## PACT Research Grant Final Report

# A NPASP-Consistent Baseline Assessment Program for the Freshwater Fishes of Belize 

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#### Abstract

The aim of this project was to meet specific objectives of the Belize National Protected Areas Systems Plan (NPASP) by accomplishing the following objectives:


1. Develop guidance on methods for assessment of fish communities in Belize as the basis for future long term monitoring.
2. Collect and publish an online baseline dataset of the freshwater fishes of Belize
3. Make spatial prediction of fish species ranges across Belize.
4. Increase the capacity of Belizeans to conduct field research in freshwater ecosystems.

In fulfilling these objectives this project helps fill a major information gap identified in the NPASP—namely our knowledge of freshwater biodiversity. The project not only extensively documented freshwater fishes, but demonstrates how existing biodiversity information can be used to make national predictions of species richness, which in turn can be used to select a network of the highest priority freshwater conservation sites.

To accomplish the above objectives it was necessary to collect freshwater fishes using standard methods in 14 of the 16 major watersheds of Belize, and to use these data in conjunction with an extensive GIS database to develop predictive models of species distributions. A total of 79 fish species were collected at 126 sites, including 4 new records for Belize (bringing the national freshwater fish count to 124). Included in the sample were two non-indigenous species of tilapias. Monkey River had the greatest combined richness ( $n=47$ ) despite being sampled far less intensively than the Belize River (second most; $n=44$ ) or other large rivers of the north. The fish fauna Temash and Moho Rivers differed strongly from their more northern counterparts, suggesting that a biogeographic boundary zone with high species turnover exists somewhere between Golden Stream and Moho River. The baseline dataset generated by this project provides an important benchmark in time with which to monitor the future of ongoing biological invasions, particularly of Nile tilapia, which was documented in 8 watersheds.

The dataset assembled for this study ( 126 sites) is being combined with past data collected in Rio Hondo ( 65 sites) and from the Belize collections done in the 1970s (109 sites) to develop range models for 87 fish species. These data will be used to demonstrate how predictive models of fish biodiversity can be utilized to select a network of the high priority freshwater conservation sites. Results from this portion of the work will be presented in the dissertation document that results from this research.

To satisfy the objectives of the NPASP it will be necessary to ensure that the protected areas system serves freshwater biodiversity at a national scale. With this in mind, three recommendations for further study are:

1. National baseline inventories of non-fish freshwater taxa, which are likely to bring freshwater species list close to 500 species.
2. Further field work to delineate distinctive freshwater biogeographic zones that contain unique faunal assemblages and unique evolutionary trajectories (e.g., Moho River).
3. Assessment of the functionality of the current protected areas system for protection of freshwater biodiversity and services.

The current study lays a solid foundation for a synthetic national understanding of freshwater biodiversity. The recommended follow-up studies will expand baseline knowledge to provide the insight necessary to expand national plans to substantively include freshwater biodiversity.

## Summary of Project Aims and Purposes

The aim of this project was to contribute to Action 3.2 of the National Protected Areas Systems Plan (NPASP; Meerman and Wilson 2005, p. 12), which calls for a "Biodiversity base-line and monitoring programme" that will:

1. Develop practical survey techniques for planning and monitoring
2. Promote research on gaps in our current information base (e.g., freshwater and open sea systems)
3. Conduct rapid ecological assessments to provide baseline information that is consistent across sites.

This project contributed to each of these for an important component of freshwater biodiversity-the fishes. As such, the overarching objectives of this project were:
5. To develop guidance on methods for assessment of fish communities in Belize as the basis for future long term monitoring.
6. To collect and publish an online baseline dataset of the freshwater fishes of Belize
7. To make spatial prediction of fish species ranges across Belize.
8. To increase the capacity of Belizeans to conduct field research in freshwater ecosystems.

The first objective was met with the development of recommended methods for assessing fishes and aquatic habitat, the second and third drew on an extensive fish field dataset collected throughout Belize in 2006 and 2007, and the fourth objective was met through training Belizeans to collect field data, holding a workshop about development of species distribution models, and publishing a website (www.belizefreshwater.com).

