	C	1.28			4.78%		

* Estimated, N- <10%, VS- 1-10%, S- 10-40%, M- 40-70%, D- 70-90% VD- >90%.

Reach 4 was a segment of the Geus that was above the first major barrier to non-gobioid streamfish, such as *Kuhlia rupestris*. The stream was bordered by extensive basalt-lined channels and the W:D ratio and mean slope were considerably higher compared to lower reach sites (Table 4). Sinuosity was low. This reach was classified as Rosgen type A.

Table 4. Geomorphic and carbon data for cross-sections 4.1-4.3

Cross-Sections	Rosgen Stream Type	Sinuosity	Entrench Ratio	W:D	Slope	Detritus Cover	Filament Algae Cover
4.1			1.59	7.8	6.54	M	S
4.2			1.12	36.4	0.63	S	S
4.3			1.30	8.2	2.51	S	D
Mean	A	1.08			6.26%		

* Estimated, N- <10%, VS- 1-10%, S- 10-40%, M- 40-70%, D- 70-90% VD- >90%.

Sediment Size Composition

The size distribution of settleable particles is a useful indicator of stream power and habitat availability for epifaunal and interstitial fauna. It also influences hyporrheic oxygen flux and redox potential. Sites from Reach 1 had consistently lower grain sizes than all other sites surveyed (Figure 3). The highest median sizes were sampled from Reach 2 and 3.

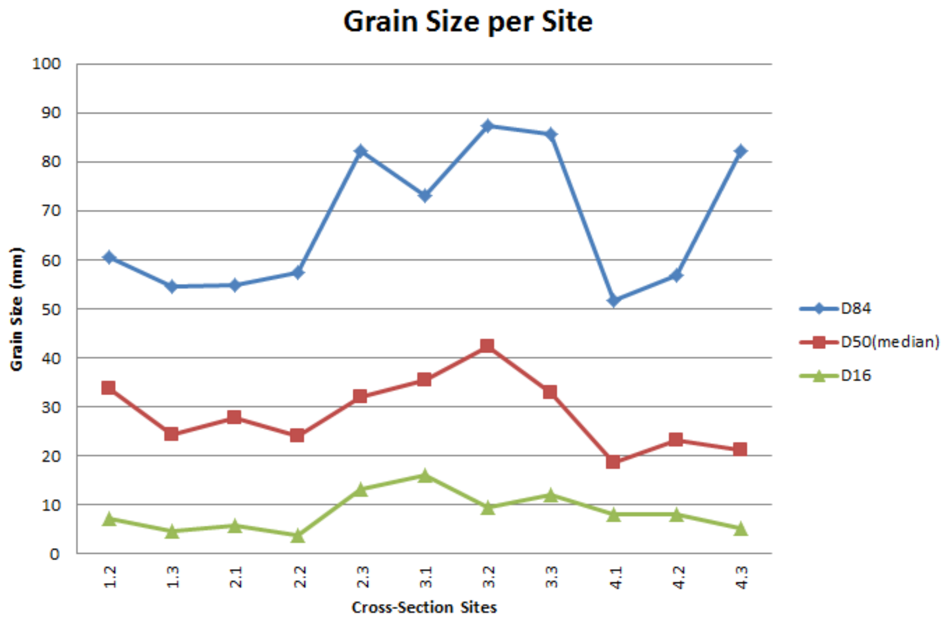

Figure 3. Percentile grain sizes (mm) from n=100 sediment particles samples haphazardly among four reaches of the Geus River. D16, D50, and D84 correspond to the 16th, 50th (i.e., median) and 84th percentile of grain size, respectively.

Riparian Community Composition

The riparian communities were sampled in reaches 1, 3 and 4. Across all three reaches, non-native species dominated the vegetative cover of the riparian zone. This was largely the result of dense stands of *Bambusa vulgaris*, which comprised 22%, 16%, and 49% of the total cover in reaches 1, 3, and 4, respectively (Table 5 a-c). Other common non-native plants included naturalized ornamental (e.g., the vine *Epipremnum aureum*) and agroforest species (e.g., *Musa acuminata*; Table 5 a).

Table 5 a. Percent cover of riparian species along 25 m line transects (n=6) in Reach 1.