## Background and Significance

The goal of the National Protected Area Policy is to "promote the sustainable use of Belize's protected areas by educating and encouraging resource users and the general public to properly conserve the biological diversity contained in these areas in order to maintain and enhance the quality of life for all" (Meerman and Wilson 2005, p. 5). The NPASP recommends 20 actions that set out an overall strategy to make the existing protected areas network function effectively. Both the NPASP and the Policy explicitly recognize the central role that biodiversity plays as a focus for the management of the protected areas network, and to provide services to society. For this reason, the NPASP recommends the promotion of a "Biodiversity baseline and monitoring programme' that will collect comprehensive data with standard methods for the whole country. Having comprehensive national biodiversity datasets will allow the Forest Department to manage the national protected areas network more effectively by giving them the necessary information to judge the relative importance of sites, to draw up appropriate management plans and to evaluate performance relative to biodiversity goals (Meerman and Wilson 2005).

Freshwater biodiversity is one of the least well documented and understood components of biodiversity in Belize. For this reason Meerman and Wilson (2005) called specifically for the promotion of research about freshwater ecosystems to fill gaps in our current information base. Given their importance to society, a well established taxonomy, and the fact that some pre-
existing data exist about their distributions from field work done in the 1970's, fishes are a logical group of organisms to begin analyzing national patterns in aquatic biodiversity. This study was undertaken to not only document fish biodiversity throughout Belize with standard methods, but to use this data to generate quantitative predictions about freshwater fish biodiversity that can be entered into national planning. Specifically, by using analytical tools that mix fish occurrence records with GIS data to predict and visualize species range limits, biodiversity maps can be created that allow us to assess the efficacy of the protected areas network for protected unique species assemblages.

The significance of this project is three-fold:

- First, the project responds directly to a major information gap identified in the NPASP to improve our knowledge of freshwater biodiversity in a systematic and consistent way throughout the country.
- Second, the project demonstrates how existing biodiversity information can be leveraged in conjunction with existing technology and GIS datasets to make national predictions of biodiversity patterns (in this case fish species richness).
- Third, the end product of this dissertation research will be to demonstrate how the national prediction of fish biodiversity can be utilized to select a network of the highest priority freshwater conservation sites.

These contributions, if effectively entered into strategic planning in the context of the national protected areas system, mark an important step forward for aquatic biodiversity conservation in Belize.

## Methods Procedures and Sampling

Methods were developed for the collection of fish, the creation of GIS datasets for use in species distribution modeling, and for creation of the models themselves. After describing the study area, the methods for each of these steps in described below.

## Study area

This research was carried out in the watersheds that drain to the Belizean coast. This includes 16 major watersheds-bounded by Rio Hondo in the north and Rio Sartsoon in the south-and numerous smaller watersheds (Figure 1). This area encompasses both the low-elevation limestone-based watersheds of the Yucatan Peninsula, and the more mountainous watersheds of southern Belize and Guatemala that originate in variable geologies and from elevations greater than 1000 m . The northern part of the study area is characterized by spring fed streams and large meandering rivers that cross an extensive coastal plain with many lagoons. The southern part of the study area is characterized by high-gradient surface and spring fed streams flowing to large meandering rivers that traverse a short distance across the coastal plain to the sea. The topographic and geologic differences between the northern and southern parts of the study area make it an excellent test site for development of predictive models that will be able to generalize to mountainous landscapes of the Caribbean slope of Central America and to the low elevation areas of Yucatan Peninsula.

Northern Mesoamerica has been called a "strong center of evolution" for freshwater fishes (Miller 1966), because of its many endemic genera and species. At least 130 species of
freshwater fishes in 34 genera and 10 families inhabit this area (Bussing 1976), a number that rises to over 200 when saltwater fishes occasionally found in freshwaters are included (Miller 1966). In Belize, we expected to catch at least 120 species that have been recorded previously throughout the country (Greenfield and Thomerson 1997, Esselman et al. 2006).

Only streams and rivers were considered in this study, and a few lagoon systems that are in line with a stream network (e.g., New River Lagoon is connected to New River). In preparation for this effort, all stream lines in the study area $(n=36,436)$ were hand digitized from scanned 1:50,000 topographic maps. The hand-digitized streamlines were used to condition a 30 m digital elevation model, which was in turn used to create a flow direction grid that allowed for the calculation of watershed variables that match the stream line layer (details given below).


Figure 1. Map showing study area (watersheds of Belize), sampling sites from this study (yellow dots), sampling sits from previous studies (Schmitter-Soto et al. 1998; Esselman et al. 2006), and point locations for collections made in the 1970s (Greenfield and Thomerson 1997). All of the data above are being used in this project to predict the distributions of fishes throughout Belize (see Figure 2).

## Description of modeling approach

As used here, a "model" can be considered a mathematical generalization of the relationships between a response variable (e.g., fish presence) and multiple predictor variables (e.g., environmental limits to a species' distribution). Once this relationship is established, the presence of a species can be predicted to places where only the environmental data held in GIS are available (e.g., where no species data exist). The power of predictive models lays in the fact that once validated, they give us the ability to understand patterns of species distributions with a known confidence (e.g., "We are 90\% confident in our predictions based on our validation exercises."). In a place like Belize, where funds are limited to sample species in all locations, predictive modeling can help fill the gaps in our knowledge so we can make more well-informed decisions.

A modeling approach was chosen that was (1) quantitatively rigorous; (2) required only presence data (not presence and absence); (3) available to the public for free; and (4) user friendly. The latter two criteria are important so that the methods reported here can be easily replicated by other users. The approach chosen is called maximum entropy or MaxEnt. MaxEnt is a mathematical approach for predicting an unknown probability distribution based on the principle that the estimated distribution must agree with everything that is known about a species' occurrence and be subject to no unfounded constraints. The approach estimates the most uniform distribution (e.g., the distribution with "maximum entropy") across a defined area subject to the constraints imposed by information available about environmental conditions at locations where a species is known to occur (Phillips et al. 2004, Phillips et al. 2006).

Inputs to the MaxEnt software include georeferenced point data of species occurrence records, and raster (pixel-based) GIS data layers of the different environmental attributes (climate, topography, etc.) hypothesized to be important to freshwater fish distributions.

The modeling process involves "model training" during which the mathematical relationship between variables is established, and "model validation" during which the trained model is tested for its predictive accuracy. In model training, MaxEnt uses a mathematical process that iteratively adjusts coefficients in an equation to maximize the likelihood that the occurrence data used to train the model are correctly predicted. By adjusting these many times (e.g., 500), the MaxEnt algorithm converges on the optimum probability distribution-the best solution to the problem given the data available. The output of a MaxEnt model is a continuous surface of values ranging between 0 and 100, with higher values indicating a higher suitability of that area for the target species. Model validation is explained below, after first describing the GIS and fish datasets assembled to enter into the modeling process.

## Environmental attributes

A total of 30 variables were prepared as individual layers for possible inclusion in the model. There were two primary data sources used as base data layers, which were then subject to resampling and processing: The Nature Conservancy's (TNC) Selva Maya Ecoregional dataset (2006) and the Inter-American Biodiversity Information Network-Development Grant Facility's (IABIN-DGF) 30 m hydrologic derivatives ${ }^{1}$. The former dataset was used as a source for temperature, precipitation, elevation, geology, soils, ecosystems, roads, and settlements data, and the latter was used for slope and flow direction (Table 1). The flow direction grid was derived from a 30 m digital elevation model by USGS using the hand-digitized streamlines generated for this project to constrain the flow direction to the location of the actual stream channel. Having a flow direction layer allows for the calculation of cumulative upstream

[^0]Table 1. Environmental variables prepared for entry into MaxEnt models of Belize fishes.