Species	Status	Percent cover
Bambusa vulgaris	Non-native	23.50%
Pennisetum polystachion	Non-native	21.13%
Epipremnum aureum	Non-native	9.34%
Canavalia rosea	Non-native	7.35%
Unknown Asteraceae	Non-native	4.85%
Pandanus tectorius	Native	4.52%
Talipariti tiliaceum	Native	4.34%
Pithecellobium dulce	Non-native	4.18%
Vitex parviflora	Non-native	4.18%

Leucaena leucocephala	Non-native	4.14%
Monstera sp.	Non-native	2.97%
Thelypteris parasitica	Native	1.57%
Luffa cylindrica	Non-native	1.56%
Musa acuminata	Non-native	1.52%
Cocos nucifera	Non-native	1.02%
Stachytarpheta jamaicensis	Non-native	0.78%
Microsorum punctatum	Native	0.59%
Syngonium sp.	Non-native	0.53%
Areca catechu	Non-native	0.47%
Synedrella nodiflora	Non-native	0.47%
Coix lachryma	Non-native	0.43%
Morinda citrifolia	Native	0.27%
Alocasia macrorrhiza	Non-native	0.23%
Colocasia esculenta	Non-native	0.04%

Table 5 b. Percent cover of riparian species along 25 m line transects (n=6) in Reach 3.

Species	Status	Percent cover
Epipremnum aureum	Non-native	22.76%
Bambusa vulgaris	Non-native	16.13%
Syngonium sp.	Non-native	13.42%
Cocos nucifera	Non-native	12.52%
Unidentified vine species	Non-native	9.89%
Leucaena leucocephala	Non-native	9.14%
Thelypteris parasitica	Native	8.86%
Nephrolepis schott	Native	7.56%
Pandanus tectorius	Native	6.90%
Talipariti tiliaceum	Native	5.86%
Areca catechu	Non-native	5.14%
Unidentified species 1	Non-native	1.90%
Polypodium scolopendria	Native	1.90%

Canavalia rosea	Native	1.43%
Monstera sp.	Non-native	1.08%
Mucuna sp.	Non-native	0.86%
Clerodendrum quadriloculare	Non-native	0.33%

Table 5 c. Percent cover of riparian species along 25 m line transects (n=6) in Reach 4.

Species	Status	Percent cover
Bambusa vulgaris	Non-native	49.62%
Canavalia rosea	Non-native	14.30%
Miscanthus floridulus	Native	11.42%
Morinda citrifolia	Native	4.92%
Pandanus tectorius	Native	4.92%
Cocos nucifera	Non-native	4.01%
Elephantopus mollis	Non-native	3.35%
Leucaena leucocephala	Non-native	2.31%
Alocasia macrorrhiza	Non-native	1.89%
Nephrolepis hirsutula	Native	1.36%
Thelypteris parasitica	Native	0.79%
Polypodium scolopendria	Native	0.47%

Faunal Surveys

Surveys of instream fauna revealed distinct differences in distribution between sites below and above the dam located at the base of reach 4 (Figure 4 & Table 5). The jungle perch *Kuhlia rupestris*, a predatory euryhaline fish, was absent from sites above the dam. Similarly, the large-bodied gobioids *Awaous guamensis* and *Eleotris fusca* were also observed in sites below the dam.