| Variable | $\begin{array}{r} \text { Min. } \\ \text { Value } \end{array}$ | Mean Value | Max. Value |
| :---: | :---: | :---: | :---: |
| Average annual catchment air temperature ( ${ }^{\circ} \mathrm{C}$ ) | 20.0 | 22.9 | 26.0 |
| Average annual catchment rainfall (mm) | 795 | 1519 | 2357 |
| Average catchment elevation (m) | 0.42 | 334 | 1047 |
| Average catchment slope (percent) | 0.00 | 8.93 | 36.03 |
| Average local annual air temperature ( ${ }^{\circ} \mathrm{C}$ ) | 20.0 | 22.6 | 26.0 |
| Average local annual rainfall (mm) | 794 | 1493 | 2391 |
| Local elevation (m) | 0 | 277.8 | 1019 |
| Local slope (percent) | 0.00 | 7.59 | 99 |
| Catchment area (km2) | 0.01 | 682 | 164728 |
| Catchment geology proportions of: |  |  |  |
| Alluvium | 0 | 0.10 | 1 |
| Andesite | 0 | 0.43 | 1 |
| Mudstones and shales | 0 | 0.43 | 1 |
| Catchment soil proportions of: |  |  |  |
| Fluvial Gleysols and Vertisols | 0 | 0.38 | 1 |
| Leptosol | 0 | 0.35 | 1 |
| Catchment terrestrial ecosystem proportions of: |  |  |  |
| Caribbean lowland season swamp forest | 0 | 0.02 | 1 |
| Central American Atlantic season and evergreen lowland forest | 0 | 0.19 | 1 |
| Meso American waterlogged savanna | 0 | 0.02 | 1 |
| Peten lowland alluvial seasonal forest | 0 | 0.01 | 1 |
| Peten seasonal evergreen forest on karstic hills | 0 | 0.30 | 1 |
| North Meso American premontane wet forest | 0 | 0.19 | 1 |
| Lowland pine forest | 0 | 0.12 | 1 |
| Catchment landuse proportions of: |  |  |  |
| Agriculture | 0 | 0.09 | 1 |
| Urban | 0 | 0.00 |  |
| Reach length ( $m$ ) | 1.90 | 821 | 35771 |
| Straight line distance to the sea (m) | 13636 | 85562 | 182776 |
| Straight line distance to next perennial lake (m) | 0 | 32442 | 118926 |
| Surface area of nearest lake (km2) | 0.52 | 6.13 | 56.78 |
| Straight line distance to nearest human settlement (m) | 69 | 8282 | 31398 |
| Proportion of catchment with roads | 0 | 0.01 | 0.51 |

influence of various environmental factors, providing a true watershed perspective.
Both local variables representing only the conditions occurring underneath each stream unit, and catchment variables representing average or cumulative conditions upstream of a given location in the stream network were used (Table 1). Four variable preparation processes were performed in ArcGIS 9.0® (ESRI Corporation) to derive the data layers: (1) clipping and resampling of raster grids to attain an equal grid extent and cell size across all layers; (2) calculation of positional metrics for each stream line in the stream and river coverage, which were then converted to individual rasters for each variable; (3) calculation of weighted flow accumulation to represent catchment proportions of geologies, soils, ecosystems, and upstream averages of catchment precipitation, temperature, slope and elevation; and (4) application of a river mask to exclude all pixels not aligned with a stream or river segment.

## Fish point occurrence data

Point occurrence data for all freshwater fishes were collected using three separate methods applied within the 14 study watersheds: backpack electrofishing, boat electrofishing, and interviews with fishermen. Back pack electrofishing involves the use of a pulsed electrical current coming from a pair of 12 volt motorcycle batteries. When the current is applied in the water, fishes are momentarily stunned and collected, then counted, and returned to the stream alive. Backpack electrofishing was used in wadable mountain streams only, where depths were frequently less than waist height. Boat electrofishing also uses a pulsed electrical current, but the current originates from a gas powered generator. Fishes are netted with long dip nets from the bow of the boat, placed in a live well, then counted and released. Boat electrofishing was used to sample fishes in non-wadable habitats of large deep rivers in the coastal plain. Electrofishing sites were selected by systematically selecting sites every 20 km along the river channel with a random starting point in the first 5000 river meters. Additionally, 38 interviews were conducted with fishermen about the presence of just African tilapias (a special topic in my dissertation) in their fishing grounds and voucher specimens were collected to confirm the identity of the species they were catching. Maps were used to help the fishermen identify the areas where they catch tilapia. For each river segment where a fish was reported as present, a point location was placed in the center of that segment.

## Model development

The model development process involves data reduction, training of the model, and validation of model results. As data analysis is ongoing, the process described here is currently being applied to the fish data collected using the methods described above.

Data reduction is necessary to eliminate redundant variables (e.g., variables carrying roughly the same information) and to yield models that are easier to interpret. The goal of data reduction is to retain no more than 10 variables that characterize the range of different environmental characteristics at all sites sampled in the watersheds (whether or not tilapias were captured there). Principal Components Analysis (PCA) is being used to assist with the variable reduction process. PCA is a statistical approach that identifies variables that are intercorrelated. The approach allows for an assessment of redundancy between variables (e.g., if 3 variables correlated strongly with the first axis, they carry the same information and can probably be reduced to only one). PCA is being performed using PC-Ord software $®$ (McCune and Mefford 1999).

After variables are reduced, the MaxEnt model is parameterized. A model for any given fish species is run for 500 iterations, using 10,000 randomly selected background points, a convergence threshold of 0.00001 , and a regularization factor of 1 (see Phillips et al. 2006 for more details). Thirty-three percent of presence points are withheld from the data set for any given species to test the accuracy of the model. The jackknife option was selected to assist with the interpretation of variable importance to the model outcome.

Three tests are used to evaluate the performance of each model: (1) the area under the curve of the receiver operating characteristic plot (ROC); (2) test prediction success; and (3) a one-tailed binomial test. A ROC plot is created by plotting the fraction of true positive predictions against the fraction of false-positive predictions across all available decision thresholds (a threshold is a point along the curve above which you assume the species is present, and below which it is assumed not present or of unknown presence). A ROC curve that maximizes true positives at low values of the false-positive fraction is considered a good model and can be quantified by calculating the area under the curve (AUC; Fielding and Bell 1997). The AUC is considered a measure of the model's overall performance and usually has values ranging from 0.5 to 1.0 ,
where a score of 0.5 implies that the predicted probability distribution does not discriminate any better than a random probability distribution, and 1 indicates that the model can discriminate between true and false positive occurrences perfectly. The other two metrics of model performance require the selection of a threshold value, which is used to classify the range of habitat suitability values into two categories (present/absent). Once a threshold is selected two additional performance metrics are applicable. Test prediction success is the percentage of sites in the test dataset that were successfully predicted as present by the model at the given threshold. The second metric, a one-tailed binomial test, determines whether a model predicts the test localities significantly better than random.

## Summary of findings (including analyses)

Baseline fish dataset
Baseline data were collected for freshwater fishes with electrofishing, and for tilapia by interviewing fishermen. A total of 24,595 fishes were collected using electrofishing at 76 different localities within the study watersheds (Figure 1). Out of the fishes captured, 79 different species were identified (Table 2). Of these, 4 are new records for the freshwaters of Belize (Marbled swamp eel, Synbranchus marmoratus, Crevalle jack, Caranx hippos, Figure 2, blue tilapia, Oreochromis aureus, and guapote cichlid Parachromis loisellei), and 2 are not native to Belize (Nile tilapia, Oreochromis niloticus, and blue tilapia, Oreochromis aureus). This brings the total number of fishes captured in freshwater habitats in Belize to 124. Vouchers for most species captured were preserved and deposited at the University of Michigan Museum of Zoology.

Combined species richness numbers for all the study watersheds reveal some interesting patterns in fish biodiversity (Table 2). Despite the fact that only 5 sites were sampled in the Monkey River, the most species were captured in this river system ( $\mathrm{n}=47$ ). The second most species were captured in the Belize River ( $n=44$ ), where 32 sites were sampled. The Sibun River had the third most diverse assemblage with 41 species captured at 10 sites (Table 2). It is somewhat surprising that so few species were captured in the Belize River given (a) the welldocumented relationship between the number of species and watershed area, and (b) the intensity of sampling effort that was expended there. However, it is unlikely that all species present were captured because of limits to the types of habitats where electrofishing is effective, thus these numbers should be interpreted conservatively.