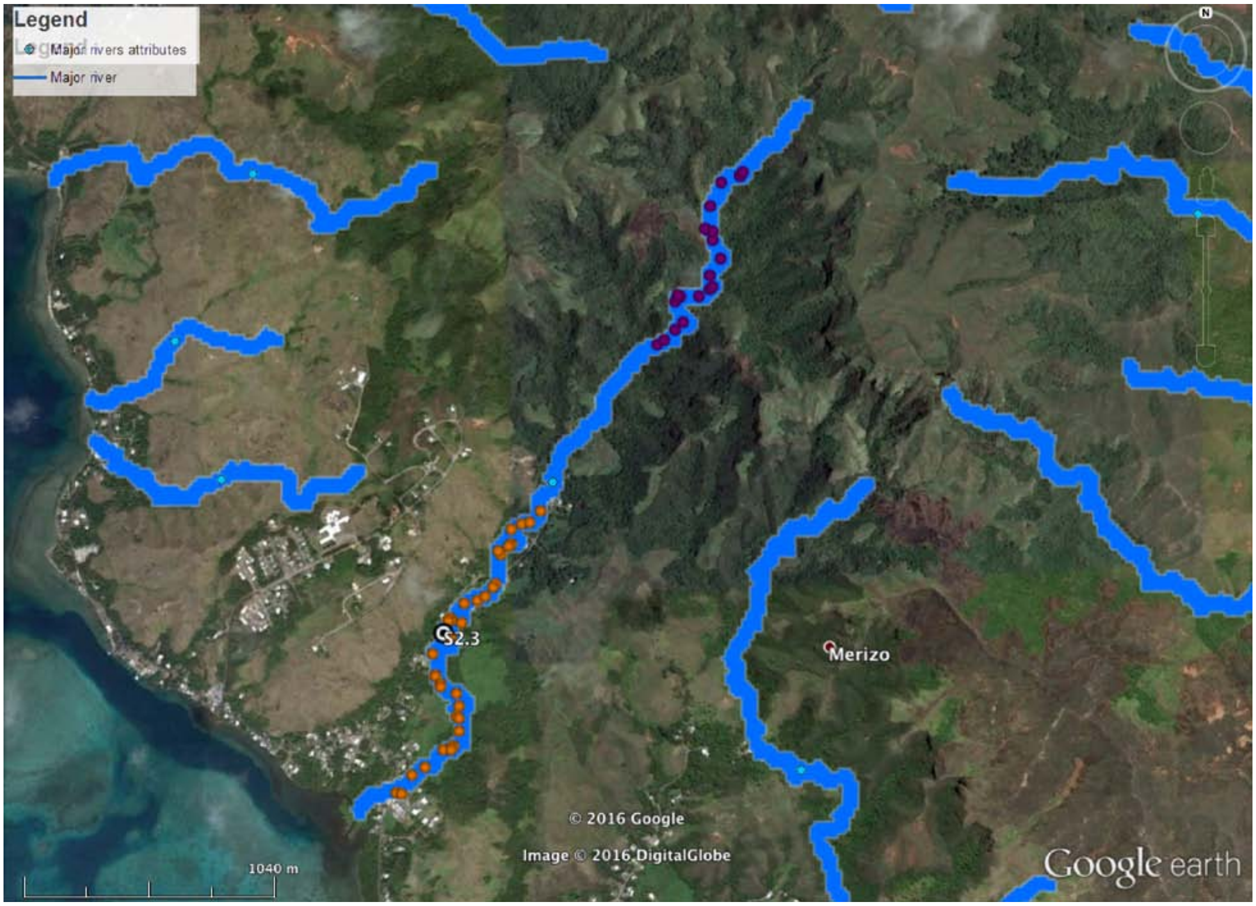

Figure 4. Survey sites for macrofaunal densities and physicochemical in the Geus River. Sites GL1 – GL37 were located in the lower reach of the river and began 100 m upstream from the main bridge. Sites GU0 – GU21 were in the upper reach of the Geus River with GU0 approximately 853 m upstream of GL37. The upper reach was surveyed above the uppermost impoundment.

In contrast, the highest densities of sicydiine gobies were generally observed above the dam. This included high densities of *Sicyopterus lagocephalus*, a species commonly occupying hard-bottomed substrates, and *Sicyopterus leprurus*, which was only observed in the upper reach. *Macrobrachium lar*, a freshwater prawn, also had higher densities above the dam. The only exception were the densities of *Stiphodon* which were much lower above than below the dam.

Table 6. Mean densities (individuals • m^{-2} ± 1 SD) of streamfish and prawns below and above the main water impoundment in the Geus River. The superstructure of this dam, while non-functional, presented a major barrier to predatory non-gobioid fishes.

	Density below dam	Density above dam
Anguilla marmorata	0	0.155 ± 0.513
Awaous guamensis	0.265 ± 1.152	0
Eleotris fusca	0.116 ± 0.399	0
Kuhlia rupestris	1.426 ± 2.820	0
Macrobrachium lar	0.063 ± 0.382	2.296 ± 2.812
Sicyopterus lagocephalus	0.224 ± 1.067	1.215 ± 3.152
Sicyopus leprurus	0	0.623 ± 1.312
Stenogobius cf. *genivittaus*	0.237 ± 0.713	0
Stiphodon sp.	1.462 ± 7.282	0.732 ± 1.316

Discussion

Sections of the channel and banks of the lower Geus River have undergone extensive modification, both in terms of man-made stabilization as well as impoundment and diversion. Actively eroding areas were relatively common among Reaches 1 through 3. All three of these reaches are classified as Rosgen class C streams. Reach 4 was classified as a Rosgen class A stream. This was due to the relatively higher gradient and lower sinuosity in Reach 4 compared to the lower reaches. Substrate composition was also comprised of more bedrock, although median particle size was variable. These physical characterizations of the Geus River channel suggest that the Rosgen stream classification system can be successfully applied to Guam watersheds. Future surveys should monitor the various parameters used in these physical assessments to evaluate the success of watershed rehabilitation efforts.