Two sites of the 76 had unusually low species diversity relative to expectations based on their locations and habitat quality. It is important to flag these sites, because low fish species diversity often reflects strong ecosystem


Figure 2. This Crevalle jack (Caranx hippos), an "ocean" jack, was captured a long way from salt water. This fish was found on 26 April 2006 in the Sibun River, just below the Coastal Road bridge. stresses. The first site is located approximately 1.5 km upstream of the Hawkesworth Bridge in San Ignacio. This site was sampled on 19 May 2006, when only 11 species were captured. A normal sample in a large river habitat like this should have yielded between 18-22 species. It is possible that hydrologic variability and water quality degradation from upstream hydropower facilities have degraded ecosystems in this area. The second site was located several kilometers upstream from the Labouring Creek mouth in the

Table 2. List of all species captured during this study, and their presence ( 1 , shaded) or absence (0) in study watersheds. Watersheds are arranged from most to least species (left to right), and the species are arranged from most to least frequent (top to bottom). MON = Monkey R., BEL = Belize, SIB = Sibun, $\mathrm{MOH}=$ Moho, NST $=$ N. Stann, TEM $=$ Temash, GOL $=$ Golden, $\mathrm{HON}=$ Hondo, NEW = New, MAN = Manatee, SIT = Sittee, SST = S. STann, MUL = Mullins.

| Family | Species name | MON | BEL | SIB | MOH | NST | TEM | GOL | HON | NEW | MAN | SIT | SST | MUL |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Characidae | Astyanax aeneus | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Poeciliidae | Belonesox belizanus | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Cichlidae | Cichlasoma salvini | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Eleotridae | Gobiomorus dormitor | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Synbranchidae | Ophisternon aenigmaticum | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Poeciliidae | Poecilia mexicana | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Cichlidae | Amphilophus robertsoni | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Cichlidae | Archocentrus spilurus | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| Gerriidae | Eugerres plumieri | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| Poeciliidae | Gambusia luma | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Poeciliidae | Heterandria bimaculata | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Pimelodidae | Rhamdia guatemalensis | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| Cichlidae | Cichlasoma octofasciatum | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| Eleotridae | Eleotris amblyopsis | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| Characidae | Hyphessobrycon compressus | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Pimelodidae | Rhamdia laticauda | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| Cichlidae | Vieja synspilum | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Gerriidae | Eucinostomus melanopterus | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Cichlidae | Petenia splendida | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 |
| Atherinidae | Atherinella sp. 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Eleotridae | Eleotris pisonis | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Lutjanidae | Lutjanus griseus | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Cichlidae | Thorichthys meeki | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| Poeciliidae | Xiphophorus helleri | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Mugilidae | Agonostomus monticola | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Centropomidae | Centropomus undecimalis | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haemulidae | Pomadasys crocro | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 |
| Gobiidae | Awaous banana | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| Cichlidae | Cichlasoma urophthalmus | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Cichlidae | Oreochromis niloticus | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Cichlidae | Parachromis friedrichsthalii | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Ariidae | Sciades assimilis | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Belonidae | Strongylura timucu | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Cichlidae | Vieja maculicauda | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Sciaenidae | Bairdiella ronchus | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Characidae | Brycon guatemalensis | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Centropomidae | Centropomus parallelus | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Centropomidae | Centropomus pectinatus | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |


| Family | Species name | MON | BEL | SIB | MOH | NST | TEM | GOL | HON | NEW | MAN | SIT | SST | MUL |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Hemiramphidae | Hyporhamphus roberti | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Lutjanidae | Lutjanus jocu | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Syngnathidae | Microphis brachyurus | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anguillidae | Anguilla rostrata | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| Centropomidae | Centropomus ensiferus | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clupeidae | Dorosoma petenense | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Poeciliidae | Gambusia sexradiata | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Megalopidae | Megalops atlanticus | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mugilidae | Mugil curema | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Poeciliidae | Poecilia petenensis | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Cichlidae | Cichlasoma bocourti | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Clupeidae | Dorosoma anale | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gerriidae | Eugerres brasilianis | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gobiidae | Evorthodus lyricus | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Poeciliidae | Gambusia yucatana | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Ictaluridae | Ictalurus furcatus | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Carangidae | Oligoplites saurus | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Synbranchidae | Synbranchus marmoratus | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| Cichlidae | Thorichthys aureus | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cichlidae | Vieja godmani | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cichlidae | Vieja intermedium | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Engraulidae | Anchoa belizensis | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Engraulidae | Anchoa lamprotaenia | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Engraulidae | Anchovia sp. | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Carangidae | Caranx hippos | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Poeciliidae | Carlhubbsia stuarti | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Paralichthyidae | Citharhichthys spilopterus | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gerriidae | Diapterus auratus | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gerriidae | Diapterus rhombeus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Eleotridae | Dormitor maculatus | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mugilidae | Joturus pichardi | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gobiidae | Lophigobius cyprinoides | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cichlidae | Oreochromis aureus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Cichlidae | Parachromis Ioisellei | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Poeciliidae | Phallichthys fairweatheri | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Poeciliidae | Poecilia orri | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Poeciliidae | Poecilia teresae | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rivulidae | Rivulis tenuis | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Achiridae | Trinectes paulistanus | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Poeciliidae | Xiphophorus maculatus | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|  | Total Species | 47 | 44 | 41 | 38 | 32 | 31 | 29 | 28 | 26 | 23 | 21 | 20 | 16 |