The riparian communities in the Geus River are comprised of a significant number of non-native species, both in terms of diversity and percent cover. The most notable species, bamboo, is emerging as a major invasive species in the watersheds of southern Guam. In addition to forming dense stands that likely outcompete other plants for resources in these riparian areas, the clumps also cleave from the riverbanks and form dams in the river channel. Thus, bamboo has the potential to physically restructure stream channel and should be eradicated in the Geus River.

The distribution and densities of streamfish in the Geus River are consistent with previous surveys of Guam streams. In those surveys, physical barriers to upstream movements of predatory *Kuhlia* have been identified as a major force structuring the distribution of gobies and decapod crustaceans. In the Geus River, *Kuhlia* were conspicuously absent from all sites above the dam. In contrast, the highest densities of much smaller gobies were in sites above the dam. Interestingly, densities of *Stiphodon* were higher in the lower reach of the stream compared to above the dam.

Objectives 5-10. Larval Drift Collection and Description, Genetic Characterization and Identification of Adult and Larvae, Relate Amphidromous Macrofaunal Reproductive Output to Instream/Landscape Parameters.

Larval Drift Collection and Description

Larval stream drift collections on the Geus river were conducted when stream flow allowed using methods previously worked out in Hawaii by one of our PIs (Lindstrom, 1998). When there was sufficient surface flow (our work was hampered by an extended drought throughout most of the summer months of 2016) we were able to deploy a Neuston net (Figure 5) from the highway bridge about 100 meters above the river mouth to intercept minute newly hatched larvae. They passively drift to the ocean where they develop in the plankton and eventually recruit back to freshwater in several months. This work had as a goal to capture examples of newly hatched drifting larvae of all amphidromous fish and shrimp which were morphologically characterized and genetically analyzed to match up with known adult species, which were also collected and genetically analyzed.

Figure 5. Nekton net employed in stream larval drift collections.

We collected several adult specimens of all known native amphidromous stream gobioid fish species (*Awaous guamensis, Awaous ocellaris, Stenogobius sp., Stiphodon percnopterygionus, Stiphodon sp2, Stiphodon sp3, Sicyopterus lagocephalus, Sicyopus sp., Eleotris fusca*) along with some species previously unknown from Guam (putatively *Sicyopus zosterophorum* and *Sicyopus cebuensis*, to be genetically confirmed with comparison to tissues previously collected in the Philippines by D. Lindstrom). Some of these were preserved for morphologically based taxonomic analysis and also had DNA extracted from a small tissue sample for the genetic work. Representatives of each adult species have been kept in aquaria procured for this project at UoG. There is some lack of taxonomic confidence for most of these fishes that will be untangled as part of this study and an ongoing inquiry by the author. For example, there is some doubt as the efficacy of there being two to three conspecifics within the genus Stiphodon in Guam where two of the "species" likely represent one that shows a high degree of chromatic plasticity that has mistakenly lead to them being identified as separate species. Our work will necessarily need to confirm or refute this hypothesis and our captive facility in conjunction with our genetic work will definitively answer these questions. We collected at least 8 morphospecies of newly hatched drifting gobioid larvae, among about a thousand captured, that have been microscopically characterized and photographed and underwent genetic analysis (Figure 6). Additionally, in the same sampling apparatus, we collected several morphospecies of newly hatched amphidromous shrimp larvae along with larvae of several crab species (Figure 7). These larvae were characterized morphologically and genetically in parallel with the larval gobies. We also collected adult shrimp of many of the known and unknown species in the same manner as the goby fish which likewise are a taxonomic conundrum awaiting resolution that this study will further help delineate. This work will continue until we have several complete sets of all species and have produced a dichotomous key to both adult, juvenile and larval species identifications. This key will allow for the discernment of any temporal patterns of reproductive output in the future that can be correlated to the findings of our ongoing instream biotic surveys.

Figure 6. Examples of larval gobies from drift sampling efforts under a dissection microscope (we found several more than depicted above that have been included in genetic analysis).

Figure 7. Examples of larval shrimp and crabs from drift sampling efforts under a dissection microscope.

<u>Genetic Characterization and Identification</u>

Select tissue biopsies from the adults and whole larvae from the above described efforts were subjected to protocols that extract and purify complete intact genomic DNA for analysis. Initial trials of Polymerase Chain Reaction (PCR) amplification of several mitochondrial DNA target regions (*Cytochrome b, Cytochrome Oxidase I, D-Loop, ATPase 8 and ATPase 8*) proved successful for both larval fishes and decapods. Initial DNA sequencing of select individuals of each adult species and larval morphotype has been promising and resulted in initial species assignment of some larval morphotypes. These protocols are being applied to a greater number and variety of larval drift specimens and each new DNA sequence is being added to a larger analysis that is allowing species distinctions to be confidently applied to larval morphospecies. Once complete, this will result in the first key to larval species identification for the newly hatched/drifting stream fauna of Guam. As yet, we have been successful in working out the protocols for DNA extraction/purification of the microscopic individual goby and shrimp larvae using a modified protocol with a commercially available kit (Qiagen DNA Micro). These extracts have been used as templates for the above-mentioned PCR amplifications of several gene loci that have proven to be successful in the delineation of species affinities for gobioids and decapods. These initial PCR amplification products were then used as templates for sequencing reactions that produce products that were visualized on a LiCor 4300 DNA Analyzer producing about 700 bidirectional base pairs of genetic sequence for each specimen. These sequences were first edited using the ESeq program (LiCor Corporation) and then exported, aligned to other sequences and analyzed in the Geneious program (Biomatters Ltd.). For the gobies, the two overlapping mitochondrial ATPase genes have been the most reliable and for the decapods, the two Cytochrome genes have proven most effective. So far we have been able to genetically characterize 5 of the putative 11 species of adult gobioids and have generated partial sequences for all 8 goby larval morphotypes. Further work is needed to complete all sequences and allow unequivocal assignment of all captured and described larval types along with all adult species present in Guam streams.

<u>Macrofaunal Reproduction and Instream/Landscape Dynamics</u>

Dr. William Klindl of Naiad of Naiad Aquatic Consultants LLC traveled Guam and oversaw our field work from May 22nd – 30th. He led out local team in an intense week of physical stream survey of the entire Geus watershed producing fine resolution data that will be combined with existing an existing GIS database which we will layer the rest of our physical and biological findings producing, to our knowledge, the most comprehensive database of its type in existence. See Objectives 1 – 4 above for further details.

Objective 11. Dissemination of Findings

Findings will be disseminated after completion of fieldwork, lab efforts and formal reporting in the form at least 2 formal scientific publications. We will also produce a detailed field guide for the identification of all native stream macrofauna and their newly hatched drifting larvae once we have a complete collection and the necessary genetic data to make confident species assignments.

Objective 12. Student Training

We hired one UoG first year undergraduate student, Mr. William Naden, from our pool of students as he has extensive knowledge of the Geus area and a propensity for exemplary field work. He assisted the PIs in various field operations and laboratory protocols. We additionally included two of our current graduate students (Jeried Calaor and Sean Moran) in field training and data gathering while Dr. Klindl was present and subsequent field work. In addition, we were fortunate enough to be chosen to mentor one of last year's winners of the Guam Science Fair Competition, Mr. Kameel Hutcherson, a local High School Junior who assisted Dr. Lindstrom in some preliminary larval drift collecting and characterization.

Acknowledgments

We would like to thank the following people for their various contributions to this study in the field, laboratory and office.

Jeried Calaor, Kameel Hutcherson, Dr. William Klindl, Cathleen Moore-Linn, Adrienne Loerzel, Dr. Kate Moots, Sean Moran, William Naden, Anna Simeon, Gloria Travis.

Citations:

Baker, J. A., and S. A. Foster. 1992. Estimating density and abundance of endemic fishes in Hawaiian streams. Division of Aquatic Resources, Hawai'i Department of Land and Natural Resources.

Garrison, G.A. 1949. Uses and modifications for the "moosehorn" crown closure estimator. Journal of Forestry. 47: 733-735.

Higashi, G.R. and R.T. Nishimoto. 2007. The Point Quadrat Method: a Rapid Assessment of Hawaiian Streams in Biology of Hawaiian Streams and Estuaries (N.L. Evenhuis and J.M. Fitzsimons, eds.). Bishop Museum Bulletin in Cultural and Environmental Studies 3: 305–312.

Lindstrom, D.L. 1998. Reproduction, Early Development and Larval Transport Dynamic of Amphidromous Hawaiian Gobioids. Ph.D. dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.

Parham, J.E. 1995. Habitat use by an assemblage of tropical oceanic island streamfishes. M.S. Thesis, University of Guam Marine Laboratory, Mangilao, Guam.

Rantz, S.E. 1982. Measurement and computation of streamflow: Volume 1. Measurement of stage and discharge. U.S. Geological Survey Water-Supply Paper 2175.

NOAA
CORAL REEF
CONSERVATION PROGRAM