Belize River drainage. This site, sampled on 14 April 2007, was virtually fishless, with only 16 individuals captured in several hours of fishing, and 8 species total. Typically, this type of habitat would yield between $300-500$ individuals and 18-24 species. These numbers are alarmingly low. It is uncertain what stressors may be present upstream of the Labouring Creek mouth, but it is worth investigating.

In terms of the distinctiveness of fish assemblages in different parts of the country, the Temash and Moho Rivers differed strongly from their more northern counterparts, including other rivers in the Toledo District such as Golden Stream and Monkey River. Based on general observations from the field, there seems to be a high degree of faunal turnover (species lost and gained) somewhere between Golden Stream and Moho River. In particular, the cichlid assemblage (the richest single family in the entire study with 17 different species captured) shows a high degree of distinction, with the southern fauna picking up species like the guapote cichlid (Parachromis loisellei), the chisel tooth cichlid (Cichlasoma boucourti), the southern checkmark cichlid (Vieja godmani), and the golden firemouth cichlid (Thorichthys aureus), while losing species like the bay snook (Petenia splendid), crana (Cichlasoma urophthalmus), and firemouth cichlid (Thorichthys meeki). This species turnover has potentially important implications for biodiversity conservation and should be studied more extensively in the future (See Recommendations below).

The baseline dataset generated by this project provides an important benchmark in time with which to monitor the future of ongoing biological invasions. Of particular interest is the ongoing invasion of Belizean waterways by the Nile tilapia (Oreochromis niloticus). Nile tilapias were captured at 20 sites with electrofishing gear, in another 50 river segments based on interviews with 38 fishermen (Figure 3a). These data were used to create a map of habitat vulnerability using the modeling technique described above (Figure 3b), which can now be used to plan for the future potential range of tilapias in Belize. From this habitat suitability prediction, it can be hypothesized that almost all coastal plains rivers are vulnerable to colonization to tilapias with a few exceptions (e.g., the smaller rivers of Port Honduras). This does not mean that they will all be dominated, but that tilapias will be able to establish populations in many coastal plain aquatic habitats.

## Fish species distribution models

As mentioned in the Methods, the ecological modeling approach employed in this study uses species occurrence points in conjunction with GIS layers about environmental conditions in watersheds to predict those habitats that are suitable for species. Having this predictive capacity gives resource managers a tool with which to visualize and understand their species of interest, and the approach is flexible in that it only requires presence localities and thus is amenable to mixing of data sets even if they were collected with different methods. In order to develop the strongest species range predictions possible for the fishes of Belize, the dataset collected in this project was combined with data from past projects in Belize and international portions of Belize's watersheds.

The dataset from the current project (126 sites including tilapia localities) were combined with past data collected in the Mexican portion of Rio Hondo ( 65 sites) and from the Belize collections done in the 1970s by Greenfield and Thomerson (109 sites). All of these were digitized and georeferenced in ArcGIS in preparation for modeling. This brings the total number of localities available for use in models to 300.

For 87 out of the 124 species that occur in the study area, sufficient point data are available to develop range models ( 5 or more points are necessary to develop a model). These point data


Figure 3. (a.)Map showing locations where tilapias were sampled during this study (left). (b) Map of habitat vulnerability to colonization by tilapias. (c) Presence/absence prediction which will allow for prediction of species richness when models for many species are combined.
are currently being used in conjunction with a subset of the 30 environmental variables (Table 1) to create MaxEnt models for each species. This process is in progress, and will be presented as part of my dissertation by the end date of my grant agreement with PACT (May 30 ${ }^{\text {th }}, 2008$ ).

For each species with sufficient point data, a model will be generated to predict (a) the suitability of habitats for the species on a continuous scale of 0-100 (e.g., Figure 3b), and (b) a prediction of range (presence or absence) (e.g., Figure 3c). While these predictions are themselves interesting for understanding the potential distributions of individual species the ultimate goal is to demonstrate how these prediction of fish biodiversity can be utilized to select a network of the highest priority freshwater conservation sites. To get to a stage where selection of freshwater conservation sites can occur, presence/absence predictions from all of the fish species for which sufficient data exist must be generated and summed together to provide a national prediction of richness. This approach is demonstrated in Figure 4 using several widely distributed cichlid species as an example.

To use this richness prediction to determine possible freshwater conservation sites, an approach similar to the one used by Meerman and Wilson (2005) in their Protected Area System Assessment and Analysis will be used. This approach (called MARXAN) relies on an algorithm that combines a "value" surface (e.g., fish richness), with a "cost" surface (e.g., human development in a watershed) to select a network of localities that does the best job conserving biodiversity relative to human pressures. Whereas Meerman and Wilson (2005) divided the country into polygons to implement this approach for terrestrial and marine systems, the current effort will focus on catchments (the portion of the watershed associated with each stream segment) to conduct the analysis so as to maintain a watershed/aquatic focus.


ENVIRONMENTAL DATA


Development of species distribution models

FISH OCCURRENCE DATABASE


FISH SPECIES RICHNESS PREDICTION
 FOR 80+ SPECIES

Selection of potential freshwater conservation sites

Figure 4. Schematic of the process for developing a fish richness surface for use in defining a network of freshwater conservation sites.

## Recommendations for further study

## "Objective 3: The national protected area system is comprehensive.

Strategy: Those areas required to obtain a fully comprehensive national protected area system are brought within its scope" (Meerman and Wilson 2005).

To satisfy the third objective of the NPASP it will be necessary to ensure that the protected areas system serves as many components of Belize's biodiversity as possible. Until this study, there has been no comprehensive analysis of freshwater biodiversity at a national scale, and there has yet to be a comprehensive analysis of how well the current protected areas network functions for freshwater biodiversity and protection of ecosystem services. To make the national protected area system truly comprehensive, such assessments will be necessary.

With Objective 3 of the NPASP as a backdrop, I recommend the following future research:
4. National baseline inventories of non-fish freshwater taxa. Fishes are a crucial component of freshwater biodiversity and this work provides a good starting point for national planning around freshwater biodiversity protection. However, this work is just that-a starting point. There are likely to be several hundred macroinvertebrate species inhabiting the freshwaters of Belize, and potentially hundreds of species of primary producers (diatoms and other algae species). Thus fishes are an important but limited starting point. Future efforts should be funded to-both literally and figuratively-put these other important aquatic taxonomic groups on the map.
5. Identification of distinctive freshwater biogeographic zones. As important as it is to identify species by sampling systematically in habitats across the country, it is important to use these data to infer faunal distinctiveness- those areas with unique assemblages and unique evolutionary trajectories. The current research will yield hypotheses about why the fauna from the Moho River south is distinctive from the northern fauna, but future research will be necessary to firm up these hypotheses. This will likely require field sampling focused specifically on hypothetical boundary areas between faunal zones.
6. An assessment of the functionality of the current protected areas system for protection of freshwater biodiversity and services. Much as Meerman and Wilson (2005) evaluated gaps in the current protected areas system for terrestrial and marine habitats, a freshwater perspective must be added to the national analysis. The important research question is: How well does the current protected areas network function for freshwater biodiversity and ecosystem service protection? This is the most fundamental question that needs to be answered before freshwater ecosystems can be incorporated into our thinking on protected areas management in Belize.

The current study, as it reaches a final form in my dissertation and published works, will begin to address these priorities with an important part of freshwater biodiversity, but more investment is needed to ensure that "The national protected area system is comprehensive".

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